J Gen Plant Pathol (2016) 82:199–211
DOI 10.1007/s10327-016-0662-y

OTHERS

Genetic diversity of *Colletotrichum coccodes* in the United States using amplified fragment length polymorphism analysis

Kholoud M. Alananbeh[1,2] · **Neil C. Gudmestad**[1]

Received: 19 February 2015 / Accepted: 12 April 2016 / Published online: 19 May 2016

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 370 single-spore isolates of *C. coccodes* were collected from naturally infected potato plants in nine states and analyzed using amplified fragment length polymorphism (AFLP) markers and three primer pairs, yielding 190 polymorphic bands with 90.7 % polymorphism. The isolates were assigned to four vegetative compatibility groups (AFLP/VCG): AFLP/VCG1, AFLP/VCG2, AFLP/VCG4/5, and AFLP/VCG6/7. No isolates tested belonged to AFLP/VCG3. The United States *C. coccodes* population structure was confirmed with a high differentiation value ($G_{ST} = 0.30$) among VCGs. AFLP/VCG2 was the dominant group in the population ($n = 262$) and was the most frequent AFLP/VCG among states, fields, farms, and plants. However, in several instances, more than one AFLP/VCG was isolated from the same plant, field, farm, and state, indicating variability within the *C. coccodes* population in the United States. A geographic pattern was found for isolates originating from Texas, Montana, North Dakota, and Wisconsin. Diversity within states accounted for 73 % of the total genetic diversity, and among populations accounted for 27 %. These results suggest that several AFLP/VCGs are widely distributed in the United States and that they form a single large population of *C. coccodes*.

Keywords Vegetative compatibility groups · AFLP · Differentiation · Genetic diversity · Potato

Introduction

Black dot disease of potato, caused by *Colletotrichum coccodes* (Wallr.) Hughes, is a fungal disease with worldwide distribution (Andrivon et al. 1997). It was named black dot due to the abundant sclerotia that are produced after infection of tubers, stolons, roots and above- and below-ground stems (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 the pathogen also contaminates soil and serves as an important source of inoculum for future potato crops (Tsror et al. 1999).

Colletotrichum coccodes is an imperfect fungus belonging to Coelomycetes, which are asexual fungi that produce fertile hyphae in specialized structures called conidiomata (Cano et al. 2004). Vegetative compatibility refers to the ability of individual fungal strains to undergo mutual hyphal anastomosis and form viable heterokaryons, thus serving as a means of genetic exchange (Leslie 1993). Characterization of the population of this fungus has been studied using nitrate non-utilizing (nit) mutants 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), Australia (Ben-Daniel et al. 2010), and South Africa (L. Tsror personnel communication). The nitrate non-utilizing (nit) mutant method for designating vegetative compatibility groups (VCGs) has divided the *C.*

✉ Neil C. Gudmestad
neil.gudmestad@ndsu.edu

[1] Department of Plant Pathology, North Dakota State University, Walster Hall 310, Fargo, ND 58102, USA

[2] Department of Plant Protection, Faculty of Agriculture, The University of Jordan, Amman 11942, Jordan

coccodes isolates into seven, eight, and six VCGs for North American, European/Israeli, and Australian populations, respectively. The VCGs from the different countries are based on each country's VC groups. For example, Eu-VCG1 is not the same as NA-VCG1 and so on for the other VCGs. Isolates from South Africa could not be assigned to any VCG due to the inability of these isolates to complement each other or other isolates of *C. coccodes* from the different continental populations (Leah Tsror, Head of Plant Pathology Unit at Gilat, personal communication). Amplified fragment length polymorphism (AFLP) markers have also been successful in differentiating the North American *C. coccodes* population (Heilmann et al. 2006) and the global population (Alananbeh et al. 2014) into five VCGs.

Many studies have reported an increased incidence of tuber infections (Johnson et al. 1997) and yield reductions with reduced tuber numbers after foliar infections by *C. coccodes* fungus (Johnson 1994). In a study of disease incidence of *C. coccodes* and *V. dahliae* in Washington State University (WSU) Experimental Station (Dung et al. 2012), neither yield reduction nor foliar symptoms was found to be related to the levels of either pathogen in seed lots. Moreover, soil-borne inoculum caused higher levels of disease than inoculum on seed tubers, where the incidence of black dot reached 57 % of crops at different levels of soil inoculum (Lees et al. 2010). Yield has been significantly reduced (30 %) by *C. coccodes* for many potato cultivars and potato early dying syndrome can also contribute to these losses especially when *V. dahliae* and *C. coccodes* are present together (Tsror et al. 1999). *C. coccodes* was found over the entire surface of tubers and in the medullary tissue and has been isolated more frequently from the stem end than from the bud end or lateral sections of infected tubers (Johnson et al. 1997). In addition, *C. coccodes* expands linearly from an inoculum source, and multiple primary infections are needed to cause symptoms on the root system (Ingram and Johnson 2010).

After the identification of *C. coccodes* VCGs, variations in morphology (Aqeel et al. 2008), physiology (Nitzan and Tsror 2003), and aggressiveness (Aqeel et al. 2008; Ben-Daniel et al. 2010; Nitzan et al. 2006; Shcolnick et al. 2007) have been documented among the VCGs. Some differences in aggressiveness may be due to the methods used to measure aggressiveness. Isolates belonging to the same VCG share common physiological traits such as growth at certain temperatures and pH (Nitzan and Tsror 2003). These differences among VCGs demonstrate that these groups are distinct in many aspects.

Some VCGs are more frequently found in different geographic regions. For example, EU/I-VCG2 is the most frequent in the Europe/Israel population (Nitzan et al. 2006; Shcolnick et al. 2007), and NA-VCG2 is most prevalent in the North American population (Heilmann et al. 2006; Nitzan et al. 2006). However, these two different populations were found to be genetically similar using the amplified fragment length polymorphism technique (Alananbeh et al. 2014). *C. coccodes* isolates from Australia appear to be predominantly 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.

Population biology has been studied in many plant pathogens (Burlakoti et al. 2008; Collado-Romero et al. 2006; Lee and Neate 2007). 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. Knowledge of VCG distribution in a population, and aggressiveness of the groups within that population, is of importance to accurately evaluate possible damage, required control measures, and isolate selection for breeding programs (Nitzan et al. 2002). Thus, the main objectives of this study were to (1) use AFLP markers to study the genetic diversity of the United States *C. coccodes* population based on their VC grouping and (2) determine the distribution of *C. coccodes* NA-VCGs in the United States population obtained from different states, fields, and plant parts.

Materials and methods

coccodes isolates

A total of 370 isolates of *C. coccodes* were isolated in 2006, 2007, 2008, and 2009 from infected plants in fields from different states in the Gudmestad laboratory in North Dakota (Table 1; Fig. 1a). Many samples (whole potato plants) were sent by farmers to the Gudmestad lab for disease identification. Each part of the potato plant was cultured to isolate fungi. The different isolated fungi were sorted by genera, and data about *C. coccodes* fungus was recorded for each isolate. The isolates were recovered from tubers, stems, roots, stolons, and leaves from 42 fields (Table 1). These isolates were the same ones used by Alananbeh et al. (2014). Plant samples were sterilized in 10 % bleach, rinsed, dried, and plated on different media: potato dextrose agar (PDA) (Edgerton 1908), clarified V8 medium (CV8) (Evans et al. 1993), water agar (WA) (Atlas 1996), and ethanol agar media (Nadakavukaren and Horner 1959). For above- and below-ground stem tissue, stems were cut at the soil line using sterile knives and scalpels. Five thin slices from both above- and below-

Table 1 Origin, source and number of *Colletotrichum coccodes* isolates collected from fields in nine states in the United States

State	Year	No. of fields/year	Plant part								Total/year	Total/State
			Stems[a]				Roots	Stolons	Tubers	Leaves		
			Above	Below	Vascular	NI						
CO	2006	3	5	8	0	0	0	5	0	3	21	
CO	2007	1	0	0	0	0	0	0	0	1	1	22
MI	2009	1	5	7	0	1	0	0	0	0	13	13
MN	2006	9	3	18	0	45	0	0	0	0	66	
MN	2007	1	0	0	0	0	0	0	1	0	1	
MN	2009	2	0	0	0	0	8	0	5	0	13	80
MO	2008	2	0	11	0	0	0	0	0	0	11	11
ND	2006	3	0	22	0	0	0	0	4	3	29	
ND	2007	3	27	22	0	8	0	0	0	0	57	86
NE	2006	6	1	21	0	0	0	0	0	2	24	
NE	2008	1	0	0	0	4	0	0	0	0	4	28
NV	2009	1	9	9	1	0	0	0	0	0	19	19
TX	2006	2	0	1	0	0	0	0	18	0	19	
TX	2009	1	0	0	0	5	0	0	0	0	5	24
WI	2006	2	5	5	0	0	0	0	0	0	10	
WI	2008	4	0	0	0	0	0	0	77	0	77	87
Total		42	55	124	1	63	8	5	105	9	370	370

CO Colorado, *MN* Minnesota, *ND* North Dakota, *NE* Nebraska, *WI* Wisconsin, *TX* Texas, *MT* Montana, *MI* Michigan, and *NV* Nevada

NI no information was available on whether they originated from above- or below-ground stems

[a] Five thin slices from both above- (Above) and below-ground (Below) parts of stems were plated. Among the 370 *C. coccodes* isolates, 31 were from both above- and below-ground portions of different plants from five fields in five states; 19 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

ground parts of stems were plated onto culture media. Among the 370 *C. coccodes* isolates, 31 were isolated from either above or below portions of the plants. These "stem" isolates originated from five fields in five states. Nineteen stems were from one field in ND, eight from one field in NV, two from one field in MI, one from one field each in WI, and MN. To determine the number of AFLP/VCGs on the same potato tuber, five random lesions were excised and plated from 22 potato tubers. Twenty of the 22 tubers were from one field in WI, and two tubers from one field in MN. Pure cultures of *C. coccodes* isolates were obtained by hyphal tip culturing. North American VCG tester isolates were from Dennis Johnson, Washington State University. Additionally a total of 105 isolates of previously assigned *C. coccodes* isolates belonging to five VCGs (Heilmann et al. 2006) were included in this study.

DNA extraction and molecular confirmation

DNA was extracted from *C. coccodes* isolates collected from the United States (Alananbeh et al. 2014) and molecularly confirmed (Cullen et al. 2002) to the species level.

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 (Alananbeh et al. 2014). To check AFLP reproducibility, representative isolates from the tester ($n = 7$) strains and the newly collected isolates ($n = 20$) were cultured five times, the DNA was extracted, and subjected to AFLP analysis. The AFLP patterns were consistent for all runs.

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 50–650 bp were scored. A total of 210 AFLP fragments were scored for the 370 *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.

Fig. 1 United States map adapted from the website http://www.democraticunderground.com/discuss/duboard.php?az=view_all&address=105x1920084. **a** UPGMA Nei-based dendrogram for *Colletotrichum coccodes* population differentiation by state (**b**)

Data analysis

A consensus tree was generated using the Phylogeny Inference Package (PHYLIP, University of Washington, Seattle, WA, USA), Statistical Analysis Software (SAS, Cary, NC, USA) using the unweighted pairgroup method with arithmetic means (UPGMA) with 1000 bootstraps (Alananbeh et al. 2014). Based on the dendogram generated, the isolates that were 95 % similar to each other were considered as clones, and clone-corrected data were used for further genetic analysis (Table 2). Clone-corrected data were used for analyses to avoid any statistical bias of the genetic structure of *C. coccodes* populations as a result of repeated sampling of the same clone in any population (Lee and Neate 2007). The *C. coccodes* isolates were classified into nine (state) populations according to their geographic origin: Colorado (CO), Minnesota (MN), North Dakota (ND), Nebraska (NE), Wisconsin (WI), Texas (TX), Montana (MT), Michigan (MI), and Nevada (NV) to test for any geographic pattern.

Clone-corrected *C. coccodes* binary matrix data generated from the 210 loci were analyzed using the programs POPGENE version 1.32 (Yeh et al. 1997), GENALEX 6.2 (Peakall and Smouse 2006), and Multilocus 1.3b (Agapow and Burt 2001; Pritchard et al. 2000). Several population statistics were generated: allele frequency, gene diversity (h, heterozygosity) (Nei 1973), Nei's unbiased genetic identity (I), genetic distance (D), percentage of polymorphic loci, total gene diversity (H_T) across populations and within populations (H_S), average differentiation among populations (ΦPT and G_{ST}; where G_{ST} ranges from zero to one, where low values indicate little variation and high values indicate a

Table 2 Clone-corrected and non-corrected data used for genetic analysis

State	Non-corrected	Corrected
Colorado	22	14
Michigan	13	10
Minnesota	80	35
Montana	11	9
Nebraska	28	18
Nevada	19	12
North Dakota	86	39
Texas	24	12
Wisconsin	87	41
Total	370	190

[a] The isolates that were 95 % similar to each other were considered as clones

[b] Clone-corrected data were used for analyses to avoid any statistical analysis bias of the genetic structure of *Colletotrichum coccodes* populations as a result of repeated sampling of the same clone in any population. Clone-corrected data were used for all genetic analyses

large amount of variation among populations; Culley et al. 2002), pairwise population differentiation, the number of different genotypes (G), genotypic diversity (GD), and linkage disequilibrium (LD). An analysis of molecular variance (AMOVA) was also run. LD values range between 0 and 1 where 0 indicates complete panmixia and 1 no recombination (Agapow and Burt 2001). This test was done with 100 randomization sets. The variance in the *C. coccodes* population was partitioned into "among populations": and "within population" variation. The significance level was tested at $\alpha = 0.05$, and was determined using 1000 permutations (Excoffier et al. 1992).

Principal coordinate analysis (PCoA) of the *C. coccodes* population was performed for both noncorrected ($n = 370$) and clone-corrected ($n = 190$) data using GenAlex 6.3 (Peakall and Smouse 2006). The PCoA analysis is characterized by a lack of correlation because the data are sorted as orthogonal according to the amount of variation they explain (Heilmann et al. 2006). The first coordinate accounts for the greatest variation followed by the second, third, and so on (Heilmann et al. 2006). Data were also grouped according to the vegetative compatibility assignment and according to fields within states, and PCoA was performed on each.

The data generated for each primer combination was tested for the number and percentage of polymorphic bands and for G_{ST} to determine whether these three primers generated close G_{ST} values. If the G_{ST} value was consistent among the three primers, overall estimation of G_{ST} value would be valid.

Results

Assigning C. coccodes isolates into AFLP/VCGs. The 370 *C. coccodes* isolates were analyzed by AFLP using the three primer pairs. In the dendogram generated, the *C. coccodes* isolates grouped with the VCGs tester isolates into five main groups: AFLP/VCG1, AFLP/VCG2, AFLP/VCG3, AFLP/VCG4/5, and AFLP/VCG6/7 (Table 3). AFLP/VCGs 6 and 7 were easily identified by their numerous bands generated by the three primer pairs and clustered in the same group. The two isolates of AFLP/VCG4, C124, and ORG1, which were used as tester strains for this VCG, were similar in their AFLP banding pattern to AFLP/VCG5. AFLP/VCG3 tester strains had a banding pattern similar to that of AFLP/VCG1, but they were in a subcluster within the main cluster. AFLP/VCG2 and AFLP/VCG5 were very similar in their AFLP banding pattern mainly with the *Eco*-RI-AC/*Mse*I-CC and *Eco*RI-AG/*Mse*I-CC primer pairs. Similarly, both VCGs formed distinct clusters within the main cluster. Of the NA-370 *C. coccodes* isolates tested, 65 isolates belonged to AFLP/VCG1, 262 isolates belonged to AFLP/VCG2, 29 isolates to AFLP/VCG4/5, and 14 to AFLP/VCG6/7 (Table 4).

AFLP/VCG distribution among states, fields, and plant tissues. At least, two AFLP/VCGs were found in each state (Table 3). AFLP/VCG2 was the most frequent AFLP/VCG found in six of the states (CO, MN, ND, NE, and NV) (Table 3). AFLP/VCG1 was the most frequent AFLP/VCG in TX, and AFLP/VCG6/7 was the most frequent from MT (Table 3).

Many VCGs were recovered from the same field in a state; i.e., for example in one field in MN, isolates belonging to AFLP/VCG1, AFLP/VCG2, and AFLP/VCG6/7 were found. Among the 370 *C. coccodes* isolates, AFLP/VCG1, AFLP/VCG2, and AFLP/VCG4/5 were the most frequently found (Table 3) and the most frequent from various plant organs (Table 4). AFLP/VCG1, AFLP/VCG2, and AFLP/VCG4/5 were the most frequently isolated AFLP/VCG from above- and below-ground stems and from tubers (Table 4).

The UPGMA Nei-based dendrogram clustered the *C. coccodes* isolates into five main groups (Fig. 1b). The first cluster included *C. coccodes* isolates from CO, MN, and ND; the second cluster included NE, WI, and MI; and the other three clusters included populations from NV, TX, and MT. Based on the non-corrected clones, the AMOVA showed that 27 % of the estimated variation originated from the variance among the state populations, and 73 % variation originated from the estimated variance (ΦPT) within the state populations (Table 5). Statistical measurement of ΦPT, within-population differentiation, was significant at $P = 0.001$. For clone-corrected data,

Table 3 Source, number of isolates of *Colletotrichum coccodes* from the United States and AFLP/VCG designations[a] using amplified fragment length polymorphism analysis

State	Organ[c]	NA-VCG[d]	No. of isolates	Cluster[e]
Colorado	S, L, St	1	0	–
$n = 22$		2	21	B15
		3	0	–
		4/5	0	–
		6/7	1	A
Michigan	S	1	4	B2, B4
$n = 13$		2	0	–
		3	0	–
		4/5	8	B11
		6/7	1	–
Minnesota	R, S, T	1	20	B3, B8, B9
$n = 80$		2	47	B15, B16
		3	0	–
		4/5	11	B11, B13
		6/7	2	A
Montana	S	1	0	–
$n = 11$		2	4	B16
		3	0	–
		4/5	0	–
		6/7	7	A
North Dakota	L, S, T	1	12	B6, B8, B9
$n = 86$		2	74	B15, B16
		3	0	–
		4/5	0	–
		6/7	0	–
Nebraska	L, S	1	4	B9
$n = 28$		2	23	B15
		3	0	–
		4/5	0	–
		6/7	1	A
Nevada	S	1	1	B6
$n = 19$		2	10	B16
		3	0	–
		4/5	7	B13
		6/7	1	A
Texas	S, T	1	20	B2, B4, B6, B8
$n = 24$		2	1	B15
		3	0	–
		4/5	3	B11
		6/7	0	–
Wisconsin	S, T	1	4	B6
$n = 87$		2	82	B15, B16
		3	0	–
		4/5	0	–
		6/7	1	A
Previously	SC	1	19	B8
$n = 105$		2	21	B17
		3	14	B10
		4/5	23	B17
		6/7	28	A
NA-testers	SC	1	6	B8
$n = 40$		2	5	B11
		3	11	B10
		4/5	5	B11
		6/7	13	A

[a] AFLP/VCG: the VCG that was determined using AFLP

[b] Previously assigned isolates were tested by Heilmann et al. (2006)

[c] Source of isolate, *L* leaf, *R* root, *S* stem, *St* Stolon, and *T* tuber. Five thin slices from both above- and below-ground parts of stems were plated; see Table 1 for details on methods

[d] AFLP/VCG identification was based on the clusters of the *C. coccodes* isolates with the North American tester isolates using UPGMA and SAS with 1000 bootstraps using PHYLIP software

[e] Cluster numbers were extracted from Table 2 in Alananbeh et al. (2014)

AMOVA showed that 17 % of the estimated variation originated from the variance among the state populations, and 83 % variation originated from the estimated variance (ΦPT) within the state populations (Table 5). Statistical measurement of ΦPT, within-population differentiation, was significant at $P = 0.001$. For among VCGs and among fields within states, ΦPT values were significant with a value of 0.39 and 0.40, respectively.

The first three axes for the noncorrected clone data and the clone-corrected data explained the most variation found in the data (Fig. 2). However, axis 1 and 2 showed the highest variation. In the non-corrected data, the percentage of variation explained was 50.72, 18.02, and 11.94 % for axis 1, axis 2, and axis 3, respectively (Fig. 2a). In the clone-corrected data, the percentage of variation explained was 51.72, 18.13, and 11.55 % for axis 1, axis 2, and axis 3, respectively (Fig. 2b). All the isolates from the nine states were mostly clustered together on the left followed by the right side of the PCoA graph (Fig. 2a, b). Similarly, based on the VC grouping, variation was similar to that of the clone-corrected data values (51.72, 18.13, and 11.55 % for axis 1, axis 2, and axis 3, respectively) (Fig. 2c). For the PCoA analysis based on fields within states, the percentage of variation explained was 39.47, 13.84, and 8.81 % for axis 1, axis 2, and axis 3, respectively (Fig. 2d).

Based on VCG differentiation, the total genetic differentiation among the five AFLP/VCGs was 0.47. However, AFLP/VCG6/7 was highly differentiated from the other

Table 4 Plant tissue origin of 370 *Colletotrichum coccodes* isolates originating from potato and tested for presumptive AFLP/VCG[a] utilizing AFLP analysis

Tissue	AFLP/VCG1	AFLP/VCG2	AFLP/VCG4/5	AFLP/VCG6/7	Total
Stem					
AG	11	39	5	0	55
BG	22	81	11	10	124
NI	20	41	–	2	63
Tuber	4	92	8	1	105
Root	7	1	–	0	8
Stolon	0	1	4	0	5
Vascular	0	0	1	0	1
Leaves	1	7	0	1	9
Total	65	262	29	14	370

AG aboveground, *BG* belowground, *NI* information on stem part not available

[a] AFLP/VCGs: vegetative compatibility groups assigned by using AFLP

AFLP/VCGs (Table 6). AFLP/VCG1 and AFLP/VCG3 were very similar, AFLP/VCG2 and AFLP/VCG4/5 were also similar and clustered together. For the genetic differentiation among states, results showed that the differentiation ranged between 0.01 between CO and MN to 0.57 between ND and TX and between MI and TX (Table 7).

There were 31 potato plants from which *C. coccodes* was isolated from above- and below-ground tissues (Table 8). AFLP/VCG2 was found in 27 potato stems, and this AFLP/VCG was found in the same above- and below-ground stem tissues in 17 of 31 plants. In the remaining 14 potato plants, the AFLP/VCG in belowground tissues, except in one tuber, was different than that from the isolates the aboveground tissues (Table 8). When multiple isolations were made from the same potato tuber, AFLP/VCG2 was recovered from 21 tubers, and 13 of 22 tubers had AFLP/VCG2 isolates from all the lesions on the same tuber. Eight tubers had two AFLP/VCGs recovered from them; these AFLP/VCGs primarily belonged to AFLP/VCG2 and NA-VCG4/5 (Table 7). Three AFLP/VCG's were recovered from one tuber: 2, 4/5, and 6/7 (Table 8).

Genetic structure of the United States C. coccodes population by state. The three primer pairs used to analyze the 370 *C. coccodes* isolates generated 210 bands; these bands were reproducible and clear for all *C. coccodes* isolates. The three primers *Eco*RI-AC/*Mse*I-CC, *Eco*RI-AG/*Mse*I-CC, and *Eco*RI-AT/*Mse*I-CC combinations generated 87.32, 96.97, and 87.67 % polymorphism among *C. coccodes* isolates. Similarly, the three primers produced consistent differences among populations with (G_{ST}) values for the three primers of 0.28, 0.27, and 0.30, respectively, indicating that the overall differentiation among the *C. coccodes* population can be averaged across the three primers (data not shown).

In the genetic diversity analysis of the data from the *C. coccodes* isolates from nine states, the total genetic diversity for all 210 loci was relatively moderate ($H_T = 0.22$), and genetic diversity within a state population was relatively low ($H_S = 0.15$) resulting in relatively high differentiation among the *C. coccodes* populations ($G_{ST} = 0.33$). Among states, gene diversity (h) ranged between a low of 0.09 (WI) to a high of 0.24 (MT) (Table 9). Both the number of distinct genotypes (G) and genotypic diversity (GD) values were high in all populations except for the TX population of *C. coccodes* (Table 9). Among the 190 *C. coccodes* clones analyzed, 176 distinct genotypes were identified (Table 9).

Linkage disequilibrium values also varied among states, ranged from 0.05 (low) in MN to 0.34 (high) in MT. LD values correlate negatively with gene flow; however, among all the populations, LD values differed significantly from zero ($P < 0.01$) (Table 7). LD was also calculated for the most frequently recovered group (AFLP/VCG2) to avoid the artifact of calculating LD among highly subdivided populations (States), and found to be 0.05, also differing significantly from zero ($P < 0.01$). Pairwise comparison of genetic identity among the nine states showed high values ranging from 0.80 (TX and MT) to 0.99 (MN and CO) (Table 10). The overall estimated differentiation among the population (G_{ST}) among the nine states was 0.33.

Discussion

The AFLP loci were highly polymorphic, and the percentage polymorphism 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. The AFLP markers were also useful in identifying the genetic diversity of this population. The results of this study are consistent with

Table 5 Summary of the AMOVA for United States *Colletotrichum coccodes* populations using clone-corrected and non-corrected data

Source	*df*	Est. var.	%	Φ Stat[a]	*P* value[b]
Clone not-corrected					
Among states (ΦPT)	8	5.59	27	0.27	0.001
Within states	362	15.2	73		
Total	370	20.79	100		
Clone corrected					
Among states (ΦPT)	8	3.45	17	0.17	0.001
Within states	181	16.83	83		
Total	189	20.29	100		
Among VCG-clone corrected[c]					
Among VCGs (ΦPT)	2	10.37	39	0.39	0.001
Within VCGs	187	16.18	61		
Total	189	26.55	100		
Among field-clone corrected[d]					
Among VCGs (ΦPT)	24	8.10	40	0.40	0.001
Within VCGs	165	12.14	60		
Total	189	20.24	100		

[a] ΦPT was calculated as the proportion of estimated variance for among population, relative to the total estimated variance

[b] Probability of obtaining low Φ value was determined by 1000 permutations

[c] Only clone-corrected data without tester strains were used. Three major groups were considered due to genetic similarity among VCG1/3, VCG2/4/5, and VCG6/7

[d] Although 42 fields were surveyed, after clone-correction, only 29 fields were included. Five of 29 fields had only one isolate; thus, the isolates from each state were merged, for a final number of 25 fields (see Fig. 2)

Table 6 Genetic differentiation among the different *C. coccodes* VCGs of the United States using clone-corrected data

VCG[a]	1/3	2/4/5	6/7
1/3	****		
2/4/5	0.25	****	
6/7	0.51	0.65	****

[a] Each VCG was compared with the other two using GENALEX 6.2 software (Peakall and Smouse 2006)

results from a previous study on *C. coccodes* (Heilmann et al. 2006) that indicated the efficiency and reproducibility of AFLP analysis in studying the genetic makeup of this fungus and in assigning a presumptive VCG to an isolate. AFLP analysis separated the 370 isolates, including the tester strains, into five main but distinct groups including AFLP/VCGs 1, 2, 3, 4/5, and 6/7. This grouping is consistent with a previous study (Heilmann et al. 2006) that generated five clusters using AFLP analysis. Isolates of *C. coccodes* belonging to AFLP/VCG4 clustered with AFLP/VCG5, isolates of AFLP/VCG6 clustered with AFLP/VCG7, and isolates of AFLP/VCG2 and AFLP/VCG5 clustered together. Furthermore, genetic analysis using gene diversity, genetic identity, population differentiation, and a UPGMA Nei-based dendrogram, clearly demonstrated that AFLP/VCG2 and AFLP/VCG5 were more closely related to each other than to the other VCGs.

AFLP/VCG2 was the most frequently recovered AFLP/VCG from potato plant tissues in most potato-producing states. When more than one AFLP/VCG was recovered within the same field or among fields on the same farm, AFLP/VCG1 was the next most frequently recovered AFLP/VCG of *C. coccodes*. These results are not surprising. Previous studies of the United States *C. coccodes* population have found a higher frequency of AFLP/VCG2 compared with the other groups when isolates from culture collections were analyzed (Aqeel et al. 2008; Heilmann et al. 2006; Nitzan et al. 2006). This high rate has been explained by the large size of the sclerotia and the small size of the conidia produced by isolates belonging to this AFLP/VCG (Aqeel et al. 2008), which suggest greater fitness (Aqeel et al. 2008; Nitzan et al. 2006) and inoculum potential. Variation in fitness among individuals promotes natural selection (McDonald 1997). In the present study, AFLP/VCG2 was dominant in 17 of 31 plants with both above- and belowground tissue parts, and in 13 of 22 tubers. The remaining plants and tubers had multiple AFLP/VCGs parasitizing the same plant organ. However, AFLP/VCG2 was also found in 10 of the 14 plants and eight of the nine tubers had more than one VCG

Table 7 Genetic differentiation among states using clone-corrected data

State	CO	MN	ND	NE	WI	TX	MO	MI	NV
CO	****								
MN	0.011	****							
ND	0.293	0.221	****						
NE	0.026	0.065	0.288	****					
WI	0.286	0.225	0.065	0.252	****				
TX	0.424	0.384	0.569	0.380	0.559	****			
MO	0.327	0.373	0.481	0.329	0.468	0.448	****		
MI	0.314	0.266	0.310	0.292	0.311	0.569	0.383	****	
NV	0.224	0.171	0.312	0.219	0.293	0.404	0.297	0.280	****

[a] The value for each state was compared with the others using GENALEX 6.2 software (Peakall and Smouse 2006)

Table 8 North American vegetative compatibility groups (AFLP/VCGs) of *Colletotrichum coccodes* isolated from above- and below-ground stem tissues from the same potato plant[a] and from lesions on same tuber[b]

No. of stems	AFLP/VCG isolated aboveground/belowground					
31	1/1	1/2	1/5	1/6.7	2/2	2/5
	1	4	2	1	17	6
No. of tubers	AFLP/VCG isolated from lesions on the same tuber[c]					
22	1	2	2/5	2/5/6.7[d]		
	1	13	7	1		

[a] Stems were from five fields in five states: 19 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

[b] Twenty of the 22 tubers were from one field in Wisconsin; two tubers were from one field in Minnesota

[c] At least two isolates were isolated for each tuber tested

[d] VCGs from three groups were isolated from the same tuber

parasitizing them. Most of our isolates were recovered from stems; only 28 tubers yielded any isolates. The data reported here demonstrates there is considerable variability of *C. coccodes* in potato plants and plant organs, as well as in its distribution within a field and within and among states. Although the sample number per field was low, these results do support the ubiquity of AFLP/VCG2 in the United States and that more than one ALFP/VCG group can colonize the same plant tissues simultaneously.

In this study, AFLP/VCG3 was not recovered from potato plants. This group is also absent from any recent collections made globally and exists primarily in VCG tester isolates used to determine VCGs within North American, Europe/Israel and Australian *C. coccodes* populations (Alananbeh et al. 2014). A close relationship between AFLP/VCG1 and AFLP/VCG3 was reported previously (Heilmann et al. 2006). Additionally, both AFLP/VCG1 and AFLP/-VCG3 were found in one cluster when a global *C. coccodes* population was studied (Alananbeh et al. 2014). This result could be due to the fitness of certain groups over the others. As discussed earlier, the short conidia and large microsclerotia of NA-VCG2 may confer greater fitness to this group, contributing to their greater frequency compared with other NA-VCGs (Aqeel et al. 2008).

Despite the climatic and geographic differences among states, there was a high degree of similarity among the AFLP/VCGs recovered from them. However, in some states such as TX, MT, ND, and WI, a geographic pattern was found. The TX and MT populations of *C. coccodes* differed from the other states when analyzing the genetic identity, genotypic diversity, and population differentiation. This finding can be partially explained according to the most frequent AFLP/VCG found within these states. In MT, AFLP/VCG6/7 accounted for half of the total isolates of this group, and this VCG was found to be very distinct from the other *C. coccodes* groups. AFLP/VCG6/7 has the highest population differentiation (G_{ST}) and the lowest genetic identity (I) compared with genotype, meaning that every individual in this group is genetically different than the other. MT is a closed seed potato state, which means seed potatoes from other states cannot go into this state. This restriction probably explains the lower genetic identity between this state and all other states. Similarly, in TX, 83 % of the *C. coccodes* isolates belonged to AFLP/VCG1. In contrast, in ND and WI, 86 % and 94 %, respectively, of the isolates belonged to AFLP/VCG2. Further genetic analysis including genetic identity (99 %) and gene diversity ($h = 0.11$) (data not shown), demonstrating that the two populations from these two states were very similar.

A geographic pattern within the United States *C. coccodes* population was further substantiated by the AMOVA, which revealed that 17 % of the estimated variation resulted from variation among states. Geographic

Table 9 Genetic variation statistics for the clone-corrected data for genetic differentiation among states

Population	Sample size	Loci[a]	% P[b]	h[c]	G	GD	LD[d]
All populations	190	185	88.10	0.19	176	0.99	0.09
Colorado	14	134	63.81	0.16	14	1.00	0.23
Minnesota	35	126	60.00	0.16	35	1.00	0.05
North Dakota	39	106	50.48	0.10	36	0.99	0.10
Nebraska	18	130	61.90	0.18	18	1.00	0.12
Wisconsin	41	100	47.62	0.09	36	0.99	0.12
Texas	12	95	45.24	0.11	6	0.68	0.33
Montana	9	118	56.19	0.24	9	1.00	0.34
Michigan	10	105	50.00	0.11	10	1.00	0.09
Nevada	12	115	54.76	0.16	12	1.00	0.17

G number of distinct genotypes, *GD* genotypic diversity

[a] Number of polymorphic loci among all isolates evaluated

[b] Percentage of polymorphic loci

[c] h = Nei's (1973) gene diversity

[d] *LD* Measure of linkage disequilibrium, all values differed significantly from zero ($P < 0.01$). LD was also calculated for members of AFLP/VCG2 (LD = 0.05) and also differed significant from zero ($P < 0.01$)

Table 10 Pairwise comparison of genetic identity (above) according to state population differentiation[a] using clone-corrected data

Pop	CO	MN	ND	NE	WI[b]	TX	MT	MI	NV
CO	****	0.99	0.94	0.99	0.94	0.86	0.86	0.92	0.93
MN		****	0.96	0.98	0.95	0.88	0.85	0.94	0.95
ND			****	0.94	0.99	0.84	0.86	0.95	0.93
NE				****	0.95	0.88	0.85	0.92	0.93
WI					****	0.85	0.86	0.95	0.94
TX						****	0.81	0.82	0.88
MT							****	0.85	0.88
MI								****	0.93
NV									****

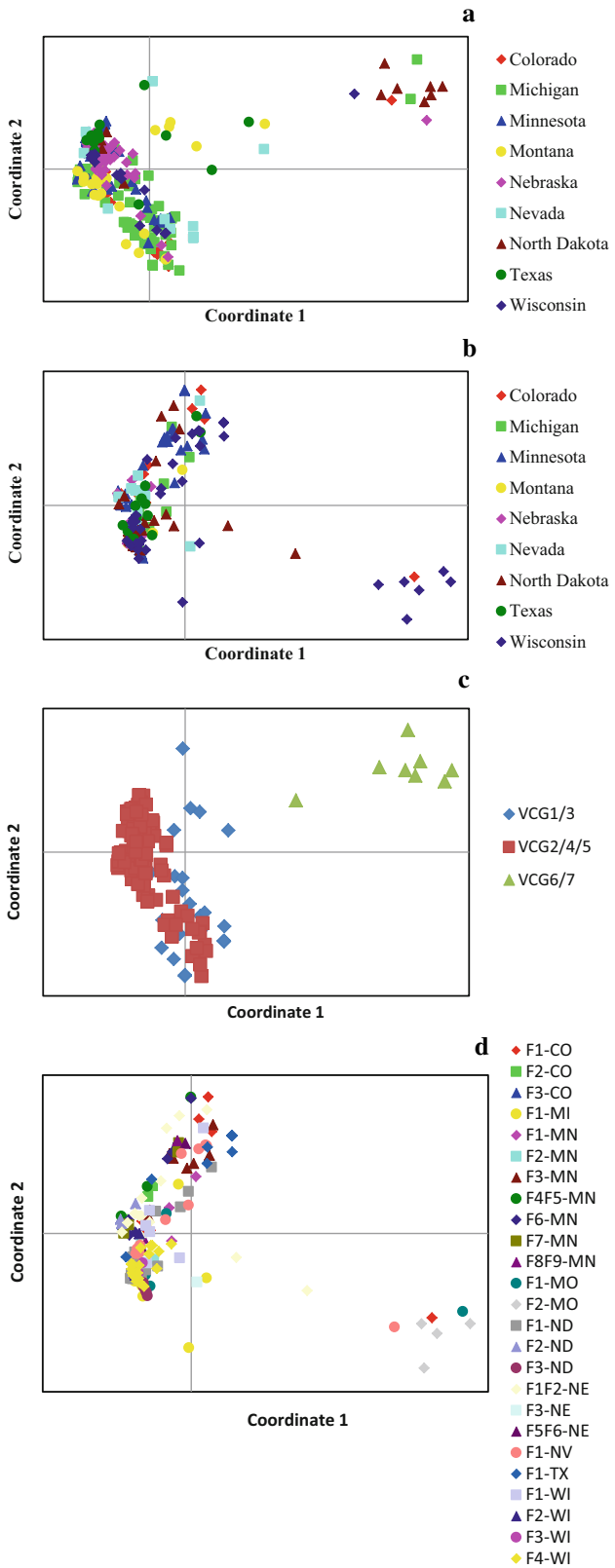
CO Colorado, *MN* Minnesota, *ND* North Dakota, *NE* Nebraska, *WI* Wisconsin, *TX* Texas, *MT* Montana, *MI* Michigan, and *NV* Nevada

[a] Each population was compared with the other eight populations (states) using POPGENE version 1.32 (Yeh et al. 1997) and GENALEX 6.2 software (Peakall and Smouse 2006)

[b] Gene flow is expected to be high between WI and ND, MN and CO, NE, and CO and MN because of their high genetic identity, the lowest between TX and the other states, MT and the other states, and MI with TX and MT because of their lower genetic identity

and climatic differences among the states could explain this variation. The remaining variation (83 %) resulted from within states. This high value of variation is probably due to the relatively high genetic diversity in the total population ($H_T = 0.22$), the high number of the distinct genotypes of *C. coccodes* ($G = 176$ of 190), and the existence of more than one AFLP/VCG within the same state, field, and plant. The variation among AFLP/VCGs was very high ($G_{ST} = 0.44$), indicating a relatively high differentiation among the *C. coccodes* AFLP/VCGs found in the United States.

We expected gene flow to be high among states that have high genetic similarities. 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 (0.80–0.99), confirming a close relationship among individuals in the entire population. If the gene flow value exceeds a value of four, then the *C. coccodes* population would have been considered as part of one population (Wright 1951), as expected among many state pairs. High gene flow suggests that high genetic exchange has been frequent among populations, and this should not be surprising. Potato is a vegetatively grown crop, and potato



◀**Fig. 2** Principal coordinate analysis for the *Colletotrichum coccodes* population in nine states. **a** Non-corrected clone data, **b**: clone-corrected data, and **c**: clone-corrected data grouped as VCGs regardless of state origin. Axes 1–3 explained most of the variation in the *C. coccodes* population either in clone-corrected or non-corrected data or based on VC grouping data. In the non-corrected data, the percentage of variation explained was 50.72, 18.02, and 11.94 % for axis 1, axis 2, and axis 3, respectively. In the clone-corrected data (**b**) and PCoA for VCG distribution (**c**), the percentage of variation explained was 51.72, 18.13, and 11.55 % for axis 1, axis 2, and axis 3, respectively. For among-fields distribution, the percentage of variation explained was 39.47, 13.84, and 8.81 % for axis 1, axis 2, and axis 3, respectively

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. For example, seed potatoes are moved frequently between ND and WI and, as discussed, the *C. coccodes* populations of these two states were very similar. The exceptions to this case, of course, are MT and TX with little opportunity for the *C. coccodes* population within the state to become intermixed, so there is very low probability for genetic exchange.

Linkage disequilibrium values were low in most of the states. The high values found in CO, TX, and MT may be due to the low number of samples from these states. However, the LD values were all significantly different from zero. The observed disequilibrium could be an artifact of calculating LD among highly subdivided populations. For that reason, AFLP/VCG2, the most frequent recovered AFLP/VCG, was selected, clone-corrected, and LD among the isolates in this group was calculated. Similarly, the LD values were low, but still significantly different from zero. The low, significant LD values in AFLP/VCG2 suggest that the members of this group may freely recombine, and there could be evidence for sexual recombination in the field (Souza et al. 2010).

Colletotrichum coccodes VCGs within different regions has variation in aggressiveness. In North America, NA-VCG2, NA-VCG5, and NA-VCG6 were the most aggressive (Aqeel et al. 2008; Nitzan et al. 2006), in Europe/Israel Eu/I-VCG5 (Shcolnick et al. 2007), and in Australia was AUS-VCG4 for (Ben-Daniel et al. 2010). Durable resistance selected for AFLP/VCG2, which is the most widely distributed VCG in North America, could provide disease control for *C. coccodes* in North America and worldwide since this VCG is genetically similar to AFLP/VCG5, that is also one of the most widely distributed groups worldwide (Alananbeh et al. 2014).

Gaining information on the genetic structure of a pathogen, its distribution, and the amount of genetic variation within and among pathogen populations are important steps for effective management (McDonald 1997). Once the genetic structure of the pathogen is known, the effect of factors such as mutation, gene flow, selection, and mating system are required to determine which factors will have the most impact on the population genetic structure (McDonald 1997). Results reported here reveal considerable genetic variation within and among *C. coccodes* populations and that this variation is structured into four main subgroups. However, this finding represents populations of *C. coccodes* from only nine states of the United States, and in some cases from a few states, very few isolates were recovered. Regardless, the data indicate that some *C. coccodes* AFLP/VCGs are widely distributed within the United States and that some states have a distinct localized population. More isolates from other potato-growing regions of 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* NA-VCG variability, biology, and epidemiology.

The AFLP analysis was efficient in evaluating large populations in a relatively short time, increasing our ability to measure the genetic relationship for global population of *C. coccodes*. The development of co-dominant markers, such as simple sequence repeats, could also be used to study genetic differences among *C. coccodes* individuals and may enhance our knowledge of diversity of this pathogen. SSR markers enabled the study of genetic diversity in *Sclerotinia sclerotiorum* (Atallah et al. 2004) and *Verticillium dahliae* (Atallah et al. 2010) where only 11 and seven microsatellite markers explained 92 and 99 % of the genetic variability in *S. sclerotiorum* and *V. dahliae*, respectively.

Acknowledgments The authors thank Curt Doetkott for his assistance in the statistical analysis. The authors also express their appreciation to the Minnesota Area II Potato Growers Association and to the North Dakota State University for funding this research. The authors also thank Jeff Miller and Dennis Johnson for providing NA-VCG testers, Barry Jacobsen for providing *C. coccodes* cultures, and J. Pasche and I. Mallik for technical support.

References

Agapow PM, Burt A (2001) Indices of multilocus linkage disequilibrium. Mol Ecol Notes 1:101–102

Alananbeh KM, Tsror (Lahkim) L, Gudmestad NC (2014) Genetic diversity of a global population of *Colletotrichum coccodes* using amplified fragment length polymorphism markers. Am J Potato Res 91:75–87

Andrivon D, Ramage K, Gurein C, Lucas JM, Jouan B (1997) Distribution and fungicide sensitivity of *Colletotrichum coccodes* in French potato-producing areas. Plant Pathol 46:722–728

Andrivon D, Lucas JM, Guerin C, Jouan B (1998) Colonization of roots, stolons, tubers and stems of various potato (*Solanum tuberosum*) cultivars by the black dot fungus *Colletotrichum coccodes*. Plant Pathol 47:440–445

Aqeel AM, Pasche JS, Gudmestad NC (2008) Variability in morphology and aggressiveness among vegetative compatibility groups of *Colletotrichum coccodes*. Phytopathol 98:901–909

Atallah ZK, Laget B, Chen X, Johnson DA (2004) High genetic diversity, phenotypic uniformity and evidence of outcrossing in *Sclerotinia sclerotiorum* in the Columbia Basin of Washington State. Phytopathol 94:737–742

Atallah ZK, Maruthachalam K, du Toit L, Koike ST, Davis RM, Klosterman SJ, Hayes RJ, Subbarao KV (2010) Population analyses of the vascular plant pathogen *Verticillium dahliae* detect recombination and transcontinental gene flow. Fungal Genet Biol 47:416–422

Atlas RM (1996) Handbook of microbiological media, 2nd edn. CRC Press, Boca Raton

Ben-Daniel B, Bar-Zvi D, Johnson D, Harding R, Hazanovsky M, Tsror (Lahkim) L (2010) Vegetative compatibility groups in *Colletotrichum coccodes* subpopulations from Australia and genetic links with subpopulations from Europe/Israel and North America. Phytopathol 100:271–278

Burdon JJ, Silk J (1997) Sources and patterns of diversity in plant pathogenic fungi. Phytopathol 87:664–669

Burlakoti RR, Ali S, Secor GA, Neate SM, McMullen MP, Adhikari TB (2008) Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato, and sugar beet in the upper Midwest of the United States. Phytopathol 98:969–976

Cano J, Guarro J, Gene J (2004) Molecular and morphological identification of *Colletotrichum* species of clinical interest. J Clin Microbiol 42:2450–2454

Collado-Romero M, Mercado-Blanco J, Olivares-García C, Valverde-Corredor C, Jiménez-Díaz RM (2006) Molecular variability within and among *Verticillium dahliae* vegetative compatibility groups determined by fluorescent amplified fragment length polymorphism and polymerase chain reaction markers. Phytopathol 96:485–495

Cullen DW, Lees AK, Toth IK, Duncan JM (2002) Detection of *Colletotrichum coccodes* from soil and potato tubers by conventional and quantitative real time PCR. Plant Pathol 51:281–292

Culley TM, Wallace LE, Gengler-Nowak KM, Crawford DJ (2002) A comparison of two methods of calculating G_{ST}, a genetic measure of population differentiation. Am J Bot 89:460–465

Dillard HR (1992) *Colletotrichum coccodes*: the pathogen and its hosts. In: Bailey JA, Jeger MJ (eds) *Colletotrichum*: Biology, pathology and control. CABI, Wallingford, pp 225–236

Dung JKS, Ingram JT, Cummings TF, Johnson DA (2012) Impact of seed lot infection on the development of black dot and Verticillium wilt of potato in Washington. Plant Dis 96:1179–1184

Edgerton CW (1908) The physiology and development of some anthracnoses. Bot Gaz 45:367–408

Evans CK, Hunger RM, Siegerist WC (1993) Enhanced production of *Pyrenophora tritici-repentis* conidial suspensions. Plant Dis 77:981–984

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Heilmann L, Nitzan N, Johnson DA, Pasche JS, Doetkott C, Gudmestad NC (2006) Genetic variability in the potato pathogen *Colletotrichum coccodes* as determined by amplified fragment length polymorphism and vegetative compatibility group analyses. Phytopathol 96:1097–1107

Ingram J, Johnson DA (2010) Colonization of potato roots and stolons by *Colletotrichum coccodes* from tuber borne inoculum. Am J Pot Res 87:382–389

Johnson DA (1994) Effect of foliar infection caused by *Colletotrichum coccodes* on yield of Russet Burbank potato. Plant Dis 78:1075–1078

Johnson DA, Rowe RC, Cummings TF (1997) Incidence of *Colletotrichum coccodes* in certified potato seed tubers planted in Washington State. Plant Dis 81:1199–1202

Lee SH, Neate SM (2007) Population genetic structure of *Septoria passerinii* in Northern Great Plains barley. Phytopathol 97:938–944

Lees AK, Hilton AJ (2003) Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. Plant Pathol 52:3–12

Lees AK, Brierley JL, Stewart JA, Hilton AJ, Wale SJ, Gladders P, Bradshaw NJ, Peters JC (2010) Relative importance of seed-tuber and soil-borne inoculum in causing black dot disease of potato. Plant Pathol 59:693–702

Leslie JF (1993) Fungal vegetative compatibility. Annu Rev Phytopathol 31:127–150

Martin FN, English JT (1997) Population genetics of soil-borne fungal plant pathogens. Phytopathol 87:446–447

McDonald BA (1997) The population genetics of fungi: tools and techniques. Phytopathol 87:448–453

McDonald BA (2004) Population genetics of plant pathogens. Plant Health Instr. doi:10.1094/PHI-A-2004-0524-01

Nadakavukaren MJ, Horner CHE (1959) An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. Phytopathol 49:527–528

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Nat Acad Sci USA 70:3321–3323

Nitzan N, Tsror (Lahkim) L (2003) Effect of temperature and pH on in vitro growth rate and sclerotial density of *Colletotrichum coccodes* isolates from different VCGs. Am J Pot Res 80:335–339

Nitzan N, Hazanovsky M, Tal M, Tsror (Lahkim) L (2002) Vegetative compatibility groups in *Colletotrichum coccodes*, the causal agent of black dot on potato. Phytopathol 92:827–832

Nitzan N, Tsror (Lahkim) L, Johnson DA (2006) Vegetative compatibility groups and aggressiveness of North American isolates of *Colletotrichum coccodes*, the causal agent of potato black dot. Plant Dis 90:1287–1292

Peakall R, Smouse PE (2006) GenAlex 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Shcolnick S, Dinoor A, Tsror (Lahkim) L (2007) Additional vegetative compatibility groups in *Colletotrichum coccodes* subpopulations from Europe and Israel. Plant Dis 91:805–808

Souza EA, Camargo OA, Pinto JM (2010) Sexual recombination in *Colletotrichum lindemuthianum* occurs on a fine scale. Genet Mol Res 9:1759–1769

Tsror L, Erlich O, Hazanovsky M (1999) Effect of *Colletotrichum coccodes* on potato yield, tuber quality, and stem colonization during spring and autumn. Plant Dis 83:561–565

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wright S (1951) The genetical structure of populations. Ann Eugen 15:323–354

Yeh FC, Yang RC, Boyle TB, Ye ZH, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada