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Genetic variation between *Phytophthora cactorum* isolates differing in their ability to cause crown rot in strawberry

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Analysis of 44 isolates of *Phytophthora cactorum*, isolated from strawberry and other hosts, by AFLP showed that the crown rot pathotype is different from leather rot isolates and from *P. cactorum* isolated from other hosts. 16 of 23 crown rot isolates, including isolates from Europe, Japan, Australia, and New Zealand, were identical in an analysis based on 96 polymorphic bands from seven primer combinations. Leather rot isolates of strawberry could not be distinguished from isolates from other hosts. The pathogenicity test of all 44 isolates on strawberry plants mostly gave unambiguous results, except for three American isolates, which seemed to have reduced aggressiveness compared to the crown rot isolates. These isolates also differed in the AFLP analysis. Comparing information on the origin of the isolates with results from the pathogenicity test, showed that isolates from strawberry fruits or petioles could be either leather rot or crown rot pathotypes. None of the isolates from hosts other than strawberry caused crown rot symptoms in strawberry.

INTRODUCTION

Phytophthora cactorum was first described on a rotting cactus in Germany in 1875. Since then, *P. cactorum* has been reported from most parts of the world as the cause of diseases in more than 200 plant species from over 60 different families (Erwin & Ribeiro 1996). In strawberry, *P. cactorum* has for a long time been known as the cause of leather rot (Rose 1924), and in 1952 was first reported as the cause of a crown rot (Deutschmann 1954). Since then, crown rot of strawberry has been detected in most of Europe, the USA, and parts of Asia and Africa. *P. cactorum* can be differentiated from other *Phytophthora* species based on morphological characteristics, and molecular markers can distinguish between *P. cactorum* isolates from different host species (Cooke *et al*. 1996, Hantula, Lilja & Parikka 1997, Lilja *et al*. 1998). Isolates of *P. cactorum* from different hosts vary slightly in the morphology of the oogonia and sporangia, but not sufficiently to be used for identification (Hantula *et al*. 2000). Several studies have examined the ability of isolates of *P. cactorum* from different hosts to cause crown rot in strawberry (van der Scheer 1971, Seemüller & Schmidle 1979, Harris & Stickels 1981, Hantula *et al*. 2000). The general conclusion of these studies was that crown rot could only be caused by strains isolated from strawberry crowns or from soil where crown rot had been present. Isolates from apple, pear, silver birch, rhododendron or strawberry fruit (leather rot), however, could all cause leather rot of strawberry fruits, but not crown rot. The causal agent of crown rot is therefore often referred to as a distinct pathotype of *P. cactorum*, but it cannot be distinguished morphologically from leather rot. Previous studies comparing isolates from strawberry crown rot from different geographic locations by random amplified microsatelite (RAMS) markers have shown little genetic variation between isolates from some European countries, while isolates from North America display a higher degree of variation (Hantula *et al*. 1997, 2000). Hantula *et al*. (2000) also concluded that isolates causing leather rot and isolates causing crown rot in strawberry could not be separated genetically by RAMS, whereas isolates from different host species were in most cases genetically separate.

In Norway, crown rot of strawberry was first detected in 1992, but has since then been found in more than 100 locations, covering most parts of the country. Leather rot is reported less frequently in Norway, probably due to less favourable climatic conditions.

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Table 1. *Phytophthora cactorum* isolates used in pathogenicity testing and AFLP analysis. Strawberry plants (cv. Inga) were inoculated with mycelium in a wound in the crown. Results from the pathogenicity test are shown as number of dead plants and plants with distinct symptoms of crown rot ($n=15$) 14 d after inoculation.

Isolate	Host (part of plant)[a]	Geographical origin, source[b]	No. of plants dead or showing crown rot symptoms[c]
1557	*Rhododendron*	Germany, *S. Werres*	0
43	Strawberry	Finland, *P. Parikka*	15
51	Apple	Brazil, *J. I. Boneti*	0
512	Apple	Brazil, *J. I. Boneti*	0
65271	Cypress	Germany, *S. Werres*	0
7178	Soil	Norway, *A. Stensvand*	0
7574	Strawberry (crown)	Norway, *J. Davik*	15
9047	Strawberry (crown)	Norway, *A. Stensvand*	15
95	Strawberry	Spain, *P. Parikka*	15
BPIC 1168	Almond	Greece, *K. Elena*	0
CAC 17	Strawberry	Scotland, *J. Duncan*	0
CAC 21	Strawberry	Scotland, *J. Duncan*	15
CH 09	Strawberry	Sweden, *C. Olsson*	15
CH 17	Strawberry	Estonia, *C. Olsson*	15
CP 669	Strawberry	South Africa, *W. A. Smit*	15
CP 722	Strawberry	South Africa, *W. A. Smit*	15
CP 786	Strawberry	South Africa, *W. A. Smit*	0
DU 3	Apple	The Netherlands, *H. Aldwinckle*	0
F-II-3	Strawberry	Japan, *S. Osada*	15
H 102	Strawberry (crown)	New Zealand, *I. Horner*	15
H 90	Strawberry (crown)	New Zealand, *I. Horner*	15
H 96	Strawberry (petiole)	New Zealand, *I. Horner*	15
H 97	Strawberry (crown)	New Zealand, *I. Horner*	15
I 1	Strawberry (crown)	Italy, *V. Faedi*	15
I 2	Strawberry (leaf/petiole)	Italy, *V. Faedi*	15
IMI 325063	Apple	New Zealand (IMI)	0
ML 95	Strawberry	Japan, *S. Osada*	15
MS 2	Apple	USA, *H. Aldwinckle*	0
NY 170	Strawberry (fruit)	USA, *W. Wilcox*	0
NY 193	Strawberry (fruit)	USA, *W. Wilcox*	4
NY 349	Cherry	USA, *H. Aldwinckle*	0
NY 411	Peach	USA, *H. Aldwinckle*	0
NY 422	Strawberry (crown)	USA, *W. Wilcox*	3
NY 568	Apple	USA, *H. Aldwinckle*	0
NY 577	Strawberry (fruit)	USA, *W. Wilcox*	4
O 29	Strawberry (fruit)	Poland, *A. Bielenin*	15
O 5	Strawberry (fruit)	Poland, *A. Bielenin*	15
P 405	Strawberry	UK, *D. Simpson*	15
P 407	Strawberry	UK, *D. Simpson*	15
PH 18	Silver Birch	Finland, *A. Lilja*	0
RP 1	Apple	USA, *H. Aldwinckle*	0
VPRI 17147	Strawberry (leaf/stem)	Australia, *W. S. Washington*	0
VPRI 22050	Strawberry (crown)	Australia, *W. S. Washington*	15
W 81	Strawberry (crown)	Poland, *A. Bielenin*	15

[a] Which part of the plant the isolates come from is only known in a few cases.
[b] Voucher isolates are preserved in the collections of the Norwegian Crop Research Institute (Aas).
[c] 15 plants were inoculated with each isolate, and number of dead plants and plants showing symptoms of crown rot were recorded 14 d after inoculation.

In Norway, *P. cactorum* is also known to cause crown and collar rot in pome fruit.

This study aims to clarify whether the genetic uniformity of crown rot isolates of *P. cactorum* seen in some European countries applies to isolates from other geographic locations as well, or if genetic variation as described in North America is more common. A further aim was to determine if AFLP could be used to distinguishing crown rot pathotypes of *P. cactorum* from those lacking the ability to cause crown rot symptoms in strawberry.

MATERIALS AND METHODS

Isolates

Isolates of *Phytophthora cactorum* were obtained from as many geographically distinct locations as possible, with emphasis on crown rot and leather rot isolates from strawberry (Table 1). To obtain a representative number of isolates without the ability to cause crown rot, isolates from other host plants were also included. The isolates were divided into three groups, based on host species or host organ: (1) isolates from strawberry

crown or stem; (2) isolates from strawberry fruit (leather rot); and (3) isolates from other hosts. The plant part where *P. cactorum* was isolated from was not known in all cases, and the isolates from strawberry could thus be either crown rot or leather rot isolates. Some of the isolates included in this test were also tested by Hantula *et al.* (2000).

Pathogenicity test

All 44 isolates (Table 1) were tested for ability to cause crown rot on strawberry plants. The test plants were of cv. 'Inga', which is known to be particularly susceptible to crown rot (Eikemo, Stensvand & Tronsmo 2000). When delivered from the producer, plants were 2 months old (after rooting), and potted into 8 cm diam disposable pots and kept in a greenhouse for 2 wk until the initiation of the experiment. Each plant was wounded in the rhizome with a scalpel, and inoculated with a V8 agar plug with mycelium from a 1 wk old culture of *P. cactorum*. For each isolate, 15 plants were inoculated, and they were kept in a greenhouse at 20 °C with a 16 h photoperiod. Artificial light was provided by high-pressure sodium lamps (SON/T, approx. 150 μE s^{-1} m^2) in periods of insufficient natural light. The plants were organised in a completely randomised design. To keep the air saturated, the plants were covered with a polyethylene sheet for 4 d after inoculation, and watered daily thereafter. After 14 d, the number of dead plants was recorded, and the crowns of the remaining plants were cut longitudinally and examined for symptoms.

DNA isolation and AFLP

Phytophthora cactorum isolates were grown in potato dextrose broth in darkness at 20 ° for 1 wk before the mycelium was harvested by vacuum filtration, washed twice with distilled water, and frozen at −20 °. Frozen mycelium was ground in a mortar with liquid nitrogen, and DNA was isolated with the Puregene® DNA Isolation Kit D-600A (Gentra Systems, MN), according to the instructions from the manufacturer, with some modifications (Bonants *et al.* 2000).

The methods used were based on Bonants *et al.* (2000) and Vos *et al.* (1995). DNA (approx. 500 ng) was digested in a 15 μl reaction volume with *Eco*RI (10 U) and the *Mse*I isoschizomer *Tru*1l (10 U) for 2 h at 37 ° followed by 2 h at 65 °. *Eco*RI (5 pmol) and *Mse*I (50 pmol) adapters (Bonants *et al.* 2000) were ligated overnight at *ca* 20 ° in a 20 μl reaction volume containing 2 μl 10 × ligase buffer (Promega, Madison, WI), 1 μl ligase (3 U μl^{-1}; Promega), 5.5 μl dH_2O and 10 μl reaction product. The cold amplification (pre-amplification) with the 0-primers (non-selective primers) was performed with 5 μl undiluted ligation product added to 5 μl 10 × PCR buffer (500 mM KCl, 100 mM Tris HCl pH 8.3, 15 mM $MgCl_2$), 1 U *Taq* DNA polymerase (Hoffmann-La Roche, Basel), 2 μl 5 mM dNTPs, 1.5 μl each of the 0-primers (50 ng μl^{-1}) in a 50 μl reaction volume (Bonants *et al.* 2000). The PCR were performed in a Gene Amp 9700 thermo cycler (Applied Biosystems, Foster City, CA) with the following programme: 30 cycles, where each cycle consisted of 30 s at 94 °, 60 s at 56 ° and 60 s at 72 °, with a final cooling to 4 °. Products of the pre-amplification were separated on a 1% agarose gel and visualised with ethidium bromide and UV illumination. The rest of the pre-amplification product was diluted 10 times and stored at −20 ° until used in the selective amplification.

Table 2. Sequences of primers used in the AFLP analysis of *Phytophthora cactorum*.

Primer	Sequence
*Eco*RI primer	
E13	5′ GACTGCGTACCAATTCAG
E14	5′ GACTGCGTACCAATTCAT
E19	5′ GACTGCGTACCAATTCGA
E20	5′ GACTGCGTACCAATTCGC
E21	5′ GACTGCGTACCAATTCGG
*Mse*I primer	
M11	5′ GATGAGTCCTGAGTAAAA
M12	5′ GATGAGTCCTGAGTAAAC
M15	5′ GATGAGTCCTGAGTAACA
M16	5′ GATGAGTCCTGAGTAACC
M17	5′ GATGAGTCCTGAGTAACG

The selective PCR was performed with 5 μl of the diluted pre-amplification product in a total reaction volume of 20 μl, containing 30 ng *Mse*I primer, 5 ng *Eco*RI primer (radioactively labelled with γ-^{33}P), 0.8 μl 5 mM dNTPs, 2 μl 10× PCR buffer and 1 U *Taq* DNA polymerase (Hoffmann-La Roche). The cycling profile for the selective PCR was as follows: 13 cycles of 30 s at 94 °, 30 s at 65 °, and 60 s at 72 °, with a lowering of the annealing temperature by 0.7 ° in the last 12 cycles; 23 cycles of 30 s at 94 °, 30 s at 56 °, and 60 s at 72 °; and finally cooling down to 4 °. Using three isolates (one crown rot isolate and two from other hosts), 40 different +2/+2 primer combinations were tested using this procedure. All 44 isolates were investigated using seven of these primer combinations (M11/E13, M12/E19, M15/E21, M16/E14, M16/E20, M16/E21, and M17/E14; Table 2). The products of the selective amplification were diluted with an equal volume (20 μl) of loading dye (98% formamide, 10 mM EDTA (pH 8), brome-phenol blue, and xylene cyanol), heated for 3 min at 90 ° and immediately put on ice. Four μl of the product was loaded on a polyacrylamide gel (5% acrylamide/bis (19:1), 7.5 M Urea, and 0.5 × TBE), and run at a constant power (70 W) until the xylene cyanol dye was 5–10 cm from the bottom of the gel. Gels were vacuum dried under a plastic cover for approx. 2 h. The dry gels were exposed to Kodak Biomax MR films for 4–7 d. The results from the different primer combinations were registered by visual evaluation and scored 1 or 0, indicating whether a band was present or not.

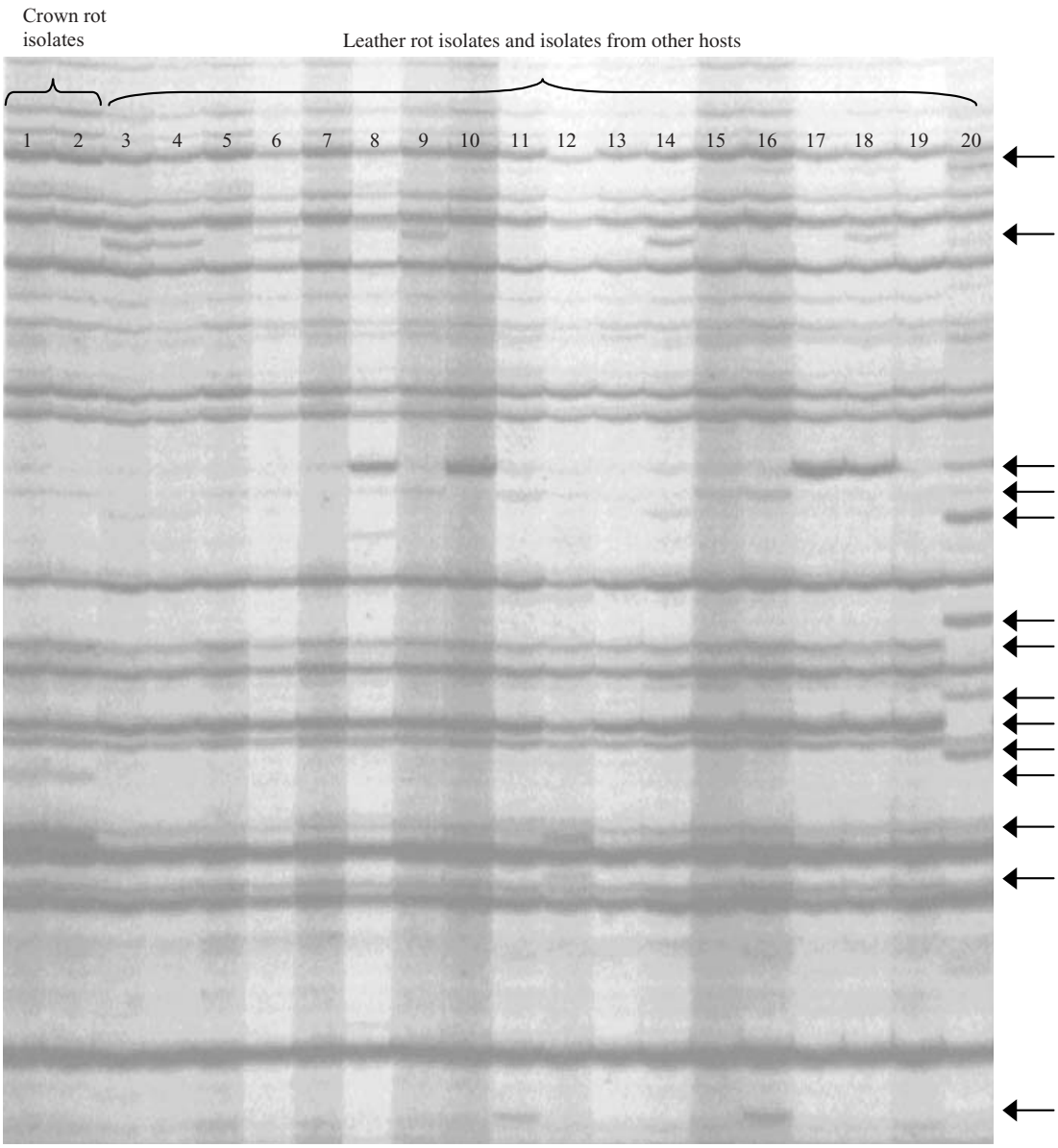

Fig. 1. Section of a gel from AFLP analysis (primer combination M16/E14) of 44 isolates of *Phytophthora cactorum* originating from different host plants, showing variation in banding pattern. Arrows indicate polymorphic bands. The following isolates are included: 1 (9047), 2 (ML 95), 3 (NY 422), 4 (NY 577), 5 (VPRI 17147), 6 (RP 1), 7 (51), 8 (BPIC 1168), 9 (MS 2), 10 (NY 411), 11 (IMI 325063), 12 (1557), 13 (512), 14 (NY 193), 15 (NY 568), 16 (CP 786), 17 (7178), 18 (CAC 17), 19 (DU 3), and 20 (PH 18). Isolates 1–5, 10, 14, 15, 17 and 18 were from strawberry.

All primer combinations were run at least twice, with new selective amplification reactions. From 20 isolates of which 14 were crown rot isolates, independent samples of DNA was extracted. Parallel AFLP amplifications on these DNA extracts were performed for some of the primer combinations.

The binary data was used to construct a similarity matrix, using the method of Nei & Li (1979). Unweighted pair group method cluster analysis of the binary data was performed with Treecon software (van de Peer & DeWachter 1997), and a similarity dendrogram was constructed with a distance scale. Bootstrap analysis was performed with 1000 replicates.

RESULTS

Pathogenicity test

All isolates that proved to be crown rot pathotypes gave unambiguous results, with at least 13 out of 15 plants dead 14 d after inoculation (Table 1). None of the plants inoculated with these isolates were free of symptoms 14 d after inoculation. Four of the isolates from strawberry proved to be leather rot pathotypes (no plants dead or showing symptoms). Three of the North American isolates (NY 193, NY 577 and NY 422 from strawberry) gave ambiguous results, with 3–4 plants dead or showing symptoms of crown rot. None

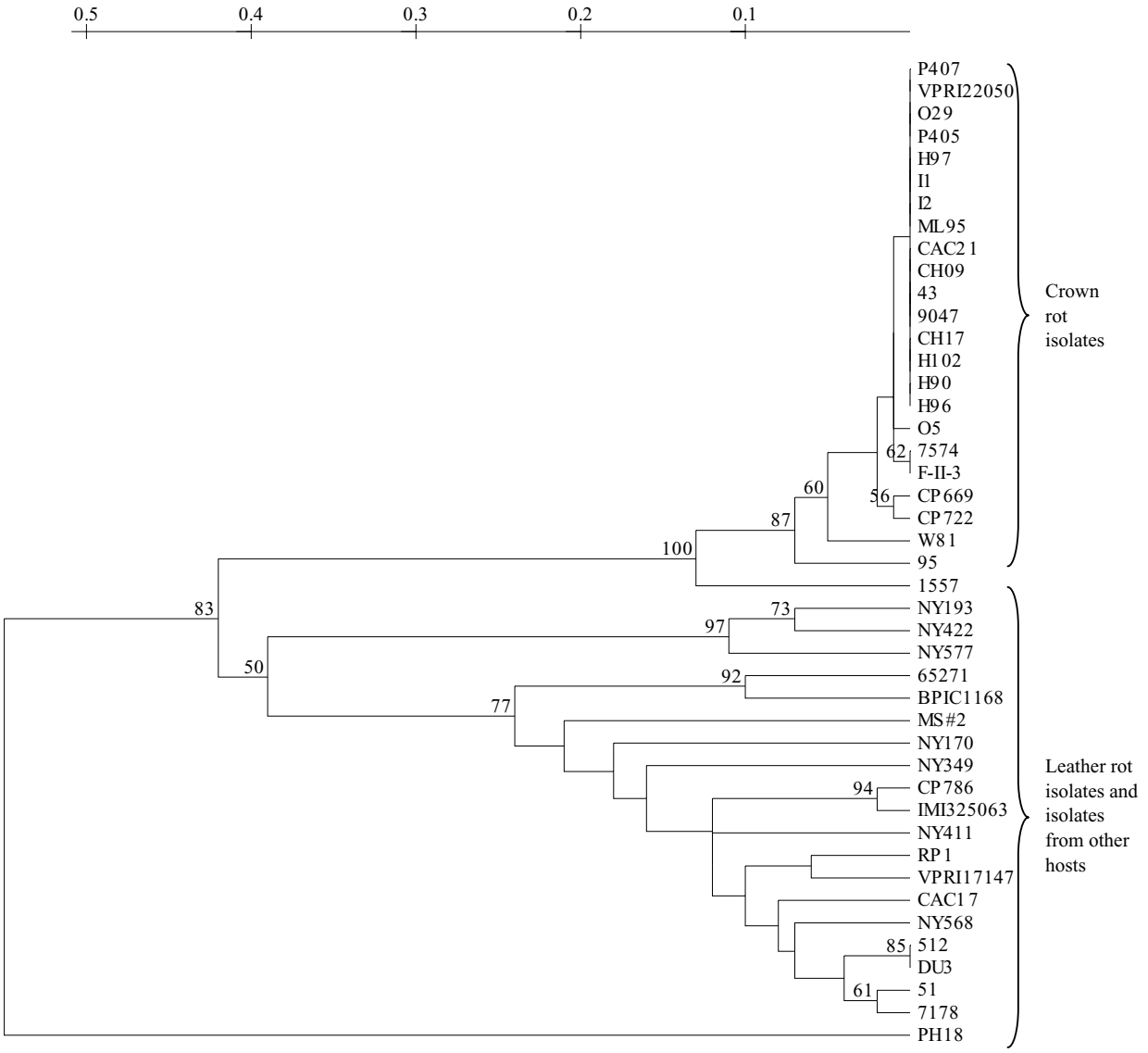

Fig. 2. AFLP similarity dendrogram of 44 isolates of *Phytophthora cactorum* originating from different hosts. UPGMA cluster analysis was performed with Treecon software. Bootstrap analysis was performed with 1000 replicates; values above 50% are indicated above branches.

of the isolates from other host plants caused crown rot symptoms in strawberry plants.

AFLP

When testing 40 different primer combinations (+2/+2) on three isolates (one crown rot isolate and two non-crown rot isolates) it was found that seven primer pairs gave multiple polymorphic bands (M11/E13, M12/E19, M15/E21, M16/E14, M16/E20, M16/E21, and M17/E14; Table 2). These combinations of primers were selected to be suitable for further studies to detect variation between isolates connected to the ability to cause crown rot.

When tested on all 44 isolates of *Phytophthora cactorum* (Table 1), the seven primer combinations all revealed variation in banding patterns following the AFLP amplification. Some primer combinations were analysed on independently extracted DNA from 20 of the isolates. On these isolates and for these primer combinations the AFLP results were found to be reproducible. A section of the AFLP gel from the reaction with primer pair M16/E14 is shown in Fig. 1. 96 polymorphic bands were obtained, and the average percentage of polymorphic bands from all primer combinations was 27%. The dendrogram (Fig. 2) showed very limited variation between crown rot isolates, but some isolates differed slightly from the rest. The isolate from Spain (95) and one from Poland (W81) deviated from the other crown rot isolates with bootstrap values of 87 and 60, respectively. The isolates from group 2 and 3, including leather rot isolates and isolates from other hosts, showed a greater degree of variation. All North American isolates were in this group, and three (NY 422, NY 577 and NY 193) grouped together, and differed from the rest with a

Table 3. Binary data from seven different primer combinations for 29 out of 44 isolates.

	Isolates																												
Marker	43	1557	7178	7574	9047	95	CAC17	CAC21	CH09	CH17	CP 669	CP 722	F-II-3	H102	I1	ML95	NY170	NY193	NY349	NY411	NY422	NY568	NY577	O29	O5	P405	VPRI17147	VPRI22050	W81
1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
9	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0
12	1	1	0	0	1	1	0	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
13	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	1	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
37	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
42	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	1	0	1	1
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	0	0	0
49	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
50	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	1	1	1	1	1	1
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1	1	0	1	1
57	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	0	0	0	1	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
64	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 3. (*Cont.*)

	Isolates																											
Marker	43	1557	7178	7574	9047	95	CAC17	CAC21	CH09	CH17	CP 669	CP 722	F-II-3	H102	I1	ML95	NY170	NY193	NY349	NY411	NY422	NY568	NY577	O29	O5	P405	VPRI17147	VPRI22050	W81
65	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1
66	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0
73	1	1	0	1	1	1	0	1	1	1	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
74	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1
75	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1
76	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0
78	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	0	1	1
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
82	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1
83	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1
84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0
87	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1
88	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0
89	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
90	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0
91	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1
94	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	1	1	1	0	1	1
95	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
96	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	1	1

bootstrap value of 50. The isolate from silver birch (PH 18) differed from all other isolates. The strict consensus tree (not shown) divided the isolates into two groups, which correlated with the ability to cause crown rot in the pathogenicity test. Leather rot isolates and all isolates from other hosts except 1557 were also clearly separated from the crown rot isolates on the distance scale. Binary data for the most variable isolates originating from strawberry (28 isolates selected) and isolate 1557 from *Rhododendron* is shown in Table 3.

DISCUSSION

The pathogenicity test showed that isolates of *Phytophthora cactorum* originating from strawberry might belong to different pathotypes. Isolates from leaf/stem (e.g. VPRI 17147 and I1) or fruit (e.g. O 5, O 29, and NY 170) can be either a crown rot or leather rot pathotype. Previous reports have shown that only *P. cactorum* originating from strawberry rhizomes can cause crown rot symptoms in strawberry, but the crown rot pathotype can also be found on berries as the cause of leather rot (van der Scheer 1971, Seemüller & Schmidle 1979, Harris & Stickels 1981). A very susceptible strawberry cultivar like 'Inga' was particularly suitable in this kind of experiments, where the purpose was to differentiate between two pathotypes of *P. cactorum* (with and without the ability to cause crown rot), and not to evaluate the aggressiveness of the isolates. There is a high degree of variation within treatment when inoculating with *P. cactorum* (Simpson *et al.* 1994, Bell *et al.* 1997, Eikemo *et al.* 2000), and distinguishing between crown rot isolates with minor differences in aggressiveness is probably impossible. The three North American isolates (NY 577, NY 422 and NY 193) that resulted in 3–4 plants dying or showing symptoms, appeared either to be intermediate between the two pathotypes, or weak variants of a crown rot isolate. Reduced aggressiveness due to a long time in storage without contact with plant material could explain this phenomenon, but the results are confirmed by Hantula *et al.* (2000) who found that two of the isolates (NY 577 and NY 193) were in a weak condition, and the third (NY 422) caused no symptoms at all.

When performing AFLP, complete digestion of DNA is critical in order to obtain reproducible results.

Incomplete DNA digestion might lead to an increased number of polymorphic AFLP bands, and hence an overestimation of the genetic variation among the isolates studied. In the present study the results were found to be reproducible when AFLP was performed on independently isolated DNA, and thus this possible artefact cannot alone explain the observed genetic variation.

The crown rot pathotype of *P. cactorum* is regarded as the specialised form in contrast to leather rot of strawberry, which also can be caused by isolates from various hosts (van der Scheer 1971, Seemüller & Schmidle 1979, Hantula *et al.* 2000). The dendrogram constructed from the AFLP results confirmed that crown rot isolates display a very low degree of variation. This has previously been shown for isolates from some European countries (Hantula *et al.* 1997). Our study shows that it also applies for isolates from Oceania, Asia, and Africa. The strict consensus tree divided the isolates according to the ability to cause crown rot, and separated them from leather rot isolates and isolates from other hosts. The crown rot isolates were also clearly separated from the other isolates (except 1557 from *Rhododendron*) on the distance scale. Hantula *et al.* (2000) concluded that leather rot of strawberry fruits and crown rot were not caused by genetically different strains of *P. cactorum*. However, our results show that crown rot is caused by a genetically distinct pathotype of *P. cactorum*, and that the two pathotypes can be separated by AFLP. The genetic uniformity of crown rot isolates over a large geographic area may indicate that it was introduced as a single clone, and the genetic variation seen in North American isolates makes North America a probable source of origin. Another possible explanation is that that the pathotypes arose independently in Europe and North America. The only North American crown rot isolate in this study (NY 422; according to the part of the plant it was isolated from) did not behave like the other crown rot isolates in the pathogenicity test. No conclusions can therefore be drawn as to the variability in North American crown rot isolates, but previous studies have shown that these isolates display a higher degree of variability than European isolates (Hantula *et al.* 2000). Among the isolates unable to cause crown rot, leather rot isolates from strawberry and isolates from apple and cherry mostly grouped together. However, three of the North American isolates (NY 577, NY 193 and NY 422) were clearly separate from the other isolates, and differed with a bootstrap value of 50. This might be indicative of a higher degree of variation in North American isolates. This, together with the reduced pathogenicity on strawberry plants, implies that these isolates are different from both crown rot and leather rot isolates.

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