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Pathogenicity of *Aphanomyces* spp. from different leguminous crops in Sweden

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Abstract

Host range and pathogenicity of a range of *Aphanomyces* spp. isolates obtained from pea roots but also from a range of other field-grown leguminous crops in southern Sweden was investigated. The *Aphanomyces euteiches* isolates originating from pea and the few obtained isolates originating from alfalfa, green bean and yellow sweet-clover were highly pathogenic only to pea. The *A. euteiches* isolated from common vetch differed from these isolates by being weakly pathogenic to pea and other legumes, but highly pathogenic to common vetch. Vetch isolates also formed a well-defined separate cluster based on principal component analysis of pathogenicity pattern on tested crops. Oospores of *A. euteiches* were observed in root tissue of pea as well as common vetch, alfalfa, green bean, broad bean, red clover and yellow sweet-clover in the greenhouse pathogenicity tests. An *Aphanomyces* sp. that morphologically differed from *A. euteiches*, was frequently isolated from several leguminous plants, but was non-pathogenic to all tested crops in the pathogenicity tests. In isozyme analysis the banding pattern of these isolates resembled the pattern of *A. cladogamus.* Another, different and so far unidentified *Aphanomyces* sp. from roots of green bean and broad bean, was also non-pathogenic to the tested legume species. Based on the isolates tested, the results obtained suggest that the population of *Aphanomyces* spp. infecting legume roots in Sweden consists of a pea-specific and a vetch-specific group of *A. euteiches*. Two other groups comprised (i) *Aphanomyces* sp. isolates that resembled *A. cladogamus*, and (ii) isolates, which resembled neither *A. euteiches* nor *A. cladogamus*. In addition, the host range of Swedish *A. euteiches* isolates was not as broad as reported for *A. euteiches* isolates from other countries.

Introduction

Pea root rot caused by *Aphanomyces euteiches*, is often regarded as the most destructive pathogen of pea (*Pisum sativum*) in areas with humid climates (Kraft and Pfleger, 2001). In Sweden, *Aphanomyces* root rot is considered a major destructive disease in peas and it causes severe losses in all Swedish pea-growing areas (Olofsson, 1967; Persson et al., 1997). Pea is also the only crop known to be affected by *A. euteiches* in Sweden, and it is therefore usually not grown in crop rotations more frequently than one in every six years (Persson et al., 1999). Other leguminous crops have also been grown in the pea-producing area, but not very frequently. However, a growing interest in organic farming has recently increased the acreage of leguminous crops as a whole and also increased the presence of legumes other than peas in crop rotations. The only practically feasible method to avoid forthcoming yield losses is presently to use greenhouse bioassay methods to assess the inoculum potential in the soil and then avoid sowing of pea in fields with a high inoculum potential (Olofsson, 1967; Sherwood and Hagedorn, 1958). Traditionally, a diversified crop rotation has been used for avoiding a build up of inocula of pea root rot and other root diseases. However, short crop rotations with other leguminous crops than pea might also propagate the pea root rot pathogen and thus increase the damage to a subsequent pea crop.

In host range studies of *A. euteiches* among leguminous crops grown in soil with known infestation in different areas around the world, alfalfa (*Medicago sativa*) (Delwiche et al., 1987), green bean (*Phaseolus vulgaris*) (Pfender and Hagedorn, 1982), broad bean (*Vicia faba*) (Lamari and Bernier, 1985), common vetch (*Vicia sativa*) (Tsvetkova and Kotova, 1985) and red clover (*Trifolium pratense*) (Holub et al., 1991; Tofte et al., 1991) were all reported as hosts for this pathogen. Furthermore, oospores of *A. euteiches* were found both in root tissue with clear symptoms as well as in symptom-free tissues of white clover (*Trifolium repens* L.) and in certain weeds such as shepherd's-purse (*Capsella bursa-pastoris*) (Chan and Close, 1987). However, some of these hosts seem to be only marginally affected by the infection.

Variations in pathogenicity among *A. euteiches* isolates has been reported. Pfender and Hagedorn (1982) found that some isolates differed in morphological characters from the typical ones as well as being pathogenic to green bean but not to pea. Therefore, these isolates were denoted *A. euteiches* f. sp. *phaseoli*. Subpopulations of *A. euteiches* have also been found (Grau et al., 1991; Holub et al., 1991; Malvick et al., 1998). Four genotypically and phenotypically different subpopulations with host preferences for bean, alfalfa and red clover/alfalfa and one non-pathogenic pathotype were proposed (Malvick et al., 1998). Moreover, different pathotypes were recently described for a French *A. euteiches* population and four pathotypes were distinguished: one with a broad host range, which was aggressive on almost all hosts; one aggressive on pea/alfalfa/vetch and weakly aggressive on broad bean; another with preferences for pea/vetch and finally one for pea/vetch/alfalfa (Wicker et al., 2001).

Both traditional and molecular methods have been used to characterize isolates of *Aphanomyces* spp. Variation in pathogenicity in combination with morphological studies is commonly used for population studies. Larsson (1994) concluded that isozyme analysis allows delineation of *A. euteiches* from *A. cochlioides* and *A. cladogamus*. PCR-based methods such as RAPD (Malvick and Grau, 2001) and sequencing parts of ribosomal DNA (Riethmueller et al., 1999) were employed to describe *Aphanomyces* spp. on the molecular level. Furthermore, Vandemark et al. (2000) identified specific sequence-characterized DNA markers, which selectively distinguish between *A. euteiches* and *A. cochlioides* even in plants and in soil samples.

The objectives of this study were (i) to compare the host range of 46 *A. euteiches* isolates obtained from pea to 10 isolates from common vetch, alfalfa, yellow sweet-clover (*Melilotus officinalis*) and green bean, and (ii) to characterize the isolates morphologically and by isozyme analysis. In addition, a group of other *Aphanomyces* spp. isolates recovered from various field-grown leguminous crops was included in the pathogenicity tests and characterized.

Materials and methods

Isolation of Aphanomyces *spp. and cultures used*

Isolates of *Aphanomyces* spp. were recovered from leguminous plants grown in fields with a history of pea root rot in southern Sweden. Pea, red clover, white clover, alfalfa, yellow sweet-clover, broad bean, green bean and common vetch were collected in early summer, from May to June. The plants were dug up with their roots, placed in plastic bags and taken to the laboratory where the roots were washed under running tap water for 1–2 h. Roots with rot symptoms were then cut into small pieces and then placed on a selective medium for *Aphanomyces* (SMA) described by Larsson and Olofsson (1994) and were incubated at room temperature overnight. Outgrown *Aphanomyces*-like colonies were then isolated and maintained on cornmeal agar plates (CMA, Difco Ltd.) at 4 °C. Their morphological features were studied by measuring the diameters of oospores and oogonia, respectively. The taxonomic classification was based on species description by Scott (1961).

For pathogenicity tests and further morphological studies, 56 Swedish isolates of *A. euteiches* isolated from pea (46 isolates), alfalfa (2 isolates), green bean (2 isolates), yellow sweet-clover (1 isolate) and common vetch (5 isolates) were selected. Eleven Swedish *Aphanomyces* spp. isolates: from alfalfa (1), green bean (2), broad bean (2), red clover (2), yellow sweet-clover (1) and from white clover (2) were, in addition, selected and tested. One Swedish reference isolate of *A. euteiches* denoted R (Larsson, 1994) was used in all tests. Reference isolates of *A. euteiches* from other countries comprised 2 pea isolates from the USA (5 and 6), 2 pea isolates from France (F5 and F46, Wicker et al., 2001), 1 pea isolate from Spain, 2 alfalfa isolates from the USA, referred in Malvick and Grau (2001), (MF 1 'race 1' and MHA 41 'race 2', here denoted 116 and 117, respectively), 2 green bean isolates from the USA (GB 210 and GB 218, here referred as 110 and 111, respectively), and 1 isolate of *A. euteiches*

f. sp. *phaseoli* from the USA (ATCC 46688, (Pfender and Hagedorn, 1982), here denoted 113).

Isozyme analysis

Seventy-seven isolates were selected for isozyme analysis. Representatives of *A. euteiches* and *Aphanomyces* sp. isolates from different host of origin were tested: 27 pea, 3 alfalfa, 4 green bean, 2 red clover, 2 broad bean, 2 sweet clover, 2 white clover, and 4 vetch isolates. In addition, 10 isolates originating from other countries were tested. Swedish isolates of *A. cladogamus* isolated from spinach and denoted K (Larsson, 1994), and *A. cochlioides* isolated from sugarbeet, were used for comparison. The tested enzyme systems were glucose-6-phosphate dehydrogenase (G6PDH) and malate dehydrogenase (MDH). Fungi were grown and isozyme analysis was performed as described by Larsson (1994), with exception of the pH of the sample buffers which was 7.4 for MDH and 7.8 for G6PDH.

Zoospore inoculum production

In order to produce a zoospore inoculum for the pathogenicity tests, the selected isolates were cultured on new CMA plates one to two weeks prior to the beginning of pathogenicity experiments. Fungal plugs were then inoculated into maltose–peptone broth in Erlenmeyer flasks that were incubated without aeration at about 20 °C for four days. Zoospores were produced by decanting the maltose–peptone broth and replacing it with water (Papavizas and Ayers, 1974).

Pathogenicity tests

Pathogenicity tests were performed on the following leguminous test plants: pea cv. 'Reco', green bean cv. 'Masai', broad bean cv. 'Aurora', common vetch cv. 'Ebena', alfalfa cv. 'Julus', red clover cv. 'Fanny', yellow sweet-clover cv. 'Norgold yellow', persian clover (*Trifolium resupinatum*) cv. 'Archibald', bird's-foot trefoil (*Lotus corniculatus*) cv. 'Leo' and white clover cv. 'Sandra'. Seeds were surface sterilised in 1.5% NaOCl for 4 min (pea and broad bean), 2 min (red clover, yellow sweet-clover, common vetch, alfalfa, persian clover and bird's-foot trefoil), or 1 min (white clover), respectively. They were not treated with fungicides with the exception of the green bean seeds, which were treated with Thiram. Plastic trays with cells of 1.5 × 1.3 cm openings and a depth of 15 cm were filled with vermiculite and placed in plastic boxes. Ten seeds of each plant (one seed per tray-cell) were then grown for nine days before being inoculated.

Prior to inoculation, trays with plants were saturated with water to provide favourable conditions for infection. The zoospore suspension (10^4 per plant in 1 ml water) from the isolates of *Aphanomyces* spp. was poured into each cell. Water was filled in the plastic boxes and kept to a depth of 2 cm. These boxes were placed in a growing chamber with a temperature of 25 °C during the light period of 16 h and 19 °C during the dark period of 8 h. The light intensity ranged between 350 and 400 μE. Boxes were moved around once in the chamber to avoid interaction between pathogenicity and environmental factors.

After two weeks, the plants were removed from the vermiculite, the roots were carefully washed in tap water and a disease severity index (DSI) was recorded using a grading scale as described by Persson et al. (1997): (0) no symptoms; (10) up to 10% of root tissue discoloured; (25) 50% of root tissue discoloured; (50) entire root system discolored; (75) root and green part of epicotyl discolored; (100) dead plant. A DSI value of >37, which corresponds to a disease severity value of 2.5 in similar investigations by Malvick et al. (1998), was chosen as a threshold value for considering an isolate to be pathogenic (Table 1). In all pathogenicity tests, four replicates per isolate and plant species were used. Sixteen isolates were also re-tested once or twice to confirm the results.

Selected root pieces with symptoms of root rot were also examined for the occurrence of oospores under the light microscope, and re-isolations of *Aphanomyces* spp. were done on SMA.

Table 1. Comparison of the scale used for grading of plant reactions in pathogenicity tests in this study and in a study by Malvick et al. (1998)

Grading used in this study	Grading by Malvick et al. (1998)	Symptom
0	0	No visible symptoms
10	1	A few, small spots visible
25	2	Discolourations covering parts of root tissue
50	3	Almost entire root surface discoloured
75	4	Entire root surface discoloured and epicotyl discoloured
100	5	Dead plant

Statistical analyses

The data obtained from DSI-readings of roots infected with zoospores were subjected for analysis of variance using the General Linear Model procedures from the Statistical Analysis System (SAS-software) (http://www.sas.com). Mean relative DSI values were compared using the Bonferroni test at $P \leq 0.05$ level. Data from Swedish isolates, which were pathogenicity tested on pea, green bean, broad bean and common vetch were subjected for principal component analysis (PCA) in order to group them based on pathogenicity pattern. The Minitab software was used (http://www.minitab.com).

Results

Isolation of Aphanomyces *spp. and morphological classification*

All isolates recovered from pea corresponded with Scott's description of *A. euteiches* (Scott, 1961). Morphologically similar isolates were also obtained from alfalfa, green bean, common vetch and yellow sweet-clover. The American *A. euteiches* isolate (No. 6) differed from the Swedish isolates in producing obviously more aerial mycelium when cultivated on CMA. Isolates from green bean and broad bean (Nos. 65, G14, B01) had oogonia and oospores within the size of *A. euteiches* (Table 2), but the aplerotic zone was generally smaller than for *A. euteiches* isolates. All isolates had two or three antheridia per oogonium. Hyphal diameter was 5–10 μm. A large number of isolates recovered from green bean, broad bean, red clover, yellow sweet-clover, white clover, alfalfa and common vetch had smaller oogonia and oospores than *A. euteiches* isolates (Table 2).

Isozyme analysis

The G6PDH system did not separate the different *Aphanomyces* spp. clearly. However, the MDH system yielded different isozyme patterns for different species (Figure 1, Table 2). The *Aphanomyces* isolates were divided into four isozyme groups denoted A, B and C according to Larsson (1994), and three isolates (Nos. 65, G14 and B01) were assigned a new group denoted E.

Pathogenicity tests

Data from the pathogenicity tests are presented in Table 2. All 46 Swedish *A. euteiches* isolates originating from pea and together 5 isolates from alfalfa, green bean and yellow sweet-clover were pathogenic to pea. About 50 % of these also induced symptoms on green bean and broad bean, but not above the threshold level for being considered as pathogenic. Symptoms of *A. euteiches* in broad bean appeared as restricted areas of black root tissue with sharp contrast to neighbouring healthy tissue. Swedish isolates of *A. euteiches* recovered from alfalfa induced also symptoms on alfalfa, however not above the threshold for being considered as pathogenic. Conversely to Swedish alfalfa isolates, the US alfalfa isolates 116 (MF 1) and 117 (MHA 41) induced clear disease symptoms on alfalfa (Table 2). They were also pathogenic to vetch (116 and 117) and broad bean (117). In a few cases there were also some symptoms on red clover, yellow sweet-clover and white clover. The DSI values obtained in tests with persian clover and bird's-foot trefoil did not differ from those of non-inoculated control plants.

The French *A. euteiches* isolates (Nos. F46 and F5), Spanish isolate (No. 104) and the US isolate (No. 5) resembled typical Swedish isolates. They were pathogenic to pea and only slightly pathogenic to some other legumes. The *A. euteiches* isolate No. 6 originating from the US induced a higher DSI on pea than all the Swedish isolates. This isolate was also clearly pathogenic to a broad range of leguminous crop plants, including red clover (Table 2). The US isolates from alfalfa, *A. euteiches* race 1 and 2 (Nos. 116 and 117), were in our tests pathogenic on alfalfa and vetch, and the *A. euteiches* f. sp. *phaseoli* (No. 113) was as expected pathogenic on green bean, but also on vetch.

Swedish *A. euteiches* isolates originating from common vetch showed a different infection range on leguminous plants than isolates from other hosts. They were all highly pathogenic to common vetch (Table 2), but were only slightly pathogenic to pea, broad bean and alfalfa. This difference in pathogenicity pattern is also illustrated in Figure 2, displaying PCA of data from DSI-readings.

All isolates denoted *Aphanomyces* sp. 1 with oogonial and oospore size similar to *A. cladogamus* were non-pathogenic to all tested plants, as was also the isolate denoted *Aphanomyces* sp. 2 (No. 65).

Oospores of *A. euteiches* were detected in root tissue of alfalfa, green bean, broad bean, pea, red

clover, yellow sweet-clover and common vetch. The re-isolations from these plants all yielded *A. euteiches*.

Discussion

Pathogenicity tests revealed that isolates of *A. euteiches* comprised two types: pea-specific and vetch-specific (Table 2). The few tested isolates of *A. euteiches* from alfalfa, green bean and yellow sweet-clover also belonged to this group. The isolates originating from vetch differed from other *A. euteiches* strains tested by being highly pathogenic to vetch, but only slightly pathogenic to pea. This type of vetch-specific isolates has not been reported before. Isolates with broader host ranges like those described from the US by e.g. Grau et al. (1991) and Malvick et al. (1998) were not found. Malvick and Percich (1998) report that isolates from pea and alfalfa tend to be most pathogenic to their own host of origin. These results are in contrast to the pathogenicity pattern of Swedish alfalfa isolates, (3 and 26) which displayed only a slight root discolouration. Temp and Hagedorn (1967) report, however, that alfalfa and sweet clover under certain condition may only act as secondary hosts for *A. euteiches* and this is probably the case for the Swedish alfalfa isolates. However, considering that *A. euteiches* is a variable pathogen, more isolates from hosts other than pea should be tested to confirm *A. euteiches* pathotypes proposed for the Swedish *A. euteiches* studied here.

None of Swedish isolates showed strong pathogenicity to alfalfa or green bean, which differs from the behaviour of many American isolates, which have been reported to be pathogenic to alfalfa, alfalfa and pea, alfalfa and red clover, alfalfa and green bean, pea, or green bean (Malvick et al., 1998). However, the alfalfa isolates obtained from the US (Nos. 116 and 117) showed pathogenicity to alfalfa. Moreover, in this study these isolates were pathogenic to vetch and isolate 117 was also pathogenic to broad bean (Table 2). Lower DSI-values obtained in this study compared to previously published work by e.g. Malvick and Grau (2001) might probably be explained by the use of lower temperatures in our study. Other explanations might be usage of different alfalfa cultivars and age of plant seedlings used for inoculation (seven days in the study by Malvick et al. (1998) and nine days in this study).

The French and Spanish isolates tested (Nos. F46, F5 and 104, Table 2) showed the same pathogenicity pattern as most of the Swedish isolates, i.e. pathogenicity only to pea. These results differ from those obtained in a similar French study reported by Wicker et al. (2001). They found that most isolates from France and also two of the Swedish isolates infected 2–5 legume species. Some of these differences can be explained by higher inoculum dosages and higher test temperatures used in the pathogenicity tests in the French study. Another difference influencing the isolate grouping in these studies is the use of different threshold levels for considering an isolate to be pathogenic. Wicker et al. (2001) used disease index values of DI $\geq$ 1 on pea, vetch, alfalfa, broad bean and DI $\geq$ 2 on green bean as threshold levels. Malvick et al. (1998) used a higher pathogenicity threshold (DI $\geq$ 2.5) for an isolate to be considered as pathogenic. In our tests we found that the higher threshold DI as was used by Malvick et al. (1998) gave more solid assessments than the lower DI used by Wicker et al. (2001) in denoting an isolate pathogenic. There are two reasons for this: (i) in our tests we found that root discolourations resulting in a DSI of 5–10 or even 25 (corresponding to DI 1 and DI 2 in the study by Wicker et al. (2001)) might be caused by other factors than *A. euteiches* infection, and (ii) the higher threshold corresponds better to pathogenicity levels found in the different legume crops in infested fields (data not shown). The threshold level of DI 2.5 used by Malvick et al. (1998) corresponds to a DSI of approximately 37 in our study (Table 1). When using this threshold, most of the Swedish *A. euteiches* isolates are considered as pea-specific and these isolates did not differ from the tested isolates from France and Spain. However, the European isolates differ from the more broad host range population in the US. The vetch-specific isolates found might be unique to Sweden. Geographical differences and/or a variation in cultivation practices might cause a selection pressure towards such divergent host preferences. It is for example well documented that vetch has been cultivated at least since the 18th century in the investigated area in Sweden (Linnaeus, 1749), which assumingly is much longer than for example in the US.

In addition to infecting pea the Swedish isolates of *A. euteiches* induced weak but discernible symptoms also in broad beans (Table 2), even though the isolate inducing the highest disease severity originated from the US (No. 6, Table 2). *A. euteiches* was earlier reported to infect broad bean in inoculation experiments with pure cultures of the pathogen (e.g. Carlson, 1965), and Lamari and Bernier (1985) reported *A. euteiches* as associated with root rot of broad beans under field conditions in North America. In this study,

Table 2. DSI for legume test plants inoculated with *Aphanomyces* spp. isolates from different leguminous hosts, isozyme patterns, and morphological and pathotype characteristics

Isol. no.	Origin	Species denotation	Original host	Isozyme pattern	DSI-values in pathogenicity tests on								Diameter in µm		Pathotype[4]
					Pea	Green bean	Alfalfa	Broad bean	Vetch	Red clover	Sweet clover	White clover	Oospore	Oogonium	
Ctrl	—	—	—		1	12	2	14	3	3	2	1			
26[2]	S	*Aphanomyces euteiches*[2]	Alfalfa	C	77*	17	15*	15	3	4*	7	nt	22.4 ± 0.6	31.2 ± 0.6	P
3[2]	S	*Aphanomyces euteiches*	Alfalfa	C	89*	39*	19*	25	7	13	12	4	22.8 ± 0.4	31.7 ± 0.5	P/B
59[2]	S	*Aphanomyces euteiches*	Green bean	C	62*	19	0	15	2	2	2	1	23.3 ± 1.0	36.2 ± 0.8	P
16	S	*Aphanomyces euteiches*	Green bean	C	76*	31*	nt	23	16	10	3	nt	22.9 ± 0.6	31.7 ± 0.7	P
116	US	*Aphanomyces euteiches*	Alfalfa	C	1	12	50*	15	48*	2	4	0	21.3 ± 0.7	26.3 ± 0.7	A/V
117	US	*Aphanomyces euteiches*	Alfalfa	C	14	17	43*	46*	51*	5	20*	0	21.1 ± 0.3	28.3 ± 0.4	A/F/V
110	US	*Aphanomyces euteiches*	Green bean	C	51*	34*	14	23	7	4	3	1	21.4 ± 0.6	26.2 ± 0.9	P
111	US	*Aphanomyces euteiches*	Green bean	C	75*	38*	nt	nt	nt	nt	nt	nt	22.8 ± 0.5	29.4 ± 0.6	P/B
113	US	*Aphanomyces euteiches* f. sp. *phaseoli*	Green bean	C	2	75*	16	16	41*	8	9	0	21.0 ± 0.5	30.9 ± 0.5	B/V
5[2]	US	*Aphanomyces euteiches*	Pea	C	64*	26	13	27	10	11	7	6	22.7 ± 0.5	31.3 ± 0.8	P
6	US	*Aphanomyces euteiches*	Pea	C	94*	42*	21*	51*	12	10	14*	3	21.3 ± 0.4	28.7 ± 0.5	P/B/F
104	ES	*Aphanomyces euteiches*	Pea	C	64*	33*	12*	12	10*	0	7	0	21.5 ± 0.2	28.2 ± 0.4	P
F46	F	*Aphanomyces euteiches*	Pea	C	62*	16	4	18	5	3	8	1	20.8 ± 0.7	27.6 ± 0.6	P
F5[2]	F	*Aphanomyces euteiches*	Pea	C	74*	16	nt	23	2	nt	nt	nt	20.2 ± 0.4	28.3 ± 0.5	P
83	S	*Aphanomyces euteiches*	Pea	C	73*	8	9	8	2	0	1	0	19.7 ± 0.3	26.6 ± 0.6	P
U	S	*Aphanomyces euteiches*	Pea	C	74*	7	3	7	11*	0	0	0	18.6 ± 0.2	25.2 ± 0.3	P
R[1,2]	S	*Aphanomyces euteiches*	Pea	C	77*	20	10	19	7	6	5	2	21.4 ± 0.9	29.8 ± 1.0	P

57[2]	S	*Aphanomyces euteiches*	Sweet clover	C	75*	13	7	19	2	1	3	1		20.2 ± 0.6	30.0 ± 0.7	P
107	S	*Aphanomyces euteiches*	Vetch	C	7	8	14*	16	74*	0	0	0		22.8 ± 0.7	35.0 ± 0.8	V
108[2]	S	*Aphanomyces euteiches*	Vetch	C	7	8	14*	28	65*	nt	7	nt		23.2 ± 0.6	34.2 ± 0.7	V
109[2]	S	*Aphanomyces euteiches*	Vetch	C	17	9	16*	30*	72*	nt	15*	nt		18.0 ± 0.4	27.5 ± 0.5	V
10[2]	S	*Aphanomyces euteiches*	Vetch	C	23*	25*	23*	30*	51*	15	9	8		22.6 ± 0.4	30.6 ± 0.6	V
V03	S	*Aphanomyces euteiches*	Vetch	nt	9	23*	36*	42*	74*	6	6	1		23.1 ± 0.3	35.5 ± 0.3	F/V
65[2]	S	*Aphanomyces* sp. 2	Green bean	E	1	13	3	16	0	nt[3]	0	nt		23.6 ± 0.8	29.8 ± 1.0	NP
G14	S	*Aphanomyces* sp. 2	Green bean	E	1	26	1	25	1	2	1	0		22.2 ± 0.6	29.4 ± 0.5	NP
B01	S	*Aphanomyces* sp. 2	Broad bean	E	5	19	3	26	3	2	2	1		20.5 ± 0.5	30.0 ± 0.7	NP
84	S	*Aphanomyces* sp. 1	Red clover	A	0	8	0	8	4	0	1	0		nt	nt	NP
14[2]	S	*Aphanomyces* sp. 1	Red clover	A	1	8	1	8	2	3	0	0		17.0 ± 0.5	23.0 ± 0.8	NP
S84	S	*Aphanomyces* sp. 1	Sweet clover	nt	3	24	2	24	1	3	2	1		19.8 ± 0.4	27.1 ± 0.6	NP
64[2]	S	*Aphanomyces* sp. 1	Sweet clover	A	2	18*	nt	25*	0	nt	nt	nt		16.9 ± 0.3	24.1 ± 0.4	NP
101[2]	S	*Aphanomyces* sp. 1	White clover	A	0	8	4	9	2	3	0	0		16.8 ± 0.3	23.4 ± 0.4	NP
102	S	*Aphanomyces* sp. 1	White clover	A	0	8	3	8	11*	9	0	0		22.9 ± 0.4	31.7 ± 0.5	NP
103[2]	S	*Aphanomyces* sp. 1	Broad bean	A	1	7	1	6	1	nt	0	nt		17.2 ± 0.3	25.6 ± 0.3	NP
7[2]	S	*Aphanomyces* sp. 1	Alfalfa	A	6	18	5*	16	4	9	2	3		18.2 ± 0.4	24.9 ± 0.4	NP
K	S	*Aphanomyces cladogamus*	Spinach	A	nt	nt	nt	nt	nt	nt	nt	nt		17.0 ± 0.5	25.0 ± 0.5	
86	S	*Aphanomyces cochlioides*	Sugar beet	B	nt	nt	nt	nt	nt	nt	nt	nt		16.3 ± 0.4	21.9 ± 0.5	
					Average DSI-values for 43 Swedish A. e isolates obtained from pea											
43 isol.	S	*Aphanomyces euteiches*	Pea	C	72	20	7	21	4	6	5	3				

[1]DSI followed by * are statistically different from the uninoculated controls according to Bonferroni-test ($P \leq 0.05$). [2]Isolates tested more than once. [3]Not tested.
[4]NP – non-pathogenic; P – pathogenic on pea; B – pathogenic on green bean; A – pathogenic on alfalfa; F – pathogenic on broad bean; V – pathogenic on vetch.

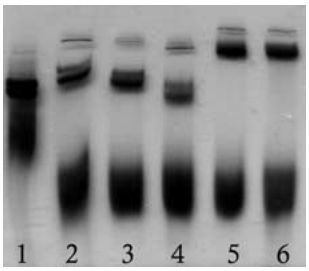

Figure 1. Isozyme banding patterns (A–C and E) of 6 isolates of *Aphanomyces* spp. for MDH. From left to right: Lane 1 – isolate '86', pattern B (*A. cochlioides*); Lane 2 – isolate '87', pattern A (*A. cladogamus*); Lane 3 – isolate '14', pattern A (*Aphanomyces* sp. 1); Lane 4 – isolate '65', pattern E (*Aphanomyces* sp. 2.); Lane 5 – isolate '63', pattern C (*A. euteiches*) pea type; Lane 6 – isolate '109', pattern C (*A. euteiches*) vetch type.

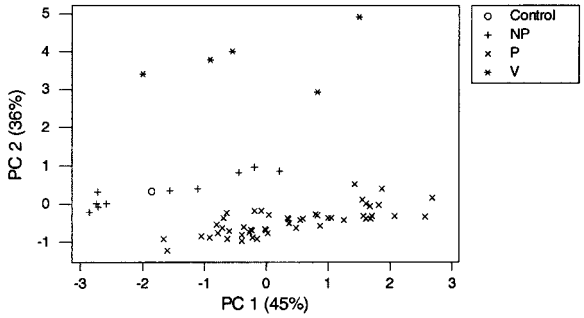

Figure 2. PCA of Swedish *Aphanomyces* isolates ($n = 65$) tested for pathogenicity on broad bean, green bean, pea and vetch. 'NP' (non-pathogenic isolates), 'P' (isolates pathogenic to pea), 'V' (isolates pathogenic to vetch).

oospores were observed in infected roots of broad beans in the greenhouse pathogenicity tests, and it was also recovered from infected root tissues. However, neither were oospores detected nor was *A. euteiches* isolated from roots of broad bean sampled in infested fields (data not shown). *A. euteiches*, thus, does not seem to be a problem in field-grown broad bean in Sweden.

In concordance to an earlier literature report (Delwiche et al., 1987), none of the *A. euteiches* isolates induced reactions different from uninoculated controls in birds-foot-trefoil, or in persian clover. Furthermore, most of the isolates induced only a weak discolouration of root tissue in red clover, white clover and sweet clover (Table 2). Thus, these legumes probably should not be regarded as true hosts of this pathogen and they may neither be readily infected in the field. Results from our field experiments support these findings, but sweet clover here seems to be an exception since oospores were found and *A. euteiches* was isolated from field-grown plants (unpublished data).

In regarding results of both the isozyme-analysis, using the enzyme MDH, the pathogenicity tests and the oospore and oogonia measurements (Table 2), four distinct groups of Swedish *Aphanomyces* isolates from leguminous hosts might be delineated: *A. euteiches*, pathogenic to pea; *A. euteiches*, non-pathogenic to pea, but pathogenic to vetch; *Aphanomyces* sp. type 1; and *Aphanomyces* sp. type 2, both non-pathogenic to pea. This, indicates that legume crop plants in the area investigated are naturally infected by at least three *Aphanomyces* spp. including: *A. euteiches,* another group resembling *A. cladogamus,* and a third one distinct from the other two species groups. However, the precise taxonomic position of the groups of *Aphanomyces* isolates non-pathogenic to pea is unclear and has to be further delineated.

From a practical disease control point of view, the host range of *A. euteiches* and the possibility of inoculum propagation in several alternative species of legumes is important, especially since the practically only applicable control measure for this severe soil-borne pea pathogen presently is minimising of susceptible hosts in the crop rotation. This should especially be the case in areas with intensive pea cropping, or where short rotations with various leguminous crops are practised, as in most types of organic farming. Furthermore, the diversity in pathogenicity among isolates found emphasise the use of suitable inocula with known pathogenicity pattern when surveying pea genotypes for resistance to *A. euteiches.*

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