STUDIES ON THE POPULATION BIOLOGY OF *COLLETOTRICHUM COCCODES* USING AFLP AND SCAR MARKERS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

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Black dot is a blemish disease of potato caused by the fungus *Colletotrichum coccodes* (Wallr.) Hughes. with worldwide distribution. It occurs in Africa, Asia, Australia, Europe, North America, South America, and Central America. *C. coccodes* has no known sexual cycle, but genetic exchange is possible through vegetative conjugation. The determination of VCG among fungal isolates is usually based on the complementation tests with nitrate nonutilizing (*nit*) mutants which enables researchers to compare strains of pathogens. Eight VCGs have been identified for the Europe/Israel population, seven for the North American population, and six for the Australian population. Variation in aggressiveness, morphological traits, and physiological traits have been detected among the different *C. coccodes* VCGs. AFLP markers have been used also to study relationships within and among North American VCGs. With this method, five VCGs were separated from *C. coccodes* isolates, coinciding almost completely with the six VCGs of *nit* mutants. Additionally, a relationship of specific AFLP bands to corresponding VCGs was reported using AFLP analysis. The objectives of this study were: to develop VCG-specific sequence characterized amplified regions (SCAR) markers, to study the population biology of *C. coccodes* of North America using the AFLP method, and to study genetic diversity of the global populations of *C. coccodes*. A total of 88 isolates representing the different *C. coccodes* VCGs were analyzed via the AFLP method to generate SCAR markers. A total of 47 primers were designed and evaluated in PCR reactions. Only one primer, AGb6F/R, with 156 bp amplification PCR product was found to be specific for NA-VCG6 and NA-

VCG7 of *C. coccodes*. For the second objective, 210 loci were generated and used to cluster the isolates into their NA-VCGs and to test the genetic structure of the North American population of *C. coccodes*. *C. coccodes* isolates recovered from potato plants were assigned to four NA-VCGs: NA-VCG1, NA-VCG2, NA-VCG4/5, and NA-VCG6/7. No isolates tested belonged to NA-VCG3. NA-VCG2 was the dominant group in the population (n=238) and was the most frequently detected NA-VCG among states, fields, farms, and plants. However, in several instances there was more than one NA-VCG recovered from the same plant, field, farm, and state, indicating variability within the *C. coccodes* population in United States. Genetic differentiation among the nine states was 0.331, and overall gene flow for the 366 isolates was (N_m =1.01). For the genetic diversity of the global *C. coccodes* population, 855 isolates were studied. Three primer pairs were used and generated 210 loci. Based on this study, the population of *C. coccodes* exists as one large population with four main groups (NA-VCG1/3; NA-VCG2; NA-VCG4/5; and NA-VCG6/7). NA-VCG5 was the most common VCG globally followed by NA-VCG2. Among the five regions studied, there was relatively low gene diversity (0.222). The overall gene flow (N_m) was 1.25, meaning that one or more individuals are exchanged among the five regions each generation and that the populations will gradually become similar. Most of the variation among the five geographic regions originated from within population differentiation. Among VCGs of *C. coccodes,* there was high VCG differentiation (G_{ST}=0.463), meaning there is a differentiation among the different *C. coccodes* VCGs and a population structure exists. AFLP analysis proved to be valuable in differentiating and studying the global population of *C. coccodes*.

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DEDICATION

This work is dedicated to

My affectionate mother, Fatimah Alananbeh, who always supported, encouraged, and prayed for me to succeed in my life.

To my beloved father, Mohammad Alananbeh, who always prayed and advised me to do the right things in my life and to depend on and think for myself. Whatever I do in my life will not be enough to pay off even a small part of what you gave and taught me.

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

LIST OF APPENDIX TABLES

LITERATURE REVIEW

The potato

The potato (*Solanum tuberosum* L.) is the fourth-most-consumed food crop after rice, wheat, and corn. The potato is an annual, herbaceous, and dicotyledonous plant. It grows in different soil types and climates, and it provides consumers with an inexpensive source of calories (USDA, 2009). World potato production has increased from 267.00 MT in 1991 to 325.30 MT in 2007. Asia and Europe are the world's major potato producing regions; accounting for more than 80% of production in 2007. Key-producers of world fresh potato production are China, Europe, Russia, India, USA, Canada, Ukraine, Poland, and Germany (FAO, 2008; Johnson et al., 2008). In the US, Idaho accounts for 33% of the 394 MHW of potato in 2009, followed by Washington (22%), Wisconsin (7%), Colorado (5.6%), North Dakota (5.4%), Minnesota (5.4), and Oregon (4.4%). This percentage includes both fresh and seed potatoes (USDA, 2010).

Potato originated in the Andes Mountains of South America, from the Lake Titicaca region, located near Peru and Bolivia (Johnson et al., 2008; Martin et al., 2006; USDA, 2009) and in Chiloe (Anynomous, 2010). It was introduced into Europe in the sixteenth century by the Spanish. By the seventeenth century, potato was distributed in many European countries, and it became an important and popular table food. In the US, potato was introduced in 1621 but it was not grown extensively until 1719 in New Hampshire. Potato is a cool temperature (21°C) crop. Ideal temperatures for tuber growth are 16-18°C. Intermediate photoperiods, cool temperatures, and sufficient nitrogen favor maximum tuber production (Martin et al., 2006). The most commonly grown potato is the tetraploid *Solanum tuberosum* L.

Propagation of potato from true seed is common in breeding programs and in some tropical climates. In commercial production, potato is propagated vegetatively via tuber pieces. Potato plants grown from tuber pieces give lateral stems arising from buds on the tuber adventitious root systems, stolons, and tubers. Stems are usually green, but can be red to purple. Leaves are compound, arranged in a spiral pattern around the stem. Different types of trichomes are present on leaves and on the above ground stems. True potato seeds produce slender taproots. Lateral roots initiate in the pericycle regions of root, and their emergence sites may serve as entry points for pathogens. Tubers form at the tip of the stolon. Its surface is covered with epidermis, on which stomata are scattered. Periderm develops below the epidermis, and it forms a protective layer over the surface of the tuber. Lenticels form below stomata and are a site of gas exchange. Flowers of the potato plant have five-parts; the corolla might be of various colors, white, yellow, pink, or lavender, with a single style and sigma. Fruits are oval, green, yellowish green or brown when they are mature, with two chambers (Stevenson et al., 2001). Potato plants have five growth stages: sprout development (Growth Stage I), vegetative growth (Growth Stage II), tuber initiation (Growth Stage III), tuber bulking (Growth Stage IV), and maturation (Growth Stage V) (Miller and Hopkins, 2008). The genetic diversity of wild and cultivated potato is greater than any other food crop. Cultivars produced today belong to three major skin types: white, red, and russet (Martin et al., 2006).

Potato is affected by many plant pathogens representing viruses, viroids, bacteria, nematodes, fungi, and phytoplasmas. Many fungal pathogens attack potatoes. For fungal pathogens *Fusarium* and *Pythium* affect potato at planting; *Rhizoctonia* and potato early dying syndrome affect crop growth; tuber quality is affected by common scab, powdery

scab, pink rot, leak, black scurf, *Fusarium* dry rot, black dot, and silver scurf (Powelson and Rowe, 2008). Insect pests also affect potato, mainly Colorado potato beetle, potato flea beetle, psyllid, tuber worm, aphids, corn maggot, leafhopper, and wireworms (Martin et al., 2006).

The disease: black dot

Black dot has been recognized as an important potato disease in Australia and Europe, and was first observed in North America in Canada in 1921. In the US, it was first reported in Indiana counties in 1974 (Stevenson et al., 1976). In North Dakota, the disease was reported for the first time in 1976 at the potato research farm in Grand Forks (Otazu et al., 1978). It is a blemish disease of potato caused by the fungus *Colletotrichum coccodes* (Dillard, 1992). Black dot refers to the abundant black sclerotia produced on tubers, stolons, roots and stems above and below ground (Andrivon et al., 1998; Dillard, 1992; Lees and Hilton, 2003). Early reports of the disease in potato and tomato date back to the early 19th century, and were described by Dickson (1926). These reports did not consider black dot as a serious problem in potato. However, there have been an increasing number of reports regarding disease incidence and crop damage caused by *C. coccodes* (Denner et al., 1998; Johnson, 1994; Johnson and Miliczky, 1993; Tsror (Lahkim) et al., 1999).

Yield reduction, weight loss, and reduced tuber numbers were observed as a result of foliage infection by *C. coccodes* (Johnson, 1994) and the incidence of black dot is increased by seed tuber infection (Johnson et al., 1997). Significant yield reductions (22-30%) were observed in many potato cultivars in a study conducted by Tsror (Lahkim) et al. (1999). Incidence of black dot reached 40% more in progeny of untreated seed planted in unfumigated soil (Denner et al., 1998), confirming the importance of soil-borne inoculum

of *C. coccodes*. In another study, high disease incidence; up to 50% and 90% in two different years were found in certified potato seed tubers collected from different geographic areas in western North America (Johnson et al., 1997). Yield losses may also result from potato early dying (PED). This syndrome is associated with complexes of soil-borne pathogens including *Verticillium dahliae* (Kleb.), *C. coccodes*, *Pratylenchus spp.*, *Erwinia carotovora* subsp. *carotovora* (Ecc), and *Rhizoctonia solani* Kuhn (Lees and Hilton, 2003; Powelson and Rowe, 1993; Rowe et al., 1987). *Verticillium* is the main causal agent for this syndrome; recently a Q-PCR approach was developed to detect and quantify this fungus (Atallah et al., 2007). PED is characterized by chlorosis, wilting, stunting, and reduced yield (Powelson and Rowe, 1993; Rowe et al., 1987). Co-inoculating *C. coccodes* with other fungi such as *V. dahliae* caused more disease than having black dot alone on certain potato cultivars (Tsror (Lahkim) and Hazanovsky, 2001). *C. coccodes* sclerotia were present in all plants inoculated with *C. coccodes* alone or in combination with *V. dahliae*, but microsclerotia of *V. dahliae* were not observed in the four cultivars tested. Co-inoculation with both fungi enhanced disease symptoms in some potato cultivars, but not in all. Black dot not only affects potato yield and quality, but also contaminates soil and serves as an important source of inoculum for future potato crops (Tsror (Lahkim) et al., 1999). *C. coccodes* has been found over the entire surface of tubers and in the medulla, and it was isolated more frequently from the stem end than from the bud end or lateral section of infected tubers (Johnson et al., 1997).

Distribution and host range. Black dot occurs in Africa, Asia, Australia, Europe, North America, South America, and Central America (Andrivon et al., 1997; Raid and Pennypaker, 1987; Rich, 1983). Tsror (Lahkim) et al. (1999) found that up to 34% of

potatoes imported from Holland in 1998 were contaminated with black dot. Read et al. (1995) also found in a survey in the UK in 1989-90 that black dot, silver scurf, and black scurf occurred in 75, 86, and 85%, respectively. Black dot symptoms were observed in 37 soil samples, and it was more frequent on roots than on tubers, in a survey conducted by Andrivon et al. (1997) in French potato crops. *C. coccodes* major hosts are potato, tomato (Ben-Daniel et al., 2009; Dillard and Cobb 1997), and pepper (Hong and Hwang, 1998), on which it causes black dot and anthracnose, respectively. *C. coccodes* has a wide host range including at least 58 species in 17 families, but primarily affect Leguminoceae, Solanaceae, Cucurbitaceae (Dillard, 1992; Raid and Pennypacker, 1987) and Fabaceae (Dillard, 1992). It also affects symptomless hosts (Dillard, 1992) and numerous species of weeds that act as alternative hosts (Raid and Pennypacker, 1987).

Symptoms. On the potato plant; symptoms develop first on underground organs then on aboveground stems (Andrivon et al., 1998). The first field symptoms are yellowing at the upper leaflets, followed by an upward rolling of leaves. Plants become stunted and mature early (Rich, 1983). Infection of potato tubers may result in silvery lesions (Rich, 1983) with poorly defined margins (Powelson and Rowe, 2008). These lesions resemble silver scurf symptoms (Pavlista et al., 1992), and can be observed at the stem end of the infected tubers. Development of the fungus on the tuber surface is characterized by production of black sclerotia (Rich, 1983). Sclerotia can also be found on roots, stems and stolons (Lees and Hilton, 2003; Pavlista et al., 1992). Producing sclerotia is the most important sign of *C. coccodes,* appearing on the below ground stem tissue. In storage, the remnants of infected stolons may remain attached to the tubers and aid in identifying the fungus. Thin-skinned cultivars are more susceptible to the disease than thick-skinned ones

(Pavlista et al., 1992). Symptoms of the fungus on foliage are associated with wounds (Johnson, 1994) and appear as water or deep sunken lesions that turn dark brown to black with time (Andrivon, 2004; Glais-Varlet et al., 2004). Lesions may look similar to those of early blight (Powelson and Rowe, 2008). Infected plants may appear wilted (Johnson, 1994; Rich, 1983) with dying foliage (Powelson and Rowe, 2008). Physiological events associated with plant senescence activate fungal growth and plant colonization of *C. coccodes* (Nitzan et al., 2006).

On tomato fruit, symptoms begin as dark small sunken lesions that have water-soaked appearance. With time these lesions increase in size and form large sunken soft area with the center became darker (Ben-Daniel et al., 2009, Byrne et al., 1997). Under favorable conditions, lesions on ripe fruit become visible after infection by 5 to 6 days (Byrne et al., 1997). On pepper, initial symptoms appear as light brown flecks, which later become slightly sunken, gradually increase in size, and form larger lesions and blights on leaves (Hong and Hwang, 1998).

Life cycle. Sclerotia are the survival structure in soil and on infected seed tubers (Powelson and Rowe, 2008), and are the source of inoculum that spread the pathogen to new fields (Pavlista et al., 1992). During the growing season, and when environmental conditions are favorable, fruiting bodies called acervuli develop from sclerotia and produce conidia that infect the below ground tissues of the potato plant (Powelson and Rowe, 2008). Conidia spread by splashing rain can infect the foliage of the plant by entering wounds caused by blowing sand. The infection remains latent untill the plant becomes senescent or stressed (Powelson and Rowe, 2008). Later, sclerotia are produced and the fungus population builds to a high level in the soil (Pavlista et al., 1992). Soil borne

inoculum of *C. coccodes* has higher potential in causing disease than tuber borne inoculum, and, severity of black dot has a sigmoid trend with soil borne inoculum (Nitzan et al., 2008). In one study, *C. coccodes* was isolated from the roots of 97% of plants at the postharvest sampedg (162 days after transplanting (Dillard and Cobb, 1997). When soil-borne inoculum exceeded 0.5-1.7g/L, disease severity does not continue to increase (Nitzan et al., 2008). Survival of conidia and sclerotia in dry and moist soil varies. Conidia have a short life in soil, just a few days, and they are sensitive to dry soil (Farley, 1976) and sclerotia remain viable for 8 years (Dillard and Cobb, 1998).

Infection process. Generally, for *Colletotrichum* spp., the first essential feature of successful pathogenesis is the attachment of the propagules to the plant surface. After that, conidia germinate to undergo differentiation to form appresoria that are essential for infection. Several modes of penetration of plant surface are possible: through natural openings and through direct penetration, which is the most common (Bailey et al., 1992). Penetration usually occurs after formation of an appresorium and penetration peg (Shen et al., 2001), and it may require enzymes to dissolve the host cuticle (Bailey et al., 1992). Appresorium formation is affected by temperature, and it is best formed at 16 and 22°C (Sanogo et al., 2003). Successful penetration of intact tissue is affected by appresorial melanization which allow a build up of the internal hydrostatic pressure necessary for cuticle penetration (Byrne et al., 1997; Kubo and Furusawa, 1998; Prusky and Plumbley, 1992). An infection vesicles formed in the penetrated host cell after 48 hr, and out of this, an infection vesicle grows (Shen et al., 2001). The interaction at this point is biotrophic. Thin secondary hyphae arise from the infection vesicles which penetrate the host cell wall and begin the necrotrophic phase (Bailey et al., 1992; Khan and Hsiang, 2003; Latunde-

Dada et al., 1997; Shen et al., 2001; Wharton et al., 2001). The infection process has been classified as either intracellular hemibiotrophic or subcuticular intramural, or a combination of both strategies (Bailey et al., 1992). Acervuli form on the plant surface with single melanized seta (Shen et al., 2001).

For *C. coccodes*, environmental factors affect development and sporulation of the fungus. Temperature, aeration, light, and wetness influence lesion development. The first study that reported the influence of temperature, aeration, and light on the occurrence and magnitude of mycelogenic and sporogenic germination of *C. coccodes* was conducted by Sanogo and Pennypacker (1997). They used sealed and non-sealed PDA-plates to resemble buried and exposed sclerotia to the environment respectively. Their results showed that temperature, aeration, and light have significant effect on sclerotial germination. Mycelia and spore germination of sclerotia occurred over a wide range of temperature (10-24°C) (Sanogo and Pennypacker, 1997). The pathogen is able to develop at low temperatures in controlled conditions, and this means that black dot symptoms can increase during storage if storages are not adequately managed (Glais-Varlet et al., 2004). The conidia and mycelia production on sclerotia are more sensitive to light and aeration than to temperature. Tsror (Lahkim) (2004) found that light duration plays an important role in *C. coccodes* infection, and may be one of the major factors responsible for the severe expression of black dot and yield losses observed in the Israeli autumn season.

The period of time necessary for conidial germination and the infection process for *C. coccodes* on tomato foliage under controlled environmental conditions has been established (Byrne et al., 1997). Results provided a chronological guide of the events involved in the infection of tomato by *C. coccodes*. Much of the infection process was

completed before 19 hr after incubation. Conidia germinated within 2 hr of inoculation. Unmelanized appresoria formation peaked after six hrs of inoculation, and the fungus successfully infected the foliage after 22 hr of inoculation, indicated by the presence of the infection vesicles (Byrne et al., 1997). Highest proportion of conidia that form appresoria was maximum at 24 °C, and it was favored by relatively low temperature (16-22 °C) (Sanogo et al., 2003). Carbon concentration and C: N ratio also affects growth, conidiation, sporulation and efficacy of *C. coccodes*. Both mycelium dry biomass and spore yield increased with increasing carbon concentration and C: N ratio (Yu et al., 1998). More spores were highly produced with C: N ratio between 7.5:1 and 10:1. However, when C: N was 15:1 and carbon concentration is 20 g/L, spore yield and mycelium biomass decreased significantly (Yu et al., 1998). Conidia germinated and produced mycelium after three days at room temperature (Glais-Varlet et al., 2004). Sclerotia and acervuli were produced 21 days after inoculation at 10 and 15 °C (Glais-Varlet et al., 2004). Calcium/calmoduline-dependant signaling pathway plays an important role on conidial germination and appresorium formation (Ahn et al., 2003). Lesions do not develop at 7 °C, and the greatest lesion diameter was found to be at 25-31 °C (Byrne et al., 1998; Dillard, 1992; Glais-Varlet et al., 2004). Wetness duration can increase disease severity and peaked at 48 hr (Dillard, 1989; Hong and Hwang, 1998).

The pathogen*: Colletotrichum coccodes*

Colletotrichum is an important fungus that causes pre- and post-harvest disease called anthracnose. There have been about 900 species assigned to this genus. Previously, *Vermicularia* was the name that was used interchangeably with *Colletotrichum*. This fungus has a telemorph stage named *Glomerella*. There have been eighty species have been

described in this genus, but only 20 of them have been reported with *Colletotrichum* anamorphs. Thirty nine species of this genus were accepted and described by Sutton in a review for the genus *Glomerella* and its anamporph *Colletotrichum* (1992).

Colletotrichum coccodes (Wallr.) Hughes, is a cosmopolitan pathogen (Dillard, 1992). It is considered to be a weak pathogen of potato roots (Otazu et al., 1978; Raid and Pennypacker, 1987) and is found in conjunction with stress conditions such as water, temperature, or due to other pathogens (Otazu et al., 1978). It was initially called *Vermicularia varians* in 1908 by Ducomet. There are numerous other synonyms for the black dot fungus such as: *C. tabificum* (Hallier), *Rhizoctonia tabifica* Hallier, *C. solanicolum* O'Gara, *Sclerotium setosum* Bewley and Shearn, *C. biologicum* Chaudhuri, *and C. atramentarium* (Berk. & Broome) Taubenh. (Dickson, 1926). *C. coccodes* is now being studied as a biological control agent for the weed velvet leaf *Abutilon theophrasti* Medicus due to longevity of the sclerotia in soil and the potential for increased inocula from colonization of velvet leaf (Dauch et al., 2003). *C. coccodes* can kill the weed when it is applied to the young seedlings (Dauch et al., 2003). Sclerotia of *C. coccodes* can remain viable from 8 yrs (Dillard and Cobb, 1998) to 13 yrs (Cullen et al., 2002). *C. coccodes* was isolated and identified from cysts and eggs of potato cyst nematode *Globodera rostochiensis* (Woll.) Skarbilovich and was found to survive as sclerotia on the cysts. It penetrated the cyst through natural openings causing 100% infection in all mature and immature cysts (Saifullah, 2000).

Morphology. Mycelium arises from a single spore with white, silky, superficial appearance. It spreads at a rate of about 8 mm/day at 12°C. Conidial masses show apricot yellow color in media of high sugar content (Dickson, 1926). The conidia were described

by Dillard (1992) to be cylindrical with obtuse ends, hyaline, aseptate and 16-24 X 2.4-4.5 μm in diameter. After the identification of six North American vegetative compatibility groups (NA-VCG's) of the fungus; differences were detected among them in conidial morphology. Parameters of conidial length, width, and length/width ratios were found to be significantly different among VCG's and medium type. Means ranged between 12.75 to 16.43, 2.99 to 3.41, and 4.15 to 4.83 μm for conidial length, conidial width, and length/width ratios, respectively, among the different VCG's using two types of medium (CV8 and PDA) (Aqeel et al., 2008). NA-VCG1 produced longer and wider conidia than other VCG's, NA-VCG2 produced shorter conidia, while NA-VCG5 produced the narrowest conidia. Across all NA-VCGs, conidia produced on CV8 were more elongated compared to the smaller and rounded conidia that were produced on PDA media. Conidia were described to be formed in orange masses, fusiform, and slightly constricted in the middle and tapered to each end (Cano et al., 2004; Sutton, 1992). Conidiophores develop free or in palisade layers and they are cylindrical, and tapering to slightly clavate, some are curved, sometimes branched (Dickson, 1926). Appresoria are cinnamon buff, ovate or elliptical, occasionally irregularly lobed, and borne on hyaline thin walled sigmoid supporting hyphae (Dillard, 1992; Sutton, 1992).

Acervuli are produced on stems, roots, and fruit. They are elongate, 200-300 μm in diameter (Dillard, 1992). In culture, acervuli are not produced (Cano et al., 2004). Sclerotia begin to appear in concentric rings in a media of high sugar content, and the superficial mycelium becomes less apparent until the whole plate is covered with sclerotia (Dickson, 1926). Sclerotia are produced in culture in high amounts (Dillard, 1992). Sclerotia are composed of three layers (Dillard, 1992). The development and structural

organization of sclerotia were studied at the ultra-structural level using scanning electron microscopy (Tu, 1980). There was no difference in the *in vitro* or *in vivo* ontogeny of the sclerotia. The colony surface of *C. coccodes* is covered with a thin film of mucilage. Beneath it acervuli were initiated. The stroma initiated by short, dark hyphal cell, differentiated into conidiophore and conidia beneath the mucilageneous film, and then a small sclerotium formed. Regardless of its size, sclerotia consist of three layers: outermost, sclerotized; the central-living, thick-walled cells; and the innermost, loosely, living hyphal cells. Isolates of *C. coccodes* vary in their sclerotial size (Dillard, 1992) and this is likely due to differences among VCGs. After the identifications of North American vegetative compatibility groups (NA-VCG) of the fungus (discussed below), significant differences in microsclerotial morphology among the six NA-VCGs grown on CV8 and PDA were reported (Aqeel et al., 2008). Isolates belonging to NA-VCG2 had longer (185.31 μm) and wider (167.03 μm) sclerotia compared to the other NA-VCGs while sclerotia produced by isolates belonging to NA-VCG5 were significantly shorter (118.2 μm) and narrower (112.13 μm) than those produced by any other NA-VCG. In addition to that, both sclerotial lengths and widths were found to be smaller when grown on PDA (135.75 μm long × 123.68 μm wide) compared to CV8 (171.46 μm long × 159.97 μm wide). Based on length and width of the sclerotia, Aqeel et al. (2008) classified sclerotia into four groups: length greater than 185 μm in NA-VCG2 isolates, length between 150 to 180 μm in NA-VCG1, 4, and 6, length between 130 to 150 μm in NA-VCG3, and length less than 130 μm in NA-VCG5. Setae also vary in length, tapering from a swollen basal cell, hyaline at the tip turning a dark color with age. Setae do not always appear in culture, especially when sub-cultured for a long period (Dickson, 1926).

Taxonomy, detection, and identification. *Colletotrichum* species are placed among the most important post-harvest pathogens because of their ability to cause latent infections (Sutton, 1992). *C. coccodes* is an imperfect fungus belonging to Coelomycetes which are asexual fungi that produce their fertile hyphae in specialized structures called conidiomata. Conidiomata are mainly two types: (i) pycnidia and (ii) acervuli (Cano et al., 2004). *Colletotrichum* belongs to the order Melanconiales (Barnett and Hunter, 1998). The key morphological features which identify the genus are its acervular conidiomata. *Colletotrichum* encompasses numerous species and their identification is based mainly on determining the plant host (Cano et al., 2004; Sutton, 1992).

A semi-selective medium was developed for the isolation of *C. gloeosporioides* (Ekefan et al., 2000), and *C. coccodes* (Farley, 1972). Media specificity for *C. coccodes* primarily was based on the selective inhibition of microorganisms by adding antimicrobial agents, and on the development of brown-color sclerotia of the fungus. Low cost and effective medium for *C. coccodes* sporulation was produced (Yu et al., 1997). Soybean and casamino acids that were equal to V8 juice for sporulation of *C. coccodes* were used.

New techniques were developed to detect *C. coccodes* from soil by using conventional and quantitative real time PCR. Polymerase chain reaction (PCR) diagnostics offer the potential to allow rapid and accurate identification of diseases and to quantify the presence of a pathogen in pre-symptomatic potato stocks and in soil (Cullen et al., 2002). PCR assay was able to distinguish *C. coccodes* from *Helminthosporium solani* Durieu & Mont. (silver scurf), although the blemish diseases black dot and silver scurf produce similar symptoms on potato (Cullen et al., 2002). Sensitive and rapid PCR diagnostic assay for *C. coccodes* was conducted. Two PCR primer sets were designed to sequence the

ribosomal internal transcribed spacer (ITS1 and ITS2) regions for use in a nested PCR. The outer primers (Cc1F1/Cc2R1) were designed in conserved regions to be genus-specific for *Colletotrichum* spp., while the nested primers (Cc1NF1/Cc2NR1) were designed to be specific for *C. coccodes* based on a unique sequence in primer Cc1NF1. Ribosomal DNA-ITS1 and ITS2 sequence data are considered as informative molecular techniques to confirm *Colletotrichum* morphological characters (Sherriff et al., 1995) and to distinguish different *Colletotrichum* species (Cano et al., 2004) without using morphology to prove its efficiency (Hsiang and Goodwin, 2001; Thaung, 2008). Molecular techniques were used to diagnose different species of *Colletotrichum* species involved in causing infections in humans (Cano et al., 2004). *C. coccodes* was one of those species. An amplicon of 130-157 bp in length was obtained using the Col-F/R primer from 20 strains of *Colletotrichum* sp were studied. This amplicon was successful in identifying the genus. Molecular techniques were useful and important tools to complement the identification based on morphological criteria for that study. Denaturing gradient gel electrophoresis (DGGE), a simple, relatively cheap method for fingerprinting to study the ability to differentiate *C. circinans* Curzi and *C. coccodes* into two distinct species was used (Fagbola and Abang, 2004). Differentiation between the two species was based on the different migration of bands of the 18S in the DGGE. Bands of *C. circinans* migrated faster than those of *C. coccodes* (Fagbola and Abang, 2004). Isolate specific markers identified and converted to SCAR markers, and RAPD markers, were used to detect the biocontrol agent *C. coccodes* (183088) strain from other isolates of the fungus (Dauch et al., 2003). This isolate is a biocontrol agent used against the velvet leaf weed.

Vegetative compatibility groups (VCGs). In many fungi, sexual and vegetative heterokaryons are quite distinct from one another. Strains that are able to form successful sexual heterokaryon may not be able to form a successful vegetative heterokaryon and vice versa. Vegetative compatibility refers to the ability of individual fungal strains to undergo mutual hyphal anastomosis and form viable heterokaryons. Strains that are vegetatively compatible with one another are described as members of the same vegetative compatibility group (VCG) (Korolov et al., 2000; Leslie, 1993). A sexually reproducing population would be expected to have a high level of VCG diversity. Since sexual recombination does not occur, members of each VCG will form a genetically isolated subpopulation that will be subjected to standard population genetic forces such as migration, selection, mutation, and drift. VCGs are used as a diagnostic tool for plant pathogens that do not regularly recombine, and this is the most useful application of VCG for many plant pathologists. In asexual species, vegetative compatibility may serve as a means of genetic exchange. Differences at the *vic* loci effectively limit the exchange of genetic information to those individuals that belong to the same VCG. Most VCGs studied are stable through space and time, including laboratory manipulation (Leslie, 1993). In addition to providing insights into cellular biology and population biology of organisms, VCGs have a number of potential applications. VCGs can determine if field isolates are clones of a common progenitor, but they are not appropriate for determining if strains belong to different biological species or for assessing differences that occur above the species level. VCGs are useful for measuring genotypic diversity, but they are not useful for assessing the levels of allele frequencies (Leslie, 1993).

Testing isolates for VCGs is usually based on complementation tests with nitrate-nonutilizing (*nit*) mutants. When the hyphae of two different *nit* mutants anastomose to form stable heterokaryons a biochemical complementation takes place in the heterokaryon which allows them to utilize nitrate when it is the sole nitrogen source in the growing medium. The result of the complementation is visible to the naked eye as a prototrophic growth at the contact zone of the mutant's colonies (Nitzan et al., 2006). The use of *nit* mutants enables researchers to compare strains of pathogens. These techniques, coupled with virulence tests provide valuable information on the genetic diversity of the natural populations of the pathogen (Correll et al., 1987). The laboratory analysis of VCGs with complementary *nit*rate-nonutilizing (*nit*) mutants is technically simple and requires little more than basic microbiological materials (Correll et al., 1987). Classification of VCGs has been used for studying the genetic structure of populations of plant-pathogenic fungi, including *Fusarium oxysporum* Schltdl. (Correll et al., 1988), *Verticillium* spp (Koroloev et al., 2000; Tsror and Levin, 2003), *Cercospora kikuchii* Matsumoto & Tomoy. (Cai and Schneider, 2005), and *Colletotrichum* spp. (Brooker et al., 1991). John Puhalla (1985) did most of the work with vegetative compatibility (VC) in pathogenic fungi. He suggested that VC could be used to subdivide populations into different VCGs and that these subdivisions were correlated with pathogenicity. He assumed that pathogens rarely participate in recombination events that could lead to yielding new VCG phenotypes. By this, each VCG is considered a clone. *nit* mutants were used to test for vegetative compatibility among 21 strains of *F. oxysporum* (Puhalla, 1985). He found a correlation between VCG and forma specialis, in which, members of the same VCG belong to the same forma speciales.

Many studies have been conducted to characterize VCGs for different fungal pathogens. *F. oxysporum* f. sp.*cubense* (E. F. Smith) Snyder & Hansen was characterized for vegetative compatibility (Ploetz and Correll, 1988). Eleven VCGs were identified. A good correlation between VCG and race among isolates for which race was known was found. Two distinct VCGs: VCG01 and VCG02 for *V. albo-atrum* from different hosts and geographical locations were identified (Correll et al., 1988). *Verticillium dahliae* from cotton, potato, olive, eggplant, chrysanthemum and tomato from 12 sites in Israel, was also studied (Bao et al., 1998). Thirty three isolates were assigned to two VCGs: VCG I contained 15 isolates from cotton, eggplant, chrysanthemum and olive; and VCG II contained 18 isolates from potato, olive and cotton. *Verticillium dahliae* VCGs using nitrate-nonutilizing (*nit*) mutants were also studied in Israel (Korolev, 2000).

Fusarium oxysporium f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hansen from watermelon and infested soil in Maryland and Delaware were characterized by cross pathogenicity to muskmelon (Zhou and Everts, 2007). Four isolates designated as race 2 and belonging to VCG0082 were moderately pathogenic to eighteen muskmelon cultivars. Three VCGs were found: 0080, 0082, and 0083. VCG0083 was newly described. VCG 0080 was the most widely distributed group followed by VCG0082, while VCG0083 had a more limited geographic distribution. Each VCG was designated to different races. Race 2 was found in all three VCGs, implying the possibility of the derivation of one VCG from the other as a result of mutation in a *vic* gene. Isolates from more than one race within the same VCG and isolates from more than one VCG were recovered from single plants and fields. No significant differences in aggressiveness were found among isolates from different VCGs of the same race.

Twenty five isolates of *C. kahawae* Waller & Bridge collected from a wide range of geographical sources in Africa were used to study the genetic variation of this fungus using pathogenicity tests, vegetative compatibility grouping, as well as RFLP (Beynon et al., 1995). All the isolates tested were able to infect the hypocotyls of the coffee plants, but there was some variation between some isolates. Five VCGs were identified, and there was no relation between RFLP and the variation found in VCGs which indicated the existence of a telemorphic stage of the pathogen.

In another study, the genetic diversity and pathogenicity of *V. dahliae* VCGs among isolates from Spain and Israel were estimated (Korolev et al., 2001). A total of 111 isolates were used, 62 from Spain and 49 from Israel. VCG analysis was studied for Spain using nitrate non-utilizing (nit) mutants, and pathogenicity tests using stem injection and root-dip method were conducted for isolates from both countries. RAPD patterns generated by single-primer PCR were used to determine relatedness among 89 isolates from both countries. Isolates from Spain found to belong to VCG1 (Defoliating (D-pathotype)), VCG2A (Non-defoliating (ND)) and VCG4B. The ND pathotype in Israel was composed of VCG2B and VCG4B. Disease reactions in pathogenicity tests varied with isolate, cultivar and inoculation method, and there was a statistically significant isolate X cultivar interaction in disease severity. RAPD amplification of DNAs form the isolates from both countries produced an average of 64 bands per isolate. All the isolates from VCG1 (defoliating isolates) from Spain were uniform and grouped together in one cluster, while the other cluster included isolates from Israel and Spain together and were non-defoliating isolates.

Three VCGs for *Colletotrichum* were identified and associated with anthracnose of anemone (*Anemone coronaria* L.) (Freeman et al., 2000). These VCGs were ANE-A, ANE-F, and NL-12. They were confirmed as *C. acutatum* Simmonds. This study of *C. acutatum* from anemone illustrated the potential of VCG analysis to reveal distinct sub-specific groups within a pathogen population that appear to be genotypically homogeneous by molecular assays.

Tester strains were identified for conducting VCG complementation tests that can be used to characterize and begin to compare populations of *C. coccodes* worldwide. Twelve were identified for Europe/Israel population (Nitzan et al. 2002), and eight isolates were selected as VCG testers for the North American populations (Garzon et al., 2005; Nitzan et al., 2006). Four multimember VCGs were observed within *C. coccodes* from 110 isolates, indicating population differentiation and genetic diversity within subpopulations (Nitzan et al., 2002). In recent studies, four new VCGs for European isolates (Shcolnick et al., 2007), seven VCGs for North American isolates (Nitzan et al., 2006), and six VCGs for Australian isolates (Ben-Daniel et al., 2010) were identified for *C. coccodes*. Interestingly, isolates of *C. coccodes* from Europe, Australia, and North America do not complement each other and VCGs from each area appear to be distinct.

The vegetative compatibility grouping method was used to study many aspects in the fungal pathogens. It was used to study genetic relatedness (Brooker et al., 1991; Pasquali et al., 2005) and diversity (Gichuru et al., 2000). It was used to determine whether a single or many species of a pathogen may cause a disease (Powell and Vargas, 2001). Virulent pathotypes were also studied for different pathogens such as *F. oxysporum* (Fiely et al., 1995) associated with spinach seed, seedlings, and mature plants; and *Verticillium*

dahliae (Ahn et al., 1998; Bao et al., 1998; Goud and Termorshuizen, 2002). Aggressiveness was another aspect that was studied using VCGs (Aqeel et al., 2008; Nitzan et al., 2002). VCGs of a number of plant pathogenic fungi have been found to vary in their morphology (Abang et al., 2004; Aqeel, et al., 2008; Correll et al., 1993; Korolev et al., 2000; Panaccione et al., 1989; Zeise and Tiedemann, 2001) and may also be affected differentially by environmental conditions (Nitzan, 2003). Morphological variations in conidia and sclerotia, as well as aggressiveness can be found among the different VCG's of a pathogen. Variation in aggressiveness among NA-VCGs of *C. coccodes* was demonstrated using foliar and root inoculations (Aqeel et al., 2008). A difference in the size of conidia and sclerotia was also detected as previously discussed. Three cultivars, Russet Burbank, Umatilla Russet, and Russet Norkotah were used to determine the aggressiveness among NA-VCGs isolates. NA-VCG2 and 6 were more aggressive and caused great reduction in tuber weight when inoculated onto foliage while NA-VCG3 and 4 were the least aggressive ones. However, there were no differences among NA-VCGs in tuber weight reductions following root inoculation.

Few studies have been conducted to cluster plant pathogen isolates according to their assignment to VCG subgroups using molecular markers. For *Verticillium* species, Collado-Romero et al. (2006) studied molecular variability among *V. dahliae* isolates by using fluorescent amplified fragment length polymorphism (AFLP) analysis and by polymerase chain reaction (PCR) assays. Isolates of VCG subgroups were molecularly more similar to each other than to isolates from the same geographic origin or host source. Isolates were assessed by DNA fingerprinting using a repetitive sequence DNA probe (pAF28) cloned from *Aspergillus flavus* Link by McAlpin et al. (2005). Thirteen distinct

DNA fingerprint groups or genotypes were identified. Twenty-four isolates including some from the United States and Japan belonging to VCG1 produced identical DNA fingerprints. Four other DNA fingerprint groups had multiple isolates sharing identical fingerprints corresponding to VCGs 2, 3, 12 and 13. Eight of the 13 fingerprint groups corresponding to VCGs 4-11 were represented by a single isolate with a unique fingerprint pattern.

AFLP markers have been used to study relationships within and among North American VCGs of *C. coccodes* (Heilmann et al., 2006). Six VCGs were obtained from 112 *C. coccodes* isolates by *nit* mutants, while for AFLP patterns, five VCGs were separated from 211 *C. coccodes* isolates, coinciding almost completely with the six VCGs of *nit* mutants. In this study, a relationship of specific AFLP bands to corresponding VCGs was reported for the first time for any plant pathogenic fungus, not only *C. coccodes*.

AFLP markers have also been used to differentiate isolates of *Colletotrichum gossypii* Southw. -causing anthracnose- from *C. gossypii var. cephalosporioides* Costa - causing ramulose disease on cotton (Silva-Mann et al., 2005). Results showed the power of AFLP technique to clearly distinguish the two fungi and cluster them into two distinct groups through the dendograms of similarities. Sixty seven isolates of *Sclerotinia homoeocarpa* F.T. Benn., which cause dollar spot disease, collected from various regions in North America were studied for their VCG identification, virulence and genetic diversity using the normal VCG-testing and AFLP fingerprinting. A total of 11 VCGs (A-K) were detected among the isolates, and five of them were new groups (G-K) (Viji et al., 2004). The minor group indicated that they might be biologically distinct and from a different species. All but five of the isolates tested were pathogenic. The isolates were classified into

four groups based on disease severity, highly virulent, moderately virulent, weakly virulent and avirulent. Overall, a relationship between VCG and virulence was observed.

Disease management. Black dot disease management in potato includes several approaches. Cultural practices are very important and include multiple years-crop rotation with cereals and non-host plants (Dillard and Cobb 1998; Pavlista et al., 1992; Rich, 1983). A three to four year crop rotation is not sufficient to reduce the viable inoculum because sclerotia can remain viable eight (Dillard and Cobb, 1998) to 13 yrs (Cullen et al., 2002). Preplant soil solarization and mouldboard ploughing to a depth of 30 cm reduced black dot incidence to 45% and 34%, respectively. Mouldboard plowing to a 30 cm depth was twice as effective as ploughing to a depth of 60cm (Denner et al., 2000). Controlling other hosts of *C. coccodes* can reduce the potential inoculum level. Fifteen weed species have been identified as hosts for *C. coccodes* (Raid and Pennypacker, 1987). Using certified seed is also a good method for reducing *C. coccodes* levels (Pavlista et al., 1992).

In storage, dry curing for two weeks after harvest has been found to be effective in reducing black dot severity as well as other potato blemish diseases such as skin spot, silver scurf, and black scurf (Hide and Boorer, 1991). Additionally, keeping relative humidity at or above 90% will also reduce the disease severity (Pavlista et al., 1992). Diseases were reduced more after early harvest compared to late harvest (Hide and Boorer, 1991). Latent infection at harvest could be found in tubers that appear healthy. Symptoms are more frequent on later harvested tubers, but latent infections are more significant in tubers from early harvests (Glais-Varlet et al., 2004).

No specific fungicides have been developed to control black dot in potatoes. Many fungicides for use against *C. coccodes* growth have been studied in vitro (Andrivon et al.,

1997; Dillard, 1988; Marais, 1990; Read and Hide, 1995; Sanogo et al., 2003; Uribe and Loria, 1994). Some of these fungicides showed promising results while others were not effective at all. Azoxystrobin has been demonstrated to reduce black dot disease severity on cv. Russet Norkotah and Russet Burbank; yields increased 13 and 23% respectively when compared to non-treated plants (Nitzan et al., 2005). Disease severity was also reduced on both cultivars by 19 - 81% and 22 - 81% on above and below ground stems sections, respectively, when plants were treated with azoxystrobin (Nitzan et al., 2005). The incidence of *C. coccodes* was studied with foliar applied fungicides, chlorothalonil (2.5 kg/ha) and mancozeb (1.68 kg/ha). The two fungicides were applied at 7, 10, or 14-day intervals to tomato plants. *C. coccodes* was recovered from root segments at harvest, and was significantly reduced by chlorothalonil and mancozeb at both interval treatments (Dillard and Cobb, 1997).

Little information is available on the levels of naturally occurring resistance to black dot in commercial potato cultivars. Most potato cultivars are susceptible to *C. coccodes* (Gudmestad et al., 2007). Bintje cultivar has symptoms more severe than those of the Roseval cultivar (Andrivon et al., 1998). Russet Norkotah was the least susceptible cultivar using foliar inoculation; while Umatilla was the most susceptible among the cultivars he studied (Aqeel et al., 2008). Quality trait loci and DNA markers to identify resistance to black dot are needed (Gudmestad et al., 2005). Recently, four selections, A0012-5, PA95B2-4, PA98NM38-1, and PO94A009-7 were found to have less black dot, and confirmed genetic stability (Nitzan et al., 2009). These selections have resistance to the powdery scab root galling stage, and they are promising for management of black dot and powdery scab (Nitzan et al., 2009).

Population biology. "Population biology is the study of patterns of distribution and variation in space and time within fungal species and the interpretation of these patterns in terms of genetic, developmental and environmental influences on phenotype and modes of proliferation" (Hawksworth et al., 1995). Population biology integrates ecological, evolutionary dynamics, and genetics of plant and pathogen populations and their interactions (Milgroom and Peever, 2003). Plant pathogenic fungi include a large group with huge diversity in strategies and ways in which they interact with their host (Burdon and Silk, 1997). The basis of evolutionary potential of species to respond to the environmental changes is diversity (Toro and Caballero, 2005). Understanding the pathogen's genetics, taxonomy, biology, ecology (Martin and English, 1997), diversity, origin, and evolution will facilitate understanding their role in shaping plant population genetic structure (Burden and Silk, 1997) and how populations evolve in response to different control strategies (McDonald, 1997).

Genetic diversity has been defined as the combination of alleles and genotypes in a population and the expression of this diversity in morphological, behavioral, and physiological differences between individuals and populations (Frankham et al., 2002). Genetic diversity can be classified as neutral, deleterious, or adaptive, from a functional view. From a descriptive view, genetic information refers to DNA sequence, individual gene, and chromosome (Toro and Caballero, 2005). Processes of mutation and recombination are the main source for genetic diversity among plant pathogenic fungi. The contribution of mutation in pathogen population diversity is affected by the size of the pathogen population, ploidy level of the pathogen, and by the inherent rate of mutation. Recombination in plant pathogens occur in sexual reproduction and in asexual reproduction

through somatic hybridization. In this process, nuclear and cytoplasmic materials may be exchanged. Sexual reproduction has more recombination possibility than an asexual one. Migration and gene flow is another source for this variation, it is the simplest, and it leads to the founding of a new pathogen in areas in which they were previously absent (Burdon and Silk, 1997). Genetic diversity is measured by the frequency of alleles and genotypes, allelic diversity, and the proportion of polymorphic loci. Differentiation through molecular measures is based on the genetic distances in allele frequencies among populations (Toro and Caballero, 2005).

Gene diversity, the probability that two alleles chosen at random from the population are different, defined by Nei (1973) is the most widely used parameter to measure the within population diversity. Genetic structure of pathogen populations is a major reason for limited disease management success. Understanding the genetic structure of pathogens would also help in breeding programs and quarantine policies (Martin and English, 1997). This understanding is achieved by studying morphological characters (Browning et al., 1999), pathogenicity tests (Balardin et al., 1997), vegetative compatibility grouping, and genetic background of a plant pathogen using molecular tools (Cubeta and Vilgalys, 1997; Martin and English, 1997). Previous techniques were used either singly or in combination in most of the studies to detect the genetic variation for plant pathogens. Morphological diversity in plant pathogens, such as culture pigmentation, spores and appresorial dimensions could help in differentiating the plant pathogen such as in *Colletotrichum* species, but this distinction could be lost after numerous transfers and long term storage. It is also not practical because of overlapping in the range of measurement for the fungus propagules (Browning et al., 1999).

Molecular markers

Before the mid 1980's, morphological, pathological, and allozyme markers were used for population studies. More recently many techniques that detect DNA sequence variation are used. A good marker is one that can detect variation in the population to be studied (Brown, 1996). Genetic diversity is one of the most studied subjects of plant pathogens (Abu-El Samen et al., 2003; Fahleson et al., 2003). This is because pathogens evolve and adapt to the environmental changes in order to survive (McDonald, 1997). Amplified fragment length polymorphism (AFLP) (Abu-El Samen et al., 2003; Dunkle and Levy, 2000; Dussle et al., 2002; Garzon et al., 2005; O'Neill et al., 1997; Vos et al., 1995), Random Amplification of Polymorphic DNA (RAPD) (Chee et al., 2006), Restriction Fragment Length Polymorphism (RFLP) (Salamati et al., 2000), Cleaved Amplified Polymorphic Sequences (CAPs) (Zhang and Stommel, 2001), Simple Sequence Repeats (SSRs) (Garcia et al., 2004), and DNA fingerprinting (DAF) (Chee et al., 2006; Cubeta et al., 1997) are examples of molecular markers used in these studies to construct genetic maps and to develop DNA fingerprints of many crops and plant pathogens. Larger sample sizes, as well as, information about the genotypic data collected would strengthen our knowledge of the pathogen genetic structure (Cubeta and Vilgalys, 1997). Molecular markers are useful in plant breeding; this is because of the presence of a gene that can be detected without waiting for the phenotypic expression of the gene. Furthermore, molecular markers can be used in the pyramiding of genes that are effective against available pathogen populations and, therefore, cannot be selected using standard biological assays (Liu et al., 1999).

Amplified fragment length polymorphism (AFLP). AFLP is a very powerful tool that saturates markers around a locus of interest (Vos et al., 1995). Yet, it is difficult to employ the AFLP method directly in a marker-assisted selection (MAS) program or for map-based gene cloning because of its high cost and complicated methodology (Dussle et al., 2002; Shan et al., 1999). The advantages of AFLP compared to RAPD and RFLP analysis are that it is fast, highly reproducible, and generates a high frequency of AFLP polymorphisms (O'Neill et al., 1997). Additionaly, these polymorphic fragments can be detected with a single PCR amplification (Brown, 1996), require only small quantities of DNA (O'Neill et al., 1997), and need no prior information about the nucleotide sequences (James et al., 2003)

One limitation of PCR-based techniques such as AFLP is the chance that unrelated sequences with similar size may be scored as 'the same' resulting in an artificial amplification of a number of similar bands. This problem may be overcome by using a large number of bands within a limited size range (O'Neill et al., 1997). Another limitation of AFLP includes their dominant type of inheritance (Dussle et al., 2002). Therefore, converting AFLP markers into other types of markers such as RFLPs or sequence-specific PCR-based markers is critical to expanding their usefulness (Shan et al., 1999) and is required for screening large populations (Dussle et al., 2002). Such conversion has already been attempted (Shan et al., 1999). Thus far, results have been discouraging, and only a few AFLP markers have been successfully converted into sequence-specific PCR-based markers (Shan et al., 1999). Many converted PCR-based markers have either lost their sequence specificity or their ability to amplify genomic DNA (Shan et al., 1999).

Polymorphisms identified by AFLP are often not transferable to more sequence-specific PCR applications. Reasons for difficulty in converting AFLPs into PCR-based markers remain unknown (Xu et al., 2001). Overall, AFLPs were found to be the best-suited molecular assay for fingerprinting and assessing genetic relationships with high accuracy (Garcia et al., 2004). AFLP is recommended by James et al. (2003) for species with small genomes. He used this technique with combinations of primers with a reduced number of selective bases to fingerprint *C. acutatum*. It was also recommended by O'Neill et al. (1997) to identify the genus *Colletotrichum*.

Sequence characterized amplified regions (SCARs). A reliable polymerase chain reaction (PCR) assay defined as sequence characterized amplified regions (SCARs) were described by Paran and Michelmore in 1993 by sequencing the two ends of the polymorphic DNA fragment and synthesizing two longer primers (24mer) homologous to each end (Liu et al., 1999). Although the AFLP technique is powerful and reliable in identifying markers closely linked to genes of interest, it has some disadvantages for use in mass assisted selection (MAS) and map-based cloning. Hence, conversion of AFLP markers into sequence-specific polymerase chain reaction markers is required for screening large populations at low costs (Brugmans et al., 2003; Dussle et al., 2002). RAPD markers are generally dominant; and heterozygous genotypes cannot be distinguished from dominant homozygous genotypes. RAPD analysis has some problems in MAS; to overcome these problems, RAPD markers can be converted into SCAR markers (Paran and Michelmore, 1993). SCAR markers are advantageous over RAPD marker as they detect only single genetically defined loci, their amplification is less sensitive to reaction conditions, and they can potentially be converted into co-dominant markers that will

increase the available information in a marker-assisted selection program (Bautista et al., 2002). Additionally, they are not aware of the presence of introns that could eliminate the priming sites, and scoring results obtained by SCAR markers are more straightforward than other PCR-based markers (Bautista et al., 2002). Consequently, SCAR markers are the most practical method for screening many samples in a time and labor-saving manner. They are accurate, practical to use and cost efficient (Kasai et al., 2000).

SCAR markers are generally allele specific. They are developed by cloning and sequencing the ends of the amplified RAPD product, generating extended primers specific to the targeted sequences, and amplifying DNA samples under higher stringency conditions (Hernandez et al., 1999; Paran and Michelmore, 1993). SCAR markers were developed in many studies from RAPD or AFLP markers.

In plants, SCARs were developed for germplasm characterization (Hernandez et al., 2001), and for locating resistance genes against certain plant pathogens. SCARs were developed to generate a linkage map of the down region of the *Vf* locus for scab resistance in apple (Huaracha et al., 2004; Xu et al., 2001), the *Ns* locus in potato (Marczewski et al., 2001), and the *Pvr4* locus for PVY resistance in pepper (Arendo-Andres et al., 2002). In pathogens, SCAR markers were used to identify pathogens such as *Verticillium albo-atrum* Reinke and Berthier isolates from hops (Radisek, et al., 2004), *Phoma sclerotioides* Preuss (Larsen et al., 2002), *Plasmodiophora brassicae* Woronin (Manzanares-Dauleux et al., 2000), *Trichoderma atroviridae* Karst 11-a (Rosa Hermosa et al., 2001), *Pseudomonas brassicacearum* (Holmberg et al., 2009), *Cryphonectria parasitica* (Murrill) M.E. Barr (Davis et al., 2005), and *Fusarium oxysporum* f. sp. *phaseoli* Kendr. & W.C. Snyder (Alves-Santos et al., 2002).

Population biology and genetic diversity studies using different molecular markers

Studies of pathogen population biology have been conducted on many plant pathogens using different molecular markers. Garzon et al. (2005) generated diagnostic AFLP fingerprints for selected *Pythium* species that were present in greenhouse crops in Pennsylvania. He explored the intraspecific variation among the isolates of *Pythium aphanidermatum* (Edson) Fitzp., *P. irregular* Buisman, and *P. ultimum* Trow in order to use them in establishing a base of information for future population studies of these species. Species-diagnostic AFLP fingerprints for *Pythium aphanidermatum, P. irregulare,* and *P. ultimum*, and tentative fingerprints for six other species were identified. Intraspecific distance analyses of *P. aphanidermatum*, *P. ultimum,* and *P. irregulare* revealed distinct patterns of intra-specific variation among the three species. *P. aphanidermatum* showed the smallest mean distance among isolates (15%), followed by *P. ultimum* (37%). *P. irregulare* had the largest mean distance among isolates (64%), which were divided into two populations with great genetic differentiation ($FST = 0.2$), suggesting the presence of a cryptic species boundary within this species.

Rhynchosporium secalis (Oudem.) Davis isolates were studied to compare the genetic structure of field populations from Australia, North America, and Northern Europe. Genetic structure in these populations was also studied to find if it is more consistent with sexual, asexual or parasexual reproduction (Salamati et al., 2000). It was found that gene and genotype diversity were high in all populations. Hierarchical gene diversity analysis showed that 13% of the total genetic variability was found among a collection station within a field, 4% among fields within continents, and 9% within continents. They also found that gene flow was significant on a regional scale, but was restricted among

continents. They postulated that *R. secalis* has a telemorph that has not yet been recognized, and that this telemorph plays an important role in both population biology and epidemiology of this fungus.

AFLP analysis was used to differentiate among South African isolates and mycelia compatibility groups (MCGs) of *Sclerotium rolfsii* Sacc. Isolates were assigned to one of nine MCGs and were defined by the letters A through I. They pooled DNA from isolates to make comparisons among MCGs. Additionally, based on the AFLP fingerprints; they concluded that genetic variation exists among isolates of a single MCG (Cilliers et al., 2000).

Two taxonomically identical, but genetically distinct sibling species, known as groups I and II, of *Cercospora zeae-maydis* Tehon and Daniels were studied (Dunkle and Levy (2000). Isolates of the fungus from Africa were compared with isolates from the United States by AFLP analysis, restriction digests of internal transcribed spacer (ITS) regions and 5.8S ribosomal DNA (rDNA), and by morphological and cultural characteristics. Analysis of restriction fragments from the ITS and rDNA regions indicated that all of the African isolates shared the profile of the *C. zeae-maydis* group II population from the eastern United States and, thus, are distinct from the group I population, which is more prevalent in the United States and other parts of the world. Cluster analysis of 85 AFLP loci proved that the African and U.S. group II populations were continental specific with limited variability. They suggested that Africa was the source of *C. zeae-maydis* group II in the United States. The overall lack of AFLP variation in this sibling species suggests that its origin is recent (Dunkle and Levy, 2000).

The population structure of *Cercospora sorghi* Ellis & Everh. isolates from wild and cultivated sorghum using the AFLP procedure was studied. Genetic variability and population structure was studied to gain insight into their potential impact on epidemics of gray leaf spot of sorghum in Africa. The fungal isolates were structured into two populations divided by their agroecological zone of origin. Their null hypothesis indicated the presence of a population structure among test isolates, and it was analyzed by Nei's coefficient of population differentiation (G_{ST}) and analysis of molecular variation (AMOVA). G_{ST} was found to be 0.03, leading to the acceptance of the null hypothesis stating an absence of population differentiation between the two populations. The results of the AMOVA analysis also revealed that the allelic variation (99.4%) was shared between populations. Average gene diversity over all loci ranged from 0.301 to 0.361, indicating higher diversity within populations rather than between populations. They concluded that Ugandan populations of *C. sorghi* were composed of one epidemiological unit and suggest that wild sorghum provides an alternative host for generating additional inocula (Okori et al., 2004).

Likewise, the population structure of *Discula destructiva* Redlin in the US was studied using AFLP analysis. Twenty genotypes were identified. Western and Eastern isolates were clearly separated. Western isolates were found to have lower genotypic diversity and more virulence than Eastern isolates. Few clones were spread among Western and Eastern isolates and the genetic diversity was low, suggesting that this fungus is still under intense selection pressure (Zhang and Blackwell, 2002).

Microsatellite markers were used to study the genetic variability, mode of reproduction, and differentiation according to host, geography, and growing system, in

several populations of *Botrytis cinerea* Pers. group II from Tunisia (Karchani-Balma et al., 2008). The isolates were collected from four different areas, and from four host plants: faba bean, grapevine, tomato, and strawberry. Isolates were completely genotyped with the microsatellites. The reproductive mode evaluation in all populations was in accordance with a significant departure from total panmixia, but not reaching complete clonality. This was explained by regular recombination in *B. cinerea,* separated by several cycles of clonal reproduction. Isolates of *B. cinerea* from grapevine that were isolated from a greenhouse were not genetically different from open field populations. However, in populations of *B. cinerea* sampled from strawberry grown under open field conditions, there were significant differences from isolates obtained from greenhouse populations in the same region. The single population from tomato exhibited the lowest genetic diversity; this was attributed to periodic bottlenecks at the entrance of external inocula and/or to genetic drift. Classical population genetic analyses showed that *B. cinerea* group II from open field populations was genetically structured because of the presence of a mountain between the fields which served as a geographic barrier. Sampling locations and host plants act together to shape the population genetic structure at the spatial scale, but it was not possible in this study to clearly separate the effect of both factors (Karchani-Balma et al., 2008).

Fifty eight isolates of *Cochliobolus sativus* (S. Ito and Kurib.) Drechsler ex Dastur from different regions of the world, and isolates of three related pathogenic *Cochilobolus* sp., were evaluated for their virulence on barley and for DNA polymorphism using AFLP marker (Zhong and Steffenson, 2001). The isolates were differentiated into three pathotypes; 0, 1, and 2. Pathotype 1 isolates were found in all regions of the world surveyed, while pathotype 0 was found only in North Dakota, Minnesota, Canada, Poland,

and Uruguay. For pathotype 2, all isolates were found only in North Dakota. The DNA banding pattern was very similar within a species, but different among species. A total of 633 AFLP bands were recorded, with 577 polymorphic ones. Cluster analysis showed that similarity among isolates within a species was high, while similarity between isolates was low. Nei's allelic diversity was 0.22 for all populations. Pathotype 0, 1, and 2 had 0.23, 0.15, and 0.10 allelic diversity, respectively. Two AFLP markers were present in pathotype 2, but none were present in pathotype 0 and 1.

AFLP was investigated for its usefulness for intra-specific variation assessment within *Alternaria brassicicola* (Schwein.) Wiltshire along the new South Wales Coast (Bock et al., 2002). Eighteen isolates of *A. brassicicola,* five of *A. alternate* (Fr.) Keissl., and a single isolate of *Rhynchosporium secalis* were used. AFLP successfully distinguished the three species. Most isolates of *A. brassicicola* were identified as separate genotypes. This fungus has a means for generating and maintaining significant variation even though it has no identified sexual stage (Bock et al., 2002).

RAPD and DAF techniques were also used to characterize the genetic structure of *Pseudoperonospora humuli* (Miyabe and Takah.) G.W. Wilson population, and to compare diversity of Oregon and Washington populations of this pathogen (Chee et al., 2006). Genetic variability was greater among Oregon isolates, than among Washington isolates. Isolates from Washington were nearly clonal, with 90% belonging to a single genotype, while 18 genotypes were found among Oregon isolates. These distinct differences in the population structure suggested that sexual reproduction might be more important in Oregon than in Washington.

Sclerotinia homoeocarpa Benn isolates were studied for their genetic variation using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis. A dendogram composed of three clusters was constructed based on 15 oligonucleotide primers. The three clusters represented different U.S. geographical locations in USA. RAPD banding pattern suggested the possibility that the east coast biotypes of the fungus were introduced into the west coast location. In addition to that, results indicated that there was a high genetic similarity between populations throughout the USA (Raina et al., 1997).

RAPD and AFLP markers were used to study the genetic variation among asexual progeny of *Phytophthora infestans* (Mont.) de Bary (Abu-El Samen et al., 2003). Polymorphism with 51 RAPD primers and with all AFLP primer pairs was detected. Cluster analysis separated the single-zoospore isolates (SZIs) from different parents into many virulence groups. There was no correlation among RAPD, AFLP and virulence groups within the progenies of SZIs studied. His study suggested that there is a considerable level of inherent genetic variability among SZIs derived asexually from the same parental isolate (Abu-El Samen et al., 2003).

Genetic variation among *Verticillium* spp. isolates was studied using AFLP (Fahelson et al., 2003). A dendrogram revealed three main groups. One group consisted of 35 European isolates derived from *Brassica napus* L. together with five Californian isolates taken from *B. oleracea* L. This group had a high degree of genetic similarity and included three isolates that were earlier were classified as *Verticillium longisporum* (Stark), indicating that all isolates in this group probably should be regarded as members of *V. longisporum*. The second group consisted of *V. dahliae* isolates, while the third group

contained four *V. albo-atrum* isolates. A cluster of six *V. nigrescens* Pethybr. isolates was also observed. Four isolates classified as *V. tricorpus* I. Isaac were highly diverse and did not cluster together. Analysis of molecular variance revealed that the isolates of *V. longisporum* were separated into four subgroups, based on geographic origin (Fahleson et al., 2003).

Many genetic diversity studies have been conducted for *Colletotrichum* spp. Pathogenic and genetic relationships among *C. lindemuthianum* (Sacc. and Magnus) Briosi and Cavara isolates from the centers of origin of the host and from distinct regions like Africa and Europe were studied (Ansari et al., 2004). A possible relationship between pathogenic and genetic variability of isolates and the geographic region was also studied. For pathogenicity tests, 74 isolates of C. *lindemuthianum* from 12 differential cultivars were used. The binary system generated via AFLP data of race classification assigned the 74 isolates into 30 races, where race 9 was the most prevalent. Most races were confined to a single country and even the most prevalent race (race 9) was only detected in four countries. Central and South American isolates were genetically diverse, but at the same time were more similar to each other than to African or European isolates. AFLP analysis clustered the isolates into three groups. Cluster A had 40 isolates from South and Central America. Cluster B included 36 isolates from a wider geographic spread, and cluster C compromised nine isolates, the majority of them from Tanzania, with other isolates from other countries. Again AFLP analysis showed that Central and South American isolates were more similar. There was some degree of linkage between the genetic diversity and country of origin of isolates when comparing molecular, pathogenic, and geographic

diversity. There was no link between race classification and AFLP profiles in this study (Ansari et al., 2004).

The genetic diversity and gene flow within and among five populations of *C. lindemuthianum* collected from different geographical regions across Himachal Pradesh in India has been investigated (Padder et al., 2007). RAPD analysis was used to study a total of 76 isolates that were grouped into five populations based on their geographic location. The fungus was found to have high genetic diversity with average genetic diversity within each population from 0.26-0.31. When they looked to the differentiation between each population pair, it was concluded that in Himachal Pradesh less frequent short distance gene flow occurs due to the hilly topography that acts as a barrier for long distance dispersal and to the farmers who grow their own bean land races (Padder et al., 2007).

Cluster analysis using virulence and RAPD polymorphism was used to study the variability of 138 *Colletotrichum lindemuthianum* isolates from six continents (Balardin et al., 1997). Based on virulence tests, 41 isolates were categorized into two groups: those found over a wide geographic area, and those restricted to a single country. No geographic pattern was evident, and no patterns correlated phonetic and virulence clusters obtained. Virulence phenotypes were clustered into 15 clusters. RAPD analyses confirmed the extensive variability in *C. lindemuthianum* virulence, and the genetic diversity of this fungus was greatest in Mexico and Honduras (Balardin et al., 1997).

The genetic diversity present in *C. graminicola* (Ces.) G.W. Wilson isolates originating on four host species: annual bluegrass (*Poa annua*), creeping bentgrass (*Agrostis palustris*), corn (*Zea mays*), and sorghum (*Sorghum halapense*) was studied (Horvath and Vargas, 2004). The study also determined whether geographic specialization

was a factor in the genetic diversity of isolates. Isolates were obtained from different sources and representing several different geographic locations. Isozymes were used to analyze the genetic diversity, where 16 alleles were resolved by five enzymes. Depending on Nei's genetic distance, isolates from creeping bentgrass and annual bluegrass were the most closely related, while isolates from maize and sorghum were the most distant. Isolates from bluegrass and creeping bentgrass had different genetic distances compared to those from maize and sorghum. Isolates from annual bluegrass had close relationship regardless of their geographic region because they had the smallest genetic distance. In conclusion, it was found that the main source for genetic diversity among *Colletotrichum* isolates used in this study was host origin not geographic origin (Horvath and Vargas, 2004).

Genetic diversity, relatedness, and pathogenicity of *C. acutatum* J.H. Simmonds from strawberry were tested using 62 isolates representing different geographic regions worldwide. Random amplified polymorphic DNA and internal transcribed spacer (ITS) was used. Clear genetic evidence of two characterized groups was obtained. CA-clonal subgroup contained isolates from strawberry, and exhibited identical RAPD patterns and identical sequence data. This might suggest a possible specialization of those isolates on strawberry. CA-variable is the other subgroup obtained, it included isolates from different hosts, and exhibited variable RAPD pattern and ITS2 sequence. The isolates representing the two groups were assigned into two pathogenicity groups. No correlation could be found between genetic and pathogenicity groups, and between genetic grouping and geographic origin for *C. acutatum*. Specific RAPD fragments were found to differentiate between the subgroups pathogenic on strawberry, and could be used to design specific PCR-primers for rapid detection (Denoyes-Rothan et al., 2003).

A study was conducted to assess the genetic relationships of *Botrytis cinerea* populations in Almería, Spain using RAPD and AFLP markers (Moyano et al., 2003). Polymorphisms were found to be more frequently detected per primer with AFLP than with RAPD markers. However, RAPD detected polymorphisms more frequently per loci than AFLP. The diversity of *B. cinerea* was higher when analyzed by RAPD than with AFLP, thus providing a better explanation of the genetic relationships between isolates (Moyano et al., 2003). Cotton anthracnose caused by *Colletotrichum gossypii* Southw. and ramulose, caused by *C. gossypii* var. *cephalosporioides* A.S. Costa, were differentiated using AFLP (Silva-Mann et al., 2005). A total of 318 polymorphic bands were selected to estimate similarities using Dice's Coefficient. The results clearly distinguished between ramulose and anthracnose isolates, which agreed with morphological and pathogenicity testing in this study.

AFLP technique was used to define the genetic relationship between *Colletotrichum trifolii* Bain and two highly aggressive *Colletotrichum* isolates, Arl-NW and 57RR, causing anthracnose on alfalfa. Nine other *Colletotrichum* species including *C. coccodes* were studied. Isolates of the same species were more similar in their genetic pattern. Isolate Arl-NW was more closely related to *C. trifolii* than with any other species, while isolate 57RR was grouped with *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. This was consistent with the morphological characters for those species. Taxonomic placement of the two alfalfa pathogens provided evidence that *Colletotrichum* species can be distinguished (O'Neill et al., 1997).

AFLP analysis has been determined to be an effective method for investigating genetic diversity among isolates and species within a number of complex fungal genera, as well as with species of *Colletotrichum*.

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CHAPTER ONE
DEVELOPMENT OF VEGETATIVE COMPATIBILITY GROUP – SPECIFIC SCAR MARKERS FOR DETECTION OF *COLLETOTRICHUM COCCODES*

Abstract

Colletotrichum coccodes (Wallr.) Hughes is an asexual fungus that has been characterized using nitrate *nit* mutants into seven vegetative compatibility groups (VCGs). At the molecular level, amplified fragment length polymorphism (AFLP) analysis has been used to differentiate the seven VCGs. Specific AFLP bands, corresponding to VCGs of *C. coccodes*, have been reported, potentially providing markers that can be converted into specific diagnostic markers such as SCAR markers. The objective of this study was to develop specific SCAR markers from AFLP bands specific for each *C. coccodes* VCG. A total of 88 isolates representing the different *C. coccodes* VCGs were analyzed via the AFLP method. Eighteen AFLP markers generated by three primer sets were successfully re-amplified and cloned. A total of 47 primers (22 forward and 25 reverse) were designed and evaluated in PCR reactions. Only one primer, AGb6F/R, with a 156bp amplification PCR product, was found to be specific for NA-VCG6 and NA-VCG7 of *C. coccodes*. Another seven primers were developed, but the PCR product was found to be present in more than one VCG of *C. coccodes* and, therefore, was not specific for the target VCG. These primers may still be used to unambiguously identify *C. coccodes* and to distinguish it from other fungal species affecting potato. Further studies into the development of PCR-markers for *C. coccodes* would simplify the identification of *C. coccodes* VCGs and aid in studying the biology, epidemiology, and ecology of this fungus.

Introduction

Colletotrichum coccodes (Wallr.) Hughes is a cosmopolitan (Dillard, 1992) weak plant pathogenic fungus (Raid and Pennypacker, 1987) causing black dot disease of potatoes (*Solanum tuberosum* L.). The disease is named due to the abundant sclerotia that are produced on different parts of the plant such as tubers, roots, stolons, stems, and leaves (Andrivon et al., 1998; Dillard, 1992; Lees and Hilton, 2003). The disease is widespread in Africa, Australia, Europe, and North, South, and Central America (Andrivon et al., 1998; Raid and Pennypacker, 1987; Rich, 1983). Its importance increased due to the growing demand of fresh pre-packed potatoes (Johnson, 1994) and the significant yield reduction resulted from infection with *C. coccodes* (Tsror (Lahkim) et al., 1999).

Potatoes are one of the most consumed crops worldwide (USDA, 2009) and the USA is the fifth largest world producer of potato (FAO, 2008). Potato early dying (PED) is an important potato disease syndrome associated with many soil-borne pathogens including *Verticillium dahliae* (Kleb.), *C. coccodes*, *Pratylenchus* spp., *Erwinia carotovora* (Jones) Bergey et al., and *Rhizoctonia solani* Kuhn (Powelson and Rowe, 1993; Rowe et al., 1987). Presence of *V. dahliae* and *C. coccodes* in some potato cultivars enhances PED development (Tsror (Lahkim) and Hazanovsky, 2001).

The identification and rapid detection of *C. coccodes* can be important in disease management. *C. coccodes* isolates have been characterized morphologically by vegetative compatibility group tests and by molecular techniques. Morphologically different structures- conidia, conidiophore, mycelia, appressoria, acervuli, sclerotia, and setae- produced by the fungus have been characterized in culture (Cano et al., 2004; Dickson, 1926; Dillard, 1992; Sutton, 1992) and by scanning electron microscopy (Tu, 1980). Semi-

selective media have been developed for the recovery of *C. coccodes* (Farley, 1972). Molecularly, polymerase chain reaction (PCR) methods have been used with primer sets (Cc1NF1 / Cc2NF1) specific for *C. coccodes* based on a unique DNA sequences (Cullen et al., 2002). Ribosomal DNA-ITS1 and ITS2 sequence data were also used to distinguish different *Colletotrichum* species including *C. coccodes* (Cano et al., 2004), confirming the morphological characters used for their identification. Ribosomal DNA has also been able to differentiate two *Colletotrichum* species; *C. circinans* (Berk.) Voglino and *C. coccodes* using denaturing gradient gel electrophoresis (DGGE) based on the migration rate of DNA fragments from each fungus. Random amplified polymorphic DNA (RAPD) and sequence characterized amplified regions (SCARs) were used to detect a *C. coccodes* strain for use as a biocontrol agent against velvet leaf weed (Dauch et al., 2003).

C. coccodes isolates have been intensively studied using the vegetative compatibility grouping method. Vegetative compatibility refers to the ability of individual fungal isolates to perform hyphal anastomosis and form a viable heterokaryon. Strains that are vegetatively compatible are described as members of the same VCG (Leslie, 1993). Fungi that reproduce sexually are expected to have higher VCG diversity compared to that of asexual reproducing fungi (Leslie, 1993). *C. coccodes* is an imperfect fungus belonging to Coleomycetes (Cano et al., 2004), thus vegetative compatibility may serve as the sole mean for genetic exchange (Leslie, 1993). The determination of VCG among fungal isolates is usually based on the complementation tests with nitrate non-utilizing (*nit*) mutants which enable researchers to compare strains of pathogens (Nitzan et al., 2006). Four multimember VCGs were identified for European/Israeli *C. coccodes* isolates with EU/IVCG-3 being the most aggressive group (Nitzan et al., 2002). An additional four new

VCGs for European/Israeli isolates were identified with EU/I-VCG-5 and EU/I-VCG-6 being the most aggressive groups (Shcolnick et al.,·2007). For North American isolates, seven VCGs were identified, with NA-VCG-2 and NA-VCG-5 being the most aggressive (Nitzan et al., 2006), and in another study NA-VCG-2 and NA-VCG-6 were found to be the most aggressive groups (Aqeel et al., 2008). Recently, six VCGs were identified for the Australian population with AUS-VCG4 being the most aggressive group (Ben-Daniel et al., 2010).

The determination of VCG is a laborious and time-consuming method. Another factor limiting the usefulness of VCG is that isolates of *C. coccodes* from Europe, North America, and Australia do not complement with each other and VCGs from each area appear to be distinct. Therefore, development of faster methods to detect each VCG and to study their biological significance is needed. Amplified fragment length polymorphism (AFLP) methods were used to study the relationships within and among North American VCGs (Heilmann et al., 2006). A total of 112 isolates were used, six VCGs were obtained using *nit* mutants, and five VCGs were obtained using AFLP markers. In this study, AFLP bands corresponding to specific VCGs were reported for the first time for *C. coccodes*, providing potential markers to be converted into specific diagnostic markers such as SCAR markers. AFLP has some limitations including, the relatively high cost, the need for expensive equipment, and may require radioactive primers, especially when used with modern instrumentation. Additionally, AFLP's are a dominant marker.

SCAR markers are a reliable polymerase chain reaction (PCR) assay. They were developed for marker assisted-selection in plant breeding programs (Paran and Michelmore, 1993). They are advantageous over RAPD and AFLP markers in that they can

detect a single locus and can be converted into codominant markers (Bautista et al., 2002). They are also more practical than AFLPs, and improve the efficiency of AFLP marker (Boukar et al., 2004; Xu et al., 2001). SCAR markers require less time and labor, and can screen numerous samples at the same time with greater accuracy and at a lower cost (Kasai et al., 2000; Shan et al., 1999, Xu et al., 2001). SCAR markers have been useful in plant breeding and genome analysis (Boukar et al., 2004) to locate resistance genes against different pathogens in crops (Arendo-Andres et al., 2002; Marczewski et al., 2001; Xu et al., 2001), and in studying population structure (Liu et al., 2009). SCAR markers have been used to identify plant pathogens such as bacteria (Holmberg et al., 2008), *Verticillium albo-atrum* Reinke & Berthier isolates from hop (Radisek, et al., 2004), and other plant pathogens such as *Phoma sclerotioides* Preuss ex Sacc. (Larsen et al., 2002), *Plasmodiophora brassicae* Woronin (Manzanares- Dauleux et al., 2000), and *Fusarium oxysporum* f. sp. *phaseoli* Kendr. & W.C. Snyder (Alves-Santos et al., 2002). SCAR markers can be designed from RAPD markers (Dauch et al. 2003; Hernandez et al., 1999; Weber et al. 2002) and AFLP markers (Radisek et al., 2004). There are no known studies on the use of SCAR markers for vegetative compatibility groups of a plant pathogen. Thus, the objective in this study was to develop specific SCAR markers from AFLP bands specific for each *C. coccodes* VCG.

Materials and Methods

***C. coccodes* isolates.** A total of 88 *C. coccodes* isolates previously collected from different locations in the USA, and assigned to different vegetative compatibility groups using a combination of nitrate non utilizing (*nit)* mutants and AFLP differentiation (Table

1.1). The isolates were assigned to six NA-VCG's (1, 2, 3, 4, 5, 6) using *nit*-mutants and to five NA-VCG's (1, 2, 3, 5, 6) using AFLP markers (Heilmann et al., 2006).

DNA extraction. Pure cultures of *C. coccodes* obtained from long term storage (-80°C) were grown on clarified CV8 medium (7.5 g agar, 50 ml sterilized CV8 juice amended with $CaCO_3$, and 450 ml ddH_2O) for 7 to 10 days until the fungus formed enough spores and sclerotia. Five milliliters of autoclaved distilled water were added to the cultures, and sterilized glass rods were used to remove the sclerotia and spores of the fungus and add them into flasks containing 100 ml of Richard's solution (10.0 g sucrose, 10.0 g potassium nitrate, 5.0 g potassium phosphate, 1.22 g magnesium sulfate, 0.02 ferric chloride, 150 ml V8 juice, and 850 ml ddH_2O) for 7 to10 days to obtain mycelial growth. The mycelia were harvested by filtering through Whatman filter papers (90 mm) using a power-operated pump, frozen at -80 °C for 30 minutes, lyophilized for 36 hrs, and ground to a fine powder with pre-cooled sterile mortar and pestle. DNA extraction was performed using the CTAB (hexadecyltrimethylammonium bromide) DNA extraction protocol (http://www.bio.utk.edu/mycology/Techniques/mt-dna_extraction.htm). Ground mycelium (250 mg) was placed in a 1.5 ml microcentrifuge tube. To this, 750 µl of Carlson Lysis Buffer (100 mM Tris pH 9.5, 20 mM EDTA, 1.4 M NaCl, 2% CTAB, 1% PEG, and 2 µl of mercaptoethanol) was added and the tubes were placed in a heating block at 74 °C for 30-40 minutes with inversion every 10 minutes. Tubes were centrifuged at 8,000 g for 10 minutes, and the supernatant was transferred to another microcentrifuge tube. One volume of chloroform: isoamyl-alcohol (24:1) was added and mixed by gently inverting each tube several times. Tubes were centrifuged again, at 8,000 g for 10 minutes, and the upper phase

Table 1.1*. *C. coccodes* isolates used in this study and their VCG assigned by *nit* mutants and AFLP markers by previous studies.

	Isolate name	Year	Location	Variety	Organ	VCG [1]	AFLP [2] Group	Source
1	00-194-20Nork	1997	Montana	Russet Norkotah	Tuber	1	1	D. Johnson
2	C19	1990	Washington	Russet Burbank	Stem	1	1	D. Johnson
3	C19-IL	1990	Washington	Russet Burbank	Stem	1	1	D. Johnson
4	C47-IL	1995	Washington	Russet Burbank	Tuber	1	1	D. Johnson
5	C57	1997	North Dakota	Russet Norkotah	Tuber	1	1	D. Johnson
6	C60	1997	Washington	Russet Norkotah	Tuber	1	1	D. Johnson
7	C138	1997	North Dakota	Russet Norkotah	Tuber	1	1	D. Johnson
8	C237-IL	1997	Nebraska	Russet Norkotah	Tuber	1	1	D. Johnson
9	CcNE02-4-1	2002	Columbus, NE	FL 1833	Stems	1	1	NCG lab
10	R-Lake-22-IL	1997	Montana	Ranger Russet	Tuber	1	1	D. Johnson
11	CcNE04-83-4	2004	O'Neill, NE	Red Norland	Tuber		1	NCG lab
12	CcNV02-24-5	2002	Winnemucca, NV	Russet Burbank	Stolons		1	NCG lab
13	CcNV04-66-3	2004	Tonopah, NV	Chipeta	AG-Stems		1	NCG lab
14	CcNV04-66-5	2004	Tonopah, NV	Chipeta	BG-Stems		1	NCG lab
15	CcNV04-66-8	2004	Tonopah, NV	Chipeta	Roots		1	NCG lab
16	CcOR04-61-1	2004	Boardman, OR	Ranger Russet	Stems		1	NCG lab
17	CcOR04-61-2	2004	Boardman, OR	Ranger Russet	Stems		1	NCG lab
18	ALB13	2002	Alberta		Tuber	2	2	D. Johnson
19	ALB15	2002	Alberta		Tuber	2	2	D. Johnson
20	C46-IL	1994	Washington	Russet Burbank	Tuber	2	2	D. Johnson
21	C112	1997	Montana	Russet Norkotah	Tuber	2	2	D. Johnson
22	C114-IL	1997	Washington	Russet Norkotah	Tuber	2	2	D. Johnson
23	C116-IL	1997	Washington	Russet Norkotah	Tuber	2	2	D. Johnson
24	C244-IL	1997	North Dakota	Russet Norkotah	Tuber	2	2	D. Johnson
25	MT11	2002	Montana	Ranger Russet		2	2	D. Johnson

Table 1.1. (continued)

	Isolate name	Year	Location	Variety	Organ	VCG [1]	AFLP [2] Group	Source
26	Coll114	1997	Washington	Russet Norkotah	Tuber	2	2	D.Johnson
27	ORG-9C	2002	Oregon	Ranger Russet	Tuber	NA	2	D. Johnson
28	ORG-2B	2002	Oregon	Ranger Russet	Tuber	2	2	D. Johnson
29	Nad2	2002	Washington			NA	2	D. Johnson
30	CcNV02-24-3	2002	Winnemucca, NV	Alturas	Stems		2	NCG lab
31	CcNV04-66-1	2004	Tonopah, NV	Chipeta	AG-Stems		2	NCG lab
32	CcOR04-61-3	2004	Boardman, OR	Ranger Russet	Stems		2	NCG lab
33	CcNE02-56	2002	Minden, NE	FL 1833	Tubers	2	2	NCG lab
34	CcMN02-22	2002	Becker, MN	Russet Burbank	Stems	3	2	NCG lab
35	MT16	2002	Montana	Ranger Russet	Tuber	2	2	D. Johnson
36	CcOR04-62-2	2004	Boardman, OR	Russet Burbank	Tuber		2	NCG lab
37	CcOR04-62-6	2004	Boardman, OR	Russet Burbank	Tuber		2	NCG lab
38	CcMN02-29	2002	East Grand Forks, MN	Red Norland	Stems	NA	2	NCG lab
39	CcOR02-53-2	2002	Hermiston, OR	Russet Burbank	Stems		3	NCG lab
40	CcNV04-66-7	2004	Tonopah, NV	Chipeta	Roots		3	NCG lab
41	C36	1992	Washington	Russet Norkotah	Stem	3	3	D. Johnson
42	C49	1995	Nebraska		Tuber	3	3	D. Johnson
43	C49-IL	1995	Nebraska		Tuber	3	3	D. Johnson
44	C61	1997	Montana	Russet Burbank	Tuber	3	3	D. Johnson
45	C242-IL	1997	Washington	Russet Norkotah	Tuber	3	3	D. Johnson
46	Coll95-IL	1997	Alberta	Russet Norkotah	Tuber	3	3	D. Johnson
47	Coll236	1997	North Dakota	Russet Norkotah	Tuber	3	3	D. Johnson
48	Coll239-IL	1997	British Columbia	Russet Norkotah	Tuber	3	3	D. Johnson
49	ORG-1	2002	Oregon	Ranger Russet	Tuber	4	2	D. Johnson
50	C124	1997	Nebraska	Russet Norkotah	Tuber	4	5	D. Johnson

Table 1.1. (continued)

	Isolate name	Year	Location	Variety	Organ	VCG [1]	AFLP [2] Group	Source
51	C43-IL	1992	Washington		Tuber	NA	5	D. Johnson
52	MT5	2002	Montana	Ranger Russet	Tuber	5	5	D. Johnson
53	R96-IL	1997	Montana	Ranger Russet	Tuber	5	5	D. Johnson
54	ORG-4	2002	Oregon			NA	5	D. Johnson
55	Nad1	2002	Washington	Russet Burbank		5	5	D. Johnson
56	CcOR04-61-4	2004	Boardman, OR	Ranger Russet	Stolons		5	NCG lab
57	CcOR04-61-5	2004	Boardman, OR	Ranger Russet	Roots		5	NCG lab
58	CcOR04-61-6	2004	Boardman, OR	Ranger Russet	Roots		5	NCG lab
59	CcOR04-62-1	2004	Boardman, OR	Russet Burbank	Tuber		5	NCG lab
60	CcOR04-62-3	2004	Boardman, OR	Russet Burbank	Tuber		5	NCG lab
61	CcOR04-62-4	2004	Boardman, OR	Russet Burbank	Tuber		5	NCG lab
62	CcOR04-62-5	2004	Boardman, OR	Russet Burbank	Tuber		5	NCG lab
63	CcWI02-8	2002	Hancock, WI	FL 1879	Tuber	5	5	NCG lab
64	CcWI02-17	2002	Antigo, WI	Pike	Tuber	5	5	NCG lab
65	CcNE02-14	2002	Kearney, NE	FL 1833	Tubers	5	5	NCG lab
66	CcOR02-57	2002					5	NCG lab
67	AN13		Ohio	Red pepper		6	6	R. Rowe
68	AN15		Ohio	Tomato		6	6	R. Rowe
69	C210	1984	Lockport, NY	Tomato	Fruit		6	H. Dillard
70	452	1988	Georgia to NY	Tomato	Roots		6	H. Dillard
71	CcMI04-70	2004	Three Rivers, MI		S,R,St		6	NCG lab
72	CcMN02-1-1	2002	Browerville, MN	Russet Burbank	Stems		6	NCG lab
73	CcMN02-1-13	2002	Browerville, MN	Russet Burbank	Stems		6	NCG lab
74	CcMN02-1-14	2002	Browerville, MN	Russet Burbank	Stems		6	NCG lab
75	CcMN02-1-15	2002	Browerville, MN	Russet Burbank	Stems		6	NCG lab

Table 1.1. (continued)

	Isolate name	Year	Location	Variety	Organ	VCG [1]	AFLP [2] Group	Source
76	CcMN02-1-19	2002	Browerville, MN	Russet Burbank	Stems		6	NCG lab
77	CcMN02-5	2002	Perham, MN	Russet Burbank	Stems		6	NCG lab
78	CcMN02-26	2002	East Grand Forks, MN	Yukon Gold	Stems		6	NCG lab
79	CcMN02-28	2002	East Grand Forks, MN	Red Norland	Stems		6	NCG lab
80	CcNE02-40	2002	O'Neill, NE	Russet Norkotah	Stems	NA	6	NCG lab
81	CcNV02-24-1	2002	Winnemucca, NV	Russet Burbank	Stems		6	NCG lab
82	CcMI04-70	2004	Three Rivers, MI		S,R,St		6	NCG lab
83	C54	1996	Wisconsin	Peppermint	Rhizome	7	6	D. Johnson
84	C55-IL	1996	Washington	Peppermint	Rhizome	7	6	D. Johnson
85	C501	1997	New York	Tomato		7	6	D. Johnson
86	C501-IL	1997	New York	Tomato		7	6	D. Johnson
87	C501iL (NY)			Culture		7		
88	C55(WA)			Culture		7		

* This table was obtained from Dr. N. C. Gudmestad laboratory.

[1] Isolates characterized to VCG's by nitrate *nit* mutants complementation test (Nitzan et al., 2006).

[2] Isolates characterized to VCG's using AFLP by Heilmann et al. (2006).

was transferred to a clean tube. The DNA was precipitated by adding an equal amount of isopropanol. Tubes were centrifuged for 10 minutes at 8,000 g to sediment DNA. The pellet was rinsed with cold 70% ethanol, and spun / centrifuged at 4 °C for 10 minutes. Ethanol was poured off, and the tubes were air-dried to get rid of traces of ethanol. DNA was eluted in a final volume of 100 µl in Tris-EDTA buffer, and 2 µl of RNAse-plus diluted (1:10) was added to the elute. Tubes were incubated at 37 °C for 30 minutes or overnight in the refrigerator. DNA was assessed for both quantity and purity. Two micro liter of DNA were mixed with low range assay solution used for 10-50 ng/µl DNA concentration (10 µl Hoechst 33258 dye stock solution (1mg/ µl) with 10 ml of 10X TNE buffer and 90 ml distilled, filtered water) and measured via TD-700 fluorometer for concentration measurement. DNA quantity was measured for representative isolates. On average, DNA quantity was found to be in a range of 50 to 70 ng/µl. For purity visualization, 5µl of DNA were mixed with 3µl of the blue juice dye (10.5 ml of 70% glycerol, 0.15 ml 0f 0.5X TAE or TBE buffer, 0.6 ml of 20 mM EDTA (0.5 M), 0.03 g of 0.2% SDS, and 0.009 g of 0.6 mg/ml bromophenol blue in 15 ml) and electrophoresed on 1% agarose gel. Assessing DNA samples of *C. coccodes* isolates for purity showed good DNA quality on 1% agarose gel (Figure 1.1).

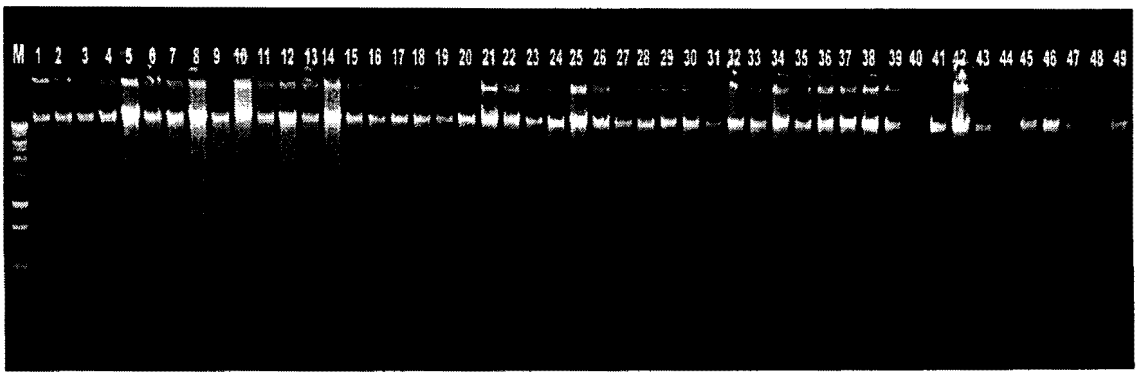

Figure 1.1. Representative purity testing on 1% agarose for DNA of *C. coccodes* isolates. M: 1kb molecular marker, 1-49 DNA of *C. coccodes*.

AFLP assays. AFLP reactions were performed according to the method of Vos et al. (1995) as modified by Heilmann et al. (2006) for *C. coccodes*. The procedure combined the digestion and ligation steps in one step. One micro liter of the genomic DNA was digested and ligated by using the following materials: 4.6 µl water, 1.1 µl ligation buffer, 1.1 µl of 1M NaCl, 0.11 µl BSA, 0.5 µl ATP, 1 µl *Eco*RI-adapter (16bp adapter-primer sequence) and 1 µl *Mse*I-adapter (16-bp adapter-primer sequence), 0.2 µl of *MseI*, 0.3 µl of *EcoRI* (New England Biolabs Inc., Beverly, MA), and 0.1 µl of T4 DNA ligase (Invitrogen, Carlsbad, CA). The reaction was digested and ligated to the fragments at 37 °C for 3.5 hrs. The amplification step was performed in a final volume of 25 µl using a PTC-200 programmable thermocycler (MJ Research, Waltham, MA). Each reaction contained 0.85 µM dNTPs, 1 µl of 25mM $MgCl_2$, 2.5 µl of 5X PCR reaction buffer, 1 µl (36 ng) of each primer set with two-base extensions, 2.0 µl of the adapter-primer DNA, and 0.1 unit of *Taq* DNA Polymerase (Promega Corp., Madison, WI). Selective amplifications were carried out with three primer pairs, *Eco*-AC/*Mse*-CC, *Eco*-AG/*Mse*-CC, and *Eco*-AT/*Mse*-CC (Table 1.2).

Table 1.2.[1] Oligonucleotides used in AFLP selective amplification polymerase chain reaction.

Primer	
Forward primers	
Eco-AC*	5′ GACTGCGTACCAATTCAC 3′
Eco-AG*	5′ GACTGCGTACCAATTCAG 3′
Eco-AT*	5′ GACTGCGTACCAATTCAT 3′
Reverse primer	
Mse-CC	5′ GATGAGTCCTGAGTAACC 3′

[1] This table was extracted from Heilmann et al. (2006) publication.
* This primer was labeled with IR-Dye700 to run via LI-COR 4300.

The PCR parameters included an initial cycle of 2 minutes at 72 °C, 30 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C. This was repeated for 39 cycles, then annealing temperature 72 °C for 5 min, and 4 °C forever. The resulting selectively amplified PCR products were electrophoresed on 2% *(m/v)* agarose gel to visualize a smearing pattern (Figure 1.2) before running the samples on polyacrylamide gel. Three microliter amplified product and 3 µl of formamide loading dye (1 X) (47.5 ml of formamide, 2 ml of 0.5 M EDTA, 40 mg of bromophenol blue, and 0.5 ml of water) were mixed and viewed for the three primer sets. If no smearing was observed in any isolate, the isolate was repeated for amplification or digestion. The amplification products were electrophoresed on 6% (w/v) polyacrylamide / 7 M urea denaturing gel in 1X TBE (1,1,2,2-tetrabromoethane) buffer for 2.30 hrs at 3000V (65W)

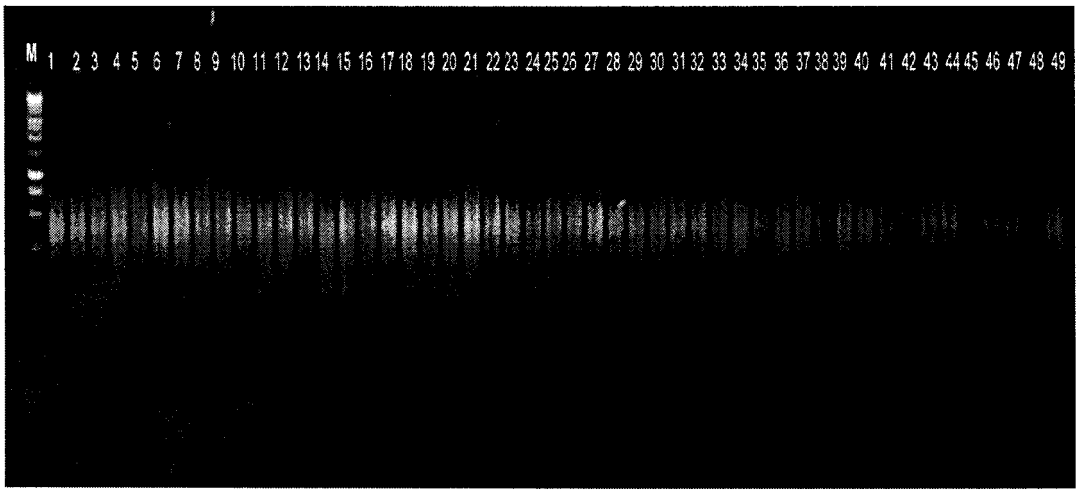

Figure 1.2. Representative of smearing pattern for selectively amplified product of *C. coccodes* DNA. M: 1kb molecular marker, 1-49 DNA of *C. coccodes* after digestion, ligation, and selective amplification steps.

for the three primer sets, and then silver stained via SILVER SEQUENCE ^{TM} DNA Sequencing System (Promega, Madison, WI, USA). The same AFLP procedure was conducted, but with IRD-700 for all the three primers sets (*Eco*-AC, *Eco*-AG, and *Eco*-AT)

(Table 1.2) and was visualized using a DNA analyzer, LI-COR model 4300. AFLP was programmed to run for 3.5 hrs, with 1500V (40W).

Cloning and sequencing of the target AFLP band. The gels were silver stained to visualize the bands. Those bands specific for a given NA-VCG were excised from the gel with a sharp-edged clean blade, transferred to 200 µL of 1 X TE (10 m*M* Tris-HCl, pH 8.0 and 1 mM EDTA pH 8.0) for 3 hrs at room temperature. The polyacrylamide gel was submerged in the water after staining for 3 hrs, and then was placed on the white light transilluminator (Model TW-43) to be able to visualize the target band. The band of interest was labeled by a marker from the bottom side of the glass plate, and was marked by a clean blade from its four edges. The gel was soaked in 2% NaOH for 30-45 minutes, and the band was removed using a clean blade without transferring the gel into Whitman filter papers. The gel fragments were placed in a heat block (95 °C for 5 minutes) and then centrifuged at 3000 rpm for 5 minutes. The eluted DNA was used as the template for PCR amplification using the same primer combination and PCR conditions as was used for selective amplification. The amplified products were electrophoresed at 180 V in a 1.2% low-melting-point agarose gel for 30 min. The critical fragments were excised from the gel and purified with Qiaquick® Gel Extraction Kit (Qiagen Sciences, Maryland) following the manufacturer's instructions. DNA concentration was measured using a flourometer. Purified DNA was diluted 1:1 depending on the sample concentration, mixed, and ligated into a pGEM-T easy vector using pGEM®-T and pGEM®-T Easy Vector Systems Kit (Promega, Madison, WI) according to the procedures described by the manufacturer. The recombinant plasmids were plated on two selective Luria-Bertani (LB) media supplemented with 100 µg/µl ampicillin (12.5 g LB media, 7.5 g agar, 500 ml distilled

water, and 1 ml ampicillin). Twenty µl of 50 µg/µl X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and 100 µl of 100 mM IPTG (isopropyl- β-D-thiogalactopyranoside) were spread over the surface of LB media plates, and allowed to absorb for 30min at 37 °C prior to use. Two plasmid concentrations were used; 50 µl on one plate and 100 µl on another plate for each fragment excised. The plates were placed at 37 °C for 18 hrs. Transformed colonies showed white color, while non-transformed showed blue color. For each VCG-specific band, six transformed plasmids were picked and streaked on LB-media plates supplemented with ampicillin, X-gal, and IPTG. The plates were placed at 37 °C for 18 hrs in order to get more quantity of the transformed colonies. After that, sterilized toothpicks were used to pick one positive transformed colony from a single colony from each AFLP fragment and dipped in a culture tube with 5 ml LB-broth supplemented with 100 µg/ml ampicillin (10g Bacto®-tryptone, 5g Bacto®-yeast extract, 5g NaCl, 100 µl of 10 M NaOH in 100 ml distilled water adjusted to 7.0 PH). The tubes were incubated at 37 °C with shaking for at least 18 hours. DNA was extracted from the plasmids using Qia prep spin miniprep Kit (Qiagen). The DNA was quantified and the concentration was adjusted to 0.25 µg/µl. Restriction digestion of the plasmid was performed to confirm the presence of the insert at 37 °C for one hr with a mixture of 6.5 µl of DEPC-treated water, 1.0 µl *Eco*RI buffer, 2 µl plasmid DNA, and 0.5 µl of *Eco*RI enzyme. The plasmids with confirmed insertswere sent to the DNA facility of Iowa State University (Ames, Iowa) for sequencing. A cloning method has advantage over direct sequencing because impurities in the PCR products can be avoided (Brugmans et al., 2003).

Designing SCAR primers. DNA sequences were analyzed using the BLAST network service of the National Center for Biotechnology Information (Bethesda,

Maryland, USA, (http:www.ncbi.nlm.nih.gov) to determine whether there were any homologous sequences in the databases. The oligonucleotide primers were designed on the basis of the identical sequence of plasmid DNA that will lead to a polymorphism between the different vegetative compatibility groups of *C. coccodes*. Primer-reverse and primer-forward were designed using Primer 3 web page (http://primer3.sourceforge.net) (Sigma Genosys Milwaukee, WI). These primers were then used to screen the isolates for the different NA-VCGs. Each primer set was tested in PCR reactions conducted with 25 µl mix containing 2 µl of template DNA, 2.5 µl of 5X *Gotaq*™ buffer, 1 µl of 1.5 mM $MgCl_2$, 1 µl of 10 mM of dNTP mix, 1 µl of 20 mM of each forward and reverse primer, and 0.3 µl of 5 u/µl of *GoTaq* DNA polymerase. Amplification was performed in a PTC-200 thermal Cycler programmed for an initial heat denaturation step at 94 °C for 2 min, DNA fragment amplification was performed for 35 cycles, comprising 30 s at 94 °C, 45s at 58 °C, and 30 s at 72 °C. Final extension was for 5 min at 72 °C. Controls with no DNA were included in each set of PCR amplifications to ensure there is no contamination of reagents; water was added instead of DNA for the negative control, and DNA from isolates where the fragment was excised was used as the positive control. Other plant pathogenic fungi such as *Colletotrichum lindemuthianum* (Sacc. & Magn.), *Streptomyces scabies* (Thaxt.) Lambert and Loria, *Verticillium dahliae* Kleb., *Acremonium strictum* W. Gams., *Fusarium graminearum* Schwabe, *F. sambucinum* Fückel, and *Rhizoctonia solani* Kühn, were used to test primer specificity for detecting *C. coccodes* only. Reaction products were viewed by electrophoresis on 2 % agarose gels in 1.0 X Tris-borate EDTA buffer stained with ethidium bromide, and then gels were visualized by illumination with ultraviolet light.

Further trials to get VCG-specific bands were conducted. For these trials, bands were targeted that were not present in the other NA-VCG groups before cloning and sequencing. For this purpose, bands were excised randomly from two other isolates belonging to different VCGs in which the target band was not present. These latter excised bands were regarded as negative controls. Negative controls were amplified with the same primer set used in the selective amplification. If the negative controls had a PCR-product with similar size to the PCR-product that was present in the original targeted VCG band, then no cloning or sequencing was conducted (Table 1.3). By using this methodology it was determined that the negative controls had a PCR-product with a similar size to the target band for all bands tested regardless of VCG. For that reason, no further steps such as cloning, sequencing, and screening were performed (Figure 1.3).

Results

Amplified fragment length polymorphism. When selective amplified products were visualized using the LI-COR model 4300, the VCG-specific bands were shown more clearly than when using the silver staining AFLP method (Figure 1.4). The AFLP banding pattern of the seven NA-VCGs demonstrated a similarity between NA-VCG6 and NA-VCG7; and NA-VCG4 and NA-VCG5. NA-VCG-6/7 was differentiated from other VCGs by numerous bands when using the three primer sets *Eco*-AC/*Mse*-CC, *Eco*-AG/*Mse*-CC, and *Eco*-AT/*Mse*-CC. These findings are consistent with previous studies, but they differ in that both isolates of NA-VCG4 (C124 and ORG1) are more similar to VCG5 than previously described (Heilmann et al., 2006). Isolate C124 (NA-VCG4) clustered with NA-VCG5 and ORG1 (NA-VCG4) clustered with NA-VCG2.

Table 1.3. Negative controls used to test for specificity of target AFLP bands for certain NA-VCGs.

Number	AFLP primer[1] combination	Presumptive band size [2]	NA-VCG for 1st negative[3]	NA-VCG for 2nd negative
1	*Eco*-AC/*Mse*-CC	AC2-500[1]	1	3
2	*Eco*-AC/*Mse*-CC	AC1,3-490	2	5
3	*Eco*-AC/*Mse*-CC	AC3-300	2	5
4	*Eco*-AC/*Mse*-CC	AC6,7-280	5	3
5	*Eco*-AC/*Mse*-CC	AC1,3-250	2	5
6	*Eco*-AC/*Mse*-CC	AC1,3-200	5	6
7	*Eco*-AC/*Mse*-CC	AC6,7-175	5	3
8	*Eco*-AC/*Mse*-CC	AC2,5-160	1	6
9	*Eco*-AC/*Mse*-CC	AC1,3-75	2	5
10	*Eco*-AC/*Mse*-CC	AC6,7-350	1	3
1	*Eco*-AG/*Mse*-CC	AG3A-320	2	5
2	*Eco*-AG/*Mse*-CC	AG3B-300	2	5
3	*Eco*-AG/*Mse*-CC	AG3C-280	2	6
4	*Eco*-AG/*Mse*-CC	AG3D-275	2	6
5	*Eco*-AG/*Mse*-CC	AG3E-200	2	6
6	*Eco*-AG/*Mse*-CC	AG3F-195	2	6
7	*Eco*-AG/*Mse*-CC	AG3G-135	2	2
8	*Eco*-AG/*Mse*-CC	AG3H-100	2	6
9	*Eco*-AG/*Mse*-CC	AG2,5-115	3	5
10	*Eco*-AG/*Mse*-CC	AG6,7A-110	5	5
11	*Eco*-AG/*Mse*-CC	AG6,7B-105	5	6
1	*Eco*-AG/*Mse*-CC	AT1-300	2	5
2	*Eco*-AT/*Mse*-CC	AT6,7-280	3	5
3	*Eco*-AT/*Mse*-CC	AT1,5-260	3	6
4	*Eco*-AT/*Mse*-CC	AT3,5-250	1	6
5	*Eco*-AT/*Mse*-CC	AT6,7-200	5	3
6	*Eco*-AT/*Mse*-CC	AT1,3-125	2	6
7	*Eco*-AT/*Mse*-CC	AT1-100	3	6
8	*Eco*-AT/*Mse*-CC	AT6,7-75	5	1

[1] Primer combination that was used in selective amplification for the target band and the negative control.

[2] The first two letters refers to the primer combination that was used to get this band; AC: *Eco*-AC/*Mse*-CC, AG: *Eco*-AG/*Mse*-CC, and AT: *Eco*-AT/*Mse*-CC. The number followed is the VCG from where the target band was excised, and the last three digits number is the presumptive band size.

[3] 1st and 2nd negatives were chosen randomly from different VCGs.

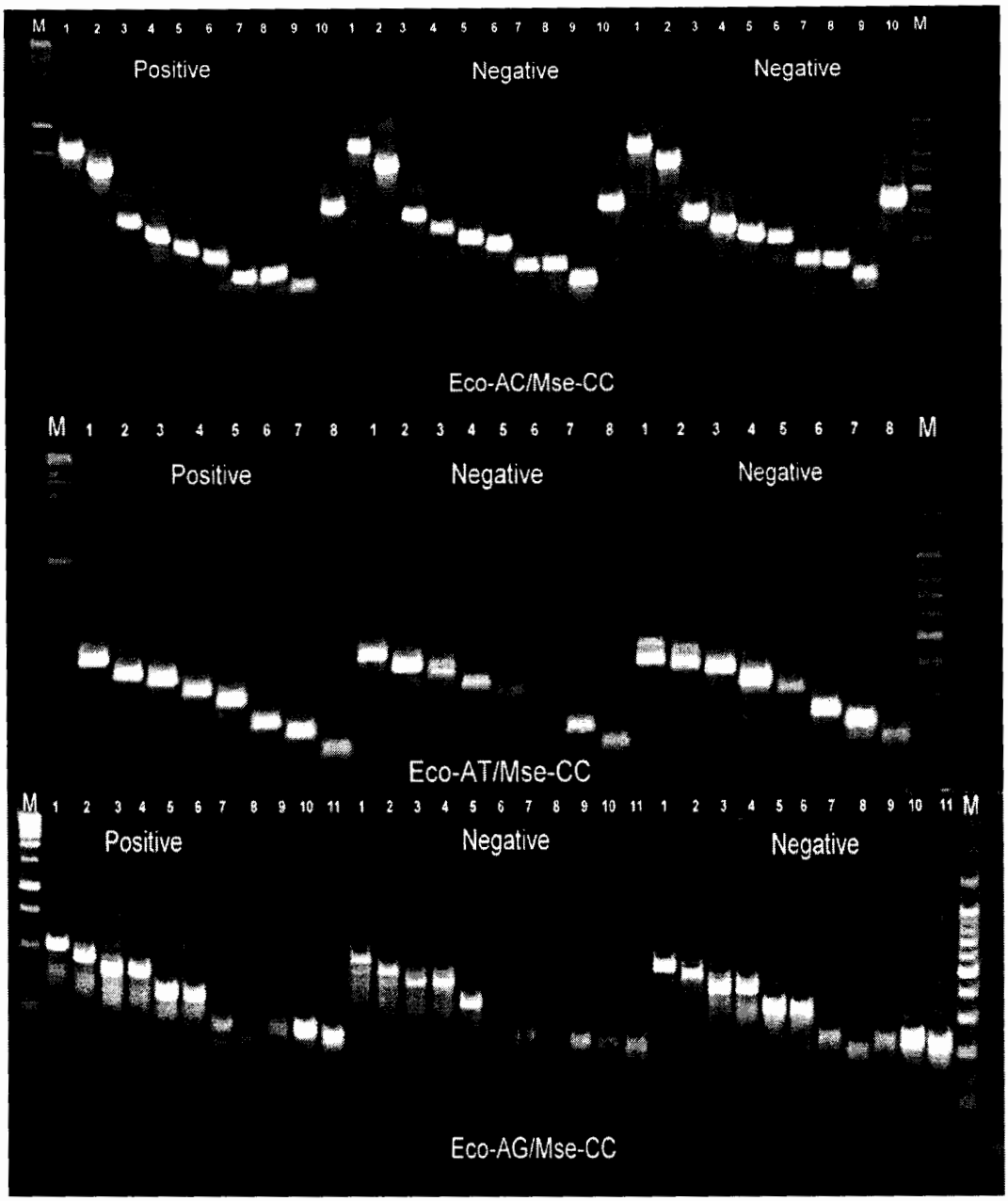

Figure 1.3. Negative controls amplification results for the three primer sets using isolates from random NA-VCG's. All the negative controls bands tested show positive PCR-product with similar size to the target band (positive). From left to right, M: 1 kb molecular marker, positives: isolates with target bands for certain NA-VCGs that have the band present in the AFLP gel, negatives: isolates that have no visible bands on the AFLP gel.

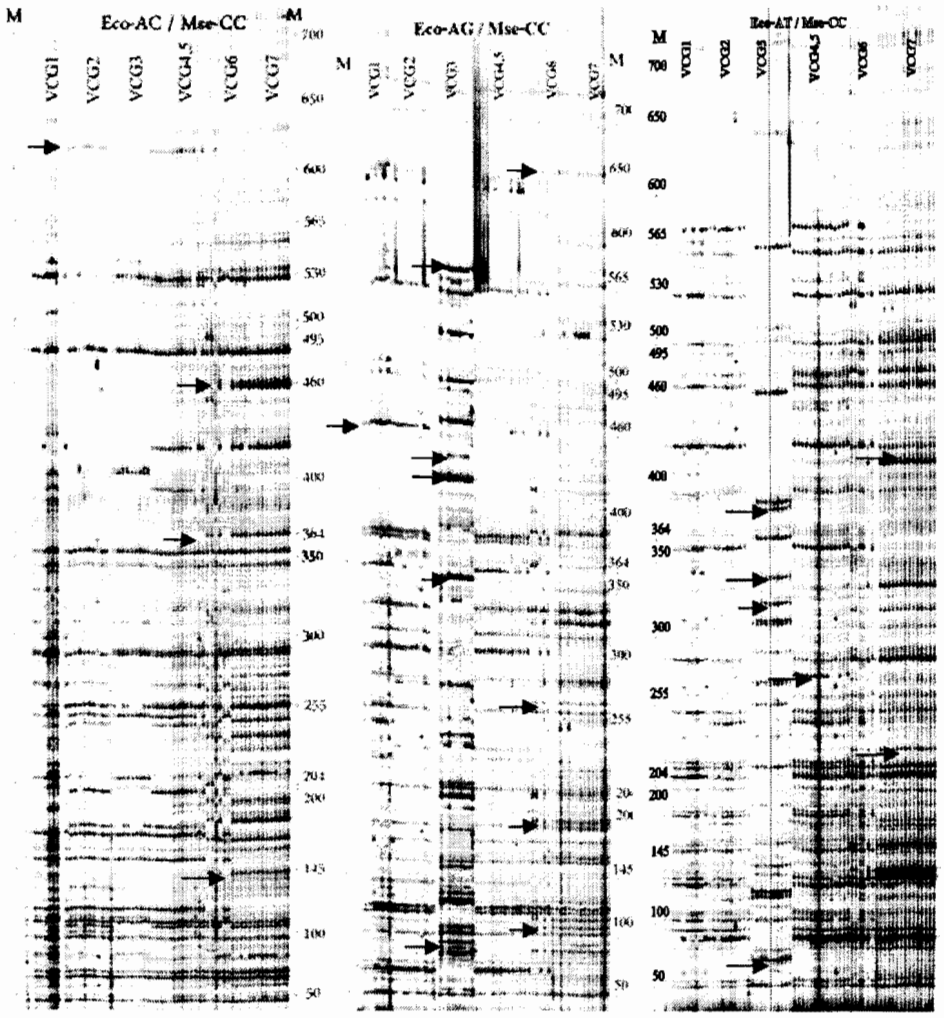

Figure 1.4. Amplified fragment length polymorphism electrophoresis of *C. coccodes* isolates for the seven NA-VCGs. The three primer sets, Eco-AC/Mse-CC, Eco-AG/Mse-CC, and Eco-AT/Mse-CC were used for selective polymerase chain (PCR) amplification reaction. PCR products were separated on 6% polyacrylamide/7 urea gel using LI-COR 4300 model. The specific AFLP bands for NA-VCGs can be detected. M: is a standardized, IRdye-700 labeled size marker of 50-bp.

Additionally, NA-VCG3 was distinct from other NA-VCGs by its numerous bands shown mainly in *Eco*-AG/*Mse*-CC, and *Eco*-AT/*Mse*-CC primer sets. These data were chosen for further study (Chapter Two).

DNA cloning and sequencing. A total of eighteen AFLP markers generated by the three primer sets were successfully re-amplified and cloned (Table 1.4). For each AFLP marker, one colony containing the cloned fragment with similar size to the AFLP marker was sequenced. Nucleotide homology searches found no sequence similarities in the GenBank database with any of the primers that have been designed in this study.

SCAR primer design and amplification specificity. A total of 47 primers (22 forward and 25 reverse) were designed and evaluated in PCR reactions (Table 1.4). DNA from seven isolates of *C. coccodes* representing the seven assigned NA-VCG's was used as template DNA for initial screening to test primer specificity. Twenty six primer pairs did not show any specificity for the VCG for which they were designed. Only one primer, AGb6F/R, with 156 bp amplification PCR product (Figure 1.5) was found to be specific for NA-VCG-6 and NA-VCG-7 of *C. coccodes* in initial screening. Further testing using additional isolates belonging to the seven NA-VCGs of *C. coccodes* and isolates belonging to other plant fungal pathogen genera including *C. lindemuthianum*, *S. scabies*, *V. dahliae*, *A. strictum*, *F. graminearum*, *F. sambucinum*, and *R. solani* was conducted. The amplicon (156 bp) was not present in any of the other NA-VCG's or in the other tested fungal plant pathogens genera (Figure 1.5). Seven primers, namely ACb6F/R (Figure 1.6), VCG175F/R (Figure 1.7), C46iLF/R (Figure 1.8), OR0613F/R (Figure 1.9), 275AG2F1/R (Figure 1.10), AG3195F/R (Figure 1.11), and AT1125F/R (Figure 1.12), with 157, 150, 229, 217, 201, 151, and 158 bp PCR products, respectively, were present in all *C. coccodes* NA-VCG representatives, but not in the other fungal plant pathogen genera, including *C. lindemuthianum*, *S. scabies*, *V. dahliae*, *A. strictum*, *F. graminearum*, *F. sambucinum*, and *R. solani*.

Table 1.4. List of SCAR markers designed from expected specific bands for different vegetative compatibility groups of *C. coccodes* using three AFLP primer sets.

AFLP primer combination	Specific for VCG	SCAR marker designation	SCAR primer sequence (5`-3`), Forward/Reverse	Product size (bp)	Ta (°C) F/R
Eco-AC/*Mse*-CC	1	AC220-F/R	ATCCAAAACCCGCTAACTGA / AAATGCGTCAACGTTGGATT	162	59..57 60..37
Eco-AT/*Mse*-CC	1	AT1125-F/R	GTTACGTCCGCTCTCCCATA / GCGACGTGAAACTTGAATGA	158	63.8 63.8
Eco-AC/*Mse*-CC	2	C46iL-F/R	CAAACGATTACGACGCCTTT / AACTCTCCACACTGCCTGCT	329	63.7 64.0
Eco-AC/*Mse*-CC	2	AC420-1F/1R	CTTCACACCAGCAATCTGGA / TTCCTGGCACTCACGTGTAA	346	63.9 64.3
Eco-AC/*Mse*-CC	2	AC420-1F/2R	CTTCACACCAGCAATCTGGA / AAGCGGCTGCTTGTTATCTC	328	63.9 63.3
Eco-AC/*Mse*-CC	2	AC420-1F/3R	CTTCACACCAGCAATCTGGA / GCAATGCTCGCAGTTCTACA	244	63.9 64.1
Eco-AG/*Mse*-CC	2	AG240-1F/R	GGGCTTCATTACGAGGTCAG / TGAGTCCTGAGTAACCCATCG	151	63.5 64.0
Eco-AG/*Mse*-CC	2	275AG2-F1/R	TCGCCTTATGGAAAGCACTC / CAGCAACCTGGATTTCCATT	201	64.1 63.7
Eco-AG/*Mse*-CC	2	275AG2-F2/R	CACCCCTCATTTCAGGTCAC / CAGCAACCTGGATTTCCATT	224	64.4 63.7
Eco-AT/*Mse*-CC	2	VCG175-F/R	GCAACCTTTTCGGAAACAAC / TGAGTAACCCGCCAGTCCTA	150	63.2 64.4
Eco-AT/*Mse*-CC	2	OR0613-F/R	CAGATGCCTCAAACTCGTGA / AACCCGAGAGCCATCTATCA	217	64.1 63.5
Eco-AC/*Mse*-CC	3	ACG3a-F/R1	TCACCAAGGTCAGTAGCCGT / GTCCATGTCAGCAATCATCG	131	64.6 64.2
Eco-AC/*Mse*-CC	3	ACG3a-F/R2	TCACCAAGGTCAGTAGCCGT / CCATGTCAGCAATCATCGAC	129	64.2 64.2
Eco-AC/*Mse*-CC	3	ACG3b-F/R1	TTCCACTCAACTCCCAAGACC / GTGAGGGGTGAGAAGTGCAT	153	64.1 64.1
Eco-AC/*Mse*-CC	3	ACG3b-F/R2	TTCCACTCACTCCCAAGACC / GAAGAAGCCAAGCAGTGAGG	167	64.1 61.2
Eco-AG/*Mse*-CC	3	AG3195-F/R	CCCCGTCAAATTACAAAGGA / GTACAGCGGTGCTGTGTTGT	151	63.4 63.9
Eco-AT/*Mse*-CC	3	ATG3a-F/R1	GAGTCCTGAGTAACCCGCAAG / GACTGCGTACCAATTCATCG	100	64.9 63.8
Eco-AT/*Mse*-CC	3	ATG3a-F/R2	GAGTCCTGAGTAACCCCGCAA / CCAATTCATCGTCCAGATCC	100	64.9 64.2
Eco-AT/*Mse*-CC	4,5	275AT4-F/R	GCGACGTGAAACTTGAATGA / GTTACGTCCGCTCTCCCATA	158	63.8 63.8
Eco-AC/*Mse*-CC	6,7	ACa6F/R	GTAACCGAGCAAGCCAATTC / CGTACCAATTCACGGCGTA	178	63.4 64.4

Table 1.4. (continued)

AFLP primer combination	Specific for VCG	SCAR marker designation	SCAR primer sequence (5`-3`), Forward/Reverse	Product size (bp)	Ta (°C) F/R
Eco-AC/*Mse*-CC	6,7	ACb6F/R	CTGCGTACCAATTCACCAAC / AACCAACGAAACCAACCTCA	157	62.9 64.2
Eco-AC/*Mse*-CC	6,7	ACG6a-F1/R	CTGAGTAACCAGTCGTGGCA / GATGAGTGTTCGGCCAGTTCT	92	63.9 62.7
Eco-AC/*Mse*-CC	6,7	ACG6a-F2/R	AGTAACCAGTCGTGGCATCA / GATGAGTGTTCGGCCAGTTCT	89	63.2 62.7
Eco-AG/*Mse*-CC	6,7	AGa6F/R	GACTGCGTACCAATTCAGGTC/ GAGTCCTGAGTAACCCAACAG	159	63.4 63.0
Eco-AG/*Mse*-CC	6,7	AGb6F/R	TGTGGTTGGTTGGTGCTAAG / ACCAATTCAGCCACGCTAAG	156	63.5 64.0
Eco-AG/*Mse*-CC	6,7	330AG6-F/R	CATCTGAAAGGCCAAGGGTA / TCGAACAGCAATACCCAACA	225	63.8 64.0
Eco-AT/*Mse*-CC	6,7	AT6200-F/R	TTTTCGCTGCAGTTGTTGTC / GTCTGATTCCTGAGCCCAAA	159	63.9 64.1

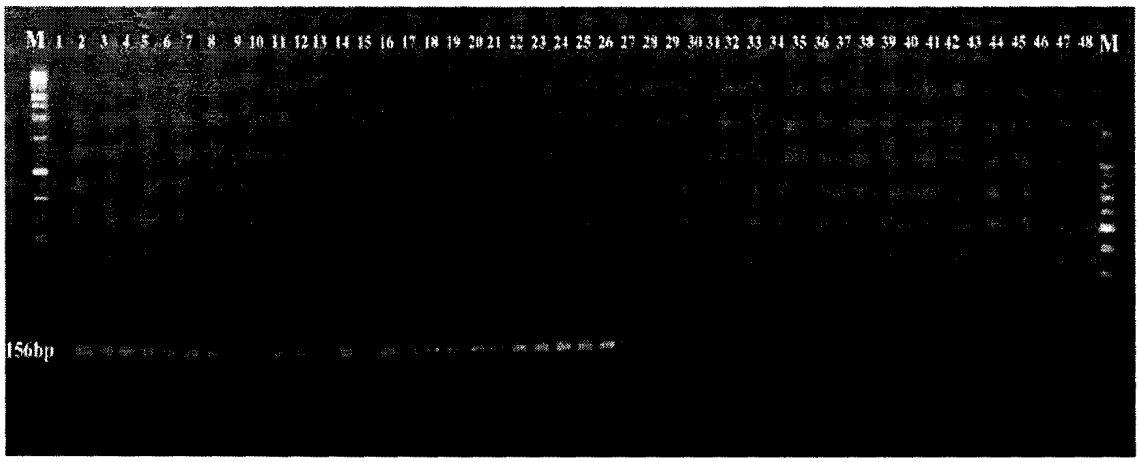

Figure 1.5. AGb6F/R primer with 156bp PCR product specific to detect NA-VCG-6 and NA-VCG-7. From the left: M: 1 kb molecular marker, lanes 1-17: NA-VCG-6 isolates, 18-27: NA-VCG-7 isolates, 28-30: NA-VCG-1, 31-33: NA-VCG-2, 34-36: NA-VCG-3, 37-38: NA-VCG-4, 39-41: NA-VCG-5, 42: *Colletotrichum lindemuthianum,* 43: *Streptomyces scabies,* 44: *Verticillium dahliae,* 45: *Acremonium strictum,* 46: *Fusarium graminearum,* 47: *Fusarium sambucinum,* 48: *Rhizoctonia solani,* M: 100bp molecular marker.

Figure 1.6. ACb6F/R primer with 157bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 1-25 are *C. coccodes* isolates, 25: *Fusarium graminearum*, 26: *Fusarium sambucinum*, 27: *Streptomyces scabies*, 28: *Rhizoctonia solani*, 29: *Verticillium dahliae*, 30: *Colletotrichum lindemuthianum*, 31: *Acremonium strictum*, and 32: blank.

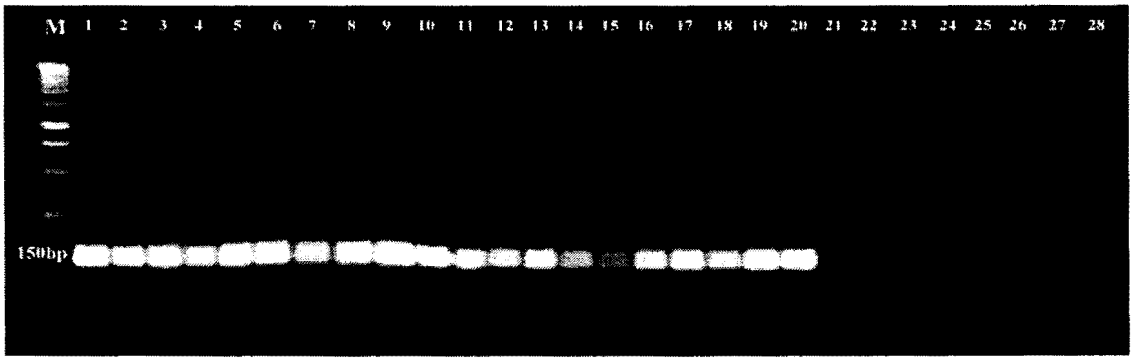

Figure 1.7. VCG175F/R with 150bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 1-20 are *C. coccodes* isolates, 21: *Fusarium graminearum*, 22: *Fusarium sambucinum*, 23: *Streptomyces scabies*, 24: *Rhizoctonia solani*, 25: *Verticillium dahliae*, 26: *Colletotrichum lindemuthianum*, 27: *Acremonium strictum*, and 28: blank.

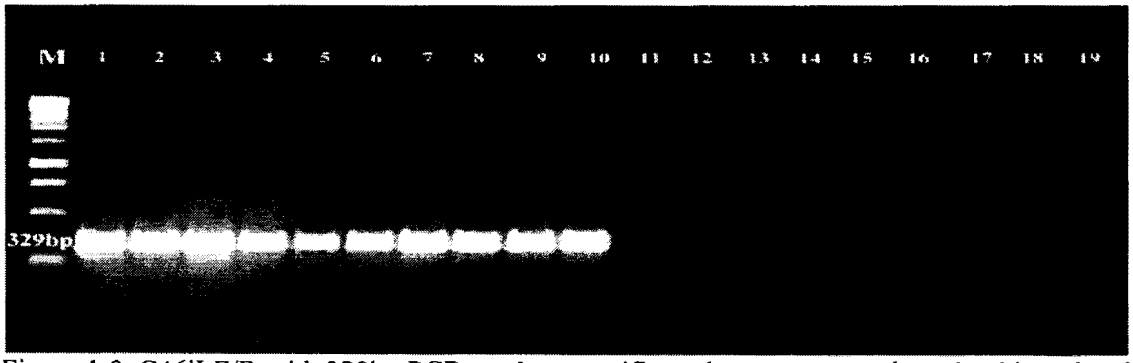

Figure 1.8. C46iLF/R with 329bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 1-10 are *C. coccodes* isolates, 11: *Fusarium graminearum*, 12: *Fusarium sambucinum*, 13: *Streptomyces scabies*, 14: *Rhizoctonia solani*, 15: *Verticillium dahliae*, 16: *Colletotrichum lindemuthianum*, 17: *Acremonium strictum*, and 18: blank.

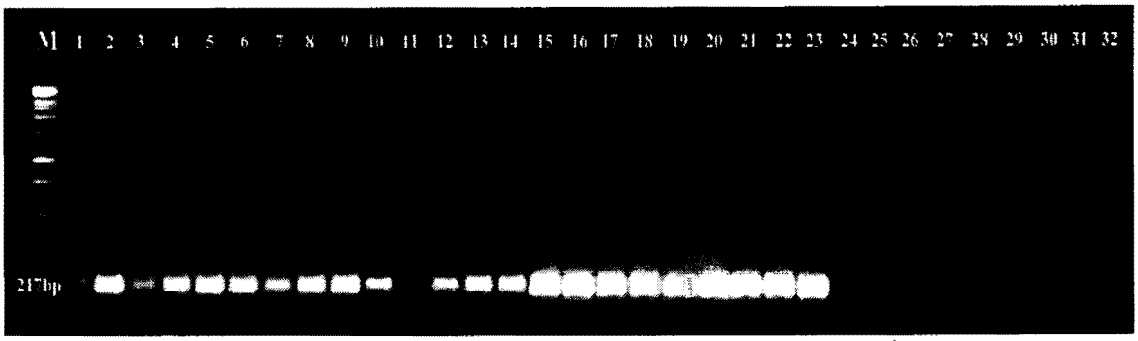

Figure 1.9. OR0613F/R with 217bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 1-23 are *C. coccodes* isolates, 24: *Fusarium graminearum*, 25: *Fusarium sambucinum*, 26: *Streptomyces scabies*, 27: *Rhizoctonia solani*, 28: *Verticillium dahliae*, 29: *Colletotrichum lindemuthianum*, 30: *Acremonium strictum*, and 31: blank.

Figure 1.10. 275AG2F/R with 201bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 1-15 are *C. coccodes* isolates, 16: *Fusarium graminearum*, 17: *Fusarium sambucinum*, 18: *Streptomyces scabies*, 19: *Rhizoctonia solani*, 20: *Verticillium dahliae*, 21: *Colletotrichum lindemuthianum*, 22: *Acremonium strictum*, and 23: blank.

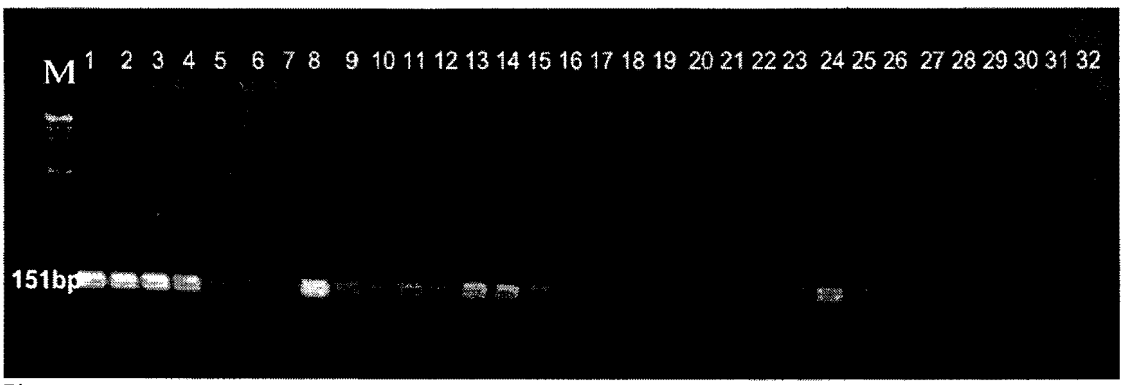

Figure 1.11. AG3195F/R with 151bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 1-25 are *C. coccodes* isolates, 26: *Fusarium graminearum*, 27: *Fusarium sambucinum*, 28: *Streptomyces scabies*, 29: *Rhizoctonia solani*, 30: *Verticillium dahliae*, 31: *Colletotrichum lindemuthianum*, 32: *Acremonium strictum*, and 33: blank.

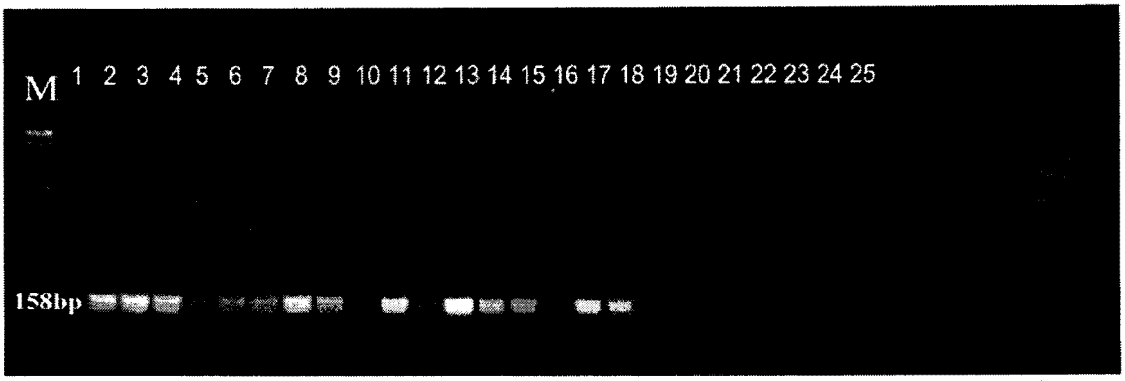

Figure 1.12. AT1125F/R with 158bp PCR product specific to detect *C. coccodes*. M: 1 kb molecular marker, lanes 2-9 and 11-18 are *C. coccodes* isolates, 1 and 10: blank, 19: *Fusarium graminearum*, 20: *Fusarium sambucinum*, 21: *Streptomyces scabies*, 22: *Rhizoctonia solani*, 23: *Verticillium dahliae*, 24: *Colletotrichum lindemuthianum*, 25: *Acremonium strictum*.

Discussion

The AFLP method was successful in separating *C. coccodes* isolates into five NA-VCGs (Heilmann et al., 2006) coinciding almost completely with the six original NA-VCGs identified using the nitrate *nit* mutants method of Nitzan et al. (2006). The two isolates, C124 and ORG1 that belong to NA-VCG4, did not cluster together. Instead, they clustered with NA-VCG2 and NA-VCG5 respectively. NA-VCG4 is rarely found in nature (Heilmann et al., 2006; Nitzan et al., 2006) and may not be a distinct VCG as originally reported (Nitzan et al., 2006). The data reported here and elsewhere (Heilmann et al., 2006) suggest that NA-VCG4 may be a group of isolates that are subgroups of other NA-VCGs.

The AFLP banding pattern showed presumptive bands specific for certain NA-VCGs. To our knowledge, this is the first study investigating the ability of converting AFLP fragments into SCAR markers specific for a VCG of a fungus species, not only for *C. coccodes*, but also for any plant pathogen. In this study, 20 AFLP bands were successfully excised, cloned and sequenced. Out of the sequences, 47 primers (22 forward, 25 reverse) were designed and screened to see their specificity for the VCG from where they were originally excised. Each primer was tested on seven isolates representing the

seven VCGs of *C. coccodes*. Unfortunately, only one primer showed specificity for a two VCGs of *C. coccodes*. For the other primers developed, each PCR product was found to be present in more than one VCG of *C. coccodes* and, therefore, found to be not specific for the target VCG. One primer set (forward/reverse), AGb6F/R, was successfully designed to be specific for NA-VCG-6 and NA-VCG-7 of *C. coccodes*. This primer was tested on broad range on all *C. coccodes* VCGs and on other fungal plant pathogens and it did not react with any fungus except all isolates of NA-VCG-6 and NA-VCG-7 of *C. coccodes*. This suggests that AGb6FR is specific to these two VCGs. These two VCGs could not be separated from each other using the three primer sets *Eco*-AC/ *Mse*-CC, *Eco*-AG/*Mse*-CC, and *Eco*-AT/*Mse*-CC, because their AFLP banding pattern was highly similar (Figure 1.4). More primer sets could be screened that could distinguish these two VCGs from each other, as well as distinguishing NA-VCG4 from NA-VCG5. Other primers were not found to be VCG-specific, but may still can be considered to identify *C. coccodes* and to distinguish it from other fungal species affecting potato. The success and failure in conversion of AFLP markers into SCAR markers has been reported previously. Two SCARs (S-9-1INT) and S-9-1EXT) out of 16 were developed to identify PG2 pathotype of *Verticillium albo-atrum* that infect hops in Slovenia (Radisek et al., 2004). The remaining SCARs lost their specificity but still could be used for the identification of *V. albo-atrum* in the hop growing regions in Solvenia (Radisek et al., 2004).

There are few reports of the successful development of SCAR markers for asexually reproducing fungi. SCAR markers have been successfully designed for a single *C. coccodes* strain 183088, a biocontrol of the velvetleaf weed species, using RAPD markers (Dauch et al., 2003). The specific primer sets (N5F/N5R, N5Fi/N5Ri) were

designed to be used in PCR assays. The primer sets amplified a product of 617 and 380 bp respectively. The SCAR primer sets were tested on the *C. coccodes* biocontrol strain 183088, other *C. coccodes* isolates, 15 phylogenetic groups of the genus *Colletotrichum*, and 11 other organisms. It was not mentioned whether these SCARs were tested on representatives of the seven VCGs of *C. coccodes*. Thus, further testing for the SCARs sets on the different *C. coccodes* VCGs is needed to truly determine the specificity of this SCAR marker.

The reasons for the unsuccessful conversion of AFLPs into PCR-based markers are unknown (Xu et al., 2001). Nevertheless, other studies have mentioned some possible explanations for the unsuccessful conversion of AFLP bands into PCR-based markers. The specificity of the AFLP marker may be lost when primers are synthesized from the internal parts of the AFLP sequence, or they might lose their ability to amplify genomic DNA (Shan et al., 1999). The small size of the AFLP marker and the possibility of contamination due to fragments from adjacent bands to the target band are other possible reasons for the unsuccessful conversion. Additionally, the possibility of migration of different AFLP bands with similar size along with the band of interest (Prins et al., 2001) may also explain the inability to convert AFLP bands into SCARs. Using pre- and selective amplification products as template DNA, instead of using the organism's DNA, can aid in designing successful SCAR markers by reducing the genomic background influence to a minimum (Xu et al., 2001).

In the study reported here both routine (silver staining) and sensitive methods (labeled primers using LI-COR model 4300) were used to run AFLP products on a 6% polyacrylamide gel. When the labeled primers were used, 0.5 µl of the amplified-PCR

product was loaded, and many specific bands for different VCGs with the three AFLP primer combination sets were clearly detected (Figure 1.4). On the other hand, using the silver staining AFLP procedure is less sensitive, and the amount of amplified product is 10 times the amount when using the labeled primers. It was not really possible to visualize precise banding patterns when using both methods. A possible solution when using a very sensitive apparatus like LI-COR® for separating the bands is to scan the polyacrylamide gel on LI-COR® Biosciences Odyssey® Infrared Imaging System with a grid pattern that would allow exact positioning of the band of interest. That band could then be cloned and sequenced for designing specific primers for that specific band. Unfortunately the Odyssey Infrared Imaging system was not available for these studies.

When the SCAR primer amplifies most of the DNA samples, that means there is a high similarity in sequences; therefore, one or more nucleotide polymorphisms should be identified using other methods before designing the SCAR primer (Xu et al., 2001). Sequencing DNA flanking regions in the *C. coccodes* genome could help in solving this problem (Bradeen and Simon, 1998; Brugmans et al., 2003; Meng et al., 2005; Xu et al., 2001). This could be done by using inverse PCR (I-PCR) (Bradeen and Simon, 1998), or by PCR-walking technology (Negi et al., 2000) which is preferable to I-PCR to identify the adjacent regions. By using either of these techniques, DNA adjacent to the AFLP fragment can be obtained, and either a single or a few nucleotide polymorphisms could be obtained, and then SCARs can be developed.

Further studies into the development of PCR-markers for *C. coccodes* would simplify the identification of *C. coccodes* VCGs and aid in studying the biology, epidemiology, and ecology of this fungus.

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CHAPTER TWO
POPULATION BIOLOGY STUDY OF *COLLETOTRICHUM COCCODES* IN NORTH AMERICA USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM

Abstract

Black dot disease of potato, caused by *Colletotrichum coccodes* (Wallr.) Hughes, is widely distributed in the United States. However, little is known regarding the population biology of this fungus. A total of 366 single spore *C. coccodes* isolates from nine states and 33 tester strains representing the seven vegetative compatibility groups of *C. coccodes* were analyzed using Amplified Fragment Length Polymorphism (AFLP). A total of 210 loci were generated and used to cluster the isolates into their NA-VCGs and to test the genetic structure of the population of *C. coccodes*. Across the three primer pairs used there were 190 polymorphic bands with 90.7% polymorphism. *C. coccodes* isolates recovered from potato plants were assigned to four NA-VCGs, NA-VCG1, NA-VCG2, NA-VCG4/5, and NA-VCG6/7. No isolates tested belonged to NA-VCG3. A total of 36 isolates were not clustered with any NA-VCG and formed distinct clusters; however, further testing showed that these isolates were more related to NA-VCG1 than to any other NA-VCG. NA-VCG2 was the dominant group in the population (n=238) and was the most frequent NA-VCG among states, fields, farms, and plants. However, in several instances there was more than one NA-VCG recovered from the same plant, field, farm, and state, indicating variability within the *C. coccodes* population in United States. A geographic pattern was observed for isolates originating from TX, MT, ND, and WI. Diversity within populations (states) accounted for 73%, and among populations there was 27% total genetic diversity. Genetic differentiation among the nine states was 0.331 and in estimating overall gene flow for the

366 isolates N_m =1.01. However, gene flow between population pairs was higher than the overall gene flow. These results suggest that several NA-VCGs are widely distributed in United States and that they form a single large population of *C. coccodes*.

Introduction

Black dot disease of potato, caused by *Colletotrichum coccodes* (Wallr.) Hughes, is a fungus with worldwide distribution. It occurs in Africa, Asia, Australia, Europe, North America, South America, and Central America (Andrivon et al., 1997; Raid and Pennypaker, 1987; Rich, 1983). It was named black dot due to the abundant sclerotia that are produced on tubers, stolons, roots and stems above and below ground (Andrivon et al., 1998; Dillard, 1992; Lees and Hilton, 2003). The disease not only affects the quantity (Johnson, 1994) and quality of potato tubers, but also it contaminates soil and serves as an important source of inoculum for future potato crops (Tsror (Lahkim) et al., 1999). *C. coccodes* is considered more important when it occurs with other fungi such as *Verticillium dahliae* (Kleb.), causing potato early dying syndrome (PED) (Lees and Hilton, 2003). Co-inoculating *C. coccodes* with *V. dahliae* caused more disease than having black dot alone on certain potato cultivars (Tsror (Lahkim) and Hazanovsky, 2001).

C. coccodes is an imperfect fungus belonging to Coelomycetes which are asexual fungi that produce their fertile hyphae in specialized structures called conidiomata (Cano et al., 2004). Vegetative compatibility serves as a sole mean of genetic exchange (Leslie, 1993). Vegetative compatibility (VC) refers to the ability of individual fungal strains to endure mutual hyphal anastomosis and form viable heterokaryons (Leslie, 1993). Characterization of the population of this fungus has been studied using nitrate non utilizing *nit* mutants method for many regions including North America (Heilmann et al.,

2006; Nitzan et al., 2006), Europe and Israel (Nitzan et al., 2002; Shcolnick et al., 2007), Austraila (Ben-Daniel et al., 2010), and South Africa (Personnel communication by L. Tsror to N.C. Gudmestad). The nitrate non utilizing *nit* mutant method used to designate vegetative compatibility groups (VCGs) has divided the *C. coccodes* population into seven, eight, and six VCGs for North American, European/Israeli, and Australian populations, respectively. Isolates from South Africa could not be assigned to any VCG due to the inability of these isolates to complement with each other or with other isolates of *C. coccodes* from the different continental populations (Personal communication of L. Tsror to N.C. Gudmestad).

Many studies before the identification of VCGs of *C. coccodes* reported an increased incidence of tuber infections (Johnson et al., 1997) and yield reductions with reduced tuber numbers after foliar infection of this fungus (Johnson, 1994). Incidence has been reported between 40% (Denner et al., 1998) to 50% and 90% in certified seed tubers (Johnson et al., 1997). Significant yield reduction (30%), have been observed in many potato cultivars (Tsror (Lahkim) et al., 1999). Yield losses may also result from potato early dying (PED) syndrome especially if pathogens, *Verticillium dahliae* and *C. coccodes* were present together (Tsror (Lahkim) et al., 1999). *C. coccodes* was found over the entire surface of tubers and in the medulla (Johnson et al., 1997). *C. coccodes* has been isolated more frequently from the stem end than from the bud end or lateral sections of infected tubers (Johnson et al., 1997). After the identification of *C. coccodes* VCGs, some VCGs have been found to be more frequently in the *C. coccodes* population among the different regions. EU/I-VCG2 was found to be the largest group in the Europe/Israel population (Nitzan et al., 2002, Shcolnick et al., 2007) and NA-VCG2 is the most prevalent in the

North American population (Heilmann et al., 2006; Nitzan et al., 2006). *C. coccodes* isolates from Australia appear to be dominated by AUS-VCG1 and AUS-VCG3 (Ben-Daniel et al., 2010). It is important to note, however, that these studies were performed on established culture collections and are not the result of a systematic survey of the fungus. Morphological variability has been reported among NA-VCGs (Aqeel et al., 2008). In that study, isolates belonging to NA-VCG2 had significantly larger sclerotia accompanied by significantly smaller conidia compared to other NA-VCGs, thereby suggesting a higher inoculum potential and perhaps greater frequency in nature. Aggressiveness is another important measurement within the population of any pathogen. Aggressiveness varied among VCGs in the different regions of the *C. coccodes* global population. EU/I-VCG3 (Nitzan et al., 2002), EU/I-VCG5 and EU/I-VCG6 (Shcolnick et al., 2007), AUS-VCG4 (Ben-Daniel et al., 2010), and NA-VCG2 (Aqeel et al., 2008; Nitzan et al., 2006), NA-VCG5 (Nitzan et al., 2006), and NA-VCG6 (Aqeel et al., 2006), from Europe/Israel, Australia, and North America respectively, were found to be the most aggressive VCGs among the three populations studied. However, some of the differences in aggressiveness may be due to the methods by which this trait is measured. Furthermore, differences in physiological traits among VCGs have been documented also (Nitzan and Tsror, 2003). Isolates belonging to the same VCG share common physiological traits such as growth at certain temperatures and pH level (Nitzan and Tsror, 2003). These differences among VCGs demonstrate that these groups are distinct in many aspects.

Population biology, the study of distribution and variation patterns in space and time within fungal species, and the analysis of these patterns in terms of genetic, developmental and environmental influences on phenotype and modes of production

(Hawksworth et al., 1995), has been studied in many plant pathogens (Burlakoti et al., 2008; Lee and Neate, 2007; Adhikari et al., 2008, and Collado-Romero et al., 2006). Population biology integrates ecological, evolutionary dynamics, and genetics of plant and pathogen populations and their interactions (Milgroom and Peever, 2003). Plant pathogenic fungi include a large group with vast diversity in ways and strategies in which they interact with their host (Burdon and Silk, 1997). The basis of evolutionary potential of species to respond to the environmental changes is diversity (Toro and Caballero, 2005). Understanding the pathogen's genetics, taxonomy, biology, ecology (Martin and English, 1997), diversity, origin, and evolution will facilitate understanding their role in shaping plant population genetic structure (Burdon and Silk, 1997) and how populations evolve in response to different control strategies (McDonald, 1997). Knowledge of VCG distribution in a population, and aggressiveness of the groups within that population, is of importance for the accurate evaluation of the possible damage, required control measures, and selection of isolates for breeding programs studies (Nitzan et al, 2002). Thus the main objectives of this study were to determine the distribution of *C. coccodes* NA-VCGs in the United States population obtained from different states, fields, and plant parts, and to study genetic diversity among NA-VCGs within this *C. coccodes* population.

Materials and Methods

***C. coccodes* isolates.** A total of 366 isolates of *C. coccodes* were collected in 2006, 2007, 2008, and 2009 from infected plants in fields from different states (Table 2.1). The isolates were recovered from tubers, stems, roots, stolons and leaves. After receiving samples they were sterilized in a 10% bleach, rinsed, dried, and plated on different media; potato dextrose agar (PDA), clarified V8 medium (CV8), water agar (H2O), and ethanol

media (ETOH). For above and below ground stem isolates; stems were divided at the soil line using sterile knifes and scalpels. Five thin slices from both above and below ground parts of stems were plated. Among the 366 *C. coccodes* isolates, there were 31 stems that had isolates originating from both above and below portions of the plant. These stems were from five fields in five states. Nineteen stems were from one field in North Dakota, eight from one field in Nevada, two from one field in Michigan, one from one field in Wisconsin, and one from one field in Minnesota. To test whether there are many NA-VCGs on the same potato tuber, five random lesions were excised and plated from 22 potato tubers. Twenty out of the 22 tubers were from one field in Wisconsin, and two tubers from one field in Minnesota. Pure cultures of *C. coccodes* isolates were obtained by hyphal tip cultures. Five agar plugs per isolate were transferred to one large Petri plate containing water agar. After 4-6 days, each plate was examined for contamination. Hyphal tipping was repeated and plated into culture plates containing 1% PDA and streptomycin. The purified cultures were checked for contamination after 5-7 days. These steps were performed to remove *Acremonium strictum* and other contaminants which fortunately were present (Rivera-Varas et al., 2007). For long term storage, four ml of 7.5% skim milk solutions was added to 14 day old culture plates, sclerotia were gently scraped from the plate surface, and were used to establish stock solutions on silica gel crystals and stored at -80°C. Isolates were also stored as colonized potato dextrose agar (PDA) plugs in 1.5 ml tubes at -20°C.

DNA extraction. Clean single spore isolates were plated from the long term storage stocks of the *C. coccodes* isolates collected. The isolates were allowed to grow for 7 to 10 days until adequate conidia and sclerotia were produced for DNA isolation. Five milliliters

Table 2.1. Origin, source and number of United States *C. coccodes*[1] isolates examined.

State	Year	Plant organ								Total/Yr	Total/state
		Stem				Root	Stolon	Tuber	Leave		
		Above	Below	Vascular	ni[2]						
CO	2006	5	8	-	-	-	5	-	3	21	
CO	2007	-	-	-	-	-	-	-	1	1	22
MI	2009	5	7	-	1	-	-	-	-	13	13
MN	2006	3	15	-	46	-	-	-	-	64	
MN	2007	-	-	-	-	-	-	1	-	1	
MN	2009	-	-	-	-	8	-	5	-	13	78
MT	2008	-	11	-	-	-	-	-	-	11	11
ND	2006	-	18	-	-	-	-	4	3	25	
ND	2007	29	22	-	8	-	-	-	-	59	84
NE	2006	1	21	-	-	-	-	-	2	24	
NE	2008	-	-	-	4	-	-	-	-	4	28
NV	2009	9	9	1	-	-	-	-	-	19	19
TX	2006	-	1	-	-	-	-	18	-	19	
TX	2009	-	-	-	5	-	-	-	-	5	24
WI	2006	5	5	-	-	-	-	-	-	10	
WI	2008	-	-	-	-	-	-	77	-	77	87
Total		57	117	1	63	8	5	105	9	366	366

[1] Isolates obtained from Dr. N. C. Gudmestad laboratory, NDSU, ND, USA.
[2] These isolates had no information regarding whether they originated from above or below ground stem parts.

sterile distilled water was added to the plates, and sclerotia and conidia were scraped using sterile glass rods, transferred to a glass flask with 100 ml Richard's solution (10.0 g Sucrose, 10.0 g potassium nitrate, 5.0 g potassium phosphate, 1.22 g magnesium sulfate, 0.02 g ferric chloride, 150 ml clarified V8 juice, and 850 ml ddH_2O) and shaken for 7 to 10 days with the cap slightly loosened and parafilmed. Before vacuum filteration, 10 µl of the solution was placed on a large Petri plate, containing 1% PDA without antibiotics, using a sterile glass rod. If the suspension was clean, vacuum filtration was performed, and the mycelium of the fungus was placed in 1.5ml plastic tubes, labeled, and stored at -80 °C for thirty minutes. Tubes were placed in a lyophilizer for 24 hrs to completely dry prior to

DNA extraction. The samples were ground using a mortar and pestle in liquid nitrogen. Cetyltrimethyl Ammonium Bromide (CTAB) protocol was followed for DNA extraction. Quantity, using TD-700 fluorometer, and purity, using 1.5% agarose gel, of DNA were assessed to ensure good DNA quality was used. DNA was also extracted from 33 multiple testers isolate, representing the seven NA-VCGs. These testers were used to assign the collected *C. coccodes* isolates into their NA-VCG according to their AFLP banding pattern.

***C. coccodes* confirmation.** The collected isolates were tested using specific primers for *C. coccodes* (Cullen et al., 2002) to confirm their identity. Two primers, Cc1NF1 and Cc2nR1, were used (Cullen et al., 2002) using a PTC-200 programmable thermocycler (MJ Research,Waltham, MA). The reaction contained 18.9 µl distilled-purified water, 2.5 µl (5X) *Gotaq* buffer, 1 µl $MgCl_2$, 1 µl dNTP mix (10 mM), 0.2 µl Cc1NF1 primer (36 ng/µl), 0.2 µl Cc1nR1primer (36 ng/µl), 0.2 µl *Gotaq* DNA Polymerase (5u/µl), and 1 µl of genomic DNA. Amplification was performed for initial denaturation cycle (95 °C for 2 min), 35 step cycles (95 °C for 45s, 72 °C for 2.15 min), and 72 °C for 5 minutes.

AFLP assays. The method of Vos et al. (1995) as modified by Heilmann et al. (2006) was used to conduct AFLP analysis for each *C. coccodes* isolate. In this method, both digestion and ligation are combined in one step. A reaction composed of 11 µl of the digestion-ligation materials was conducted for each isolate. In this reaction, 4.6 µl water, 1.1 µl ligation buffer, 1.1 µl of 1M NaCl, 0.11 µl BSA, 0.5 µl ATP, 1 µl *Eco*RI-adapter (16 bp adapter-primer sequence) and 1 µl *Mse*I-adapter (16-bp adapter-primer sequence), 0.2 µl of *Mse*I, 0.3 µl of *Eco*RI, and 0.1 µl of T4 DNA ligase and one micro liter of the genomic DNA, were added. The reaction was performed at 37 °C for 3.5 hrs. Selective amplification step was performed with a final reaction volume of 25 µl for each sample. Each reaction

contained 0.85 µM dNTPs, 1 µl of 25mM $MgCl_2$, 2.5 µl of 5X PCR reaction buffer, 1 µl (36 ng) of each primer set with two-base extensions, 2.0 µl of the adapter-primer DNA, and 0.1 unit of *Taq* DNA Polymerase (Promega Corp., Madison, WI). *Eco*RI-AC/*Mse*I-CC (5' GACTGCGTACCAATTCAC 3'), *Eco*RI-AG/*Mse*I-CC (5'GACTGCGTACCAATTCAG 3'), and *Eco*RI-AT/*Mse*I-CC (5' GACTGCGTACCAATTCAT 3') were the three primer pairs (Heilmann et al., 2006) that were used for the selective amplification. The forward primers were labeled with IRDye-700. Selective amplification was carried out in PTC-200 programmable thermocycler (MJ Research,Waltham, MA) for 39-repeated cycles with initial cycle of 2 minutes at 72 °C, 30 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C, and a final cycle at 72 °C for 5 minutes. The selective amplicon was viewed on 2% *(m/v)* agarose gel to see a smearing pattern indicating the success of selective amplification of the sample. The amplicons were electrophoresed on 6% (w/v) polyacrylamide /7 M urea denaturing gel in 1X TBE (1,1,2,2-tetrabromoethane) buffer for the three primers sets, and were visualized using LI-COR model 4300, a DNA analyzer. Default AFLP program was used to run the gels for 3.5 hours, at 1500 V (40W).

Scoring of AFLP fragments. AFLP images were printed, and scored manually for presence or absence (1 = presence or 0 = absence) of single band for each isolate. Fragments within the range of 50 to 650 bp were scored for the three primer pairs used. A total of 210 AFLP fragments were scored for the 366 *C. coccodes* isolates including 71 DNA fragments with *Eco*RI-AC/*Mse*I-CC primer, 66 with *Eco*RI-AG/*Mse*I-CC primer, and 73 with *Eco*RI-AT/*Mse*I-CC primer. The binary matrices with scores for the presence or absence of bands were combined and used for data analysis.

Data analysis. A consensus tree was generated via Phylogeny Inference Package (PHYLIP, University of Washington). A bootstrap analysis of 1000 permutations was used to test tree branche reliability in SEQBOOT tool. Statistical Analysis Software (SAS) was used to calculate the distance matrix which was used by the NEIGHBOR tool. An Unweighted Pairgroup Method with Arithmetic Means (UPGMA) was used with 1,000 bootstrap replicates to generate an out tree for CONSENSE tool of PHYLIP. The output file generated via CONSENSE was then used via the DRAWGRAM tool to generate the dendogram. TASSEL2.1 stand alone software (Trait Analysis by aSSociation, Evolution, and Linkage) (Bradbury et al., 2007) was also used to generate trees based on UPGMA. The *C. coccodes* isolates were classified into nine (state) populations according to their geographic origin: Colorado, Minnesota, North Dakota, Nebraska, Wisconsin, Texas, Montana, Michigan, and Nevada to test for geographic pattern. The *C. coccodes* population (n=366) was also divided into five sub-populations according to the assignment of the isolates into a NA-VCG. These groups included NA-VCG1, NA-VCG2, NA-VCG4/5, NA-VCG6/7, and a group that had the non-assigned isolates according to PHYLIP software output. It is important to note that the AFLP analysis, as explained later, cannot distinguish NA-VCG 4 & 5 and NA-VCG 6 & 7 at the molecular genetics level.

Data generated from the 210 loci were combined to identify a multilocus genotype (G) for each isolate. Statistics for AFLP loci used in this study were calculated via POPGENE version 1.32 (Yeh et al., 1997). The statistical measurements included allele frequency, gene diversity (h) (Nei, 1973), Nei's unbiased genetic identity (I), genetic distance (D) and percentage of polymorphic loci. Total gene diversity (H_T) for all 210 loci across populations, within populations (H_S), and the average differentiation among

populations (G_{ST}), as well as gene flow (N_m) were also estimated using this software. Gene flow was estimated from G_{ST}, using the formula $N_m = (1-G_{ST})/2G_{ST}$, where 2 was used in the denominator because *C. coccodes* is a haploid fungus (McDermott and McDonald, 1993). The data generated for each primer combination was tested for number and percentage of polymorphic bands, and for G_{ST} in order to determine whether these three primers generate close G_{ST} values. If the G_{ST} value was consistent among the three primers; overall estimation of G_{ST} value would be applicable. The analysis of molecular variance (AMOVA) for the 366 isolates of *C. coccodes* and pairwise population differentiation were also calculated by GENALEX 6.2 software (Peakall and Smouse, 2006). The variance in the *C. coccodes* population was partitioned into 'among populations' and 'within population variation' (ΦPT). The significance level was tested at $P<0.05$, and was determined using 1,000 permutations (Excoffier et al., 1992). The number of different genotypes (*G*), genotypic diversity (*GD*), and linkage disequilibrium (LD) were calculated by Multilocus 1.3b software (Agabow and Burt, 2001; Pritchard et al., 2000). Linkage disequilibrium is defined as the nonrandom association between two markers. It ranges between 0 and 1. If the LD value is significantly different from zero, that means there is less gene flow, recombination, and mutation.

Results

***C. coccodes* isolates confirmation.** All 366 isolates of *C. coccodes* yielded a 349 bp amplicon (Cullen et al., 2002) confirming *C. coccodes* as the identity of the fungus used in these studies.

Assigning *C. coccodes* isolates into NA-VCGs. The 366 *C. coccodes* isolates were analyzed by AFLP using the three primer pairs. The AFLP analysis provided a clear

banding pattern that could distinguish five NA-VCGs of *C. coccodes* representing United States populations. NA-VCGs 6 and 7 were easily identified by their numerous bands generated by the three primer pairs, but could not be distinguished from each other due to a high similarity in the banding pattern for both of these vegetative compatibility groups. The two isolates of NA-VCG4, C124 and ORG1, which were used as tester strains for this VCG, were similar in their AFLP banding pattern to NA-VCG5. NA-VCG3 tester strains had distinctive banding pattern that could differentiate them from other NA-VCGs, but this VCG was not found among any of the *C. coccodes* isolates tested. NA-VCG2 and NA-VCG5 had relatively high similarity in their AFLP banding pattern mainly in *Eco*-RI-AC/*Mse*I-CC and *Eco*RI-AG/*Mse*I-CC primer pairs. Cluster analysis based on UPGMA analysis separated the United States *C. coccodes* isolates into ten distinct clusters (Figure 2.1). In the dendogram generated, the *C. coccodes* isolates grouped efficiently with the NA-VCGs tester isolates into five groups. Out of the 366 *C. coccodes* isolates tested, 69 isolates belonged to NA-VCG1, 229 isolates belonged to NA-VCG2, 19 isolates to NA-VCG4/5, and 13 to NA-VCG6/ 7 (Table 2.2). A total of 36 isolates could not be assigned to any NA-VCG and these isolates formed six distinct clusters (Figure 2.1). Cluster analysis alone was insufficient to assign these isolates into their VCG. Further analysis (Chapter 3) showed that these isolates were subsequently assigned with 90% probability to a group closely related to NA-VCG1 and NA-VCG3 isolates.

NA-VCG's distribution among states, fields, and plant tissue. In all states, NA-VCG2 was the most frequent VCG recovered from fields (Table 2.2). It accounted for 62.5% of the total isolates collected. On the other hand, isolates belonging to NA-VCG3 had the lowest percentage (0%) recovery (Table 2.2).

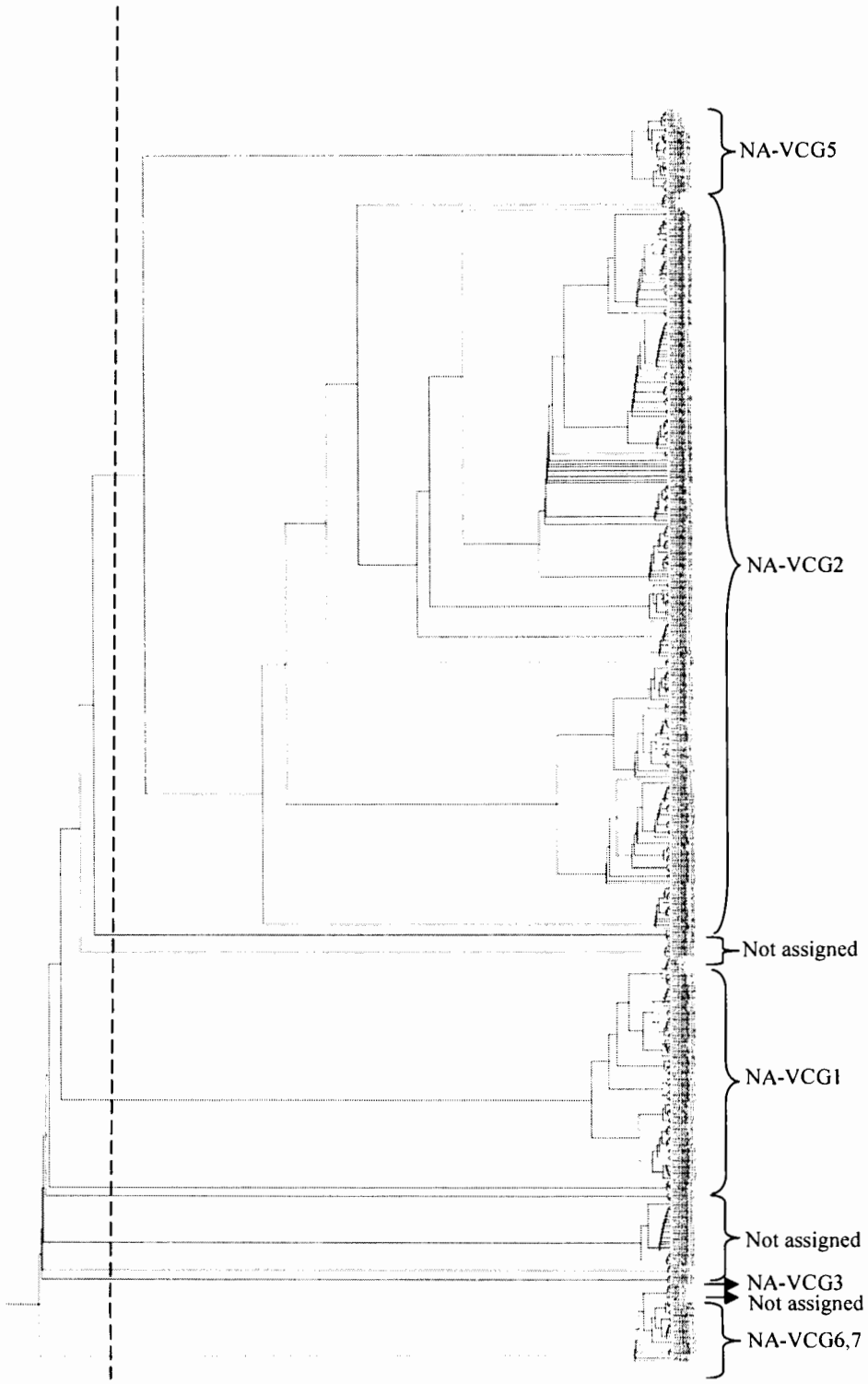

Figure 2.1. Dendogram generated by 1,000 bootstraps reiterations using PHYLIP application to analyze AFLP data of 366 *C. coccodes* North American isolates. Unweighted pair group method with arithmetic cluster analysis was used to generate the dendogram using a similarity matrix of 210 AFLP bands that were scored as present or absent. The dashed vertical line designates the separation point for assigning the clusters.

Table 2.2. Designation of *C. coccodes* isolates collected in the United States into NA-VCGs.

State	NA-VCG1[1]	NA-VCG2	NA-VCG3	NA-VCG5	NA-VCG6/7	Not assigned	Total
Colorado	5	12	-	4	1	-	22
Michigan	1	8	-	-	-	4	13
Minnesota	43	32	-	1	2	-	78
Montana	-	3	-	1	7	-	11
North Dakota	8	72	-	-	-	4	84
Nebraska	5	17	-	1	1	4	28
Nevada	4	10	-	3	1	1	19
Texas	1	3	-	1	-	19	24
Wisconsin	2	72	-	8	1	4	87
Total	69	229	-	19	13	36	366

[1] NA-VCG: north American vegetative compatibility groups

None of the *C. coccodes* isolates collected in the USA clustered with NA-VCG3. Of the nine states represented, five had isolates from four VCGs, namely, NA-VCG1, 2, 4/5, and 6/7 (Table 2.2). Nineteen isolates originating from Texas did not cluster with any NA-VCG but were together in one cluster.

NA-VCG2 was the most frequent VCG within fields in Colorado, Michigan, North Dakota, Nebraska, Nevada, and Wisconsin (Table 2.3). On the other hand, NA-VCG1 was the most frequent VCG in Minnesota, and NA-VCG6/7 was the most frequently recovered VCG from Montana (Table 2.3). Within fields, 20% of the isolates recovered from potato plants belonged to more than one VCG (Table 2.3). NA-VCG1 and NA-VCG2 were the most frequently recovered VCGs from potato plants within fields (Table 2.3) and from various plant organs (Table 2.4). NA-VCG2 was the most frequently recovered VCG from above and below ground stems and tubers (Table 2.4). Interestingly, 24 isolates recovered from tubers could not be assigned to any known VCG (Table 2.4).

Table 2.3. *C. coccodes* NA-VCGs[1] distribution within fields in nine states of United States.

State	Field	NA-VCGs						Total/field	Total/State
		VCG1	VCG2	VCG3	VCG4/5	VCG6/7	na[2]		
CO	F1	5	8	-	-	-	-	13	22
	F2	-	1	-	4	-	-	5	
	F3	-	3	-	-	-	-	3	
	F4	-	-	-	-	1	-	1	
	Total	5	12	-	4	1	-		
MI	F1	1	8	-	-	-	4	13	13
	Total	1	8	-	-	-	4		
MN	F1	7	1	-	-	-	-	8	
	F2	3	2	-	-	-	-	5	
	F3	2	1	-	-	2	-	5	
	F4	-	1	-	-	-	-	1	
	F5	-	3	-	-	-	-	3	
	F6	1	-	-	-	-	-	1	
	F7	12	2	-	-	-	-	14	
	F8	1	3	-	-	-	-	4	
	F9	2	5	-	-	-	-	7	
	F10	7	6	-	-	-	-	13	
	F11	6	6	-	-	-	-	12	
	F12	2	2	-	1	-	-	5	
	Total	43	32		1	2			
MT	F1	-	3	-	1	7	-	11	11
	Total		3		1	7			
ND	F1	-	12	-	-	-	-	12	84
	F2	1	-	-	-	-	3	4	
	F3	6	44	-	-	-	1	51	
	F4	-	8	-	-	-	-	8	
	F5	-	3	-	-	-	-	3	
	F6	1	5	-	-	-	-	6	
	Total	8	72				4		
NE	F1	-	1	-	-	-	-	1	
	F2	-	1	-	-	-	1	2	
	F3	4	15	-	1	-	-	20	
	F4	1	-	-	-	-	-	1	
	F5	-	-	-	-	1	3	4	
	Total	5	17		1	1	4		
NV	F1	4	10	-	3	1	1	19	19
	Total	4	10		3	1	1		
TX	F1	-	3	-	-	-	2	5	24
	F2	1	-	-	-	-	17	18	
	F3	-	-	-	1	-	-	1	
	Total	1	3		1		19		
WI	F1	-	64	-	8	1	4	77	87
	F2	2	8	-	-	-	-	10	
	Total	2	72		8	1	4		
Total		69	229	-	19	13	36	366	366

[1] NA-VCGs: North American vegetative compatibility groups

[2] na: non assigned isolates

Table 2.4. Plant tissue origin from 366 *C. coccodes* isolates originated from potato tested for presumptive NA-VCG[1] utilizing AFLP method analysis.

Plant tissue[2]	NA-VCG1	NA-VCG2	NA-VCG4/5	NA-CG6/7	na[3]	Total
Stem						
AG	11	39	2	-	3	55
BG	26	77	3	9	4	119
n.i	20	36	-	2	5	63
Tuber	4	68	8	1	24	105
Root	7	1	-	-	-	8
Stolon	-	1	4	-	-	5
Vascular	-	-	1	-	-	1
Leaves	1	7	-	1	-	9
Total	69	229	18	13	36	366

[1] NA-VCGs: North American vegetative compatibility groups.
[2] AG: Above ground, BG: below ground, n.i: stem part not identified.
[3] na: isolates not assigned to any presumptive NA-VCG using the three primer sets.

There were 31 stems from which isolates of *C. coccodes* were recovered from above and below ground tissues (Table 2.5). NA-VCG2 was found in 27 stems and was found in the same above and below ground stem parts in 17 of 31 plants. In the remaining 14 potato plants, the VCG in below ground tissues was different than that recovered from the above ground tissues (Table 2.5). When multiple isolations were made from the same potato tuber, NA-VCG2 was recovered from 21 tubers and 13 of 22 tubers had NA-VCG2 recovered from all the lesions on the same tuber. Eleven tubers had two VCGs recovered from them; these VCGs primarily belonged to NA-VCG1 and NA-VCG5. Only one tuber was found infected with three NA-VCGs (2, 5, 6/7) (Table 2.5).

Genetic structure of the United States *C. coccodes* population by states. The three primer pairs used to analyze the 366 *C. coccodes* isolates generated 210 bands; these bands were reproducible and clear over all *C. coccodes* isolates. The three primers

Table 2.5. North American vegetative compatibility groups (NA-VCGs) recovered from above and below ground stem parts from the same plant[1], and from lesions on same tuber[2].

# of stems	Above ground/below ground[3]							
	1/1	1/2	1/na	1/6,7	2/2	2/na	2/5	na/na
31	1	4	1	1	17	4	2	1

# of tuber	NA-VCG recovered from lesions on the same tuber[4]				
	1	2	2/na	2/5	2/5/6,7
22	1	13	2	5	1

[1] The stems were from five fields in five states. Ninteen stems were from one field in ND, eight from one field in NV, two from one field in Michigan, one from one field in Wisconsin, and one from one field in Minnesota.
[2] Twenty out of the 22 tubers were from one field in Wisconsin, and two tubers from one field in Minnesota.
[3] Isolates were recovered from both above and below ground parts for the same plant. na: non assigned isolates.
[4] At least two isolates were recovered for each tuber tested.

combinations generated a high degree of polymorphism among *C. coccodes* isolates (87 to 97%) (Table 2.6). Differences among populations (G_{ST}) obtained by the three primers were consistent, indicating that the overall differentiation among the *C. coccodes* population can be averaged across the three primers (Table 2.6).

Table 2.6. Total number of AFLP bands scored, number and percentage of polymorphisms with three primer combinations used in analysis of 366 *C. coccodes* isolates collected in 2006-2009 from United States, and G_{ST} estimation.

Primer combination	No. of bands[1]	No. of polymorphic bands	Polymorphism (%)	G_{ST}[1]
*Eco*RI-AC/*Mse*I-CC	71	62	87.32	0.28
*Eco*RI-AG/*Mse*I-CC	66	64	96.97	0.27
*Eco*RI-AT/*Mse*I-CC	73	64	87.67	0.30
Total	210	190	90.65	

[1] Population differentiation for each primer was tested. If the value varied among the three primers; overall estimation will not be applicable.

When gene diversity analysis was performed on the data from the *C. coccodes* isolates recovered from nine states, total genetic diversity for all 210 loci was relatively moderate (H_T = 0.23) and gene diversity within a state population was relatively low (H_S=0.15) (Table 2.7). This resulted in a relatively high differentiation among the *C. coccodes* populations (G_{ST}=0.33) (Table 2.7). Among states, gene diversity (h) ranged between 0.107 (ND) to 0.23 (MT) (Table 2.8). Both the number of distinct genotypes (*G*) and genotypic diversity (*GD*) values were high in all populations except for the Texas population of *C. coccodes* (Table 2.8). Among the 366 *C. coccodes* isolates analyzed, 307 distinct genotypes were identified (Table 2.8).

Table 2.7. Gene diversity analysis in *C. coccodes* population according to states differentiation.

Population[1]	Sample size	Ht[1]	Hs[2]	G_{ST}[3]	N_m[4]
All States	366				
Mean		0.23	0.15	0.33	1.01
St. Dev		0.026	0.013		

[1] Genetic diversity in the total population.
[2] Genetic diversity within population.
[3] Estimates Nei's (1973) G_{ST}, average differentiation among populations.
[4] N_m = estimate of gene flow for overall population from G_{ST}, $N_m = 0.5(1 - G_{ST})/G_{ST}$.

Linkage disequilibrium values also varied among states. It ranged from 0.09 (low) in Minnesota to 0.41 (high) in Montana. Linkage disequilibrium values correlate negatively with gene flow. Among all the populations, LD values were significantly different from zero (P<0.01) (Table 2.8). Pairwise comparison of genetic identity among the nine populations (states) showed high values ranging from 0.79 (TX and MT) to 0.99 (WI and ND) (Table 2.9). The overall estimated differentiation among the population (G_{ST}) and gene flow (N_m) values among the nine populations (states) were 0.33 and 1.01, respectively. The overall estimated value of N_m means that there is one migrant per

generation among the nine populations. Since the N_m value was approximately 1.0, this means that gene flow and gene drift among the nine populations are balanced.

Table 2.8. Genetic variation statistics for the 210 loci according to state-population differentiation.

Population	Sample size	Loci[1]	%[2]	h[3]	*G*[4]	*GD*[5]	LD[6]
Colorado	22	134	63.81	0.15	20	0.99	0.21
Minnesota	78	161	76.67	0.19	75	0.99	0.09
North Dakota	84	129	61.43	0.11	72	0.99	0.11
Nebraska	28	131	62.38	0.17	27	0.99	0.13
Wisconsin	87	155	73.81	0.11	62	0.97	0.14
Texas	24	144	68.57	0.14	10	0.61	0.32
Montana	11	118	56.19	0.23	11	1.00	0.41
Michigan	13	119	56.67	0.14	13	1.00	0.12
Nevada	19	116	55.24	0.13	17	0.98	0.21

[1] Number of polymorphic loci among all isolates evaluated
[2] The percentage of polymorphic loci
[3] h = Nei's (1973) gene diversity
[4] *G*: number of distinct genotypes
[5] *GD*: genotypic diversity
[6] LD: Measurement of linkage disequilibrium, all values are significantly different from zero ($P<0.01$)

The gene flow between the ND and WI population pair showed the highest N_m value (N_m=11.01), while the MT and ND populations pair showed the lowest N_m (1.02) (Table 2.9). A high gene flow value suggests genetic exchange among states such as WI and ND and also within those respective states.

Table 2.9. Pairwise comparison of gene flow (N_m)[1] (above diagonal) and genetic identity (below diagonal) according to states population differentiation.

Pop[1]	CO	MN	ND	NE	WI	TX	MT	MI	NV
CO	****	9.38	2.27	7.87	2.33	1.27	1.18	1.66	2.19
MN	0.98	****	3.80	7.06	3.6	1.83	1.45	2.88	4.20
ND	0.94	0.96	****	2.30	11.01	1.04	1.02	2.25	2.58
NE	0.98	0.97	0.94	****	3.06	1.48	1.27	1.93	2.38
WI	0.94	0.95	0.99	0.95	****	1.07	1.12	2.30	2.80
TX	0.87	0.89	0.86	0.88	0.86	****	1.07	1.07	1.27
MT	0.81	0.82	0.81	0.81	0.82	0.79	****	1.23	1.19
MI	0.90	0.94	0.94	0.91	0.94	0.85	0.82	****	1.07
NV	0.93	0.96	0.95	0.93	0.95	0.88	0.83	0.93	****

[1] N_m = estimate of gene flow from G_{ST}, $N_m = 0.5(1 - G_{ST})/G_{ST}$.

The UPGMA Nei-based dendogram clustered the *C. coccodes* isolates into 5 main groups (Figure 2.2). The first cluster included *C. coccodes* isolates from CO, MN, and NE, the second cluster included ND, WI, and NV, and the other 3 clusters included populations from MI, TX, and MT.

Analysis of molecular variance showed that 27% of the estimated variation originated from the estimated variance among the state populations, and 73% variation originated from the within the state population estimated variance (ΦPT) (Table 2.10). Statistical measurement of ΦPT, within population differentiation, was significant at $P = 0.001$.

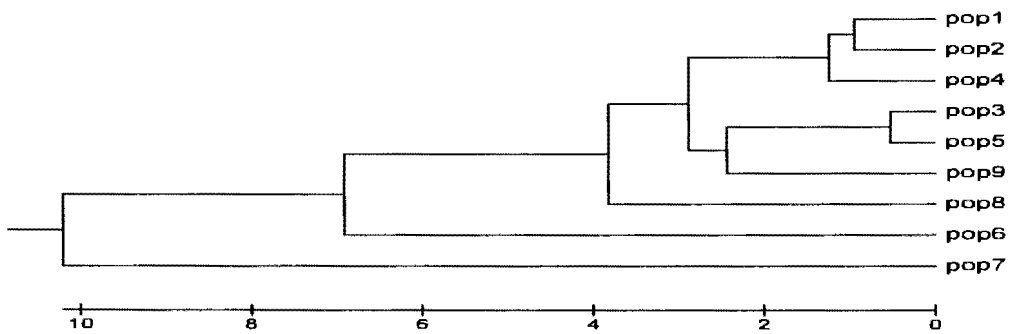

Figure 2.2. UPGMA Nei based dendogram for *C. coccodes* population differentiation according to states. Populations were divided according to states: pop 1: Colorado, pop 2: Minnesota, pop 3: North Dakota, pop 4: Nebraska, pop 5: Wisconsin, pop 6: Texas, pop 7: Montana, pop 8: Michigan, and pop 9: Nevada.

Table 2.10. Analysis of molecular variance (AMOVA) summary for United States *C. coccodes* population according to states origin.

Source	df	Est. Var.	%	Φ Stat[1]	*P*-Value[2]
Among states	8	5.59	27%		
Within states ΦPT	357	15.2	73%	0.27	0.001
Total	365	20.79	100%		

[1] ΦPT was calculated as the proportion of estimated variance for among states, relative to the total estimated variance.
[2] Probability of obtaining low Φ value was determined by 1,000 permutations.

Genetic structure of United States *C. coccodes* VCGs. Average differentiation among NA-VCGs (G_{ST}) obtained by the three primers was very high (G_{ST}=0.45) (Table 2.11). Gene diversity analysis was performed on data from the five groups obtained based on PHYLIP analysis. The total genetic diversity for all the 210 loci was relatively high (H_T = 0.28). The genetic diversity within a NA-VCG population was relatively low (H_S=0.15), resulting in a high differentiation among the NA-VCG populations (G_{ST}=0.45). The gene diversity (h) ranged between 0.10 (NA-VCG2) to 0.21 (the group with the non-assigned isolates) (Table 2.12). Both the number of distinct genotypes and genotypic diversity values were high among all NA-VCGs (Table 2.12). The linkage disequilibrium value among the entire NA-VCG population was low (0.09), but varied among VCGs, from low in NA-VCG2 (0.09) to high in NA-VCG1 (0.21). Among all NA-VCGs, LD values were significantly different from zero (P<0.01) (Table 2.12), indicating nonrandom association among loci.

Table 2.11. Gene diversity analysis in *C. coccodes* population according to NA-VCG- differentiation.

Population	Sample size	Ht[1]	Hs[2]	G_{ST}[3]	*Nm*[4]
All VCGs	366				
Mean		0.28	0.15	0.45	0.61
St. Dev		0.029	0.013		

[1] Genetic diversity in the total population.
[2] Genetic diversity within population.
[3] Estimates Nei's (1973) Gst, average differentiation among populations.
[4] *Nm* = estimate of gene flow from *G*st, $N_m = 0.5(1 - G_{ST})/G_{ST}$.

Pairwise comparison of genetic identity (*I*) among NA-VCGs showed values ranging from low (*I*= 0.65) between NA-VCG2 and NA-VCG6/7, to high (0.94) between VCG2 and NA-VCG4/5 isolates. The non assigned isolates had genetic identity (*I* = 0.92) with NA-

VCG1. These results indicate that the non-assigned isolates are more related to NA-VCG1 than any other VCG (Table 2.13).

Table 2.12. Genetic variation statistics for the 210 loci according to NA-VCG population differentiation.

Population	Sample size	#Pol. Loci[1]	%[2]	h[3]	*G*[4]	*GD*[5]	LD[6]
NA-VCG1	69	140	66.67	0.18	66	0.99	0.21
NA-VCG2	229	143	68.10	0.10	18	0.99	0.09
NA-VCG4/5	18	77	63.67	0.11	15	0.99	0.11
NA-VCG6/7	13	125	59.52	0.16	13	0.99	0.13
Not assigned	36	166	79.05	0.21	25	0.97	0.14

[1] The number of polymorphic loci
[2] The percentage of polymorphic loci
[3] h = Nei's (1973) gene diversity
[4] *G*: number of distinct genotypes
[5] *GD*: genotypic diversity
[6] LD: Linkage disequilibrium, all values are significantly different from zero ($P<0.01$).

Table 2.13. Nei's unbiased measures of genetic identity (above diagonal) and differentiation among NA-VCGs (below diagonal) according to NA-VCG population differentiation.

Pop	VCG1	VCG2	VCG4/5	VCG6/7	na[1]
VCG1	****	0.90	0.92	0.68	0.92
VCG2	0.23	****	0.94	0.65	0.89
VCG4,5	0.19	0.20	****	0.66	0.90
VCG6,7	0.44	0.54	0.53	****	0.73
N.A	0.15	0.23	0.21	0.40	****

[1]Non assigned isolates group

The UPGMA Nei-based dendogram grouped the *C. coccodes* isolates across VCGs into three main clusters (Figure 2.3). The first cluster included NA-VCG1 and the non assigned isolates, the second included NA-VCG2 and NA-VCG5, and the third cluster included isolates from NA-VCG6/7.

The AMOVA demonstrated that 44% of variation originated from the estimated variance among VCGs and 56% of variation originated from the within the VCG estimated

variance (Table 2.14). The statistical measurement of ΦPT, within VCGs differentiation, was significant at $P = 0.001$. This means that the *C. coccodes* NA-VCGs are different from each other.

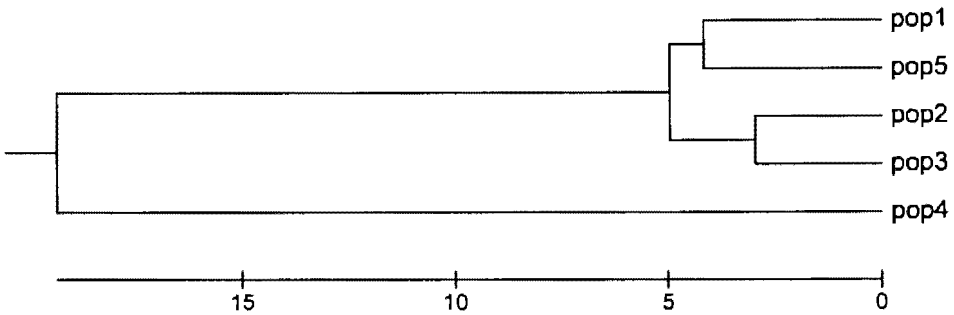

Figure 2.3. UPGMA Nei-based dendogram for *C. coccodes* population differentiation across VCGs. Pop1: NA-VCG1, pop2: NA-VCG2, pop3: NA-VCG4/5, pop4: NA-VCG6/7, and pop5: the non-assigned isolates. This dendogram was generated via POPGENE 1.32 software. The dendogram was refined using MEGA4 software (Kumar et al., 2008).

Table 2.14. Analysis of molecular variance (AMOVA) summary for United States *C. coccodes* VCGs.

Source	df	Est. Var.	%	ΦStat	*P*-value[2]
Among VCGs	4	10.86	44%		
Within VCGs (ΦPT)[1]	361	13.76	56%	0.44	0.001
Total	365	24.62	100%		

[1] ΦPT was calculated as the proportion of estimated variance for among populations, relative to the total estimated variance.
[2] Probability of obtaining low Φ value was determined by 1,000 permutations.

Discussion

AFLP loci were highly polymorphic and polymorphism percentage among all isolates in the population was high (90.48%), indicating the efficiency and effectiveness of these loci in differentiating the *C. coccodes* population in the United States and in identifying the genetic diversity of this population.

The results of this study are consistent with results from a previous study on *C. coccodes* (Heilmann et al., 2006) indicating the efficiency and reproducibility of AFLP analysis in studying the genetic makeup of this fungus. Results reported here also showed distinct groups within the United States population, indicating genetic diversity within this species. AFLP analysis separated the 399 isolates, including the tester strains, into five main distinct groups including the NA-VCGs 1, 2, 3, 4/5, and 6/7. This delineation could be demonstrated as distinct groups within the large population (Heilamnn et al., 2006), and most of the NA-VCGs could be separated successfully using AFLP molecular markers. This is consistent with a previous study (Heilmann et al., 2006) where five clusters were generated with the AFLP method. Isolates of *C. coccodes* belonging to NA-VCG4 clustered with NA-VCG5, isolates of NA-VCG6 clustered with NA-VCG7, and isolates of NA-VCG2 and NA-VCG5 clustered together. In the genetic analysis performed in this study, including gene diversity, genetic identity, population differentiation measurement, and a UPGMA Nei-based dendogram, NA-VCG2 and NA-VCG5 were shown to be more closely related to each other compared to the other VCGs.

The dominance of NA-VCG2 as the most frequently recovered VCG from potato plant tissues in most potato-producing states is not surprising. Previous studies of the United States *C. coccodes* population have found this group to be in higher frequency compared to the other groups when isolates from culture collections were analyzed (Aqeel et al., 2008; Heilmann et al., 2006; and Nitzan et al., 2006). This has been explained by the large size of the sclerotia and the small size of the conidia produced by isolates belonging to this VCG (Aqeel et al., 2006), suggesting a greater fitness (Aqeel et al., 2008; Nitzan et al., 2006) and inoculum potential. Variation in fitness among individuals promotes natural

selection (McDonald, 1997). NA-VCG2 was dominant in 17 of 31 plants with both above and below ground tissue parts, and in 13 of 22 tubers, the remaining plants (14) and tubers (9) tested had multiple VCGs parasitizing the same plant organ. However, NA-VCG2 was also found in eight of the 14 plants and eight of the nine tubers that had more than one VCG parasitizing them. Similarly, NA-VCG2 was the most frequently recovered VCG of *C. coccodes* from potato plants in fields of most states. In instances when more than one VCG was recovered within the same field, or among fields on the same farm, NA-VCG1 was the next most frequently recovered VCG of *C. coccodes*. Perhaps most interesting was the finding that the *C. coccodes* recovered from potato plants in Texas could not be assigned to any known or described VCG. Whether or not these isolates represent a distinct NA-VCG or are simply a sub-group of a previously characterized NA-VCG has yet to be determined. Regardless, the data reported here demonstrates the variability of the NA-VCGs distributed within an organ, a plant, within a field, and within and among states.

Despite the climatic and geographic differences among states, there was a high degree of similarity among the VCGs recovered from them. However, in some states a geographic pattern was observed, such as in Texas, Montana, North Dakota, and Wisconsin. The Texas and Montana populations of *C. coccodes* were different than the other states when looking to the genetic identity, genotypic diversity, population differentiation, and gene flow values of the isolates recovered from these states. This can be explained by the most frequent VCG found within these states. In Montana, NA-VCG6/7 accounted for half of the total isolates of this group (n=7). NA-VCG6/7 was found to be very distinct from the other groups. It had the highest population differentiation (G_{ST}), the lowest genetic identity (I) compared with the other VCGs, and a 100% distinct

genotype, meaning that every individual in this group is genetically different than the other. Montana is a closed seed potato state. Potato seeds from other states cannot go into Montana. This may explain the low gene flow between this state and all the other states.

Similarly, in Texas, 80% of the *C. coccodes* isolates did not cluster with any known VCG, demonstrating a geographic pattern in the VCGs distribution. However, further analysis of these isolates, in addition to the ones that were not assigned to any VCG tester, showed that they are more related to NA-VCG1 with a genetic identity of 92%. Furthermore, based on the UPGMA Nei's unbiased dendogram, the Texas *C. coccodes* isolates clustered with NA-VCG1. In contrast, *C. coccodes* isolates from North Dakota and Wisconsin had more than 83% of the isolates recovered belonging to NA-VCG2. Further genetic analysis including genetic identity (99%), gene flow (N_m=11), and gene diversity (h=0.11), demonstrating that the two populations from these two states were very similar.

A geographic pattern within the United States *C. coccodes* population is further substantiated by AMOVA which revealed that 27% of the estimated variation was a result of variation among states. Geographic and climatic differences among the states could explain this variation value. The remaining variation (73%) resulted from within states variation. This high value of variation can be explained by the relatively high genetic diversity in the total population (Ht=0.23), the high number of the distinct genotypes of *C. coccodes* (*G*=307 out of 366), and the existence of more than one VCG within the same state, field, and plant. However, overall an average of one or more migrants per generation (N_m => 1) between each pair of populations (states) appears to indicate genetic similarities among the nine populations. When *N*m=1, gene flow and gene drift are balanced, if N_m >1,

which is the case in most of the population (states) pairs, isolation by distance is nonexistent (Milgroom and Lipari, 1995). If an average of one or more migrants is exchanged per generation between populations, then the populations will not diverge by genetic drift, and the populations will gradually become more similar (McDonald, 2004). Additionally, genetic distances among the populations were quite low and genetic identity was relatively high (0.80 to 0.99) confirming a close relationship among individual in the entire population. If the gene flow value had exceeded a value of 4.0, then the *C. coccodes* population would have been considered as part of one population (Wright, 1951), as is the case among many population pairs (states pairs). High gene flow suggests that high genetic exchange has been frequent among populations (Yan et al., 2007) and this should not be surprising. Potato is a vegetatively grown crop and potato seed is frequently moved among states. Since *C. coccodes* infects potato tubers there has been ample opportunity for genetic exchange among the populations of this pathogen throughout the potato-producing states of United States.

A number of other studies have examined the genetic diversity within a population of a plant parasitic fungus. The genetic diversity and population differentiation of the fungus *Cryphonectria parasitica* (Murrill) M.E. Barr has been studied in China (Yan et al., 2007). The fungus populations were divided based on their geographic origin. Seventy-four percent of the diversity originated from the within populations diversity. G_{ST}, population differentiation was found to be 0.26, and considered a moderate value. Gene flow was found to be 1.4 among the populations. In that study, Southwest China was suggested as the center of origin of *C. parasitica*. Geographic distances, climatic differences, and human activities were suggested to affect the structure of *C. parasitica* populations. In other

studies, there was a lack of a geographic pattern found with the global population of *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur which was explained as evidence of gene flow within the population (Zhong and Stevenson, 2001). Genetic structure of *Septoria passerinii* Sacc. was examined from nine field populations at within lesions, among lesions in a leaf, among leaves in a field, and among fields in both North Dakota and Minnesota using the AFLP method (Lee and Neate, 2007). One to four genotypes were found within a lesion and six to nine genotypes found from lesions on a leaf. In fields, genetic diversity within a field was similar to that within a leaf. G_{ST}, the differentiation among populations was determined to be 0.24. This was due to the high differentiation of *Septoria passerinii* in certain areas. Data for that study suggested that the population structure of this fungus is consistent with a sexually reproducing fungus. In our study G_{ST} was relatively high in the *C. coccodes* population (G_{ST}= 0.332). This could be explained by the distribution of more than one VCG in each population, the variation among the isolates within each population, and the variation among the NA-VCGs where the G_{ST} value among VCGs was very high (0.451), indicating relatively high differentiation among the VCGs found (McDermott and McDonald, 1993).

Phaeosphaeria nodorum (E. Müll.) Hedjar. population genetic structure was studied in the North-Central and Midwestern US using RAPD and SSR markers (Adhikari et al., 2008). High levels of gene (0.18-1.52) and genotype diversity (0.60-0.97) for most populations, and gametic disequilibrium for four populations in North Dakota were reported. Sexual reproduction was suggested for this fungus in the major growing areas of the United States. Additionally, geographically separated populations were genetically differentiated indicating restricted gene flow or selection.

Genetic structure of a pathogen, the distribution, and the amount of genetic variation within and among pathogen populations, is an important step for effective managements (McDonald, 1997), after which the effect of different factors including mutation, gene flow, selection, and mating system, are required for determining the factors that have most impact on population genetic structure (McDonald, 1997). The results reported in this study revealed the considerable genetic variation within and among *C. coccodes* populations, and that this variation is structured into four main subgroups. However, this finding represents only nine states of the United States population of *C. coccodes* and, in some cases, very few isolates were recovered from some states. Nonetheless, there was more than one VCG recovered from some states, fields, and plants, indicating that *C. coccodes* VCGs are widely distributed within the United States. More isolates from the United States, representing all the main potato growing regions, as well as isolates of *C. coccodes* from different hosts and weeds, would enhance our understanding of the *C. coccodes* VCG variability, biology, and epidemiology. AFLP analysis was efficient in evaluating large populations in a relatively short period of time, increasing our ability to measure the genetic relationship for global population of *C. coccodes*. However, using co-dominant markers would give another view about the genetic diversity of this fungus. Molecular markers such as simple sequence repeat (SSR) could be used to study genetic differences among *C. coccodes* individuals. This marker was successful in studying genetic diversity in *Verticillium dahliae* (Subbarao et al., 2008) where only seven microsatellite markers explained 99% of the genetic variability in *V. dahliae* strains.

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CHAPTER THREE
GENETIC DIVERSITY STUDY OF THE GLOBAL POPULATION OF *COLLETOTRICHUM COCCODES* USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM MARKERS

Abstract

Colletotrichum coccodes (Wallr.) Hughes is an imperfect fungus, thus vegetative compatibility may serve as a means of genetic exchange and the useful for measuring genotypic diversity. Many vegetative compatibility groups (VCGs) have been identified for this fungus in different regions of the world, and variations in aggressiveness, morphological, and physiological traits have been detected among them. Although vegetative compatibility grouping is a widely used technique to study the genetic diversity among populations of asexually reproducing fungi, it has certain limitations that reduce its effectiveness and utility. In the case of *C. coccodes*, isolates from different continents do not anastomose thereby preventing an analysis of the global population of this plant pathogen. Molecular markers have proven very effective in studying the genetic diversity of several plant pathogens. The main objective of this study was to study the genetic diversity of a global population of *C. coccodes*, including North American, European, Middle Eastern, Australian, and South African isolates, using AFLP markers. A total of 855 *C. coccodes* isolates were studied. Three primer pairs were used and generated 210 loci. Based on this study, the population of *C. coccodes* exists as one large population with four main groups (NA-VCG1/3; NA-VCG2; NA-VCG4/5; and NA-VCG6/7). NA-VCG5 was the most common VCG globally, followed by NA-VCG2. Among the five regions studied, there was a relatively low gene diversity (0.222) and relatively high population differentiation G_{ST} (0.285). The overall gene flow (N_m) was 1.25, meaning that one or more

individuals are exchanged among the five regions each generation. Most of the variation among the five geographic regions originated from the within population differentiation (ΦPT) (79%). Nineteen percent of the estimated variation originated from among regions (ΦRT), and 2% originated from among the *C. coccodes* population within the regional (ΦPR) variation. Among VCGs of *C. coccodes,* there was also high VCG differentiation (G_{ST}=0.463) and high genetic diversity. AFLP markers proved valuable in differentiating and studying the global population of *C. coccodes*.

Introduction

Colletotrichum coccodes (Wallr.) Hughes is a cosmopolitan pathogen (Dillard, 1992) that has wide distribution and host range (Andrivon et al., 1997). It is primarily found on vegetables in the *Solanaceae*, *Cucurbitaceae* and *Fabaceae* families (Dillard, 1992). *C. coccodes* causes black dot which is a blemish disease on potato, it is characterized by the presence of black sclerotia produced on tubers, stolons, roots and stems (Andrivon et al., 1998; Lees and Hilton, 2003). Earlier reports did not consider black dot as a serious problem on potato, however, there have been an increasing number of reports regarding disease incidence and crop damage caused by *C. coccodes* (Johnson, 1994; Johnson and Miliczky, 1993; and Tsror (Lahkim) et al., 1999). *C. coccodes* is an imperfect fungus (Cano et al., 2004), thus vegetative compatibility may serve as a means of genetic exchange and be useful for measuring genotypic diversity (Cole and Kendricks, 1981). Vegetative compatibility refers to the ability of individual fungus to undergo mutual hyphal anastomosis and form viable heterokaryons. Strains that are vegetatively compatible with one another are described as members of the same vegetative compatibility group (VCG) (Leslie, 1993).

Characterization of the *C. coccodes* population has been studied using nitrate *nit* mutants method in many regions. Eight VCGs were identified for the Europe/Israel population (Nitzan et al., 2002; Shcolnick et al., 2007), 7 VCGs for the North American population (Nitzan et al., 2006), and 6 VCGs for the Australian population (Ben-Daniel et al. 2010). Variation in aggressiveness, morphological, and physiological traits have been detected among the different *C. coccodes* VCGs. Isolates belonging to NA-VCG2 (Aqeel et al., 2008; Nitzan et al., 2006), NA-VCG5 (Nitzan et al., 2006) and NA-VCG6 (Aqeel et al., 2008) have been found to be the most aggressive in North America. In other regions of the world, *C. coccodes* isolates belonging to EU/I-VCG5 for Europe/Israeli (Shcolnick et al., 2007), and AUS-VCG4 for Australian population (Ben-Daniel et al., 2010) are the most aggressive. Conidial and sclerotial variations have also been detected among NA-VCGs of *C. coccodes.* NA-VCG2 was found to have the shortest and narrowest conidia and largest sclerotia compared to other VCGs (Aqeel et al., 2008). Furthermore, isolates belonging to the same VCG share common physiological traits, including optimum growth at certain temperatures and pH levels (Nitzan and Tsror, 2003).

Though the vegetative compatibility grouping method is a widely used technique to study genetic diversity among populations of different fungi, it has certain limitations that can reduce its effectiveness and utility. Such limitations include the inability of some isolates to form *nit* mutants on chlorate medium (Strausbaugh et al., 1992). Some mutant isolates are also not able to anastomose with selected tester isolates and form stable heterokaryons (Joaquim and Rowe, 1990; Puhalla, 1979). Having several tester strains with the ability to yield strong heterokaryons was suggested to avoid the complementation problem (Joaquim and Rowe, 1990). The inability to form stable heterokaryon is attributed

to allelic and non allelic compatibility that are governed by *vic* (vegetiative incompatibility) and *het* (heterokaryon) loci (Leslie, 1993).

In *C. coccodes*, isolates from different continents have been reported to be vegetatively incompatible (Nitzan et al., 2006). More recently, a very limited complementation has been reported among a few North American, Australian, and Europe/Israeli isolates (Ben-Daniel et al., 2010). The amount of variability present in some populations is another factor that has been determined to affect the usefulness of VCGs in studying population genetic diversity (Leslie, 1993). Additionally, VCG analysis is a laborious and time consuming technique (Joaquim and Rowe, 1990). These limitations for studying genetic diversity by traditional methods could be enhanced by the use of molecular techniques.

Genetic diversity of a pathogen, the combination of alleles and genotypes in a population and the expression of this diversity in morphological, behavioral, and physiological differences between individuals and populations (Frankham, et al., 2002), is an important factor that facilitates our understanding of their role in shaping the genetic structure of a population (Burdon and Silk, 1997). Genetic diversity is measured by the frequency of alleles and genotypes, allelic diversity, and the proportion of polymorphic loci. Differentiation through molecular measures is based on the genetic distances in allele frequencies among populations (Toro and Caballero, 2005). Gene diversity, the probability that two alleles chosen at random from the population are different, as defined by Nei (1973), is the most widely used parameter to measure the within population diversity.

Molecular markers such as restriction fragment length polymorphism (RFLP) (Garcia et al., 2004), random amplified polymorphic DNA (RAPD) (Abu-El Samen et al.,

2003), and amplified fragment length polymorphism (AFLP) (Abu-El Samen et al., 2003; Heilmann et al., 2006; O'Neill et al., 1997; Zhong and Steffenson, 2001) have been widely used to study genetic diversity of plant pathogens. Markers based on AFLPs have the potential for sensitive detection (Hynes et al., 2006) and offer many advantages. They are reproducible (Hynes et al., 2006; Majer et al., 1996) and can be applied to a wide range of DNA sources (Hynes et al., 2006; Savelkoul et al., 1999; Mueller and Wolfenbarger, 1999) when compared with other methods such as RAPD (Hynes et al., 2006). Additionally, small amounts of DNA are required (Majer et al., 1996; Savelkoul et al., 1999; Mueller and Wolfenbarger, 1999) and even partially degraded samples can be used, but DNA should be highly purified with no PCR inhibitors to obtain optimal results (Mueller and Wolfenbarger, 1999). AFLP is an attractive method to study the genetic diversity among isolates of *Colletotrichum* species (O'Neill et al., 1997). AFLP was efficient in studying relationships within and among North American VCGs of *C. coccodes* (Heilmann et al., 2006). It can evaluate a large population efficiently and rapidly, and can measure the genetic relationships of populations distributed worldwide. Thus, the main objectives of this study were to study the global population of *C. coccodes*, including North American, European, Middle Eastern, Australian, and South African isolates, to determine genetic relationships using AFLP markers, and to determine whether geographic origin is a factor in the genetic diversity of *C. coccodes*.

Materials and Methods

***C. coccodes* isolates.** A total of 855 *C. coccodes* isolates originating from five continents were used in this study (Appendix 1). Out of the 855 isolates, there were 515 isolates belonging to the North American population. These isolates included 366 recently

collected isolates from 2006 to 2009 (Gudmestad lab, used in Chapter 2), 109 previously assigned isolates (Heilmann et al., 2006), and 40 tester isolates representing the seven NA-VCGs (Table 3.1). A total of 224 isolates originating from Europe/Israel, including the tester isolates (Nitzan et al., 2002; Shcolnick et al., 2007), 102 from Australia (Ben-Daniel et al., 2010), and 14 isolates from South Africa (Appendix 1). Australian, Europe/Israeli, and South African isolates were received as cultures on PDA or as sclerotia grown in sterilized soil. For these isolates, mycelium plugs were transferred onto CV8 medium (7.5g agar, 50 ml sterilized CV8 juice amended with $CaCO_3$, and 450 ml ddH2O) and left for a few days to check for any contamination. If contamination was detected, hyphal tips were obtained for each isolate and re-cultured on CV8. Soon after, when sclerotia formed, isolates were prepared for long term storage as previously described (Chapter 2). Three species, including *Fusarium graminearum* Schwabe, *Acremonium strictum* W. Gams., and *Colletotrichum lindemuthianum* (Sacc.& Magnus) Lams.-Scrib. were used as out-groups (Appendix 1). The tester isolates used in this study were composed of multimember isolates representing the different VCGs for each continental population (Table 3.1).

DNA extraction. Pure cultures of *C. coccodes* were grown in Richard's solution (10.0 g sucrose, 10.0 g potassium nitrate, 5.0 g potassium phosphate, 1.22 g magnesium sulfate, 0.02 ferric chloride, 150 ml V8 juice, and 850 ml ddH_2O), filtered through Whatman filter papers (90 mm) by a power-operated pump, frozen at -80 °C for 30 minutes, lyophilized for 24 hrs, and ground to a fine powder in liquid nitrogen using pre-cooled sterile mortar and pestle. DNA extraction was performed using the cetyltrimethyl ammonium bromide (CTAB) DNA extraction protocol (http://www.bio.utk.edu/mycology/Techniques/mt-dna_extraction.htm).

Table 3.1. List of multimember tester isolates of *C. coccodes* representing VCGs for each population.

	Origin	Isolate	VCG	Source
1	Europe/Israel	Si-6/9	EU/I-VCG1	Leah Tsror[1]
2	Europe/Israel	#24.3	EU/I-VCG2	Leah Tsror
3	Europe/Israel	Si-110/7	EU/I-VCG2	Leah Tsror
4	Europe/Israel	Si-72/8	EU/I-VCG3	Leah Tsror
5	Europe/Israel	Si-88/3	EU/I-VCG3	Leah Tsror
6	Europe/Israel	#46/7	EU/I-VCG4	Leah Tsror
7	Europe/Israel	#184/6	EU/I-VCG5	Leah Tsror
8	Europe/Israel	Si-20/9	EU/I-VCG6	Leah Tsror
9	Europe/Israel	Si-1/7	EU/I-VCG7	Leah Tsror
10	Europe/Israel	Si-70/15	EU/I-VCG7	Leah Tsror
11	Europe/Israel	Si-28/4	EU/I-VCG8	Leah Tsror
12	Australia	7.7	AUS-VCG1	Leah Tsror
13	Australia	79.3	AUS-VCG1	Leah Tsror
14	Australia	90/07AH.18	AUS-VCG1	Leah Tsror
15	Australia	55.2	AUS-VCG1	Leah Tsror
16	Australia	77.2	AUS-VCG1	Leah Tsror
17	Australia	115.14	AUS-VCG2	Leah Tsror
18	Australia	95/07AH.4	AUS-VCG2	Leah Tsror
19	Australia	114.3	AUS-VCG3	Leah Tsror
20	Australia	6.15	AUS-VCG3	Leah Tsror
21	Australia	C6.1	AUS-VCG3	Leah Tsror
22	Australia	120/07PI.1	AUS-VCG3	Leah Tsror
23	Australia	15/07P.2	AUS-VCG4	Leah Tsror
24	Australia	2.5	AUS-VCG4	Leah Tsror
25	Australia	59/07TA.12	AUS-VCG4	Leah Tsror
26	Australia	12/07.7	AUS-VCG5	Leah Tsror
27	Australia	97/07AH.8	AUS-VCG6	Leah Tsror
28	USA	1.7	NA-VCG1	Leah Tsror
29	USA	6.1	NA-VCG1	Leah Tsror
30	USA	26.2	NA-VCG2	Leah Tsror
31	USA	47.6	NA-VCG2	Leah Tsror
32	USA	10.10b	NA-VCG2	Leah Tsror
33	USA	30.8	NA-VCG3	Leah Tsror
34	USA	27.9	NA-VCG3	Leah Tsror
35	USA	N-124/12	NA-VCG4	Leah Tsror
36	USA	48.4	NA-VCG4	Leah Tsror
37	USA	33.6	NA-VCG5	Leah Tsror
38	USA	35.5	NA-VCG5	Leah Tsror
39	USA	39.6	NA-VCG6	Leah Tsror
40	USA	45.17	NA-VCG7	Leah Tsror
41	USA	C60	NA-VCG1	Johnson, D[2]
42	USA	C19	NA-VCG1	Johnson, D
43	USA	C60-9 nit M	NA-VCG1	Johnson, D
44	USA	ALB15	NA-VCG2	Johnson, D
45	USA	ALB13	NA-VCG2	Johnson, D

Table 3.1. (continued)

	Origin	Isolate	VCG	Source
46	USA	ALB14-6 nit M	NA-VCG2	Johnson, D
47	USA	ALB14	NA-VCG2	Johnson, D
48	USA	MT11-5 nit M	NA-VCG2	Johnson, D
49	USA	236-14,M	NA-VCG3	Johnson, D
50	USA	COLL236	NA-VCG3	Johnson, D
51	USA	ORG1	NA-VCG4	Johnson, D
52	USA	C124	NA-VCG4	Johnson, D
53	USA	MT5-6 nit M	NA-VCG5	Johnson, D
54	USA	MT5	NA-VCG5	Johnson, D
55	USA	AN-13	NA-VCG6	Johnson, D
56	USA	C501	NA-VCG7	Johnson, D
57	USA	C54	NA-VCG7	Johnson, D
58	USA	C55	NA-VCG7	Johnson, D

[1] Isolates obtained as PDA cultures from Leah Tsror, Israel.
[2] Isolates obtained as sclerotia on sterilized soil from Johnson, D. WA, USA.

DNA was assessed for quantity and purity. Two microliters of DNA were mixed with low range assay solution used for 10-50 ng/µl DNA concentration (10 µl Hoechst 33258 dye stock solution (1 mg/µl) with 10 ml of 10X TNE buffer and 90 ml distilled filtered water) and measured via TD-700 fluorometer to determine the concentration. DNA quantity was measured for representative isolates. DNA quantities were found to be in a range of 50 to 70 ng/µl. For purity visualization 5 µl of DNA were mixed with 3µl of the blue juice loading dye (10.5 ml of 70% glycerol, 0.15 ml 0f 0.5X TAE of TBE buffer, 0.6 ml of 20 mM EDTA (0.5M), 0.03 g of 0.2% SDS, and 0.009 of 0.6 mg/ml bromophenol blue, in 15 ml) and electrophoresed on 1% agarose gel. Assessing DNA samples of *C. coccodes* isolates for purity showed good DNA quality on 1% agarose gel (Figure 3.1). DNA of three genera including *Fusarium graminearum* Schwabe, *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, and *Acremonium strictum* Gams was also extracted. These genera were used as out-groups to test the success of separating *C. coccodes* isolates from other fungi using the AFLP method.

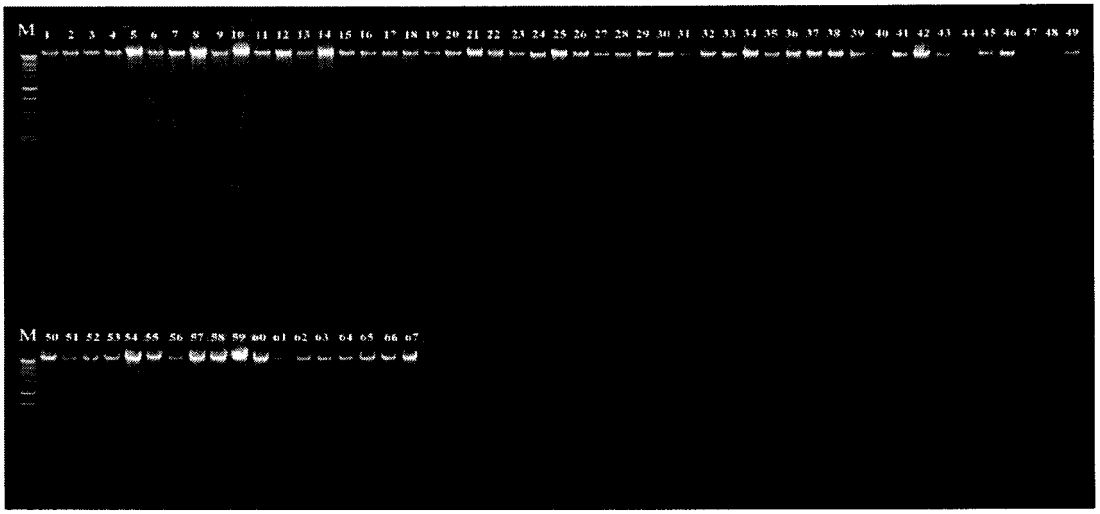

Figure 3.1. Representative of DNA purity testing of *C. coccodes* isolates on 1.5% agarose gel. M: 1kb molecular marker, 1-67: DNA of *C.coccodes* isolates.

Specific PCR assays. PCR was performed on DNA from the newly collected isolates from North America, and for isolates obtained from Australia, Europe, Israel, and South Africa to ensure species identity by using *C. coccodes* specific primers Cc1NF1 and Cc2nR1 (Cullen et al., 2002). A reaction contained 18.9 µl distilled-purified water, 2.5 µl (5X) Gotaq buffer, 1 µl of 25mM $MgCl_2$,, 1 µl dNTP mix (10 mM), 0.2 µl Cc1NF1 primer (36 ng/ µl), 0.2 µl Cc1nR1primer (36 ng/ µl), and 0.2 µl *Gotaq* DNA Polymerase (5u/ µl); 1 µl of genomic DNA was prepared. Amplification was performed in a PTC-200 programmable thermocycler (MJ Research,Waltham, MA), programmed for 1 initial denaturation cycle (95 °C for 2 min), 35 step cycles (95 °C for 45 s, 72 °C for 2.15 min), 72 °C for 5 min, and forever cycle for 4 °C.

AFLP assays. AFLP reactions were performed according to the method of Vos et al. (1995) as modified by Heilmann et al. (2006) for *C. coccodes*. In this method, the procedure was modified by combining digestion and ligation into one step. The following quantities from the different materials were used for digestion and ligation step: 4.6 µl water, 1.1 µl ligation buffer, 1.1 µl of 1M NaCl, 0.11 µl BSA, 0.5 µl ATP, 1 µl *Eco*RI-

adapter (16bp adapter-primer sequence) and 1 µl *Mse*I-adapter (16-bp adapter-primer sequence), 0.2 µl of *MseI*, 0.3 µl of *EcoRI*, and 0.1 µl of T4 DNA ligase and one micro liter of the genomic DNA. This step was done at 37 °C for 3.5 hours. The selective amplification step was performed using a PTC-200 programmable thermocycler (MJ Research, Waltham, MA). A final reaction volume of 25 µl was prepared for each sample. Each reaction contained the following amounts of materials: 0.85 µM dNTPs, 1 µl of 25mM $MgCl_2$, 2.5 µl of 5X PCR reaction buffer, 1 µl (36 ng) of each primer set with two-base extensions, 2.0 µl of the adapter-primer DNA, and 0.1 unit of *Taq* DNA Polymerase (Promega Corp., Madison, WI). Three primer pairs were used to carry out the selective amplification, *Eco*-AC/*Mse*-CC, *Eco*-AG/*Mse*-CC, and *Eco*-AT/*Mse*-CC (Table 3.2). The PCR parameters used for selective amplification included a 39-repeated cycles with initial cycle of 2 minutes at 72 °C, 30 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C. A final cycle at 72 °C for 5 minutes was the last cycle for annealing. The selective amplified PCR products were viewed on 2% *(m/v)* agarose gel to see the smearing pattern.

Table 3.2[1]. Oligonucleotides used in AFLP selective amplification polymerase chain reaction.

Primer	
Forward primers	
* *Eco*-AC	5′ GACTGCGTACCAATTCAC 3′
* *Eco*-AG	5′ GACTGCGTACCAATTCAG 3′
* *Eco*-AT	5′ GACTGCGTACCAATTCAT 3′
Reverse primer	
Mse-CC	5′ GATGAGTCCTGAGTAACC 3′

[1] This table was extracted from Heilmann et al., 2006.
* *Eco*-AC, *Eco*-AG, and *Eco*-AT are labeled by IRDye700.

Three microliters of amplified product and 3 µl of formamide loading dye (1X) (47.5 ml of formamide, 2 ml of 0.5 M EDTA, 40mg of bromophenol blue, and 0.5 ml of water) were

mixed and viewed for the three primer sets. The amplification products were electrophoresed on 6% (w/v) polyacrylamide / 7 M urea denaturing gel in 1× TBE (1,1,2,2-tetrabromoethane) buffer for all of the three primers sets (*Eco*-AC, *Eco*-AG, and *Eco*-AT) and was visualized using a DNA analyzer, LI-COR model 4300. Default AFLP program was used to run the gels. It was programmed to run for 3.5 hrs, at 1500V (40W).

Scoring of AFLP fragments. AFLP images were scored for presence or absence (1 = presence or 0 = absence) of a single band. The bands were scored manually. All DNA fragments within the range of 50 to 650bp were scored for the three primer sets, *Eco*-AC/*Mse*-CC, *Eco*-AG/*Mse*-CC, and *Eco*-AT/*Mse*-CC for each isolate, then combined and used to develop a binary matrix. A total of 210 AFLP fragments were obtained from the 855 isolates of *C. coccodes*, including 71 DNA fragments with *Eco*RI-AC/*MseI*-CC, 66 with *Eco*RI-AG/*MseI*-CC, and 73 with *Eco*RI-AT/*MseI*-CC primers. The matrices with scores for the presence or absence of bands contained 180180, 56628, and 62634 data points for the three primer sets, respectively. An additional 130 bands were scored for the three primer sets. These bands were found in the three out-groups that were used in this study, but not in *C. coccodes* isolates. These bands included 36 *Eco*-AC/*Mse*-CC, 45 *Eco*-AG/*Mse*-CC, and 49 *Eco*-AT/*Mse*-CC. When repeating runs, the polymorphic banding pattern was consistent in each run for *C. coccodes* isolates.

Population analysis. The consensus tree was generated via Phylogeny Inference Package (PHYLIP, University of Washington). The binary matrix data was run using SEQBOOT tool with 1000 replicates. The output generated by this tool was used in Statistical Analysis Software (SAS) to calculate the distance matrix. Distance matrix output was used by NEIGHBOR tool. In NEIGHBOR tool, an Unweighted Pairgroup Method

with Arithmetic Means (UPGMA) base was used with 1000 bootstrap replicates to generate an out tree to be used in CONSENSE tool of PHYLIP. An out tree file that was generated via CONSENSE was then run via DRAWGRAM tool to generate the dendogram. TASSEL2.1 standalone (Trait Analysis by aSSociation, Evolution, and Linkage) software (Bradbury et al., 2007) was also used to generate trees based on UPGMA. In TASSEL, data were loaded as a polymorphism alignment. Alignment was converted to genotypic states without removing the minimum frequency from the genotype output. Preliminary analysis showed that the dendogram generated by PHYLIP package had few clusters with non-assigned isolates. Further analysis using STRUCTURE 2.3. X software (Pritchard et al., 2000) was conducted for multi-locus genotype data to examine the population structure. It was used to infer the presence of distinct populations and assign individuals to populations based on their genotype using a Bayesian distribution (Pritchard, et al., 2000). In this analysis; the 855 *C. coccodes* isolates were tested, assuming an admixture model, with an independent allele frequency in the population, with 10,000 replicates and a 100,000 burn-in period. The admixture model assumes that the individual has inherited a fraction of its genome from ancestors in the population *k*. The smallest value of *k* that captured the major structure in the data was chosen. STRUCTURE 2.3.X software estimates the population structure by using the model based approach (Pritchard et al., 2000). Using this analysis, the number of subpopulations (*k*) that consist of loci in Hardy-Weinberg and linkage equilibrium are determined. Multiple runs were performed for many *k* values, and probabilities for each run of a given *k* were also determined. For our analysis, probabilities were determined for five runs with the number of subpopulations from one to seventeen. In order to determine the appropriate *k* value, Wilcoxon two-sample test was

used to determine which number of the subpopulations better identified the population as a whole. This was accomplished by comparing the probabilities for all runs of a given k, with the probabilities from the run that has a k-value one larger (k=1 vs k=2; k=2 vs k=3, k=3 vs k=4 etc). The smaller k value, in which the first non-significant Wilcoxon two-sample test was obtained, was the best estimate of the subpopulations number (Wang et al., 2008). This software also has the ability to infer an individual's ancestry (Pritchard, et al., 2000). The *C. coccodes* isolates were classified into populations based on geographic origin and based on their assignment to a population based on ancestry (STRUCTURE software output). Based on geographic origin, *C. coccodes* populations were divided as: North America, Australia, Europe, Middle East, and South Africa. Based on ancestry, *C. coccodes* isolates were divided into six populations. The following analysis was performed for both. Data generated from 210 loci for *C. coccodes* isolates were combined to identify a multilocus genotype (G). Genetic variation statistics for all loci including Nei's gene diversity (h), number and percentage of polymorphic loci were calculated using POPGENE version 1.32 (Yeh et al., 1997). H, which is a function of the number and frequency of alleles at each locus was estimated in the within population (H_S) and the total population (H_T). G_{ST}, the population differentiation across the 210 loci was measured among populations. G_{ST} values range from zero to one. Low values indicate that little variation is proportioned among populations and high values indicate that amount of variation is found among populations is large (Culley et al., 2002). Gene flow (N_m) estimation between populations and for the entire population was calculated from average G_{ST} across the three AFLP primer combinations (Yeh et al., 1997). If $N_m < 1$, then local population differentiation will result, and if $N_m > 1$, then there will be little differentiation among populations. In other words,

one individual movement per generation between populations will be sufficient to prevent differentiation between those populations (McDermott and McDonald, 1993). Pairwise population matrix of Nei genetic distance and identity was also calculated using POPGENE version 1.32. A dendogram based on Nei's genetic distance using UPGMA method with 1,000 replications was also generated.

Allele frequency by locus and by population was calculated. Number and percentage of polymorphic loci, pairwise population matrix of Nei genetic distance and identity, were calculated using GENALEX 6.3 software (Peakall and Smouse, 2006). Analysis of molecular variance (AMOVA) (Peakall and Smouse, 2006) was performed to determine the genetic variation in each population. The population was classified based on geographic origin and were divided into four regions. The number of regions was chosen based on a previously generated dendogram using Nei's genetic distance and the UPGMA method with 1,000 replications generated by POPGENE (version 1.32). The variance was partitioned into covariance components. Covariance components were used to calculate three PHI (Φ) fixation indices (ΦPR, ΦRT, and ΦPT) and are defined as follows. Variance was partitioned into variation among regions (ΦRT), variation among populations within regions (ΦPR), and variation within a population (ΦPT). The level of significance for those indices was ($P<0.05$), and was determined using 1000 permutations (Excoffier et al., 1992). AMOVA analysis was conducted for populations based on their ancestors. The variance was partitioned into variations among populations and within populations.

The number of different genotypes (*G*), genotypic diversity (*GD*), and linkage disequilibrium (LD) were calculated by Multilocus 1.3b software (Agabow and Burt, 2001). LD is a statistical measure of the nonrandom association of alleles at different loci

in a population. LD measurement (the statistic rBarD) used in this analysis is independent of the number of loci and includes a standardization for the covariance. This term (rBarD) is similar to correlation coefficient, with values between 0 (complete panmixia) and 1 (no recombination) (Agabow and Burt, 2001). This test was done with 100 randomization sets. Genotypic diversity versus number of loci was also tested using Multilocus 1.3b software for each population and for the 855 *C. coccodes* population. This calculation allows one to see whether scoring more loci would increase the genotypic diversity, or whether it has reached a plateau. The program randomly sampled 100 times from 1 to *m*-1 (*m* is the total number of loci used) from *C. coccodes* population and calculate the different genotypes and genotypic diversity. The *m* AFLP loci are considered to be powerful if the genotypic diversity recovered for 1 to 209 loci reaches a plateau, so if we increase the number of loci scored, this will not increase the genotypic diversity recovered.

In some of the analyses conducted (h, Ht, Hs, G_{ST}, N_m, polymorphic loci number and percentage), the population structure among the five regions was studied in two ways. First, data from all the 855 isolates were considered as one population to quantify H_T, H_S, and G_{ST}, AMOVA and other statistics. Second, data for each population pair (NA vs AUS, NA vs EU, NA vs ME, NA vs SA, AUS vs EU, AUS vs ME, AUS vs SA, EU vs ME, EU vs SA) and for each pair of populations that were identified based on ancestors, were statistically tested using POPGENE V 1.32 to determine the gene flow estimate between each population pair.

Results

Molecular characterization of isolates by specific PCR assay. All *C. coccodes* isolatesused in this study yielded the species-specific 349 bp polymorphic DNA band in PCRassayusing the Cc1NF1 and Cc2NR1 primer pair (Cullen et al., 2002) (Figure 3.2).

AFLP analysis. The three primer sets used in this study generated 210 loci. The primers were highly polymorphic among *C. coccodes* isolates. Polymorphism ranged from 93 to 100% (Table 3.3). The population differentiation estimate G_{ST} was consistent among the three primer pairs; therefore, data from all three primer pairs could be combined

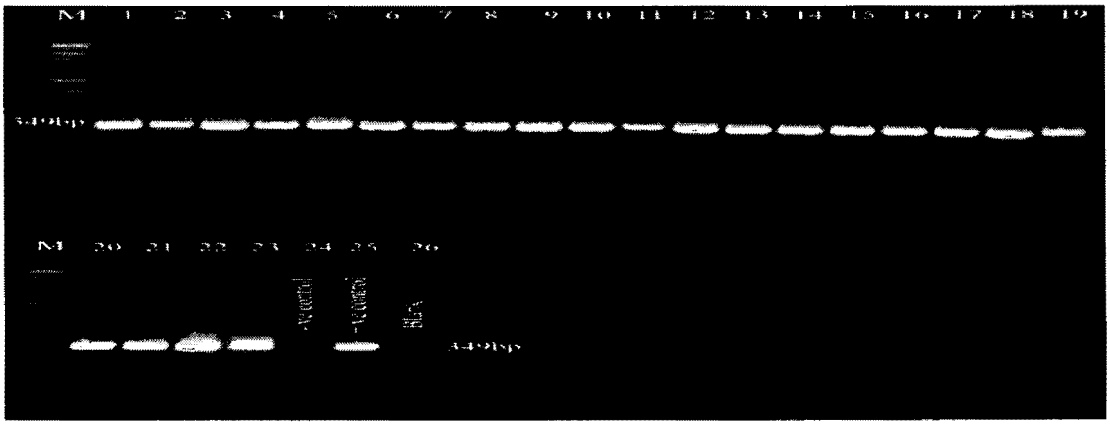

Figure 3.2. Confirmation of representative *C. coccodes* isolates using species-specific primers derived from Cullen et al., 2002. M: 1 kb DNA molecular marker, 1-23 *C. coccodes* isolates, 24: negative control, 25: positive control, 26: blank.

for further analysis. Cluster analysis based on UPGMA separated *C. coccodes* isolates from the three out-groups, *F. graminarum*, *C. lindemuthianum*, and *A. strictum* (Figure 3.3) and the out-groups were separated into two clusters. When analyzing VCGs of *C. coccodes*, UPGMA- based analysis separated *C. coccodes* isolates into 11 clusters. North American isolates belonging to NA-VCG6 and NA-VCG7 could not be separated and all isolates in these two VCGs clustered together and were distinct from all other isolate clusters (Figure 3.3).

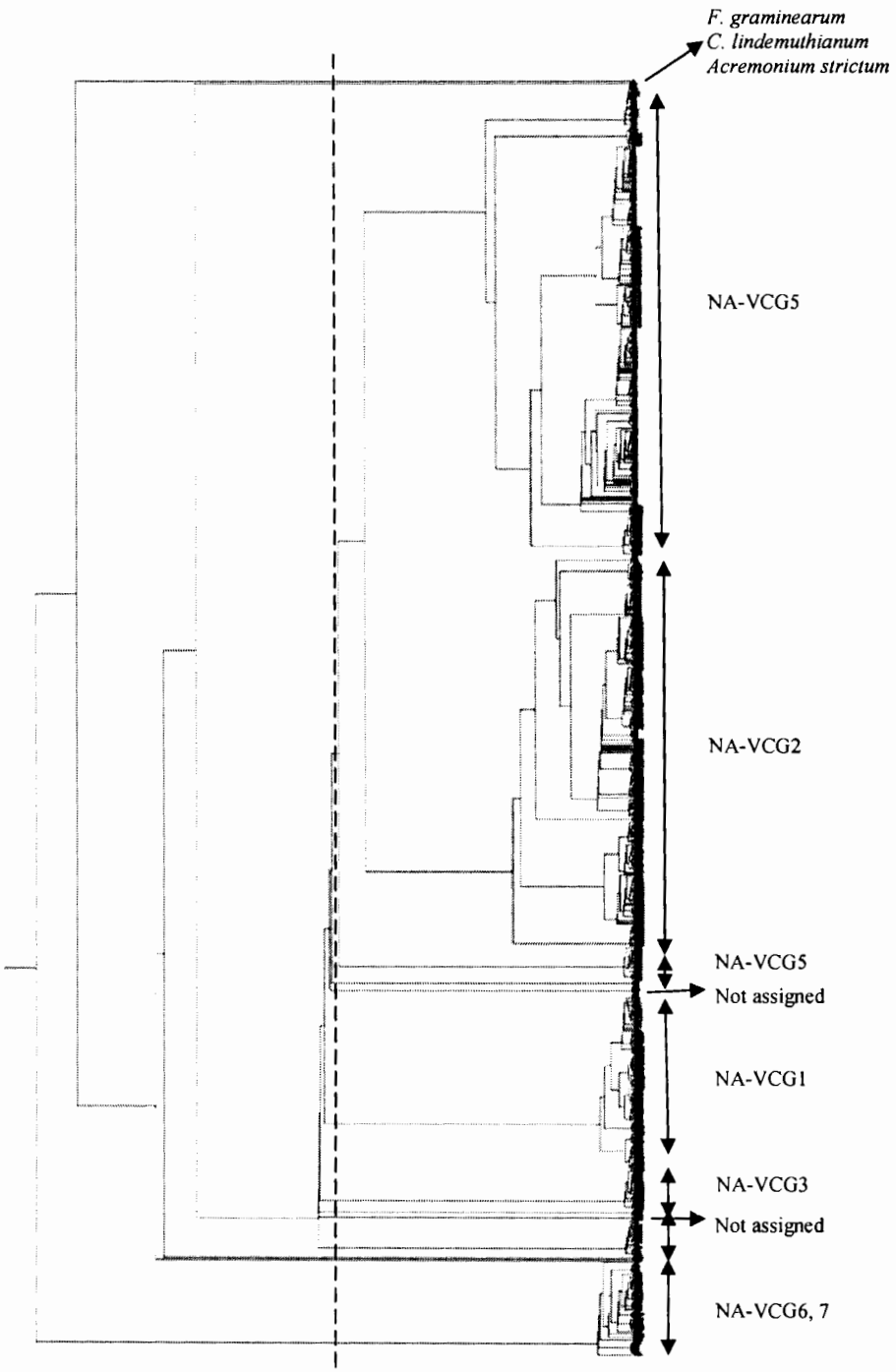

Figure 3.3. Dendogram generated by 1,000 bootstraps reiterations using TASSEL and PHYLIP applications to analyze AFLP data of 855 *Colletotrichum coccodes* isolates. Unweighted pair group method with arithmetic cluster analysis was used to generate the dendogram using a similarity matrix of 340 AFLP bands that were scored as present or absent. The dashed vertical line designates the separation point for assigning the clusters.

Table 3.3. Total number of AFLP bands scored, number and percentage of polymorphisms with three primer combinations used in analysis of 855 *C. coccodes* isolates from North America, Australia, Europe, Israel, and South Africa.

Primer combination	No. of bands[1]	No. of polymorphic bands	Polymorphism (%)	G_{ST}
*Eco*RI-AC/*Mse*I-CC	71	70	98.59	0.29
*Eco*RI-AG/*Mse*I-CC	66	66	100	0.27
*Eco*RI-AT/*Mse*I-CC	73	68	93.15	0.30
Total	210	204	97.25*	0.29*

[1] Bands between 50 and 650bp were included.
* This is the mean of the values in the all three primer sets.

A total of 64 isolates clustered into NA-VCG6/7. Interestingly, NA-VCG7 isolates formed a distinct sub-cluster within the NA-VCG6/7 cluster. Those included 33 previously assigned North American isolates, 8 NA-tester isolates for this group, 13 recently collected isolates from North America, as well as 9 isolates from South Africa, and 1 isolate from Scotland. The nine South African isolates that clustered with NA-VCG7 were separated from the other isolates into a distinct sub-cluster (Figure 3.3).

NA-VCG3 was also distinct from the other groups and had one small cluster that included 14 previously assigned NA-VCG3 isolates (Heilmann et al., 2006), 4 AUS-VCG3 testers, and one AUS-VCG5 tester isolate. The Australian isolates formed a distinct cluster within the NA-VCG3 cluster (Figure 3.3). No isolates recently collected from the United States clustered with this group.

The NA-VCG1 cluster included 120 isolates, 20 of them were previously assigned as NA-VCG1 (Heilmann et al., 2006), 69 new previously unassigned *C. coccodes* isolates from the USA, 2 isolates from Scotland, and 22 isolates from Australia (Figure 3.3). The 22 Australian isolates formed a distinct sub-cluster within this cluster.

C. coccodes isolates belonging to NA-VCG2 and NA-VCG5 clustered together in one large cluster, forming the largest cluster of isolates for this pathogen. Twenty four

previously assigned NA-VCG2 isolates (Heilmann et al., 2006) from North America assigned with 6 isolates from Europe, and 229 recently collected NA-isolates (Figure 3.3). NA-VCG5 isolates formed one large and one small cluster within this larger cluster. The largest cluster included 316 isolates including the 11 European isolates that represent the 8 EU/I tester isolates, 10 Australian testers belonging to AUS-VCGs 1 (5), 2 (2), and 4 (3), 12 NA-VCG5 testers, three isolates were previously assigned as NA-VCG2, 2 *C. coccodes* isolates previously assigned to NA-VCG4, and seven isolates previously assigned to NA-VCG5. A total of 148 *C. coccodes* isolates from Europe, 64 isolates from Israel, 63 isolates from Australia, and 19 unassigned isolates from North America also clustered with NA-VCG5. Isolates originating from Israel and Europe formed three distinct clusters within the NA-VCG5 cluster (Figure 3.3). Two of them include isolates from Israel and *C. coccodes* isolates from the Netherland, France, and Germany. One cluster included only isolates from Scotland. The 63 Australian isolates also formed a distinct sub-cluster within the larger NA-VCG5 cluster. The smallest sub-cluster within the NA-VCG5 cluster included 24 isolates of the previously assigned isolates (Heilmann et al., 2006).

NA-VCG5 was the most common VCG globally, containing 340 of the 810 isolates assigned, followed by NA-VCG2 containing 263 isolates (Table 3.4). On the other hand, NA-VCG3 was the least common group globally (Table 3.4). From the global population of *C. coccodes*, 45 isolates did not cluster with any of the VCGs testers in the generated dendogram. These isolates formed seven small distinct clusters. One cluster included 25 isolates from North America and one isolate from Australia. The remaining six clusters had very few isolates within them. Three clusters had three, three, and four isolates, respectively, from North America; another cluster had a single isolate from Australia,

represented by the AUS-VCG6 tester isolate. The sixth cluster had three isolates, two of them from Israel and one from Europe. The last cluster included five isolates from South Africa (Figure 3.3).

Table 3.4. Origin and designation of the 855 *C. coccodes* isolates to the vegetative compatibility groups using AFLP marker.

Population	NA-VCG1	NA-VCG2	NA-VCG3	NA-VCG5	NA-VCG6,7	Total assigned	Not assigned
North America	96	257	18	55	54	480	35
Europe	2	6	-	148	1	157	1
Israel	-	-	-	64	-	64	2
Australia	22	-	5	73	-	100	2
South Africa	-	-	-	-	9	9	5
Total	120	263	23	340	64	810	45

Cluster analysis did not assign *C. coccodes* efficiently into their presumptive VCGs. Further testing based on Bayesian distribution to estimate the best population number (k) of the global *C. coccodes* population depending on ancestry, showed that k=6 was the best estimate (Table 3.5). In this classification, isolates were assigned to a certain sub-population based on the P-value (0.50-0.99). A total of 854 *C. coccodes* isolates were efficiently assigned to the six sub-populations. Only one isolate had a P value partitioned among the six sub-populations, and thus, was not assigned to any group. The output of the best k was visualized when plotting each individual's estimated membership coefficients (Q-hat) (Figure 3.4). Each color represents each sub-population (Figure 3.4, Table 3.6), and whether there are isolates having ancestors from other groups. Furthermore, each isolate is represented by a single vertical line. This vertical line is partitioned into K colored segments representing the estimated membership fraction of that isolate in each of the K inferred clusters (Figure 3.5).

Table 3.5. Wilcoxon two-sample test analysis for estimating the best sub-population number of *C. coccodes* global population.

Subpop[1]	N	Sum of scores	Expected under H_0	Std. Dev. under H_0	Mean score	Pr > |Z| [2]
1	5	15	27.5	4.77	3	
2	5	40	27.5	4.77	8	0.01
2	5	15	27.5	4.78	3	
3	5	40	27.5	4.78	8	0.01
3	5	15	27.5	4.78	3	
4	5	40	27.5	4.78	8	0.01
4	5	15	27.5	4.78	3	
5	5	40	27.5	4.78	8	0.01
5	5	15	27.5	4.78	3	
6	5	40	27.5	4.78	8	0.01
6	5	15	27.5	4.78	3.6	
7	5	40	27.5	4.78	7.4	0.06[2]

[1] The numbers represent *k* values. Each *k* represents a subpopulation that is a mix of *C. coccodes* isolates from different geographic origin. Only k=2 and k=5 had isolates that are originated from EU/I and NA respectively (Table 3.7).

[2]This was accomplished by comparing the probabilities for all runs of a given *k*, with the probabilities from the run that has a *k*-value one larger (k=1 vs k=2; k=2 vs k=3, k=3 vs k=4 etc) (Wang et al., 2008).

[3]At this point, there is no significant difference between k=6 and k=7 at P=0.05, so k=6 Is the value that is considered as the best estimate for the population number (Wang et al., 2008).

Genetic structure of global *C. coccodes* population based on geographic origin.

Genetic diversity of the 855 *C. coccodes* isolates among the five geographic regions was analyzed for the 210 loci. Total genetic diversity was relatively high (H_t = 0.22) and gene diversity within a regional population was relatively low (H_S=0.16) (Table 3.7). The overall differentiation among populations was also relatively high (G_{ST}=0.29). This value was similar to the mean G_{ST} value obtained from merging the three primer pairs (Table 3.3).

Table 3.6. Population structure based on individual's ancestry.

Subpopulation	VCG-representing	# of isolates	Represented color
1	NA-VCG6, 7	66	Red
2	EU/I isolates only[1]	122	Green
3	NA-VCG1, 3	151	Dark blue
4	NA-VCG5	222	Yellow
5	NA-VCG1,2,5[2]	100	Pink
6	NA-VCG2	193	Light blue
	Not-assigned	1	
Total		855	

[1] All of the isolates in this group were from Europe/Israel populations.
[2] All of the isolates in this group were from North America. It included isolates assigned to NA-VCG1, NA-VCG2, and NA-VCG5 based on its clustering with the different VCG testers.

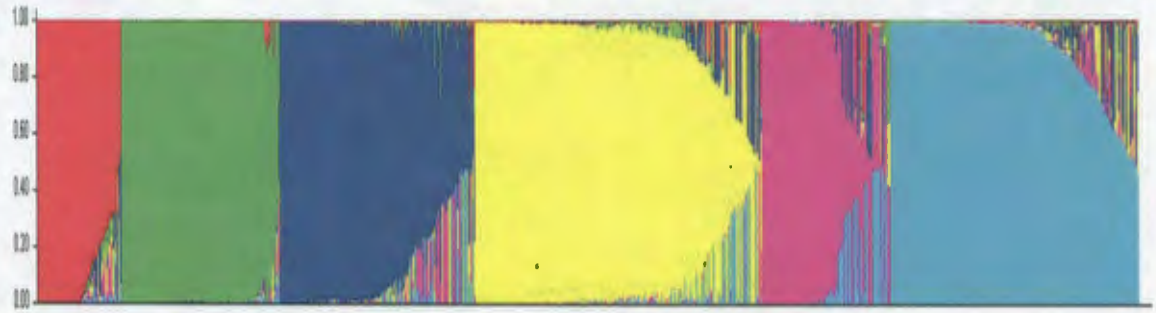

Figure 3.4. Plots of ancestry estimates. The six subpopulations of *C. coccodes* are represented by different colors; red color: subpopulation 1, green: subpopulation 2, dark blue: subpopulation 3, yellow: subpopulation 4, pink: subpopulation 5, and light blue: subpopulation 6. Each isolate is represented by a single vertical line. This vertical line is partitioned into *K* colored segments; represent the estimated membership fraction of that isolate in each of the *K* inferred clusters.

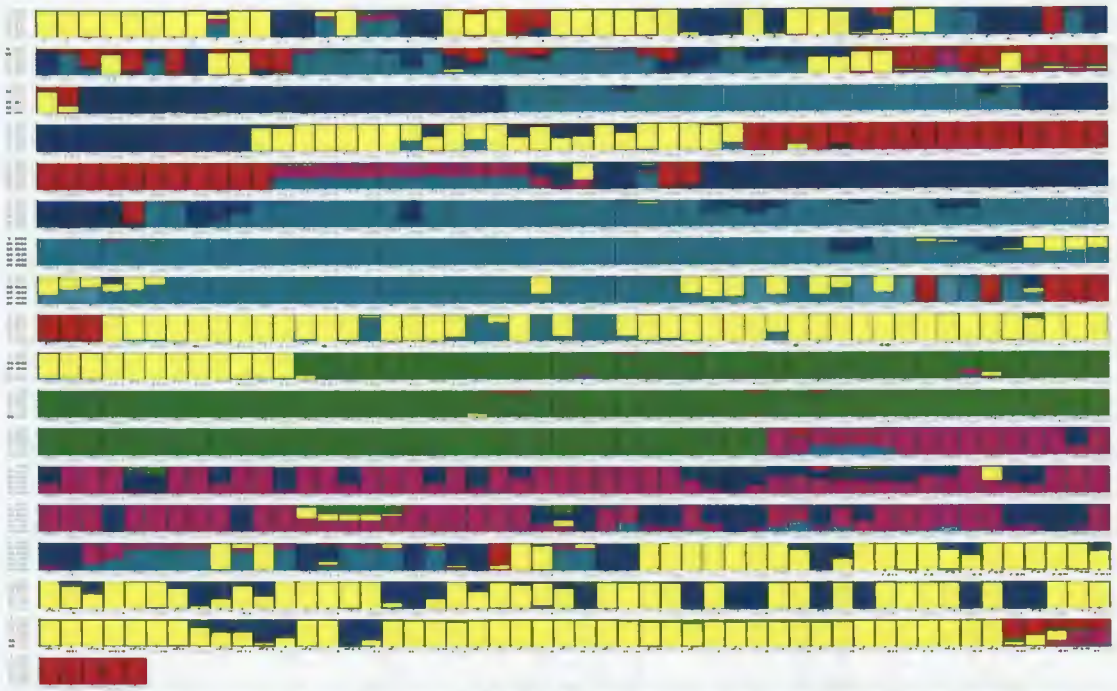

Figure 3.5. Assigning of each isolate of the 854 *C. coccodes* to its subpopulation based on their ancestry. Isolates were assigned to their population based on the *P*-values (0.50-1.00). Each color represents one subpopulation. The isolates that had more than one color had ancestors from the sub-population of the color that it has. Each isolate is represented by a single vertical line. This vertical line is partitioned into *K* colored segments; represent the estimated membership fraction of that isolate in each of the *K* inferred clusters. The different colors represent subpopulations; red color: subpopulation 1, green: subpopulation 2, dark blue: subpopulation 3, yellow: subpopulation 4, pink: subpopulation 5, and light blue: subpopulation 6.

Table 3.7. Genetic diversity analysis for the 855 *C. coccodes* isolates based on their geographic origin.

Population[1]	size	h[2]	Ht[3]	Hs[4]	G_{ST}[5]	N_m[6]	Pol. loci[7]	%[8]
All pop.	855							
Mean		0.22	0.22	0.16	0.29	1.25	204	97.14
St. Dev.		0.16	0.02	0.01				

[1] Populations include North America (NA), Australia (Aus), Europe (EU), Middle East (ME), and South Africa (SA).
[2] h = Nei's (1973) gene diversity.
[3] Total genetic diversity in the pooled population.
[4] Inter-population genetic diversity: the average genetic diversity within each population.
[5] Estimates Nei's (1973) G_{ST}, average differentiation among populations.
[6] N_m = estimate of gene flow from G_{ST}. $N_m = 0.5(1 - G_{ST})/ G_{ST}$.
[7] The number of polymorphic loci.
[8] The percentage of polymorphic loci.

Overall gene diversity was relatively low (0.22). It ranged between 0.09 (low) in the Middle East to 0.23 (moderate) in North America (Table 3.8). The number of distinct genotypes and genotypic diversity was high in all regions *C. coccodes* (Table 3.8). There were 715 distinct genotypes recognized out of 855 isolates. Genotypic diversity ranged from 0.98 to 1.00. When plotting the genotypic diversity versus the 210 loci used in this study, *GD* diversity reached a plateau, indicating that these markers were adequate to estimate the existing diversity (Figure 3.6). Linkage disequilibrium values were low among the five regions, ranging from 0.06 to 0.08, except in South Africa were it was 0.22. The small sample numbers for the South African *C. coccodes* isolates could explain this large value. Though LD values were low, they were all significantly different from zero ($P<0.01$) (Table 3.8).

Pairwise comparison of population genetic identity and genetic distance among the five regions showed that the European and Middle Eastern population pair had the highest genetic similarity (0.99) (Table 3.9), while South Africa had the least similarity with any of the other regions (Table 3.9).

The overall estimated differentiation (G_{ST}) and gene flow (N_m) values among the five regions were 0.29 and 1.25, respectively (Table 3.7). Gene flow values greater than 1.0 indicate migration of genes which ranged from one migrant in South Africa to the Middle East, to 16 migrants in the Middle East and Europe (Table 3.10). The high gene flow value suggests genetic exchange among the five regions.

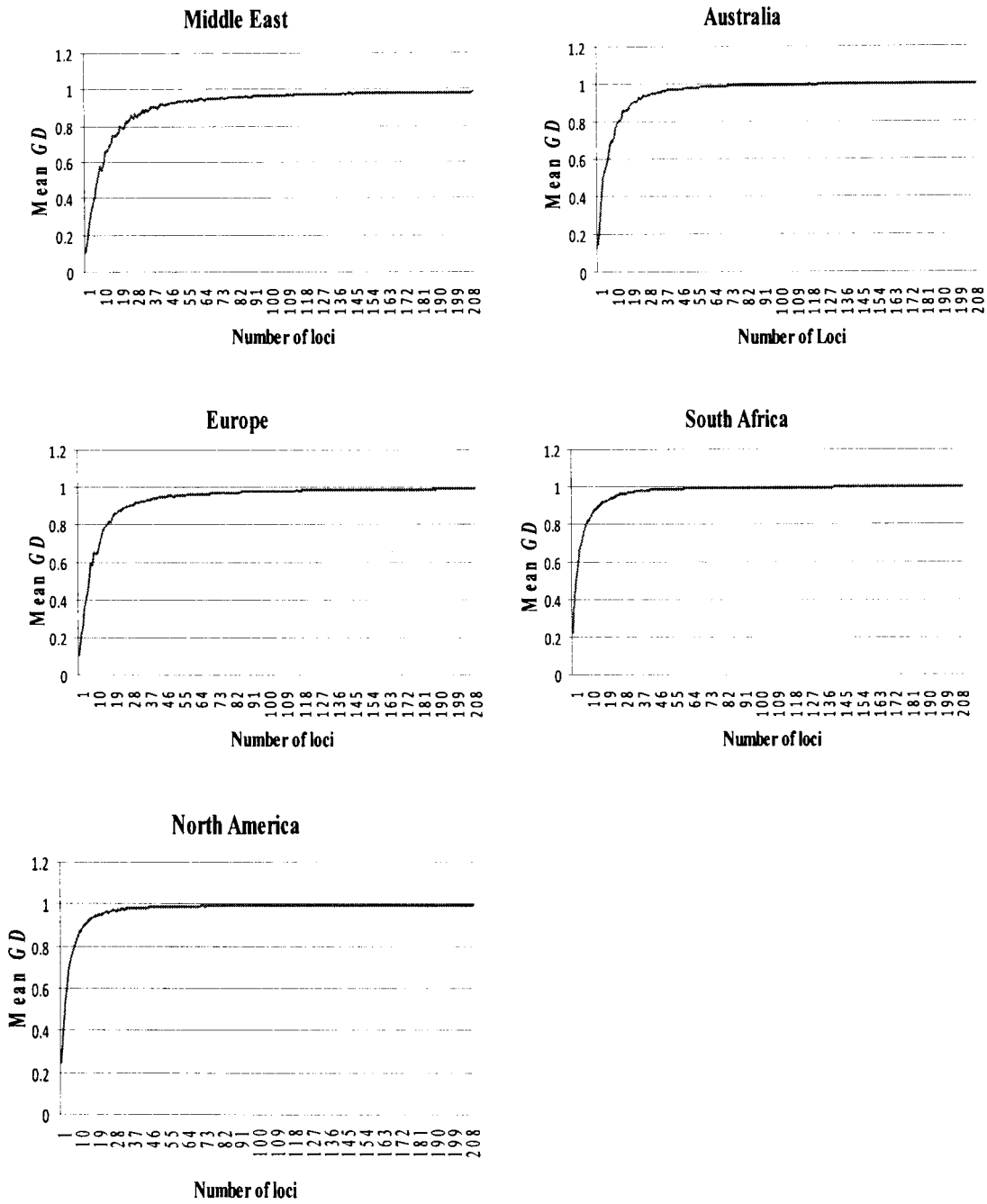

Figure 3.6. The power of the 210 loci generated via the three primer pairs in this study. The number of the different genotypes and the genotype diversity were calculated after 100 random samples of 1 to 210 loci.

Table 3.8. Genetic variation statistics for the 210 loci of *C. coccodes* based on their geographic differentiation.

Population	Sample size	#Pol. Loci[1]	%[2]	h[3]	*G*[4]	*GD*[5]	LD[6]
North America	515	200	95.24	0.24	430	0.99	0.07
Australia	102	132	62.86	0.14	98	0.99	0.08
Europe	158	157	74.76	0.11	124	0.99	0.06
Middle East	66	96	45.71	0.09	49	0.98	0.08
South Africa	14	113	53.81	0.21	14	1.00	0.22

[1] The number of polymorphic loci
[2] The percentage of polymorphic loci
[3] h = Nei's (1973) gene diversity
[4] *G*: number of distinct genotypes
[5] *GD*: genotypic diversity
[6] Measurement of linkage disequilibrium (LD), all values are significant from zero ($P<0.01$)

Table 3.9. Pairwise comparison matrix of Nei genetic identity (above) and Nei genetic distance (below).

Pop ID[1]	1	2	3	4	5
1	****	0.94	0.95	0.93	0.86
2	0.06	****	0.96	0.95	0.84
3	0.05	0.04	****	0.99	0.83
4	0.07	0.05	0.01	****	0.83
5	0.15	0.17	0.19	0.19	****

[1] Population ID: 1: North America, 2: Australia, 3: Europe, 4: Middle East, and 5: South Africa.

Table 3.10. Pairwise population matrix of average differentiation among populations (G_{ST}[1]) (below) and gene flow (N_m[2]) (Above).

Pop ID[3]	NA	AUS	EU	ME	SA
NA	****	3.91	3.94	2.83	2.04
AUS	0.11	****	3.35	2.38	1.29
EU	0.11	0.13	****	16.11	1.10
ME	0.15	0.17	0.03	****	1.00
SA	0.20	0.28	0.31	0.33	****

[1] Estimates Nei's (1973) G_{ST}, average differentiation among populations.
[2] N_m = estimate of gene flow from G_{ST}. E.g., $N_m = 0.5(1 - G_{ST})/G_{ST}$.
[3] Populations include North America (NA), Australia (Aus), Europe (EU), Middle East (ME), and South Africa (SA).

When analyzing the global populations of *C. coccodes* by continent, the UPGMA Nei-based dendogram clustered the five populations into two main clusters (Figure 3.6). The first cluster included *C. coccodes* isolates from North America, Australia, Europe, and Middle East, and the second cluster had only *C. coccodes* populations from South Africa. Populations from Europe and the Middle East were more similar compared to the other populations (Figure 3.7). North American and Australian populations were distinct from the populations of Middle East and Europe.

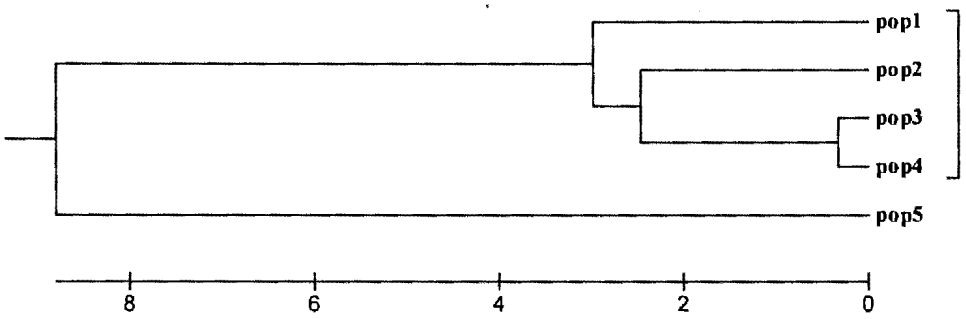

Figure 3.7. Dendrogram based on Nei's genetic distance using the UPGMA method for the five regions. Population ID: pop1: North America, pop2: Australia, pop3: Europe, pop4: Middle East, and pop5: South Africa.

Based on UPGMA dendogram, populations were divided into 4 regions for AMOVA analysis, where both Middle Eastern and European populations were considered as one region. AMOVA showed that 19% of the estimated variation originated from variation among regions (ΦRT, $P = 0.001$), 2% among populations within regions (ΦPR, $P = 0.001$), and 79% of the variation originated from within the population estimated variance (ΦPT, $P = 0.001$) (Table 3.11).

Genetic structure of global *C. coccodes* populations based on their ancestry. Genetic diversity of the 854 *C. coccodes* isolates among the six groups was differentiated according to their ancestors and was analyzed among the 210 loci. Total genetic diversity

was relatively high (H_t = 0.25) and genetic diversity within a group population was relatively low (H_S=0.16) (Table 3.12). Furthermore, the overall differentiation among populations was high (G_{ST}=0.46). This high value indicates a high differentiation among the various population groups.

Table 3.11. Analysis of molecular variance (AMOVA) for *C. coccodes* populations from five regions.

Source of variation[1]	df	Est. Var.	%	ΦValue	*P* value[6]
Among population	4	5.05	20%		
Within population (ΦPT)[2]	850	20.15	80%	0.20	0.001
Total	854	25.20	100%		
Among Regions (ΦRT)[3]	3	4.86	19%	0.19	0.001
Among Populations/Regions (ΦPR)[4]	1	0.43	2%	0.02	0.001
Within Populations (ΦPT)[5]	850	20.15	79%	0.21	0.001
Total	854	25.44	100%		

[1] Variance was partitioned into among regions; regions included four groups (North America, Australia, Europe/Israel, and South Africa), among populations within regions, and within populations.
[2] ΦPT was calculated as the proportion of the estimated variance of among population relative to the total variance.
[3] ΦRT was calculated as the proportion of the estimated variance of among regions, relative to the total variance.
[4] ΦPR was calculated as the proportion of estimated variance of among populations relative to the estimated variance among population/regions and among populations variance.
[5] ΦPT was calculated as the proportion of estimated variance for among regions and Among populations, relative to the total estimated variance.
[6] Probability of obtaining low Φ value was determined by 1,000 permutations.

Table 3.12. Gene diversity analysis in *C. coccodes* population based on their ancestor's differentiation.

Population	Sample size	h	Ht[1]	Hs[2]	Gst[3]	N_m[4]
All groups	854					
Mean		0.22	0.25	0.14	0.46	0.58
St. Dev		0.16	0.03	0.01		

[1] Genetic diversity in the total population
[2] Genetic diversity within population
[3] Estimates Nei's (1973) GST, average differentiation among populations
[4] *Nm* = estimate of gene flow from G_{ST}, $N_m = 0.5(1 - G_{ST})/G_{ST}$

Overall gene diversity (h) was 0.22. It ranged between 0.07 in group two to 0.21 in group three (Table 3.13). Genotypic diversity values for *C. coccodes* were high in all subpopulations (*GD* = 0.99). LD values were low among the six subpopulations. Though LD values were low, they were all significantly different from zero ($P < 0.01$) (Table 3.13). The larger the LD, the more likely the population is involved in nonrandom mating. Low LD refers to the presence of an evolutionary force such as mutation, selection or migration. In our study, gene flow is the force that affects the population. Results suggest that there is limited random mating for certain group.

Table 3.13. Genetic variation statistics for the 210 loci based on the populations assigned according to their ancestors.

Population	Sample size	#Pol. Loci[1]	%[2]	h[3]	*G*[4]	*GD*[5]	LD[6]
1	66	170	84.29	0.19	58	0.99	0.08
2	122	108	51.43	0.07	101	0.99	0.05
3	151	182	86.67	0.21	135	0.99	0.04
4	193	173	82.38	0.09	186	0.99	0.04
5	100	136	64.76	0.12	83	0.99	0.07
6	222	174	82.86	0.14	148	0.99	0.05

[1] The number of polymorphic loci
[2] The percentage of polymorphic loci
[3] h = Nei's (1973) gene diversity
[4] *G*: number of distinct genotypes
[5] *GD*: genotypic diversity
[6] Linkage disequilibrium, all values are significant from zero ($P<0.01$)

Pairwise comparison of population genetic identity among the six subpopulations showed that the subpopulation with isolates belonging to VCG6/7 had less identity and was more differentiated than the other subpopulations (Table 3.14). Additionally, subpopulation two, which included isolates from Europe/Middle East, was more similar (95%) to subpopulation 4 which included isolates belonging to NA-VCG5. Furthermore, group 5 isolates were more similar (92%) to group 6 which includes isolates belonging to NA-VCG2 (Table 3.14). This similarity among groups is very clear in the UPGMA-based dendogram (Figure 3.8). In this dendogram, there are four main subpopulations in the global *C. coccodes* population. One subpopulation included isolates belonging to NA-VCG1/3, the second included isolates belonging to NA-VCG2, and the third subpopulation includes isolates belonging to NA-VCG5. The fourth and final group included isolates belonging to NA-VCG6/7.

Analysis of molecular variance showed that 43% of the estimated variation originated among subpopulations and 57% of the variation originated from the within the population estimated variance (Table 3.15). Statistical Measurement, ΦPT, was significant at $P = 0.001$.

Table 3.14. Nei's unbiased measures of genetic identity (above diagonal) and genetic differentiation among VCGs (below diagonal) based on their ancestry differentiation.

Pop	1	2	3	4	5	6
1	****	0.68	0.73	0.70	0.66	0.67
2	0.51	****	0.88	0.95	0.92	0.88
3	0.36	0.28	****	0.92	0.89	0.88
4	0.44	0.18	0.17	****	0.92	0.93
5	0.48	0.27	0.22	0.22	****	0.95
6	0.51	0.40	0.26	0.22	0.18	****

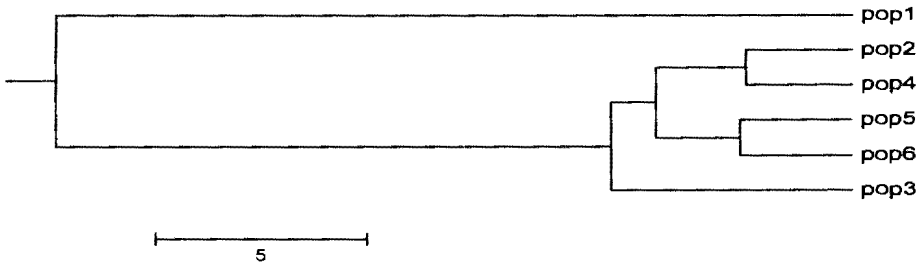

Figure 3.8. Dendrogram based on Nei's genetic distance using UPGMA method for the six populations. Populations 1 to 6 represent the six populations differentiated based on their ancestors.

Table 3.15. Analysis of molecular variance (AMOVA) summary for *C. coccodes* population based on their ancestors.

Source	df	Est. Var.	%	Φ Stat[1]	*P*-Value[2]
Among pop (ΦPT)	5	10.77	43%	0.43	0.001
Within pop	848	14.37	57%		
Total	853	25.14	100%		

[1] ΦPT was calculated as the proportion of estimated variance for among groups relative to the total estimated variance.
[2] Probability of obtaining low Φ value was determined by 1,000 permutations.

Discussion

The studies reported here are the first attempts to investigate the global population of *C. coccodes* at the molecular genetics level using a molecular marker. Only one previous study has evaluated *C. coccodes* isolates using AFLP markers and that study only included isolates from North America (Heilmann et al., 2006). The study reported here used a much larger population and includes isolates from five continents including North America, Australia, Europe, Middle East, and South Africa. The high number and percentage of the polymorphic loci obtained using the AFLP method on the global population showed the effectiveness of these loci to differentiate the *C. coccodes* population efficiently. The number of loci used in this study was demonstrated to be powerful in detecting the genetic diversity of the global population.

The AFLP method was effective in separating *C. coccodes* from the three out-groups, *F. graminearum, A. strictum, and C. lindemuthianum,* and was also successful in separating *C. coccodes* isolates into distinct populations. The data presented here clearly demonstrate that AFLP analysis is informative at the species level for *C. coccodes* (Savelkoul et al., 1999; Mueller and Wolfenbarger, 1999). Cluster analysis by itself was insufficient to analyze the data generated via the AFLP method. The overall analysis using different genetic and statistical analyses was capable of differentiating the global *C. coccodes* population.

Although the VCG technique has been widely used to characterize *C. coccodes* populations, it has proven limited in its ability to study a global population of this fungus due to a lack of vegetative compatibility among continental populations. Many isolates in previous studies were not assigned to any VCG due to the inability of these isolates to anastomose with tester isolates and form stable hetrokaryons (Nitzan et al., 2002; Nitzan et al., 2006, Shcolnick et al., 2007). Successful anastomosis has been reported among a very limited number of North American, Australian, and European *C. coccodes* isolates (Ben-Daniel et al., 2010). However, four AUS-VCG4 isolates anastomosed successfully with one NA-VCG5 isolate, six AUS-VCG2 anastomosed with two EU/I isolates, four AUS-VCG4 anastomosed with two isolates of EU/I VCG7, 18 isolates of NA-VCG2 anastomosed with one EU/I VCG6, and five isolates of NA-VCG5 anastomosed with EU/IVCG6 group (Ben-Daniel et al., 2010). While these data indicate that there are similarities among the global population of *C. coccodes*, vegetative compatibility grouping alone has been ineffective in studying a much larger population.

C. coccodes populations from various geographic origins appear intermingled with the VCGs of the North American *C. coccodes* population. North American population had values of gene diversity (h) and polymorphic loci number and percentage similar or greater than that found in the remainder of the global population, suggesting that the North American population is a mix of populations that are found worldwide. The South African population had high values of gene diversity and number of polymorphic loci, also indicating it has a mix of populations that are found globally. The South African isolates clustered with NA-VCG6/7 (n=9), and 5 isolates of this population belonged to the groups that had isolates from NA-VCG5 based on their ancestry. The high gene diversity values in the South African population could be attributed to the small number of isolates (n=14) for this population which likely affects the genetic analysis. This interpretation is supported by the fact that most of the South African isolates belonged to NA-VCG6/7, a group which was found to have the highest genetic diversity and highest genetic differentiation of any *C. coccodes* VCG.

C. coccodes populations originating from different geographic regions formed distinct sub-clusters or groups within the main clusters. Genetic analysis showed that there is geographic pattern found for the global *C. coccodes* population. Based on the ancestry, one group included 150 isolates originating from North America and one isolate from South Africa while another group included only isolates from Europe/Israel. Interestingly, the group of 150 North American isolates clustered with NA-VCG2 which has been shown to be the most common group in North America (see chapter 2). Similarly, further analysis grouped the European/Israel isolates with NA-VCG5 which is consistent with a previous study where most of the isolates originating from EU/I tested clustered with NA-VCG5

(Heilmann et al., 2006). Perhaps more interesting is that there were VCGs found in only two geographic origins but no others. For example, NA-VCG3 was detected in populations from North America and Australia while NA-VCG6/7 was identified only in populations originating from North America and South Africa, clearly demonstrating a geographic pattern for the global *C. coccodes* population. Genetic analysis supporting this found that 20% of variation detected was among the geographic populations supported by a relatively high population differentiation value G_{ST} (0.29). In a previous study conducted to determine genetic diversity and gene flow for *Colletotrichum lindemuthianum* Sacc. & Magnus) Briosi & Cavara across Himachal Pradesh in India, 50% of the variation was explained by isolation resulting from geographic isolation due to the topography of that area. In that study, the G_{ST} value was 0.12, indicating less gene flow occurs in Himachal Pradesh (Padder et al., 2007). Little correlation between geographic origin and VCGs was found for *Alternaria solani* Sorauer (Van der Waals et al., 2004). However, geographic isolation was not reported in *C. lindemuthianum* isolates from South, Central, and North America (Balardin et al., 1997) or in *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur isolates from the United States, Canada, Brazil, Poland, Uruguay, China, and Japan.

The data demonstrate that linkage disequilibrium estimates are low in all populations except for the South African population, where the sample size was small. However all LD values were significant from zero. The higher the LD values are, the lower the gene flow is, and the higher the differentiation among the population is. The significant values of LD, indicating that pairs of paired loci which are in linkage disequilibrium could pass to the second generation. Further confirmation of low genetic variation among populations was revealed via the AMOVA test. Most of the estimated variation was a result

of within population variation (79%) as there were high numbers of distinct genotypes, high genotypic diversity, and the existence of sub-populations (VCGs) within the one population. In a study conducted to find the genetic relationships for *Gibberella zeae* (Schwein.) among five hosts, 90% of the variation in the fungus resulted from the within population (hosts) differentiation; and low LD values (0.003 to 0.04), significant from zero, were reported, suggesting that genetic drift or sample size are possible mechanisms for deviation from equilibrium (Burlakoti et al., 2008). In our study, LD values were low but significant from zero. Genetic drift has no effect on the *C. coccodes* population because there was gene flow. Very little gene flow is needed to counteract the genetic drift effect.

The *C. coccodes* population analysis showed strong evidence for relatively high genetic diversity for an asexual fungus, low genetic differentiation among populations, and the existence of gene flow, indicating high genetic exchange among the five populations to maintain one large population. Expected distance between populations from different geographic origin would be large if there is a complete geographic specialization. However, the genetic distances from the five regions, North America (NA), Australia (AUS), Europe (EU), Middle East (ME), and South Africa (SA), revealed that *C. coccodes* isolates from the five regions share a close relationship. Distance analysis profiles in *A. solani* Sorauer provided no evidence for clustering of isolates from different geographic origins (Van der Waals et al., 2004). Similarly, the lack of genetic variation among the five populations confirms the presence of a large population that limits the genetic differentiation via gene flow (Horvath and Vargas, 2004). Overall gene flow in all populations was greater than 1 (Nm=1.255), indicating little differentiation among populations (McDermott and McDonald, 1993). An average of one or more migrants per

generation is considered to be sufficient to prevent population differentiation (McDermott and McDonald, 1993). Gene flow between populations is the most important factor in preventing isolation through geographical distances. Moreover, there were only two private alleles, or alleles that are found in only one population but not in the other, among the five regions of *C. coccodes* isolates tested. Additionally, common AFLP alleles were shared across the five continents, indicating one large population and suggesting there has been no restriction of gene flow among continents. Gene flow among the five geographic origins ranged between one to 16 migrants per generation indicating the potential for long-distance dispersal of *C. coccodes*. Potato tubers are exchanged between Europe and Israel (Shcolnick et al., 2007), explaining the low population differentiation and high gene flow between these two regions. The first potato introduction to South Africa came from Holland for planting purposes (Anonymous, 2003). This could explain the gene flow between South Africa and Europe.

The G_{ST}, population differentiation value, obtained in our study among the five regions (0.03 to 0.33) was more than what was obtained among five populations of *C. lindemuthianum* where it ranged from 0.03 to 0.21 (Padder et al., 2007). Pathogen- induced selection may have a strong role in generating variability within populations, especially for pathogens having vegetative compatibility systems (Burdon and Silk, 1997). In these species, conditions that support new genotype generation would also support the establishment of new VCGs, whether through gene flow or other forces (Burdon and Silk, 1997). In another study, high values for H_T (0.26) and H_S (0.22), and a relatively low level of G_{ST} (0.14) were found in *Aspergillus niger* van Tiegh that were considered to be similar to species with known sexual life cycles (Pekarek et al., 2006). The populations used in that

study were all belonged to the same VCG based on pairing the isolates with tester isolates and them selves on complete yeast medium. G_{ST} value indicated gene flow occurrence for *A. niger*. The AMOVA and clustering analysis for this pathogen revealed that most of the variation in their population is from among individual fungal isolates, and low but significant variation from the between plant and between site levels. These results indicated that isolates from each site are referred to be genetic subpopulations. Parasexuality was proposed to be the reason of the extreme levels of variation in populations and subpopulations of this fungus (Pekarek et al., 2006). In our study, we had high variation originating from within the populations due to the gene flow, richness of our population with the distinct genotypes, and due to the many VCGs included. In a genetic structure study for *Rhynchosporium secalis* (Oud.) Davis, field populations from three continents, moderate gene flow explained by moderate population differentiation among continents, and regular recombination explained by non-significant linkage disequilibrium and low degree of clonality, were suggested. The pathogen population structure was found to be similar among continents, because common RFLP alleles were shared across continents, and allele frequencies were similar (Salamati et al., 2000). In that study the sexual stage for *R. secalis* was not yet identified. Later on, the sexual stage for this fungus was proposed (Linde et al., 2003). In the current study moderate to high gene flow was reported among the five continents for the asexual *C. coccodes* fungus, explained by relatively moderate population differentiation among continents and low, but significant linkage disequilibrium values. Similarly, AFLP alleles in *C. coccodes* from different regions were shared across continents, indicating similarity in the population structure.

These very similar populations of *C. coccodes* from different geographic origins are generally not able to anastomose with each other. Allelic compatibility, which is simply a system in which individuals have to be similar at all the vegetiative compatibility (*vic*) or heterokaryon (*het*) loci in order to form stable heterokaryon, could explain the inability of these population to anastomose with each other using the nitrate *nit* mutant technique. Our data suggest that populations of *C. coccodes* are from one large global population that is closely related, and probably of the same origin. We postulated that at one time, the population was isolated and all the same then became established on each continent as the potato was moved around the global; due to the lack of intermixing it became bottle-necked. This explains the existence of certain VCGs on some continents, as revealed by our data, such as in South Africa, Europe, and Australia. Molecular markers, such as AFLP, are not limited to the number of isolates tested. The use of AFLP markers and a wide collection of isolates in this study differentiated the global population, and assigned the isolates into different groups efficiently regardless of origin, to which VCG it belongs, and whether it can form a stable heterokaryon or not. AFLP analysis could evaluate a large population efficiently and provided a more rapid measure of the *C. coccodes* genetic relationships worldwide with only three primer sets. Screening more primers to test this global population could provide addittional differentiation among VCGs and populations. Similarly, more primers pairs were successful in confirming differences between nine mycelial compatibility groups of *Sclerotium solfsii* Sacc. (Cilliers et al., 2000). AFLPs are dominant and multilocus markers that proved to be useful in assessing genetic differences among populations, individuals, and species (Mueller and Wolfenbarger, 1999). Based on our study, the population structure of *C. coccodes* isolates appear to have originated as one

large population with four main groups (NA-VCG1/3; NA-VCG2; NA-VCG4/5; and NA-VCG6/7). However, the dominance character of AFLP marker limits their usefulness in viewing heterozygosity. Thus, using co-dominant markers which can detect heterozygosity alleles, such as simple sequence repeat markers (SSR) would be more informative at the genetic level, and could differentiate *C. coccodes* populations more effectively than the dominant markers (Atallah et al., 2009). These markers are relatively easy to score, making them a valuable tool that can be applied in different laboratories. SSR markers have been successfully used to study the evolutionary and migratory histories of *Verticillium dahliae* (Kleb.) (Atallah, et al., 2009), and to examine the genetic variability and differentiation according to geography, host, and growing system in *Botrytis cinerea* Pers. from Tunisia (Karchani-Balma et al., 2008).

Variation in aggressiveness among *C. coccodes* VCGs within different regions has been reported. NA-VCG2, NA-VCG5, and NA-VCG6 (Aqeel et al., 2008, Nitzan et al., 2006) for North American, EU/I-VCG5 (Shcolnick et al., 2007) for Europe/Israeli, and AUS-VCG4 for Australian populations (Ben-Daniel et al., 2010) were the most aggressive groups. EU/I-VCG5 and AUS-VCG4 for Europe/Israeli and the Australian population cluster with the North American aggressive group NA-VCG5. NA-VCG5 was found to be the most important globally distributed group. Durable resistance selected against this collective group could possibly mean that cultivars developed with resistance in one region would also be resistant to black dot in other regions thereby providing disease control for *C. coccodes* worldwide.

In summary, the data presented here reveal that *C. coccodes* is a global population that is closely related. This is likely due to this fungus being dispersed with the potato as it

was distributed worldwide and propagated as an agricultural crop. This hypothesis could be tested by collecting isolates of *C. coccodes* from the center of origin of the potato in South America and by developing more robust molecular markers such as SSR markers to determine more precisely the origin, relatedness and ancestry of the global population.

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GENERAL CONCLUSIONS

Colletotrichum coccodes (Wallr.) Hughes is an asexual fungus that causes black dot disease on potato. This fungus is morphologically characterized by the production of sclerotia on potato tubers, stems, and stolons. Since *C. coccodes* has no known sexual cycle, genetic exchange is possible only through vegetative conjugation. The determination of VCG among fungal isolates is usually based on the complementation tests with nitrate nonutilizing (*nit*) mutants which enables researchers to compare strains of pathogens (Nitzan et al., 2006). A total of seven VCGs have been identified for North American *C. coccodes* populations (Nitzan et al., 2002, Nitzan et al., 2006), eight for European/Israeli populations, and six for Australian populations (Ben-Daniel et al., 2010). These VCGs differ in their aggressiveness, morphology, and physiology. Interestingly, isolates of *C. coccodes* from Europe, Australia, and North America do not complement with each other and VCGs from each area appear to be distinct. Recently, however, complementation between a few isolates from Australia, North America, and Europe has been reported (Ben-Daniel et al., 2010). AFLP analysis of *C. coccodes* DNA has proven effective in assigning North American populations of this fungus into their VCGs (Heilmann et al., 2006). A relationship of specific AFLP bands to corresponding VCGs was reported for the first time for any plant pathogenic fungus.

In this study, the AFLP method was successful in separating *C. coccodes* isolates into five NA-VCGs; NA-VCG1, NA-VCG2, NA-VCG3, NA-VCG4/5, AND NA-VCG6/7. The AFLP banding pattern showed presumptive bands specific for several NA-VCGs. Many SCAR markers were tested for their success to be VCG-specific markers. Only one SCAR, AGB6F/R, was successful in identifying NA-VCG6 /7 from the other VCGs. Other

SCARs have been developed but were not VCG specific. Though not VCG specific, these SCARs can be used to differentiate *C. coccodes* from other fungal plant pathogens. More primer sets could be screened that could distinguish NA-VCG6 from NA-VCG7, as well as distinguishing NA-VCG4 from NA-VCG5. Further studies into the development of PCR-markers for *C. coccodes* are needed.

A total of 366 recently collected *C. coccodes* isolates were used to study the population biology of the North American population. The AFLP method was used for this study. Two hundred and ten loci were generated and used to cluster the isolates into their NA-VCGs and to study the genetic structure of the North American population of *C. coccodes*. Recently collected *C. coccdes* isolates were assigned into four VCGs, NA-VCGs, NA-VCG1, NA-VCG2, NA-VCG4/ 5, and NA-VCG6/ 7. No isolates tested belonged to NA-VCG3. The dominant group in the population was NA-VCG2. It was the most frequent NA-VCG among states, fields, farms, and plants. In several instances, more than one NA-VCG was recovered from the same plant, field, farm, and state. Despite the climatic and geographic differences among states, there was a high degree of similarity among the VCGs recovered from them. However, in some states a geographic pattern was observed, such as in Texas, Montana, North Dakota, and Wisconsin. A geographic pattern within the United States *C. coccodes* population was further substantiated by AMOVA which revealed that 27% of the estimated variation was a result of variation among states. Geographic and climatic differences among the states may explain this variation. The remaining variation (73%) resulted from within states variation. The results revealed the considerable genetic variation within and among *C. coccodes* populations, and that this

variation is structured into four main subgroups. Results also suggest that the NA-VCGs are distributed in United States and they form a single large population of *C. coccodes*.

The global population of *C. coccodes* was studied at the molecular genetics level using AFLP markers for the first time. The global population study used a much larger population and includes isolates from five continents including North America, Australia, Europe, Middle East, and South Africa (n=855). The data clearly demonstrate that AFLP analysis is informative at the species level for *C. coccodes*. The number of loci generated via AFLP analysis was powerful in detecting the genetic diversity of the global population. *C. coccodes* populations from various geographic origins appear intermingled with the VCGs of the North American *C. coccodes* population. *C. coccodes* populations originating from different geographic regions formed distinct sub-clusters or groups within the main clusters.

The *C. coccodes* population analysis showed strong evidence for relatively high genetic diversity for an asexual fungus, low genetic differentiation among populations, and the existence of gene flow, indicating high genetic exchange among the five populations to maintain one large population. The data revealed that *C. coccodes* is a global population that is closely related. This is likely due to this fungus being dispersed with the potato as it was distributed worldwide and propagated as an agricultural crop. Based on this study, the population structure of *C. coccodes* isolates indicated one large population with four main groups (NA-VCG1/3; NA-VCG2; NA-VCG4/5; and NA-VCG6/7). NA-VCG5 was the most common VCG globally followed by NA-VCG2. The dominant character of AFLP markers limits their usefulness in viewing heterozygosity. Furthermore, using co-dominant markers such as simple sequence repeat markers (SSR) would be more informative at the

genetic level, and could differentiate *C. coccodes* population more effectively than the dominant AFLP marker (Atallah et al., 2009).

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APPENDIX A

Table A.1. Origin, source and number of isolates used for genetic diversity study of the global populations of *C. coccodes*.

Origin	No. of isolates	Total	Source
USA		515	NCG lab[1]
Recently collected	366		
Previously assigned	109[2]		NCG lab
NA-testers	40		Johnson, D[3]
Europe/ Israel	213	224	Leah Tsror[4]
EU/I testers	11		Leah Tsror
Australia	86	102	Leah Tsror[5]
Aus-testers	16		
South Africa	14	14	Leah Tsror
Out-group species[5]		3	
Total		858[6]	

[1] Isolates obtained from Dr. N. C. Gudmestad laboratory, ND, USA.
[2] Isolates characterized to VCG's using AFLP by Heilmann et al. (2006).
[3] Isolates obtained as sclerotia on sterilized soil from Johnson, D., WA, USA.
[4,5] Isolates obtained as cultures from Leah Tsror, Israel. These isolates included 147 isolates from Europe and 66 isolate from Israel. All the isolates received from Europe/Israel and Australia had no information about the VCG to which they belong, using *nit* mutant technique
[5] Out groups used were *Fusarium graminearum, Acremonium strictum,* and *Colletotrichum lindemuthianum.*
[6] For more details regarding the samples used in this study, see appendix 2.

Table B.1. Global *C. coccodes* isolates information for the genetic diversity study.

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group[1] A	B	C	Source[2]
1	Cc06CO-1	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
2	Cc06CO-2	2006	USA, Colorado		Potato	Stem	1	3	-	NCG lab
3	Cc06CO-3	2006	USA, Colorado		Potato	Stem	1	3	-	NCG lab
4	Cc06CO-4	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
5	Cc06CO-5	2006	USA, Colorado		Potato	Stem	1	3	-	NCG lab
6	Cc06CO-6	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
7	Cc06CO-7	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
8	Cc06CO-8	2006	USA, Colorado		Potato	Stem	1	3	-	NCG lab
9	Cc06CO-9	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
10	Cc06CO-10	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
11	Cc06CO-11	2006	USA, Colorado		Potato	Stem	1	3	-	NCG lab
12	Cc06CO-12	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
13	Cc06CO-13	2006	USA, Colorado		Potato	Stem	2	5	-	NCG lab
14	Cc06CO-14	2006	USA, Colorado	FL1833	Potato	Stolon	5	5	-	NCG lab
15	Cc06CO-15	2006	USA, Colorado	FL1833	Potato	Stolon	5	5	-	NCG lab
16	Cc06CO-16	2006	USA, Colorado	FL1833	Potato	Stolon	5	5	-	NCG lab
17	Cc06CO-17	2006	USA, Colorado	FL1833	Potato	Stolon	5	5	-	NCG lab
18	Cc06CO-18	2006	USA, Colorado	FL1833	Potato	Stolon	2	5	-	NCG lab
19	Cc06CO-19	2006	USA, Colorado		Potato	Leaves	2	5	-	NCG lab
20	Cc06CO-20	2006	USA, Colorado		Potato	Leaves	2	5	-	NCG lab
21	Cc06CO-21	2006	USA, Colorado		Potato	Leaves	2	5	-	NCG lab
22	Cc07CO-1	2007	USA, Colorado	Umatilla Russet	Potato	Leaves	6,7	1	-	NCG lab
23	Cc09MI-1	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
24	Cc09MI-2	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
25	Cc09MI-3	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
26	Cc09MI-4	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
27	Cc09MI-5	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
28	Cc09MI-6	2009	USA, Michigan		Potato	Stem	1	3	-	NCG lab
29	Cc09MI-7	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
30	Cc09MI-8	2009	USA, Michigan		Potato	Stem	na	3	-	NCG lab
31	Cc09MI-9	2009	USA, Michigan		Potato	Stem	na	1	-	NCG lab
32	Cc09MI-10	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
33	Cc09MI-11	2009	USA, Michigan		Potato	Stem	na	6	-	NCG lab
34	Cc09MI-12	2009	USA, Michigan		Potato	Stem	na	6	-	NCG lab
35	Cc09MI-13	2009	USA, Michigan		Potato	Stem	2	6	-	NCG lab
36	Cc06MN-1	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
37	Cc06MN-2	2006	USA, Minnesota	Russeet Burbank	Potato	Stem	2	5	-	NCG lab
38	Cc06MN-3	2006	USA, Minnesota	Russeet Burbank	Potato	Stem	2	5	-	NCG lab
39	Cc06MN-4	2006	USA, Minnesota	Russeet Burbank	Potato	Stem	2	5	-	NCG lab
40	Cc06MN-5	2006	USA, Minnesota	Russeet Burbank	Potato	Stem	1	5	-	NCG lab
41	Cc06MN-6	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
42	Cc06MN-7	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
43	Cc06MN-8	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
44	Cc06MN-9	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
45	Cc06MN-10	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
46	Cc06MN-11	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
47	Cc06MN-12	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
48	Cc06MN-13	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
49	Cc06MN-14	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
50	Cc06MN-15	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
51	Cc06MN-16	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
52	Cc06MN-17	2006	USA, Minnesota		Potato	Stem	1	5	-	NCG lab
53	Cc06MN-18	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
54	Cc06MN-19	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
55	Cc06MN-20	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
56	Cc06MN-21	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
57	Cc06MN-22	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
58	Cc06MN-23	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
59	Cc06MN-24	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
60	Cc06MN-25	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
61	Cc06MN-26	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
62	Cc06MN-27	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
63	Cc06MN-28	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
64	Cc06MN-29	2006	USA, Minnesota		Potato	Stem	5	5	-	NCG lab
65	Cc06MN-30	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
66	Cc06MN-31	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
67	Cc06MN-32	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
68	Cc06MN-33	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
69	Cc06MN-34	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
70	Cc06MN-35	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
71	Cc06MN-36	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
72	Cc06MN-37	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
73	Cc06MN-38	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
74	Cc06MN-39	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
75	Cc06MN-40	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
76	Cc06MN-41	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
77	Cc06MN-42	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
78	Cc06MN-43	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
79	Cc06MN-44	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
80	Cc06MN-45	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
81	Cc06MN-46	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
82	Cc06MN-47	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
83	Cc06MN-48	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
84	Cc06MN-49	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
85	Cc06MN-50	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
86	Cc06MN-51	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
87	Cc06MN-52	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
88	Cc06MN-53	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
89	Cc06MN-54	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
90	Cc06MN-55	2006	USA, Minnesota		Potato	Stem	2	5	-	NCG lab
91	Cc06MN-56	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
92	Cc06MN-57	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
93	Cc06MN-58	2006	USA, Minnesota		Potato	Stem	2	4	-	NCG lab
94	Cc06MN-59	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
95	Cc06MN-60	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
96	Cc06MN-61	2006	USA, Minnesota		Potato	Stem	2	6	-	NCG lab
97	Cc06MN-62	2006	USA, Minnesota		Potato	Stem	6,7	1	-	NCG lab
98	Cc06MN-63	2006	USA, Minnesota		Potato	Stem	6,7	1	-	NCG lab
99	Cc06MN-64	2006	USA, Minnesota		Potato	Stem	1	3	-	NCG lab
100	Cc07MN-1	2007	USA, Minnesota	FL1867	Potato	Tuber	2	6	-	NCG lab
101	Cc09MN-1	2009	USA, Minnesota	Vin02	Potato	Tuber	5	4	-	NCG lab
102	Cc09MN-2	2009	USA, Minnesota	Vin03	Potato	Tuber	2	6	-	NCG lab
103	Cc09MN-3	2009	USA, Minnesota	Vin04	Potato	Tuber	2	6	-	NCG lab
104	Cc09MN-4	2009	USA, Minnesota	Vin05	Potato	Tuber	1	3	-	NCG lab
105	Cc09MN-5	2009	USA, Minnesota	Vin06	Potato	Tuber	1	3	-	NCG lab
106	Cc09MN-7	2009	USA, Minnesota		Potato	Root	1	3	-	NCG lab
107	Cc09MN-8	2009	USA, Minnesota		Potato	Root	1	3	-	NCG lab
108	Cc09MN-9	2009	USA, Minnesota		Potato	Root	2	6	-	NCG lab
109	Cc09MN-10	2009	USA, Minnesota		Potato	Root	1	3	-	NCG lab
110	Cc09MN-11	2009	USA, Minnesota		Potato	Root	1	6	-	NCG lab
111	Cc09MN-12	2009	USA, Minnesota		Potato	Root	1	3	-	NCG lab
112	Cc09MN-13	2009	USA, Minnesota		Potato	Root	1	4	-	NCG lab
113	Cc09MN-14	2009	USA, Minnesota		Potato	Root	1	4	-	NCG lab
114	Cc08MO-1	2008	USA, Montana	Atlantic	Potato	Stem, BG	2	6	-	NCG lab
115	Cc08MO-2	2008	USA, Montana	Atlantic	Potato	Stem, BG	2	6	-	NCG lab
116	Cc08MO-3	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
117	Cc08MO-4	2008	USA, Montana	Atlantic	Potato	Stem, BG	2	6	-	NCG lab
118	Cc08MO-5	2008	USA, Montana	Atlantic	Potato	Stem, BG	5	3	-	NCG lab
119	Cc08MO-6	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
120	Cc08MO-7	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
121	Cc08MO-8	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
122	Cc08MO-9	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
123	Cc08MO-10	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
124	Cc08MO-11	2008	USA, Montana	Atlantic	Potato	Stem, BG	6,7	1	-	NCG lab
125	Cc06ND-1	2006	USA, North Dakota		Potato	Leaves	2	5	-	NCG lab
126	Cc06ND-2	2006	USA, North Dakota		Potato	Leaves	2	5	-	NCG lab
127	Cc06ND-3	2006	USA, North Dakota		Potato	Leaves	2	5	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
128	Cc06ND-4	2006	USA, North Dakota		Potato	Stem	1	3	-	NCG lab
129	Cc06ND-5	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
130	Cc06ND-6	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
131	Cc06ND-7	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
132	Cc06ND-8	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
133	Cc06ND-9	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
134	Cc06ND-10	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
135	Cc06ND-11	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
136	Cc06ND-12	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
137	Cc06ND-13	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
138	Cc06ND-14	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
139	Cc06ND-15	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
140	Cc06ND-16	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
141	Cc06ND-17	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
142	Cc06ND-18	2006	USA, North Dakota		Potato	Stem	2	6	-	NCG lab
143	Cc06ND-19	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
144	Cc06ND-20	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
145	Cc06ND-21	2006	USA, North Dakota		Potato	Stem	2	5	-	NCG lab
146	Cc06ND-22	2006	USA, North Dakota		Potato	Tuber	na	3	-	NCG lab
147	Cc06ND-23	2006	USA, North Dakota		Potato	Tuber	1	3	-	NCG lab
148	Cc06ND-24	2006	USA, North Dakota		Potato	Tuber	na	3	-	NCG lab
149	Cc06ND-25	2006	USA, North Dakota		Potato	Tuber	na	3	-	NCG lab
150	Cc07ND-1	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
151	Cc07ND-2	2007	USA, North Dakota		Potato	Stem/Root	1	3	-	NCG lab
152	Cc07ND-3	2007	USA, North Dakota		Potato	Stem/Root	1	3	-	NCG lab
153	Cc07ND-4	2007	USA, North Dakota		Potato	Stem/Root	1	3	-	NCG lab
154	Cc07ND-5	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
155	Cc07ND-6	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
156	Cc07ND-7	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
157	Cc07ND-8	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
158	Cc07ND-9	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
159	Cc07ND-10	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
160	Cc07ND-11	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
161	Cc07ND-12	2007	USA, North Dakota		Potato	Stem/Root	1	3	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
162	Cc07ND-13	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
163	Cc07ND-14	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
164	Cc07ND-15	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
165	Cc07ND-16	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
166	Cc07ND-17	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
167	Cc07ND-18	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
168	Cc07ND-19	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
169	Cc07ND-20	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
170	Cc07ND-21	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
171	Cc07ND-22	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
172	Cc07ND-23	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
173	Cc07ND-24	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
174	Cc07ND-25	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
175	Cc07ND-26	2007	USA, North Dakota		Potato	Stem/Root	1	6	-	NCG lab
176	Cc07ND-27	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
177	Cc07ND-28	2007	USA, North Dakota		Potato	Stem/Root	1	6	-	NCG lab
178	Cc07ND-29	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
179	Cc07ND-30	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
180	Cc07ND-31	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
181	Cc07ND-32	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
182	Cc07ND-33	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
183	Cc07ND-34	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
184	Cc07ND-35	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
185	Cc07ND-36	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
186	Cc07ND-37	2007	USA, North Dakota		Potato	Stem/Root	1	6	-	NCG lab
187	Cc07ND-38	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
188	Cc07ND-39	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
189	Cc07ND-40	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
190	Cc07ND-41	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
191	Cc07ND-42	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
192	Cc07ND-43	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
193	Cc07ND-44	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
194	Cc07ND-45	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
195	Cc07ND-46	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
196	Cc07ND-47	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
197	Cc07ND-48	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
198	Cc07ND-49	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
199	Cc07ND-50	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
200	Cc07ND-51	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
201	Cc07ND-52	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
202	Cc07ND-53	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
203	Cc07ND-54	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
204	Cc07ND-55	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
205	Cc07ND-56	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
206	Cc07ND-57	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
207	Cc07ND-58	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
208	Cc07ND-59	2007	USA, North Dakota		Potato	Stem/Root	2	6	-	NCG lab
209	Cc06NE-1	2006	USA, Nebraska	FL1833	Potato	Leaves	2	5	-	NCG lab
210	Cc06NE-3	2006	USA, Nebraska		Potato	Stem	na	5	-	NCG lab
211	Cc06NE-4	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
212	Cc06NE-5	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
213	Cc06NE-6	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
214	Cc06NE-7	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
215	Cc06NE-8	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
216	Cc06NE-9	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
217	Cc06NE-10	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
218	Cc06NE-11	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
219	Cc06NE-12	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
220	Cc06NE-13	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
221	Cc06NE-14	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
222	Cc06NE-15	2006	USA, Nebraska		Potato	Stem	1	3	-	NCG lab
223	Cc06NE-16	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
224	Cc06NE-17	2006	USA, Nebraska		Potato	Stem	1	3	-	NCG lab
225	Cc06NE-18	2006	USA, Nebraska	FL1867	Potato	Leaves	1	3	-	NCG lab
226	Cc06NE-19	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
227	Cc06NE-20	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
228	Cc06NE-21	2006	USA, Nebraska		Potato	Stem	1	3	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
229	Cc06NE-22	2006	USA, Nebraska		Potato	Stem	5	2,3, 4	-	NCG lab
230	Cc06NE-23	2006	USA, Nebraska		Potato	Stem	2	3	-	NCG lab
231	Cc06NE-24	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
232	Cc06NE-25	2006	USA, Nebraska		Potato	Stem	2	5	-	NCG lab
233	Cc08NE-1	2008	USA, Nebraska	Russet Burbank	Potato	Stem	6,7	1	-	NCG lab
234	Cc08NE-2	2008	USA, Nebraska	Russet Burbank	Potato	Stem	na	4	-	NCG lab
235	Cc08NE-3	2008	USA, Nebraska	Russet Burbank	Potato	Stem	na	5	-	NCG lab
236	Cc08NE-4	2008	USA, Nebraska	Russet Burbank	Potato	Stem	na	5	-	NCG lab
237	Cc09NV-1	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
238	Cc09NV-2	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
239	Cc09NV-3	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
240	Cc09NV-4	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
241	Cc09NV-5	2009	USA, Nevada	Russet Norkotah	Potato	Stem	5	4	-	NCG lab
242	Cc09NV-6	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
243	Cc09NV-7	2009	USA, Nevada	Russet Norkotah	Potato	Stem	5	4	-	NCG lab
244	Cc09NV-8	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
245	Cc09NV-9	2009	USA, Nevada	Russet Norkotah	Potato	Stem	1	3	-	NCG lab
246	Cc09NV-10	2009	USA, Nevada	Russet Norkotah	Potato	Stem	na	3	-	NCG lab
247	Cc09NV-11	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
248	Cc09NV-12	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
249	Cc09NV-13	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
250	Cc09NV-14	2009	USA, Nevada	Russet Norkotah	Potato	Stem	1	3	-	NCG lab
251	Cc09NV-15	2009	USA, Nevada	Russet Norkotah	Potato	Stem	2	6	-	NCG lab
252	Cc09NV-16	2009	USA, Nevada	Russet Norkotah	Potato	Stem	1	3	-	NCG lab
253	Cc09NV-17	2009	USA, Nevada	Russet Norkotah	Potato	Stem	1	3	-	NCG lab
254	Cc09NV-18	2009	USA, Nevada	Russet Norkotah	Potato	Stem	6,7	1	-	NCG lab
255	Cc09NV-19	2009	USA, Nevada	Russet Norkotah	Potato	Stem	5	4	-	NCG lab
256	Cc06TX-1	2006	USA, Texas	FL2101	Potato	Stem	5	4	-	NCG lab
257	Cc06TX-2	2006	USA, Texas		Potato	Tuber	1	3	-	NCG lab
258	Cc06TX-3	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
259	Cc06TX-4	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
260	Cc06TX-5	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
261	Cc06TX-6	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
262	Cc06TX-7	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
263	Cc06TX-8	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
264	Cc06TX-9	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
265	Cc06TX-10	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
262	Cc06TX-7	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
263	Cc06TX-8	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
264	Cc06TX-9	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
265	Cc06TX-10	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
266	Cc06TX-11	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
267	Cc06TX-12	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
268	Cc06TX-13	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
269	Cc06TX-14	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
270	Cc06TX-15	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
271	Cc06TX-16	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
272	Cc06TX-17	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
273	Cc06TX-18	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
274	Cc06TX-19	2006	USA, Texas		Potato	Tuber	na	3	-	NCG lab
275	Cc09TX-1	2009	USA, Texas		Potato	Stem	2	6	-	NCG lab
276	Cc09TX-2	2009	USA, Texas		Potato	Stem	2	6	-	NCG lab
277	Cc09TX-3	2009	USA, Texas		Potato	Stem	2	6	-	NCG lab
278	Cc09TX-4	2009	USA, Texas		Potato	Stem	na	6	-	NCG lab
279	Cc09TX-6	2009	USA, Texas		Potato	Stem	na	1	-	NCG lab
280	Cc06WI-1	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	2	5	-	NCG lab
281	Cc06WI-2	2006	USA, Wisconsin		Potato	Stem	2	5	-	NCG lab
282	Cc06WI-3	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	1	3	-	NCG lab
283	Cc06WI-4	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	1	3	-	NCG lab
284	Cc06WI-5	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	2	5	-	NCG lab
285	Cc06WI-6	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	1	5	-	NCG lab
286	Cc06WI-7	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	2	5	-	NCG lab
287	Cc06WI-8	2006	USA, Wisconsin	Silverton Russet	Potato	Stem	2	5	-	NCG lab
288	Cc06WI-9	2006	USA, Wisconsin	Freedom Russets	Potato	Stem	2	5	-	NCG lab
289	Cc06WI-10	2006	USA, Wisconsin	Freedom Russets	Potato	Stem	2	5	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
290	Cc08WI-1	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
291	Cc08WI-2	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
292	Cc08WI-3	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
293	Cc08WI-4	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
294	Cc08WI-5	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
295	Cc08WI-6	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
296	Cc08WI-7	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
297	Cc08WI-8	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
298	Cc08WI-9	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
299	Cc08WI-10	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
300	Cc08WI-11	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
301	Cc08WI-12	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
302	Cc08WI-13	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
303	Cc08WI-14	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
304	Cc08WI-15	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
305	Cc08WI-16	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
306	Cc08WI-17	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
307	Cc08WI-18	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
308	Cc08WI-19	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
309	Cc08WI-20	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
310	Cc08WI-21	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
311	Cc08WI-22	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
312	Cc08WI-23	2008	USA, Wisconsin		Potato	Tuber 4	na	3	-	NCG lab
313	Cc08WI-24	2008	USA, Wisconsin		Potato	Tuber 4	na	6	-	NCG lab
314	Cc08WI-25	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
315	Cc08WI-26	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
316	Cc08WI-27	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
317	Cc08WI-28	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
318	Cc08WI-29	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
319	Cc08WI-30	2008	USA, Wisconsin		Potato	Tuber 3	na	3	-	NCG lab
320	Cc08WI-31	2008	USA, Wisconsin		Potato	Tuber 3	na	6	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
321	Cc08WI-32	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
322	Cc08WI-33	2008	USA, Wisconsin		Potato	Tuber 1	2	4	-	NCG lab
323	Cc08WI-34	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
324	Cc08WI-35	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
325	Cc08WI-36	2008	USA, Wisconsin		Potato	Tuber 2	2	4	-	NCG lab
326	Cc08WI-37	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
327	Cc08WI-38	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
328	Cc08WI-39	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
329	Cc08WI-40	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
330	Cc08WI-41	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
331	Cc08WI-42	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
332	Cc08WI-43	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
333	Cc08WI-44	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
334	Cc08WI-45	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
335	Cc08WI-46	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
336	Cc08WI-47	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
337	Cc08WI-48	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
338	Cc08WI-49	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
339	Cc08WI-50	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
340	Cc08WI-51	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
341	Cc08WI-52	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
342	Cc08WI-53	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
343	Cc08WI-54	2008	USA, Wisconsin		Potato	Tuber 2	2	6	-	NCG lab
344	Cc08WI-55	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
345	Cc08WI-56	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
346	Cc08WI-57	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
347	Cc08WI-58	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
348	Cc08WI-59	2008	USA, Wisconsin		Potato	Tuber 3	5	4	-	NCG lab
349	Cc08WI-60	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
350	Cc08WI-61	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
351	Cc08WI-62	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
352	Cc08WI-63	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
353	Cc08WI-64	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
354	Cc08WI-65	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
355	Cc08WI-66	2008	USA, Wisconsin		Potato	Tuber 1	2	6	-	NCG lab
356	Cc08WI-67	2008	USA, Wisconsin		Potato	Tuber 1	5	4	-	NCG lab
357	Cc08WI-68	2008	USA, Wisconsin		Potato	Tuber 2	5	4	-	NCG lab
358	Cc08WI-69	2008	USA, Wisconsin		Potato	Tuber 2	5	4	-	NCG lab
359	Cc08WI-70	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
360	Cc08WI-71	2008	USA, Wisconsin		Potato	Tuber 3	5	4	-	NCG lab
361	Cc08WI-72	2008	USA, Wisconsin		Potato	Tuber 3	2	6	-	NCG lab
362	Cc08WI-73	2008	USA, Wisconsin		Potato	Tuber 3	5	4	-	NCG lab
363	Cc08WI-74	2008	USA, Wisconsin		Potato	Tuber 4	5	6	-	NCG lab
364	Cc08WI-75	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
365	Cc08WI-76	2008	USA, Wisconsin		Potato	Tuber 4	5	4	-	NCG lab
366	Cc08WI-77	2008	USA, Wisconsin		Potato	Tuber 4	2	6	-	NCG lab
367	C-60	1997	USA, Washington	Russet Norkotah	Potato	Tuber	1	3	1	NCG lab
368	CcNE02-4-1	2002	Columbus, NE	FL 1833		Stems	1	3	1	D. Johnson
369	R-LAKE-22-IL	1997	USA, Montana	Ranger Russet	Potato	Tuber	1	3	1	NCG lab
370	C138	1997	USA, North Dakota	Russet Norkotah	Potato	Tuber	1	3	1	D. Johnson
371	MN24-1		USA				1	3	1	D. Johnson
372	CcNE04-83-4	2004	USA, Nebraska	Red Norland	Potato	Tuber	1	3	1	NCG lab
373	CcNV04-66-3	2004	USA, Nevada	Chipeta	Potato	AG-stems	1	3	1	NCG lab
374	CcOR04-61-1	2004	USA, Oregon	Ranger Russet	Potato	Stems	1	3	1	NCG lab
375	CcNV04-66-8	2004	USA, Nevada	Chipeta	Potato	Roots	1	3	1	NCG lab
376	CcNV02-24-1	2002	Winnemucca, NV	Russet Burbank		Stems	1	3	1	NCG lab
377	CcNV04-66-5	2004	USA, Nevada	Chipeta		BG-stems	1	3	1	NCG lab
378	CcNV02-24-5	2002	USA, Nevada	Russet Burbank		Stolons	1	3	1	NCG lab
379	00-194-20Nork	1997	Montana	Russet Norkotah		Tuber	1	3	1	D. Johnson
380	C47iL	1995	USA, Washington	Russet Burbank		Tuber	1	3	1	D. Johnson
381	C19iL	1990	USA, Washington	Russet Burbank		Stem	1	3	1	D. Johnson
382	C57	1997	USA, North Dakota	Russet Norkotah		Tuber	1	3	1	D. Johnson
383	C19	1990	USA, Washington	Russet Burbank		Stem	1	3	1	D. Johnson
384	C237iL	1997	USA, Nebraska	Russet Norkotah			1	3	1	D. Johnson

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
385	CcOR04-61-2	2004	USA, Oregon	Ranger Russet		Stems	1	3	1	NCG lab
386	CcNE02-4-1	2002	Columbus, NE	FL 1833		Stems	1	3	1	NCG lab
387	ALB13	2002	USA, Alberta			Tuber	2	6	2	D. Johnson
388	MT11	2002	Montana	Ranger Russet			2	6	2	D. Johnson
389	C116iL	1997	USA, Washington	Russet Norkotah		Tuber	2	6	2	D. Johnson
390	ORG-9C	2002	Oregon	Ranger Russet		Tuber	2	6	2	D. Johnson
391	BR302		USA				2	6	2	NCG lab
392	Nad2	2002	Washington				2	6	2	D. Johnson
393	C112	1997	USA, Montana	Russet Norkotah		Tuber	2	6	2	D. Johnson
394	C114iL	1997	USA, Washington	Russet Norkotah		Tuber	2	6	2	D. Johnson
395	MT16	2002	Montana	Ranger Russet		Tuber	2	6	2	D. Johnson
396	ALB15	2002	USA, Alberta			Tuber	2	6	2	D. Johnson
397	CcNV04-66-1	2004	Tonopah, NV	Chipeta		AG-Stems	2	6	2	NCG lab
398	CcOR04-62-6	2004	Boardman, OR	Russet Burbank		Tuber	2	6	2	NCG lab
399	CcOR04-61-3	2004	Boardman, OR	Ranger Russet		Stems	2	6	2	NCG lab
400	CcOR04-61-8	2004	Boardman, OR	Ranger Russet			2	6	2	NCG lab
401	C244iL	1997	USA, North Dakota	Russet Norkotah		Tuber	2	6	2	D. Johnson
402	CcOR04-61-7	2004	Boardman, OR	Ranger Russet		Roots	2	6	2	NCG lab
403	CcMN02-22	2002	Becker, MN	Russet Burbank		Stems	2	6	2	NCG lab
404	C46iL	1994	USA, Washington	Russet Burbank		Tuber	2	6	2	D. Johnson
405	Coll114	1997	Washington	Russet Norkotah		Tuber	2	6	2	D.Johnson
406	CcMN02-29	2002	East Grand Forks, MN	Red Norland		Stems	2	6	2	NCG lab
407	CcNE02-56	2002	Minden, NE	FL 1833		Tubers	2	6	2	NCG lab
408	ORG-2B	2002	Oregon	Ranger Russet		Tuber	2	6	2	D. Johnson
409	CcOR04-62-2	2004	Boardman, OR	Russet Burbank		Tuber	2	6	2	NCG lab
410	CcOR04-62-6	2004	Boardman, OR	Russet Burbank		Tuber	2	6	2	NCG lab
411	Coll236	1997	North Dakota	Russet Norkotah		Tuber	3	3	3	D. Johnson
412	ORG-3	2002	Oregon				3	3	3	D. Johnson
413	C242-1L	1997	Washington	Russet Norkotah		Tuber	3	3	3	D. Johnson
414	Coll95-IL	1997	Alberta	Russet Norkotah		Tuber	3	3	3	D. Johnson
415	BR40-5		USA				3	3	3	NCG lab
416	CcOR02-53-2	2002	Hermiston, OR	Russet Burbank		Stems	3	3	3	NCG lab
417	C11-1L	1990	Washington	Russet Burbank		Stem	3	3	3	D. Johnson
418	CcNV04-66-7	2004	Tonopah, NV	Chipeta		Roots	3	3	3	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
419	Coll239-1L	1997	British Columbia	Russet Norkotah		Tuber	3	3	3	D. Johnson
420	C61	1997	Montana	Russet Burbank		Tuber	3	3	3	D. Johnson
421	CcNV04-66-4	2004	Tonopah, NV	Chipeta		AG-Stems	3	3	3	NCG lab
422	C49	1995	Nebraska			Tuber	3	3	3	D. Johnson
423	C49-1L	1995	Nebraska			Tuber	3	3	3	D. Johnson
424	C36	1992	Washington	Russet Norkotah		Stem	3	3	3	D. Johnson
425	C124	1997	Nebraska	Russet Norkotah		Tuber	5	4	4	D. Johnson
426	ORG-1	2002	Oregon	Ranger Russet		Tuber	5	4	4	D. Johnson
427	MT5	2002	Montana	Ranger Russet		Tuber	5	4	5	D. Johnson
428	CcOR04-61-4	2004	Boardman, OR	Ranger Russet		Stolons	5	4	5	NCG lab
429	MT5	2002	Montana	Ranger Russet		Tuber	5	4	5	D. Johnson
430	ORG-2	2002	Oregon				5	4	5	D. Johnson
431	R96-1L	1997	Montana	Ranger Russet		Tuber	5	4	5	D. Johnson
432	CcOR04-61-6	2004	Boardman, OR	Ranger Russet		Roots	5	4	5	NCG lab
433	C43-1L	1992	Washington			Tuber	5	4	5	D. Johnson
434	Nad1	2002	Washington	Russet Burbank			5	4	5	D. Johnson
435	CcOR04-62-5	2004	Boardman, OR	Russet Burbank		Tuber	5	4	5	NCG lab
436	CcWI02-8	2002	Hancock, WI	FL 1879		Tuber	5	4	5	NCG lab
437	CcOR04-62-1	2004	Boardman, OR	Russet Burbank		Tuber	5	4	5	NCG lab
438	CcNE02-14	2002	Kearney, NE	FL 1833		Tubers	5	4	5	NCG lab
439	CcOR04-62-3	2004	Boardman, OR	Russet Burbank		Tuber	5	4	5	NCG lab
440	CcOR04-61-5	2004	Boardman, OR	Ranger Russet		Roots	5	4	5	NCG lab
441	CcWI02-17	2002	Antigo, WI	Pike		Tuber	5	4	5	NCG lab
442	CcOR04-62-4	2004	Boardman, OR	Russet Burbank		Tuber	5	4	5	NCG lab
443	Nad3	2002	Washington	Russet Burbank		Tuber	5	4	5	D. Johnson
444	CcNE02-14	2002	Kearney, NE	FL 1833		Tubers	5	4	5	NCG lab
445	ORG-3	2002	Oregon				5	4	5	D. Johnson
446	ORG-4	2002	Oregon				5	4	5	D. Johnson
447	CcOR02-57	2002					5	4	5	NCG lab
448	AN13-1		Ohio	Red pepper			6,7	1	6	R. Rowe
449	AN13-2		Ohio	Red pepper			6,7	1	6	R. Rowe
450	CcNE02-45	2002	O'Neill, NE	Russet Norkotah		Stems	6,7	1	6	NCG lab
451	CcNE02-40	2002	O'Neill, NE	Russet Norkotah		Stems	6,7	1	6	NCG lab
452	CcNE02-41	2002	O'Neill, NE	Russet Norkotah		Stems	6,7	1	6	NCG lab

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
453	C210	1984	Lockport, NY	Tomato		Fruit	6,7	1	6	H. Dillard
454	CcMN02-1-15	2002	Browerville, MN	Russet Burbank		Stems	6,7	1	6	NCG lab
455	CcMN02-1-19	2002	Browerville, MN	Russet Burbank		Stems	6,7	1	6	NCG lab
456	CcMI04-70	2004	Three Rivers, MI			S,R,St	6,7	1	6	NCG lab
457	CcMN02-1-13	2002	Browerville, MN	Russet Burbank		Stems	6,7	1	6	NCG lab
458	CcMN02-1-14	2002	Browerville, MN	Russet Burbank		Stems	6,7	1	6	NCG lab
459	CcMN02-1-1	2002	Browerville, MN	Russet Burbank		Stems	6,7	1	6	NCG lab
460	CcMN02-28	2002	East Grand Forks, MN	Red Norland		Stems	6,7	1	6	NCG lab
461	CcMN02-26	2002	East Grand Forks, MN	Yukon Gold		Stems	6,7	1	6	NCG lab
462	CcMN02-5	2002	Perham, MN	Russet Burbank		Stems	6,7	1	6	NCG lab
463	CcNE04-67	2004	Cody, NE			S,R,St,L	6,7	1	6	NCG lab
464	AN15		Ohio	Tomato			6,7	1	6	R. Rowe
465	452	1988	Georgia to NY	Tomato		Roots	6,7	1	6	H. Dillard
466	CcNE02-40	2002	O'Neill, NE	Russet Norkotah		Stems	6,7	1	6	NCG lab
467	CcNE02-43	2002	O'Neill, NE	Russet Norkotah		Stems	6,7	1	6	NCG lab
468	CcNE02-40	2002	O'Neill, NE	Russet Norkotah		Stems	6,7	1	6	NCG lab
469	C55-IL	1996	Washington	Peppermint		Rhizome	6,7	1	7	D. Johnson
470	C501	1997	New York	Tomato			6,7	1	7	D. Johnson
471	C55-IL	1996	Washington	Peppermint		Rhizome	6,7	1	7	D. Johnson
472	C54	1996	Wisconsin	Peppermint		Rhizome	6,7	1	7	D. Johnson
473	C501-IL	1997	New York	Tomato			6,7	1	7	D. Johnson
474	C501iL (NY)			Culture			6,7	1	7	
475	C55(WA)			Culture			6,7	1	7	
476	Aus136/07k	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
477	Aus59/07Ta	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
478	Aus104/07AH	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
479	Aus-118	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
480	Aus88/07AH	2009	Australia		Potato	Culture	5	4	3	Leah Tsror
481	Aus-67/07AH	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
482	Aus-4	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
483	Aus-62	2009	Australia		Soil	Culture	5	4	2	Leah Tsror
484	Aus157/06	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
485	Aus/114/07K	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
486	Aus84/07K	2009	Australia		Potato	Culture	5	4	na	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
487	Aus-59/07Tb	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
488	Aus-77/07K	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
489	Aus-2	2009	Australia		Soil	Culture	5	4	4	Leah Tsror
490	Aus-45/07WA	2009	Australia		Potato	Culture	5	4	na	Leah Tsror
491	Aus-118/07K	2009	Australia		Potato	Culture	1	4	1	Leah Tsror
492	Aus-135/07K	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
493	Aus-109/07K	2009	Australia		Potato	Culture	5	4	na	Leah Tsror
494	Aus-85/07AH	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
495	Aus-54	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
496	Aus-79/07K	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
497	Aus-84/07K	2009	Australia		Potato	Culture	5	4	na	Leah Tsror
498	Aus-92/07AH	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
499	Aus-42/07PI	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
500	Aus-C12	2009	Australia		Potato	Culture	1	4	na	Leah Tsror
501	Aus-130/07K	2009	Australia		Potato	Culture	5	4	na	Leah Tsror
502	Aus-5	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
503	Aus-90/07AH	2009	Australia		Potato	Culture	5	4	3	Leah Tsror
504	Aus-98/07AH	2009	Australia		Potato	Culture	5	4	3	Leah Tsror
505	Aus-80/07K	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
506	Aus-72/07K3	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
507	Aus-89/07P	2009	Australia		Potato	Culture	5	4	-	Leah Tsror
508	Aus-72/07K	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
509	Aus-78/07K	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
510	Aus-67/07AH	2009	Australia		Potato	Culture	5	4	-	Leah Tsror
511	Aus-86/07AH	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
512	Aus-123/07AH	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
513	Aus-113/07K	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
514	Aus-C4	2009	Australia		Potato	Culture	1	3	na	Leah Tsror
515	Aus-156/06	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
516	Aus-6	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
517	Aus-13/07K	2009	Australia		Potato	Culture	5	4	-	Leah Tsror
518	Aus-125/07AH	2009	Australia		Potato	Culture	1	4	3	Leah Tsror
519	Aus-5	2009	Australia		Potato	Culture	5	4	2	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
520	Aus-112/07K	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
521	Aus-C9	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
522	Aus-120/07PI	2009	Australia		Potato	Culture	5	4	3	Leah Tsror
523	Aus-9/07W	2009	Australia		Potato	Culture	na	3	na	Leah Tsror
524	Aus-77	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
525	Aus-12	2009	Australia		Potato	Culture	5	4	na	Leah Tsror
526	Aus-69/07P	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
527	Aus-24	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
528	Aus-19/07T	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
529	Aus-111/07K	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
530	Aus-11/07P	2009	Australia		Potato	Culture	1	3	1	Leah Tsror
531	Aus-122/07P	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
532	Aus-18/07T	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
533	Aus-13	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
534	Aus-C6	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
535	Aus-115	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
536	Aus-12/07P	2009	Australia		Potato	Culture	1	3	6	Leah Tsror
537	Aus-8/07W	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
538	Aus-96/07AH	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
539	Aus-14	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
540	Aus-20/07T	2009	Australia		Potato	Culture	5	4	3	Leah Tsror
541	Aus-97/07AH	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
542	Aus-2/07K	2009	Australia		Potato	Culture	5	4	6	Leah Tsror
543	Aus-114	2009	Australia		Potato	Culture	1	3	2	Leah Tsror
544	Aus-9	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
545	Aus-15/07P	2009	Australia		Potato	Culture	5	4	4	Leah Tsror
546	Aus-131/07K	2009	Australia		Potato	Culture	5	4	5	Leah Tsror
547	Aus-106	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
548	Aus-79	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
549	Aus-26	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
550	Aus-83/07K	2009	Australia		Potato	Culture	5	4	2	Leah Tsror
551	Aus-13/07P	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
552	Aus-15	2009	Australia		Potato	Culture	5	4	1	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
553	Aus-126/07AH	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
554	Aus-112	2009	Australia		Potato	Culture	5	4	na	Leah Tsror
555	Aus-11	2009	Australia		Potato	Culture	5	4	3	Leah Tsror
556	Aus-81/07K	2009	Australia		Potato	Culture	1	4	na	Leah Tsror
557	Aus-55	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
558	Aus-16	2009	Australia		Potato	Culture	1	3	na	Leah Tsror
559	Aus-81	2009	Australia		Potato	Culture	1	3	3	Leah Tsror
560	Aus-16/07AH	2009	Australia		Potato	Culture	5	4	1	Leah Tsror
561	Aus-7	2009	Australia		Soil	Culture	5	4	1	Leah Tsror
562	SA-H1	2009	South Africa		Potato	Culture	na	4	-	Leah Tsror
563	SA-K1	2009	South Africa		Potato	Culture	na	4	-	Leah Tsror
564	SA-K3a	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
565	SA-K3b	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
566	SA-K4	2009	South Africa		Potato	Culture	na	5	-	Leah Tsror
567	SA-R4	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
568	SA-R6	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
569	SA-K7	2009	South Africa		Potato	Culture	na	4	-	Leah Tsror
570	SA-R7	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
571	SA-R12	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
572	SA-R13	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
573	SA-R14	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
574	SA-K14	2009	South Africa		Potato	Culture	na	4	-	Leah Tsror
575	SA-R16	2009	South Africa		Potato	Culture	6,7	1	-	Leah Tsror
576	SCRI-C2	1997	Washington State U.		Potato		1	3	1	A. Lees
577	SCRI-C3	1997	Washington State U.		Potato		1	3	-	A. Lees
578	SCRI-C4	1997	Washington State U.		Potato		5	4	-	A. Lees
579	SCRI-C5	1985	SCRI, Dundee, Scotland		Maris Piper		5	4	-	A. Lees
580	SCRI-C6	1989	SCRI, Dundee, Scotland		Maris Piper		5	4	-	A. Lees
581	SCRI C7	1988	SCRI, Dundee, Scotland		Record		5	4	-	A. Lees
582	SCRI-C8	1997	Dept of Ag., N. Ireland		Navan		5	4	-	A. Lees
583	SCRI-C9	1997	SASA, Montrose, Scotland		Potato		5	4	-	A. Lees

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
584	SCRI-C10	1998	SASA, Newmill, Scotland		Potato		5	4	-	A. Lees
585	SCRI-C11	1998	SASA, Brichie, Scotland		Potato		5	4	-	A. Lees
586	SCRI-C12	1998	SASA, Edinburgh, Scotland		Potato		5	4	-	A. Lees
587	SCRI-C13	1998	Dept. of Ag., N. Ireland		Anna		5	4	-	A. Lees
588	SCRI-C14	1998	Dept. of Ag., N. Ireland		Avalanche		5	4	-	A. Lees
589	SCRI-C15	1998	Dept. of Ag., N. Ireland		Sante		5	4	-	A. Lees
590	SCRI-C16		SCRI, Dundee, Scotland		Potato		5	4	-	A. Lees
591	SCRI-C17		TESCO Supermarket-Scotland		Charlotta		5	4	-	A. Lees
592	SCRI-C18		TESCO Supermarket-Scotland		Charlotta		5	4	-	A. Lees
593	SCRI-C19	1983	Hertfordshire, England		P. Crown		5	4	-	A. Lees
594	SCRI-C20	1988	Hertfordshire, England		Desiree		5	4	-	A. Lees
595	SCRI-C21	1988	Hertfordshire, England		Desiree		5	4	-	A. Lees
596	SCRI-C22	1991	Rosefarm, Scotland		Desiree		5	4	-	A. Lees
597	SCRI-C23	1991	Dalreoch Farm, Scotland		Desiree		5	4	-	A. Lees
598	SCRI-C24	1997	Sutton Bridge, England		Fianna		5	4	-	A. Lees
599	SCRI-C25	1997	Sutton Bridge, England		Maris Piper		5	4	-	A. Lees
600	SCRI-C26	1997	Sutton Bridge, England		Maris Piper		5	4	-	A. Lees
601	SCRI-C27	1997	Sutton Bridge, England		Navan		5	4	-	A. Lees
602	SCRI-C28	1997	Sutton Bridge, England		Potato		5	4	-	A. Lees
603	SCRI-C29	1997	Sutton Bridge,England			Tuber	5	4	-	A. Lees
604	SCRI-C30	1998	SCRI, Dundee, Scotland		Estima	Tuber	5	4	-	A. Lees
605	SCRI-C31	1998	SCRI, Dundee, Scotland		Maris Piper	Tuber	5	4	-	A. Lees
606	SCRI-C32	1998	SCRI, Dundee, Scotland		Maris Piper	Tuber	5	4	-	A. Lees
607	SCRI-C33	1999	(Argentina)		Strawberry		6,7	1	6,7	A. Lees
608	Si-1	2006	Netherland			Dried samples	5	4	-	Leah Tsror
609	Si-2	2006	Netherland			Dried samples	5	4	-	Leah Tsror
610	Si-3	2006				Dried samples	5	4	-	Leah Tsror
611	Si-4	2006	Netherland			Dried samples	5	4	-	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
612	Si-5	2006	Netherland			Dried samples	5	4	-	Leah Tsror
613	Si-6	2006	Netherland			Dried samples	5	4	-	Leah Tsror
614	Si-7	2006	Netherland			Dried samples	5	4	-	Leah Tsror
615	Si-8	2006				Dried samples	5	4	-	Leah Tsror
616	Si-9	2006				Dried samples	5	4	-	Leah Tsror
617	Si-10	2006	Netherland			Dried samples	5	4	-	Leah Tsror
618	Si-11	2006	Netherland			Dried samples	5	4	-	Leah Tsror
619	Si-12	2006				Dried samples	5	4	-	Leah Tsror
620	Si-13	2006	Netherland			Dried samples	2	6	-	Leah Tsror
621	Si-15	2006	Netherland			Dried samples	5	4	-	Leah Tsror
622	Si-16	2006				Dried samples	5	4	-	Leah Tsror
623	Si-17	2006	Netherland			Dried samples	5	4	-	Leah Tsror
624	Si-18	2006				Dried samples	5	4	-	Leah Tsror
625	Si-19	2006	Netherland			Dried samples	2	6	-	Leah Tsror
626	Si-20	2006	Netherland			Dried samples	2	6	-	Leah Tsror
627	Si-21	2006	Netherland			Dried samples	5	4	-	Leah Tsror
628	Si-22	2006	Netherland			Dried samples	2	6	-	Leah Tsror
629	Si-23	2006	Netherland			Dried samples	5	4	-	Leah Tsror
630	Si-24	2006	Netherland			Dried samples	2	6	-	Leah Tsror
631	Si-25	2006	Netherland			Dried samples	2	6	-	Leah Tsror
632	Si-26	2006	Netherland			Dried samples	5	4	-	Leah Tsror
633	Si-27	2006	Netherland			Dried samples	5	4	-	Leah Tsror
634	Si-28	2006				Dried samples	5	4	-	Leah Tsror
635	Si-29	2006	Netherland			Dried samples	5	4	-	Leah Tsror
636	Si-30	2006	Netherland			Dried samples	5	4	-	Leah Tsror
637	Si-31	2006	Scotland			Dried samples	5	4	-	Leah Tsror
638	Si-32	2006	Scotland			Dried samples	5	4	-	Leah Tsror
639	Si-33	2006	Scotland			Dried samples	5	4	-	Leah Tsror
640	Si-34	2006	Netherland			Dried samples	5	4	-	Leah Tsror
641	Si-35	2006				Dried samples	5	4	-	Leah Tsror
642	Si-36	2006	Netherland			Dried samples	5	4	-	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
643	Si-37	2006	Scotland			Dried samples	5	4	-	Leah Tsror
644	Si-38	2006	Scotland			Dried samples	5	4	-	Leah Tsror
645	Si-39	2006	Scotland			Dried samples	5	4	-	Leah Tsror
646	Si-40	2006				Dried samples	5	4	-	Leah Tsror
647	Si-41	2006				Dried samples	5	4	-	Leah Tsror
648	Si-42	2006	Netherland			Dried samples	5	4	-	Leah Tsror
649	Si-43	2006	Scotland			Dried samples	5	4	-	Leah Tsror
650	Si-44	2006	Scotland			Dried samples	5	4	-	Leah Tsror
651	Si-45	2006	Scotland			Dried samples	5	4	-	Leah Tsror
652	Si-46	2006				Dried samples	5	4	-	Leah Tsror
653	Si-47	2006				Dried samples	5	4	-	Leah Tsror
654	Si-48	2006				Dried samples	5	4	-	Leah Tsror
655	Si-49	2006	Netherland			Dried samples	5	4	-	Leah Tsror
656	Si-50	2006	Netherland			Dried samples	5	4	-	Leah Tsror
657	Si-51	2006	Netherland			Dried samples	5	4	-	Leah Tsror
658	Si-52	2006	Scotland			Dried samples	5	4	-	Leah Tsror
659	Si-53	2006	Scotland			Dried samples	5	4	-	Leah Tsror
660	Si-54	2006	Scotland			Dried samples	5	4	-	Leah Tsror
661	Si-55	2006	Netherland			Dried samples	5	4	-	Leah Tsror
662	Si-56	2006	Netherland			Dried samples	5	4	-	Leah Tsror
663	Si-57	2006	Netherland			Dried samples	5	4	-	Leah Tsror
664	Si-58	2006	Netherland			Dried samples	5	4	-	Leah Tsror
665	Si-59	2006	Netherland			Dried samples	5	4	-	Leah Tsror
666	Si-60	2006	Netherland			Dried samples	5	4	-	Leah Tsror
667	Si-61	2006	Netherland			Dried samples	5	2	-	Leah Tsror
668	Si-62	2006				Dried samples	5	2	-	Leah Tsror
669	Si-63	2006	Israel			Dried samples	5	2	-	Leah Tsror
670	Si-64	2006				Dried samples	5	2	-	Leah Tsror
671	Si-65	2006				Dried samples	5	2	-	Leah Tsror
672	Si-66	2006				Dried samples	5	2	-	Leah Tsror
673	Si-67	2006				Dried samples	5	2	-	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
674	Si-68	2006				Dried samples	5	2	-	Leah Tsror
675	Si-69	2006	Netherland			Dried samples	5	2	-	Leah Tsror
676	Si-70	2006	Scotland			Dried samples	5	2	-	Leah Tsror
677	Si-71	2006				Dried samples	5	2	-	Leah Tsror
678	Si-72	2006	Netherland			Dried samples	5	2	-	Leah Tsror
679	Si-73	2006	Netherland			Dried samples	5	2	-	Leah Tsror
680	Si-74	2006	Netherland			Dried samples	5	2	-	Leah Tsror
681	Si-76	2006	France			Dried samples	5	2	-	Leah Tsror
682	Si-77	2006				Dried samples	5	2	-	Leah Tsror
683	Si-78	2006	France			Dried samples	5	2	-	Leah Tsror
684	Si-80	2006	Netherland			Dried samples	5	2	-	Leah Tsror
685	Si-81	2006				Dried samples	5	2	-	Leah Tsror
686	Si-83	2006	Netherland			Dried samples	5	2	-	Leah Tsror
687	Si-85	2006	Germany			Dried samples	5	2	-	Leah Tsror
688	Si-86	2006				Dried samples	5	2	-	Leah Tsror
689	Si-87	2006	Netherland			Dried samples	5	2	-	Leah Tsror
690	Si-88	2006	Netherland			Dried samples	5	2	-	Leah Tsror
691	Si-89	2006	Netherland			Dried samples	5	2	-	Leah Tsror
692	Si-91	2006				Dried samples	5	2	-	Leah Tsror
693	Si-92	2006	Scotland			Dried samples	5	2	-	Leah Tsror
694	Si-93	2006	Scotland			Dried samples	5	2	-	Leah Tsror
695	Si-95	2006				Dried samples	5	2	-	Leah Tsror
696	Si-96	2006	Netherland			Dried samples	5	2	-	Leah Tsror
697	Si-97	2006	Netherland			Dried samples	5	2	-	Leah Tsror
698	Si-99	2006	Netherland			Dried samples	5	2	-	Leah Tsror
699	Si-101	2006	Netherland			Dried samples	5	2	-	Leah Tsror
700	Si-102	2006	Scotland			Dried samples	5	2	-	Leah Tsror
701	Si-103	2006	Scotland			Dried samples	5	2	-	Leah Tsror
702	Si-104	2006	Netherland			Dried samples	5	2	-	Leah Tsror
703	Si-105	2006	Netherland			Dried samples	5	2	-	Leah Tsror
704	Si-106	2006	Netherland			Dried samples	5	2	-	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
705	Si-107	2006	Netherland			Dried samples	5	2	-	Leah Tsror
706	Si-108	2006	Netherland			Dried samples	5	2	-	Leah Tsror
707	Si-109	2006	Netherland			Dried samples	5	2	-	Leah Tsror
708	Si-110	2006	Netherland			Dried samples	5	2	-	Leah Tsror
709	Si-112	2006	Scotland			Dried samples	5	2	-	Leah Tsror
710	Si-113	2006				Dried samples	5	2	-	Leah Tsror
711	Si-114	2006				Dried samples	5	2	-	Leah Tsror
712	Si-115	2006	Israel			Dried samples	5	2	-	Leah Tsror
713	Si-116	2006				Dried samples	5	2	-	Leah Tsror
714	Si-117	2006	Israel			Dried samples	5	2	-	Leah Tsror
715	Si-118	2006				Dried samples	5	2	-	Leah Tsror
716	Si-119	2006	Israel			Dried samples	5	2	-	Leah Tsror
717	Si-120	2006	Israel			Dried samples	5	2	-	Leah Tsror
718	Si-121	2006	Israel			Dried samples	5	2	-	Leah Tsror
719	Si-122	2006	Israel			Dried samples	5	2	-	Leah Tsror
720	Si-123	2006	Israel			Dried samples	5	2	-	Leah Tsror
721	Si-124	2006	Israel			Dried samples	5	2	-	Leah Tsror
722	Si-125	2006	Israel			Dried samples	5	2	-	Leah Tsror
723	Si-126	2006	Israel			Dried samples	5	2	-	Leah Tsror
724	Si-127	2006				Dried samples	5	2	-	Leah Tsror
725	Si-128	2006	Israel			Dried samples	5	2	-	Leah Tsror
726	Si-129	2006	Israel			Dried samples	na	2	-	Leah Tsror
727	Si-130	2006				Dried samples	na	2	-	Leah Tsror
728	Si-131	2006	Israel			Dried samples	5	2	-	Leah Tsror
729	Si-132	2006	Israel			Dried samples	5	2	-	Leah Tsror
730	Si-133	2006	Israel			Dried samples	5	2	-	Leah Tsror
731	Si-134	2006	Israel			Dried samples	5	2	-	Leah Tsror
732	Si-136	2006				Dried samples	5	2	-	Leah Tsror
733	Si-137	2006				Dried samples	5	2	-	Leah Tsror
734	Si-138	2006	Israel			Dried samples	5	2	-	Leah Tsror
735	Si-139	2006	Israel			Dried samples	5	2	-	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
736	Si-142	2006	Netherland			Dried samples	5	2	-	Leah Tsror
737	Si-143	2006	France			Dried samples	5	2	-	Leah Tsror
738	Si-144	2006	France			Dried samples	na	2	-	Leah Tsror
739	Si-145	2006	France			Dried samples	5	2	-	Leah Tsror
740	Si-146	2006				Dried samples	5	2	-	Leah Tsror
741	Si-147	2006	Germany			Dried samples	5	2	-	Leah Tsror
742	Si-148	2006	Germany			Dried samples	5	2	-	Leah Tsror
743	Si-149	2006				Dried samples	5	2	-	Leah Tsror
744	Si-150	2006	France			Dried samples	5	2	-	Leah Tsror
745	Si-151	2006	France			Dried samples	5	2	-	Leah Tsror
746	Si-152	2006	Netherland			Dried samples	5	2	-	Leah Tsror
747	Si-153	2006	Netherland			Dried samples	5	2	-	Leah Tsror
748	Si-154	2006	Netherland			Dried samples	5	2	-	Leah Tsror
749	Si-155	2006	Scotland			Dried samples	5	2	-	Leah Tsror
750	Si-156	2006	Scotland			Dried samples	5	2	-	Leah Tsror
751	Si-157	2006	Scotland			Dried samples	5	2	-	Leah Tsror
752	Si-159	2006				Dried samples	5	2	-	Leah Tsror
753	Si-160	2006	France			Dried samples	5	2	-	Leah Tsror
754	Si-161	2006	Scotland			Dried samples	5	2	-	Leah Tsror
755	Si-162	2006	Scotland			Dried samples	5	2	-	Leah Tsror
756	Si-163	2006	Scotland			Dried samples	5	2	-	Leah Tsror
757	Si-164	2006	Scotland			Dried samples	5	2	-	Leah Tsror
758	Si-165	2006				Dried samples	5	2	-	Leah Tsror
759	Si-166	2006	Netherland			Dried samples	5	2	-	Leah Tsror
760	Si-167	2006	Scotland			Dried samples	5	2	-	Leah Tsror
761	Si-168	2006	Scotland			Dried samples	5	2	-	Leah Tsror
762	Si-169	2006	Scotland			Dried samples	5	2	-	Leah Tsror
763	Si-170	2006	Scotland			Dried samples	5	2	-	Leah Tsror
764	Si-171	2006	Scotland			Dried samples	5	2	-	Leah Tsror
765	Si-172	2006	Scotland			Dried samples	5	2	-	Leah Tsror
766	Si-173	2006	Scotland			Dried samples	5	2	-	Leah Tsror
767	Si-174	2006	Scotland			Dried samples	5	2	-	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
768	Si-175	2006				Dried samples	5	2	-	Leah Tsror
769	Si-176	2006	France			Dried samples	5	2	-	Leah Tsror
770	Si-177	2006	Netherland			Dried samples	5	2	-	Leah Tsror
771	Si-178	2006	Netherland			Dried samples	5	2	-	Leah Tsror
772	Si-179	2006	Netherland			Dried samples	5	2	-	Leah Tsror
773	Si-180	2006				Dried samples	5	2	-	Leah Tsror
774	Si-182	2006	Netherland			Dried samples	5	2	-	Leah Tsror
775	Si-183	2006				Dried samples	5	2	-	Leah Tsror
776	Si-184	2006				Dried samples	5	2	-	Leah Tsror
777	Si-185	2006	Netherland			Dried samples	5	2	-	Leah Tsror
778	Si-186	2006	Netherland			Dried samples	5	2	-	Leah Tsror
779	Si-188	2006				Dried samples	5	2	-	Leah Tsror
780	Si-189	2006				Dried samples	5	2	-	Leah Tsror
781	Si-190	2006	Netherland			Dried samples	5	2	-	Leah Tsror
782	Si-191	2006	Netherland			Dried samples	5	2	-	Leah Tsror
783	Si-192	2006	Netherland			Dried samples	5	2	-	Leah Tsror
784	Si-193	2006	Netherland			Dried samples	5	2	-	Leah Tsror
785	Si-197	2006	Israel			Dried samples	5	2	-	Leah Tsror
786	Si-198	2006				Dried samples	5	2	-	Leah Tsror
787	Si-199	2006				Dried samples	5	2	-	Leah Tsror
788	Si-200	2006				Dried samples	5	2	-	Leah Tsror
789	Si-6/9	2009	Europe			Culture	5	4	1	Leah Tsror
790	#24.3	2009	Europe			Culture	5	4	2	Leah Tsror
791	Si-110/7	2009	Europe			Culture	5	4	2	Leah Tsror
792	Si-72/8	2009	Europe			Culture	5	4	3	Leah Tsror
793	Si-88/3	2009	Europe			Culture	5	4	3	Leah Tsror
794	#46/7	2009	Europe			Culture	5	4	4	Leah Tsror
795	Si-28/4	2009	Europe			Culture	5	4	8	Leah Tsror
796	#184/6	2009	Europe			Culture	5	4	5	Leah Tsror
797	Si-20/9	2009	Europe			Culture	5	6	6	Leah Tsror
798	Si-1/7	2009	Europe			Culture	5	4	7	Leah Tsror
799	Si-70/15	2009	Europe			Culture	5	4	7	Leah Tsror
800	1.7	2009	USA			Culture	1	3	1	Leah Tsror
801	6.1	2009	USA			Culture	1	3	1	Leah Tsror

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
802	26.2	2009	USA			Culture	5	6	2	Leah Tsror
803	N-124/12	2009	USA			Culture	5	4	4	Leah Tsror
804	47.6	2009	USA			Culture	5	6	2	Leah Tsror
805	10.10b	2009	USA			Culture	5	6	2	Leah Tsror
806	30.8	2009	USA			Culture	3	3	3	Leah Tsror
807	27.9	2009	USA			Culture	3	3	3	Leah Tsror
808	48.4	2009	USA			Culture	5	4	4	Leah Tsror
809	33.6	2009	USA			Culture	5	4	5	Leah Tsror
810	35.5	2009	USA			Culture	5	4	5	Leah Tsror
811	39.6	2009	USA			Culture	6,7	1	6	Leah Tsror
812	45.17	2009	USA			Culture	6,7	1	7	Leah Tsror
813	7.7	2009	Australia			Culture	5	4	1	Leah Tsror
814	79.3	2009	Australia			Culture	5	4	1	Leah Tsror
815	90/07AH.18	2009	Australia			Culture	5	4	1	Leah Tsror
816	55.2	2009	Australia			Culture	5	4	1	Leah Tsror
817	77.2	2009	Australia			Culture	5	4	1	Leah Tsror
818	115.14	2009	Australia			Culture	5	4	2	Leah Tsror
819	114.3	2009	Australia			Culture	3	3	3	Leah Tsror
820	6.15	2009	Australia			Culture	3	3	3	Leah Tsror
821	C6.1	2009	Australia			Culture	3	3	3	Leah Tsror
822	95/07AH.4	2009	Australia			Culture	5	4	2	Leah Tsror
823	120/07PI.1	2009	Australia			Culture	3	3	3	Leah Tsror
824	15/07P.2	2009	Australia			Culture	5	4	4	Leah Tsror
825	2.5	2009	Australia			Culture	5	4	4	Leah Tsror
826	59/07TA.12	2009	Australia			Culture	5	4	4	Leah Tsror
827	12/07.7	2009	Australia			Culture	3	3	5	Leah Tsror
828	97/07AH.8	2009	Australia			Culture	na	3	6	Leah Tsror
829	MT5	2009	USA			Culture	5	4	5	Johnson, D
830	C19	2009	USA			Culture	1	3	1	Johnson, D
831	236-14,M	2009	USA			Culture	3	6	3	Johnson, D
832	C501	2009	USA			Culture	6,7	1	6,7	Johnson, D
833	ALB14	2009	USA			Culture	2	6	2	Johnson, D
834	C60	2009	USA			Culture	1	3	1	Johnson, D
835	C60-9 nit M	2009	USA			Culture	1	3	1	Johnson, D

Table B.1. (Continued)

	Isolate designation	Year	Origin	Cultivar	Host	Organ	Group A	Group B	Group C	Source
836	ALB14-6 nitM	2009	USA			Culture	2	6	2	Johnson, D
837	AN-13	2009	USA			Culture	6,7	1	6,7	Johnson, D
838	MT5-6 nit M	2009	USA			Culture	5	4	5	Johnson, D
839	C54	2009	USA			Culture	6,7	1	6,7	Johnson, D
840	MT11-5 nit M	2009	USA			Culture	2	6	2	Johnson, D
841	C55		USA			Culture	6,7	1	6,7	Johnson, D
842	COLL236		USA			Culture	3	3	3	Johnson, D
843	C124		USA			Culture	5	4	5	Johnson, D
844	MT5		USA			Culture	5	4	5	Johnson, D
845	AN-13		USA			Culture	6,7	1	6,7	Johnson, D
846	ORG1		USA			Culture	5	4	5	Johnson, D
847	ALB15		USA			Culture	2	6	2	Johnson, D
848	ALB13		USA			Culture	2	6	2	Johnson, D
849	C60		USA			Culture	6,7	1	6,7	Johnson, D
850	C55		USA			Culture	6,7	1	6,7	Johnson, D
851	C501-2	2007	USA			Culture	6,7	1	6,7	Johnson, D
852	C501iL(NY)-2	2007				Culture	6,7	1	6	Johnson, D
853	C501il(WA)-2	2007				Culture	6,7	1	6	Johnson, D
854	C55iL-2	2007				Culture	6,7	1	6	Johnson, D
855	C55il(WA)-2	2007				Culture	6,7	1	6	Johnson, D

[1] A: isolates were assigned based on UPGMA clustering with the different *C. coccodes* tester isolates. B: isolates assigned to a population based on their ancestry. na: not assigned with NA-VCGs based on the dendogram generated via UPGMA. Further analysis on the isolates ancestry showed that subpopulations 2 and 4 clustered together and were belonged to NA-VCG5, and subpopulations 5 and 6 clustered together and were belonged to NA-VCG2. C: isolates previously assigned to VCGs (Ben-Daniel et al., 2010; Heilmann et al., 2006, Nitzan et al., 2006).

[2] Isolates obtained from Dr. Neil Gudmestad laboratory, ND, Leah Tsror, Israel; and Johnson, D., WA, USA.