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Specific behaviour of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand

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Abstract

The pathogenic variability of *Aphanomyces euteiches* on pea was investigated using a collection of 88 pea-infecting isolates from France and 21 isolates from Denmark, Sweden, Norway, USA, Canada and New Zealand. Aggressiveness and virulence were assessed by scoring the root symptoms on a differential set of six pea genotypes. Eleven virulence types were characterised. The virulence type I, previously described as virulent on the whole set, was predominant and included the most aggressive isolates of all geographical origins. The other types were much less prevalent, existing as one to five isolates. Three virulence types (III, IV and V) contained no French isolates. The type III, avirulent on MN313, was composed of American isolates only, and resembled the 'major group' recently described in the USA. A wide range of aggressiveness was found within the virulence type I, and the French isolates appeared globally more aggressive than the foreign isolates. These findings indicate that isolates from the virulence type I should be used as references in breeding programs, and that pea lines PI180693 and 552 may be the most interesting resistance sources to date, despite their only partial resistance.

Introduction

Common root rot of pea, caused by *Aphanomyces euteiches* Drechs., has been one of the major yield-reducing factors for pea production worldwide over the last eighty years (Papavizas and Ayers, 1974; Hagedorn, 1984; Persson et al., 1997). Despite many attempts to control the disease, the only efficient measure is to avoid cultivation of pea in infested fields. Host resistance has been one of the most promising ways of reducing damage to the pea crops, but the development of an effective breeding strategy requires knowledge of the pathogenic variability of the fungus.

The first studies on the pathogenic variability of *A. euteiches* towards pea were conducted on a limited number of isolates from a single country. In the USA, Beute and Lockwood (1967) characterised two physiological races (races 1 and 2) among 15 American isolates according to the sum of mortalities they caused on a set of six pea genotypes. Sundheim (1972), using the same set, but assessing resistance as number of dead plants 10 days after inoculation, established, on 14 isolates from Norway, that race 1 was also present in Norway, and described three new races (3, 4 and 5). However, Manning and Menzies (1984) characterised 16 of 17 isolates from New Zealand as race 5 *sensu* Sundheim, but also pointed out the ambiguous nature of the pathogenicity assessment, and the wide variation in pathogenicity within races defined by both Beute's and Sundheim's methods.

The most recent study was conducted on 114 isolates from Central and Western United States on

a new differential set (Malvick and Percich, 1998) and assessed disease through root symptoms scores on a 0–5 scale. Four virulence groups were found (Malvick and Percich, 1999). Two groups were distinguished by RAPD markers and by their behaviour on two pea genotypes, MN313 and MN314. The major group was avirulent, whereas the minor group was virulent. These groups were found to coexist within the same disease nursery.

In France, Aphanomyces root rot has recently developed, causing major damages on the pea crops (Didelot and Chaillet, 1995). Genetics appeared as one of the most promising control means, but the first field screening results, showing mixed behaviours of the resistant American lines, raised questions about the possible pathogenic variability existing within the French pathogen populations, and between French and foreign (notably American) populations. The objectives of the present study were thus to assess whether the French *A. euteiches* populations displayed specific virulence/aggressiveness features, compared with some foreign isolates (including American ones), and whether virulence variability exists within the French populations. For that purpose, the pathogenic variability of a collection of representative French isolates, and some foreign isolates, including reference strains was assessed. The study was based on a differential set of pea genotypes (Wicker et al., 2001b), taking into account the different resistance sources available and the different isolate–genotype interaction types.

Materials and methods

Aphanomyces euteiches *isolates*

One hundred and nine isolates of *A. euteiches*, all described as pathogenic on pea (Sundheim, 1972; Wicker et al., 2001a), were tested. The 21 foreign isolates (Table 1), kindly provided by other researchers, were all isolated from pea except Ae173, which was isolated from diseased green bean (*Phaseolus*). Nine were from Northern Europe (Sweden, Norway and Denmark), six from the USA, three from Canada and three from New Zealand. The 88 French isolates (Table 2) were isolated from

Table 1. Foreign *A. euteiches* isolates

Code	Country	Source*	Original name	Characteristics and reference
Ae134	Norway	CBS	CBS155.73	Race 3 (Sundheim, 1972)
Ae135	Norway	CBS	CBS156.73	Race 4 (Sundheim, 1972)
Ae122	Sweden	LG Engqvist	94OI96	
Ae145	Sweden	L Persson	LP R	(Larsson, 1994)
Ae146	Sweden	L Persson	LP U	(Larsson, 1994)
Ae147	Sweden	L Persson	LP 83	
Ae148	Sweden	L Persson	LP97040	
Ae149	Sweden	L Persson	LP96024	
Ae181	Denmark	S Rosendahl	S63	
Ae87	USA	JM Kraft	SP7	Idaho (Kraft et al., 1994)
Ae109	USA	CR Grau	467	Wisconsin (Malvick and Percich, 1998; 1999)
Ae110	USA	CR Grau	IHP30	Wisconsin
Ae142	USA	ATCC	ATCC46691	*A. euteiches* f.sp. *pisi* S11 (Pfender and Hagedorn, 1982)
Ae172	USA	CR Grau	P54	Pea pathotype (Malvick et al., 1998)
Ae173	USA	CR Grau	B16	
Ae163	Canada	L Couture	SRSF-0671	
Ae164	Canada	L Couture	SRSF-0672	
Ae165	Canada	L Couture	SRSF-0673	
Ae121	New Zealand	A Stewart	NZ1143	
Ae138	New Zealand	ICMP	ICMP12291	
Ae139	New Zealand	ICMP	ICMP12294	

ATCC = American Type Culture Collection, Manassas (VA), USA; ICMP = International Collection of Microorganisms from Plants – Landcare Research Auckland, New Zealand.
*CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

Table 2. A. euteiches isolates with code number, geographical origin, disease severity of six pea genotypes, and virulence type

Code	Origin[3]	Disease severity on the differential set of pea genotypes[4]						Virulence type[5]
		Baccara	Capella	MN313	90-2131	552	PI180693	
Part 1: Isolates from France								
Ae21	EPB	4.70	4.30	3.45	3.10	2.95	2.83	I
Ae116	EPB	4.60	3.45	3.35	3.35	3.05	2.85	I
Ae126	EPB	4.42	3.55	3.40	3.23	3.10	2.58	I
Ae19	EPB	4.35	3.25	3.30	3.20	2.95	3.00	I
Ae169	EPB	4.33	4.88	3.40	3.50	3.15	3.00	I
Ae132	EPB	4.32	3.35	3.30	3.33	2.88	2.95	I
Ae18	EPB	4.20	3.05	3.10	2.90	2.60	1.80	I
Ae125	EPB	4.05	3.15	3.23	3.40	2.90	2.88	I
Ae167	EPB	4.05	3.45	3.15	3.22	2.65	2.90	I
Ae28	EPB	4.00	3.60	3.55	3.20	3.05	2.40	I
Ae48	EPB	4.00	3.10	3.07	3.38	2.95	2.75	I
Ae63	EPB	4.00	3.35	3.00	3.02	2.70	2.75	I
Ae81	EPB	3.95	3.40	3.19	3.00	2.88	2.32	I
Ae5[1]	EPB	3.91	3.14	3.14	3.06	2.77	2.84	I
Ae123	EPB	3.90	3.05	3.05	3.18	2.40	2.65	I
Ae43	EPB	3.85	2.55	3.00	1.93	2.10	1.30	I
Ae103	EPB	3.85	3.10	3.07	3.00	2.83	2.75	I
Ae24	EPB	3.78	3.75	3.55	3.30	3.05	2.60	I
Ae107	EPB	3.75	3.00	3.05	3.00	2.85	2.45	I
Ae115	EPB	3.75	2.90	3.15	3.00	2.80	2.10	I
Ae65	EPB	3.70	3.00	3.00	2.75	2.85	2.40	I
Ae117	EPB	3.60	3.20	3.10	2.58	2.90	2.58	I
Ae166	EPB	3.55	3.00	2.65	2.60	2.40	2.33	I
Ae12	EPB	3.50	2.75	2.80	2.85	2.25	1.75	I
Ae124	EPB	3.50	2.70	2.25	3.07	1.70	0.90	II
Ae127	EPB	3.50	3.37	3.25	3.30	3.00	2.93	I
Ae44	EPB	3.45	2.65	2.70	2.77	2.05	2.05	I
Ae46	EPB	3.45	2.95	3.00	2.98	2.45	2.05	I
Ae118	EPB	3.43	3.00	3.00	2.95	2.35	1.90	I
Ae128	EPB	3.40	3.20	3.10	3.30	2.95	2.95	I
Ae168	EPB	3.35	2.40	2.80	3.15	2.05	1.40	I
Ae1	EPB	3.28	3.10	3.00	2.95	2.90	2.45	I
Ae85	EPB	3.27	2.85	2.80	2.73	1.90	2.50	I
Ae55	EPB	3.20	3.00	3.00	3.00	2.55	2.45	I
Ae78	EPB	3.20	2.55	1.90	1.55	1.45	0.60	II
Ae130	EPB	3.05	2.65	2.95	2.63	1.70	1.90	I
Ae170	EPB	3.05	2.50	2.45	2.00	1.80	1.40	I
SOL49	EPB	3.05	3.05	3.05	3.05	2.45	2.25	I
Ae131	EPB	3.00	2.25	2.15	1.85	1.75	1.70	I
Ae6	EPB	2.65	1.93	1.05	1.20	1.10	0.73	II
Ae106	EPB	2.60	1.75	1.00	1.20	2.10	1.60	I
Ae16	EPB	2.25	1.75	1.45	1.65	0.65	0.57	VII
Ae37	EPB	2.15	1.35	0.85	1.10	0.75	0.35	VIII
Ae2	EPB	1.90	1.45	1.30	0.85	0.78	1.02	XI
Ae66	EPB	1.80	0.65	0.30	0.10	0.10	0.00	VI
Ae36	EPB	0.75	0.15	0.35	0.35	0.45	0.00	NA
Ae25	EPB	0.15	0.05	0.00	0.00	0.00	0.00	NA
Ae23	EPB	0.00	0.05	0.10	0.00	0.15	0.05	NA
Ae45	EPB	0.00	0.00	0.00	0.00	0.00	0.00	NA
Ae70	WPB	4.78	3.60	3.60	3.40	3.15	1.75	I
Ae33	WPB	4.68	3.78	3.05	3.00	3.05	2.30	I
Ae57	WPB	4.50	3.85	3.25	3.48	3.05	3.20	I
Ae76	WPB	4.50	3.20	3.30	3.30	3.05	2.75	I

Table 2. (*Continued*)

Code	Origin[3]	Disease severity on the differential set of pea genotypes[4]						Virulence type[5]
		Baccara	Capella	MN313	90-2131	552	PI180693	
Ae31	WPB	4.48	3.28	3.20	3.27	2.75	2.90	I
Ae59	WPB	4.25	3.30	3.44	3.13	3.32	2.55	I
Ae41	WPB	4.23	2.90	3.20	2.85	1.57	1.95	I
Ae34	WPB	4.05	3.30	3.42	3.25	2.90	3.00	I
Ae30	WPB	3.95	3.35	3.20	2.95	3.00	3.00	I
Ae144	WPB	3.95	3.10	3.00	3.00	2.90	2.85	I
Ae120	WPB	3.90	3.15	3.05	2.77	2.85	2.75	I
Ae75	WPB	3.60	2.55	2.63	1.97	2.25	2.25	I
Ae60	WPB	3.45	2.90	3.15	2.73	2.75	2.72	I
Ae22	WPB	2.00	1.30	1.22	0.45	0.43	0.15	X
Ae10	WPB	1.93	1.75	0.88	1.13	0.48	0.65	VIII
Ae32	WPB	1.10	0.40	0.10	0.10	0.10	0.00	VI
Ae53	Brittany	4.67	3.30	3.50	3.15	3.20	3.05	I
Ae29	Brittany	4.22	3.00	3.07	3.10	2.53	2.35	I
Ae52	Brittany	4.10	3.05	3.25	2.73	1.87	2.83	I
Ae82	Brittany	3.40	2.95	2.68	2.45	1.57	2.22	I
Ae171	Brittany	3.35	3.05	3.10	2.95	2.80	2.85	I
Ae83	Brittany	3.25	3.20	3.32	2.85	2.55	2.30	I
Ae7	Brittany	3.10	3.27	3.15	3.05	2.80	2.85	I
Ae54	Brittany	3.05	2.95	2.83	2.00	2.05	2.10	I
Ae92	Brittany	2.70	2.30	1.65	1.43	1.65	1.50	I
Ae51	Brittany	2.00	0.88	1.40	1.40	0.20	0.67	IX
Ae119	Centre	5.00	3.70	3.75	3.33	3.15	2.98	I
Ae71	Centre	4.15	3.40	3.50	3.40	3.10	2.50	I
Ae50	Centre	3.20	2.90	2.95	2.45	2.45	1.60	I
Ae72	Centre	3.20	3.25	3.05	2.50	2.90	2.00	I
Ae47	North	4.85	3.60	3.25	3.33	3.10	3.00	I
Ae27	North	4.70	3.20	3.30	3.17	2.65	2.40	I
Ae11	North	4.30	3.45	3.00	2.97	2.85	2.67	I
Ae26	North	4.15	3.10	3.00	2.47	2.95	1.65	I
Ae42	North	2.92	2.40	2.65	1.15	2.07	1.05	I
Ae49	North	0.05	0.00	0.00	0.00	0.40	0.05	NA
Ae84	South-West	3.55	3.73	2.80	3.00	2.80	1.92	I
Ae40	South-West	3.40	3.27	2.72	2.75	2.70	1.61	I
Ae39	South-West	0.00	0.00	0.00	0.05	0.10	0.00	NA
Mean (std)[6]		3.60 (0.79)	2.93 (0.75)	2.79 (0.78)	2.65 (0.81)	2.38 (0.81)	2.14 (0.81)	
Part 2: Isolates from other countries								
Ae122	Sweden	3.40	3.20	3.15	3.17	2.93	2.75	I
Ae147	Sweden	3.38	3.05	3.00	3.05	3.00	2.95	I
Ae148	Sweden	3.25	3.30	3.20	2.97	2.68	1.85	I
Ae149	Sweden	3.23	2.95	2.70	2.97	2.95	2.80	I
Ae146	Sweden	2.95	3.00	2.85	2.93	2.50	2.45	I
Ae145	Sweden	2.90	2.05	2.37	2.05	1.57	0.80	II
Ae181	Denmark	3.70	3.15	3.25	3.00	3.05	2.25	I
Ae134	Norway	0.00	0.00	0.00	0.00	0.00	0.00	NA
Ae135	Norway	0.00	0.00	0.00	0.00	0.00	0.00	NA
Ae142	USA	3.65	3.15	1.20	3.32	3.20	2.58	I
Ae87	USA	3.45	3.35	2.73	2.02	1.88	1.45	I
Ae109	USA	3.37	3.15	0.95	3.00	2.75	2.85	III
Ae110	USA	3.00	2.95	1.90	3.15	2.65	2.80	I
Ae172	USA	3.00	2.25	2.05	1.80	1.60	1.25	I
Ae173[2]	USA	2.62	2.23	0.20	2.12	1.43	1.55	III
Ae163	Canada	1.95	1.42	1.78	0.95	1.65	0.35	V

Table 2. (*Continued*)

Code	Origin[3]	Disease severity on the differential set of pea genotypes[4]						Virulence type[5]
		Baccara	Capella	MN313	90-2131	552	PI180693	
Ae165	Canada	1.70	1.65	1.20	0.15	0.00	0.00	X
Ae164	Canada	1.30	0.93	0.50	0.35	0.80	0.15	VI
Ae138	New Zealand	3.28	2.90	2.80	2.78	2.85	2.65	I
Ae139	New Zealand	2.30	2.45	0.95	2.10	0.81	1.60	IV
Ae121	New Zealand	2.15	1.85	1.65	1.85	1.15	0.40	II
Mean (std)[6]		2.87 (0.69)	2.58 (0.72)	2.02 (0.97)	2.30 (0.96)	2.08 (0.94)	1.76 (1.02)	

[1]The reference-isolate Ae5 was tested at each experiment (eight times). The result is the mean of the eight experiments.
[2]Isolates tested twice. The result is the mean of two experiments.
[3]Country or region of origin of the isolate: Eastern Parisian Basin (EPB) comprises Seine-et-Marne, Aisne, Marne, Val de Marne; Western Parisian Basin (WPB) comprises Eure, Eure-et-Loir, Loir-et-Cher, Seine-Maritime, Yvelines; Brittany comprises the departments Finistère and Morbihan; North comprises Somme and Oise; Centre comprises Indre, Cher, Creuse, Côte d'Or; South-West comprises Charente-Maritime and Pyrénées-Atlantiques.
[4]The disease severity index (DSI) is assessed according to a scale from 0 to 5.
[5]The virulence types were determined according to a pathogenicity threshold of DSI = 1. NA = non-aggressive isolates in the test conditions.
[6]The mean and standard deviation were calculated on the DSI values of the infective isolates (82 from France and 19 from other countries).

soil by baiting with pea seedlings (*Pisum sativum* L., cv. Baccara) (Wicker et al., 2001a), except Ae166, Ae167 and Ae169 which were baited on lentil (*Lens culinaris* L.) from a field naturally infested with *A. euteiches*, and Ae106 and Ae107, which were baited on alfalfa seedlings (*Medicago sativa* L.) grown under controlled conditions. Six main zones of origin were defined: 'Eastern Parisian Basin' (EPB, 49 isolates), 'Western Parisian Basin' (WPB, 16 isolates), 'Brittany' (10 isolates), 'North' (6 isolates), 'Centre' (4 isolates) and 'South-West' (3 isolates). These isolates were characterised as *A. euteiches* according to the key of (Scott, 1961), maintained on corn meal agar (CMA) (Difco, Detroit, MI) slants at 10 °C, and transferred every six months to fresh CMA slants.

Differential set of pea genotypes

The virulence and aggressiveness of the *A. euteiches* isolates were assessed on a set of six pea genotypes: Baccara (Ets Desprez), Capella (Svalof Weibull AB), 902131, MN313, 552 and PI180693. A brief description of the elaboration of this set is given: a screening of many resistance sources allowed a preset of 13 pea genotypes of different resistance levels to be selected. This preset was inoculated with 14 French and foreign isolates to search for genotype–isolate interactions. Five resistance patterns were characterised during this confrontation, leading to the choice of these five partially resistant lines and the susceptible control Baccara (Wicker et al., 2001b).

Testing conditions

Zoospores of *A. euteiches* were produced (Wicker et al., 2001a), counted with a Malassez haemocytometer, and their concentration adjusted to 200 zoospores/ml of suspension. Each treatment consisted of a tray with five randomised plastic pots containing 250 ml of vermiculite (Vermex M, Bretagne Matériaux, 35570 L'Hermitage, France). These five pots were considered as replicates. Four seeds of pea were sown in each pot and the vermiculite was dampened with tap water. Plants were grown in a growth chamber with a 16 h light : 8 h dark regime, with a temperature regime of 25 and 23 °C respectively.

When seedlings were seven days old, 20 ml of a zoospore suspension were dispensed on the vermiculite over the entire area of the pot, providing a total of 10^3 zoospores per plant. The same quantity of water was dispensed on the uninoculated control. Vermiculite was then saturated with water to optimise infestation, and was watered as required over the following days to keep the substrate moist.

The entire study was comprised of eight batches of 7–20 isolates; Ae5 was included as a reference isolate in each batch. As the Ae5 pathogenicity data did not differ significantly between dates (data not presented), the eight batches were considered as the same experiment.

Pathogenicity assessment

Seven days after inoculation, the plants were removed from the vermiculite and root symptoms were assessed visually on a 0–5 scale (Wicker et al., 2001a), giving a disease severity index (DSI).

A virulence threshold was established at DSI = 1: each genotype × isolate interaction resulting in a DSI equal or superior to 1 was considered as compatible, whereas interactions giving a DSI inferior to 1 were considered incompatible, since the fungus could not successfully infect its host (reisolation from the roots was impossible) nor reproduce itself in the roots (no oospore were visible within the roots) (Wicker et al., 2001b). The response of the six differential pea genotypes allowed the virulence types to be characterised.

Data analysis

The genotype, isolate and genotype × isolate interaction effects on the DSI were analysed for variance (ANOVA) using the software SAS (SAS Institute Inc., Cary, NC, USA). The frequency distributions of the virulence types within the different geographical origins (countries, and regions of France) were compared, using χ^2 test (Microsoft Excel 97).

Results

At the inoculum level applied, eight of the 109 *A. euteiches* isolates tested (Table 2) caused no symptoms on all the set, including the susceptible control Baccara. These non-aggressive isolates (NA) included the reference strains CBS155.73 (Ae134) and CBS156.73 (Ae135), previously named 'race 3' and 'race 4' respectively (Sundheim, 1972), as well as the isolates Ae25, Ae39, Ae45, Ae49, previously characterised as pea–vetch (PV) isolates, and Ae23 and Ae36 characterised as pea–vetch–alfalfa (PVA) isolates (Wicker et al., 2001a). Great variability in virulence and aggressiveness was observed among the 101 infective isolates, including the isolates from lentil, alfalfa and green bean.

Analysis of variance

The genotype, isolate and genotype–isolate interaction effects were highly significant (Table 3). However, the contributions to the total variance of the genotype and isolate effects were much higher than the contribution of the interaction, indicating that the differences of resistance between pea genotypes, and of aggressiveness between isolates, were predominant over the differential effects.

Table 3. Analysis of variance for DSI assessed on six pea genotypes, after inoculation with 101 aggressive *A. euteiches* isolates

Source	Degree of freedom	Mean square
Genotype	5	121.68**
Isolate	100	20.73**
Isolate × genotype	500	0.61**
Error	2735	0.09

**Significant at $P < 0.01$.

Variability in virulence

Virulence types

On the basis of the threshold DSI = 1, 11 virulence types were established (Table 4). Most isolates (82) were virulent on all the genotypes, i.e. virulence type I. Five isolates were virulence type II, avirulent on PI180693. Three isolates were virulence type VI, only virulent on Baccara. The other virulence types were minor groups: the types III (avirulent on MN313), VIII (avirulent on MN313, 552 and PI180693) and X (avirulent on 902131, 552 and PI180693) contained two isolates each. The types IV (avirulent on MN313 and 552), V (avirulent on 902131 and PI180693), VII (avirulent on 552 and PI180693), IX (VII with avirulence on Capella) and XI (avirulent on 902131 and 552) were represented by a single isolate.

Situation in France

Within the 82 French isolates, eight virulence types were found (Table 5). Type I was highly predominant, and found in all regions (Table 2). Type II grouped three isolates (all in Eastern Parisian Basin), and all the other virulence types (VI–XI) were minor. The isolates from lentil (Ae166, Ae167, Ae169) and from alfalfa (Ae106 and Ae107) were all of type I. The distributions of each virulence type (I–XI) within regions were not different from their expected values (χ^2 test, $P = 0.16$ (type IX) to 0.99 (type I)) which meant that no geographical virulence specificity appeared among the French isolates, as far as sampling allowed us to investigate this regional variability.

Table 4. Virulence types defined within the *A. euteiches* collection (101 infective isolates)

Virulence type	Disease response on the pea genotypes*						Number of isolates
	Baccara	Capella	MN313	902131	552	PI180693	
I	+	+	+	+	+	+	82
II	+	+	+	+	+	−	5
III	+	+	−	+	+	+	2
IV	+	+	−	+	−	+	1
V	+	+	+	−	+	−	1
VI	+	−	−	−	−	−	3
VII	+	+	+	+	−	−	1
VIII	+	+	−	+	−	−	2
IX	+	−	+	+	−	−	1
X	+	+	+	−	−	−	2
XI	+	+	+	−	−	+	1

*The isolates causing a DSI < 1 are considered avirulent, and noted $-$. Those causing a DSI ≥ 1 are considered virulent and noted $+$.

Table 5. Distribution of the *A. euteiches* isolates from France and from other countries within the different virulence types

Virulence type*	Country of origin						Total isolates/ virulence type
	France	Sweden	Denmark	USA	Canada	New Zealand	
I	71	5	1	4	—	1	82
II	3	1	—	—	—	1	5
III	—	—	—	2	—	—	2
IV	—	—	—	—	—	1	1
V	—	—	—	—	1	—	1
VI	2	—	—	—	1	—	3
VII	1	—	—	—	—	—	1
VIII	2	—	—	—	—	—	2
IX	1	—	—	—	—	—	1
X	1	—	—	—	1	—	2
XI	1	—	—	—	—	—	1
Total isolates/ country	82	6	1	6	3	3	101

*The virulence types were determined according to the virulence threshold DSI $= 1$.

Comparison between situations in France and in other countries

Four virulence types were present both in France and foreign countries (Table 5). The type I was comprised of isolates from France and from Northern Europe (Sweden and Denmark), USA and New Zealand. The type II was comprised of French, Swedish and New Zealand isolates. Types VI and X consisted of French and Canadian isolates. Conversely, three virulence types were not found in France. The type III was only displayed by American isolates (Ae109, Ae173). The type IV was represented in New Zealand only (Ae139) and type V in Canada (Ae163). Three virulence types were displayed by French isolates only: VII, VIII and XI.

Variability in aggressiveness

Aggressiveness levels in the French and foreign situations

The relative aggressiveness of the *A. euteiches* isolates was estimated by the mean DSI caused on the susceptible control Baccara. The French isolates caused a mean DSI of 3.60 ± 0.79 on this genotype, and ranked the differential pea lines, in term of decreasing susceptibility, as: Baccara $>$ Capella $>$ MN313 $>$ 902131 $>$ 552 $>$ PI180693 (Table 2). The foreign isolates were less aggressive (DSI on Baccara of 2.87 ± 0.69). Moreover, they ranked the differential pea lines in a different way: Baccara $>$ Capella $>$ 902131 $>$ 552 $>$ MN313 $>$ PI180693.

Table 6. Distribution of the 101 infective *A. euteiches* isolates within four levels of aggressiveness on Baccara, as related to their geographical origin and virulence type

Origin	Aggressiveness classes*				Total
	[1.0–2.0] ($n = 7$)	[2.0–3.0] ($n = 13$)	[3.0–4.0] ($n = 52$)	[4.0–5.0] ($n = 29$)	
France					
Virulence type					
I	—	3	39	29	71
II	—	1	2	—	3
VI	2	—	—	—	2
VII	—	1	—	—	1
VIII	1	1	—	—	2
IX	—	1	—	—	1
X	—	1	—	—	1
XI	1	—	—	—	1
Total	4	8	41	29	82
Foreign countries					
Virulence type					
I	—	1	10	—	11
II	—	2	—	—	2
III	—	1	1	—	2
IV	—	1	—	—	1
V	1	—	—	—	1
VI	1	—	—	—	1
X	1	—	—	—	1
Total	3	5	11	—	19

*The 101 isolates were dispatched into four classes according to their aggressiveness, assessed by DSI on the cv. Baccara: [1.1–2.0], [2.0–3.0], [3.0–4.0], [4.0–5.0]. The total number of isolates per interval are given within brackets.

The isolates were also classified in four aggressiveness subdivisions, corresponding to each interval of DSI from 1 to 5 ([1.0–2.0], [2.0–3.0], [3.0–4.0] and [4.0–5.0]). No foreign isolate was present in the interval [4.0–5.0], corresponding to the most aggressive isolates, whereas the French isolates were present in each of the four intervals (Table 6). The frequency distributions of the foreign isolates within the aggressiveness intervals were significantly different from their expected distributions (χ^2 test, $P = 0.01$), whereas the frequency of very aggressive isolates (interval [4.0–5.0]) is significantly different from the expected value (χ^2 test, $P < 0.01$). So, the foreign isolates seem to be significantly less aggressive than the French isolates.

Within the French collection, the distributions of aggressiveness intervals within regions were not different from their expected values (χ^2 test, $P = 0.07$–0.82), implying that no geographical aggressiveness specificity appeared among the French isolates. Very aggressive isolates were found within all regions (Table 2).

Aggressiveness levels and virulence types

When both French and foreign isolates were considered, the relative aggressiveness was highest for the isolates of the virulence type I (Table 6), since the DSIs on Baccara ranged from 2.6 (interval [2.0–3.0]) to 5.0. The isolates of the types II and III were found in the intervals [2.0–3.0] and [3.0–4.0], whereas these of the other virulence types were in the two intervals of lesser aggressiveness.

Differential effects within the virulence type I

Analysis of variance showed highly significant genotype, isolate and interaction effects within the isolates of type I (Table 7), indicating that differential interactions, although not predominant, were also found within this type. Most of the isolates ranked the genotypes, from susceptible to resistant, as: Baccara > Capella > MN313 > 902131 > 552 > PI180693 (Figure 1A). PI180693, the most resistant line, displayed a DSI from 3.2 (Ae57, WPB) to 1.05 (Ae42, North) (Table 2). However, nine isolates did not rank the genotypes in the same way. Ae110 and Ae142

Table 7. Analysis of variance for DSI assessed on six pea genotypes, after inoculation with 82 *A. euteiches* isolates of the virulence type I

Source	Degree of freedom	Mean square
Genotype	5	89.49**
Isolate	81	4.93**
Isolate × genotype	405	0.49**
Error	2143	0.08

**Significant at $P < 0.01$.

(from the USA, Figure 1B and C) were much less aggressive on MN313 (having a behaviour closer to the virulence type III). Ae106 (Eastern Parisian Basin (EPB), France) was least aggressive on MN313 and 902131 (Figure 1D). Ae75 (Western Parisian Basin (WPB), France) was least aggressive on 902131, whereas Ae42 (North, France) was least aggressive on 902131 and PI180693, displaying a behaviour close to the virulence type V (Figure 1J and G, respectively). Ae85 (EPB, France), Ae41 (WPB, France), Ae52 and Ae82 (both from Brittany, France), were least aggressive on the pea line 552 (Figure 1E,F,H and I respectively).

Discussion

This study is a large-scale comparison of the diversity in virulence and aggressiveness on pea of *A. euteiches* isolates from Europe, America and New Zealand.

Eleven virulence types were described, but one (type I) largely predominated. The other types were much less prevalent, at least in France, and existed of isolates that were also moderately to weakly aggressive on the susceptible control Baccara. The non-pathogenicity of the reference strains CBS155.73 and CBS156.73, representative respectively of the Sundheim's races 3 and 4 (Sundheim, 1972), made it impossible to compare Sundheim's results with ours. The inconsistency of behaviour of these reference strains has been reported (Manning and Menzies, 1984), and may be explained by the differences of methodologies (inoculum dose, incubation conditions), or by a loss of the pathogenic characteristics of the strains due to long storage.

The virulence type I was characterised by its ability to infect the whole set, which is composed of the most efficient resistance sources known, and was found within all the geographical origins (different countries and French regions). The reference strain from Kraft (Ae87 = SP7) (Kraft et al., 1994), as an example, was in this group. Moreover, the most aggressive isolates were from this type. Breeding programmes for resistance to *A. euteiches* should use isolates from this virulence type, particularly in the greenhouse screening trials. Some breeders are now using a strain of this type (Ae5) as an aggressive and representative reference of the French *A. euteiches* populations (Moussart et al., 2001). Furthermore, the most partially resistant line, PI180693, can be used as a control; this line, as well as 552, seem to be the most interesting sources of resistance to date, despite their partial resistance. Their field behaviour has been considered as interesting: they both express disease symptoms later than the other lines (M Duparque, pers. comm., 2000). However PI180693 has a 'wild-type' phenotype, which makes its use difficult in breeding programmes; 552 has the advantage of being partially resistant, and having desirable agronomical traits. The aim of resistance breeding programmes should be, on the one hand, to obtain germplasm of a much better resistance level, with desirable agronomical traits, and on the other hand to identify new potential sources of more resistant germplasm.

Three virulence types were neither found in France, nor in Europe: type III (avirulent on MN313) which was similar to the 'major group' described in the USA by Malvick and Percich (1998); type IV (avirulent on MN313 and 552) and type V (avirulent on 902131 and PI180693). The interesting point concerns the avirulence or low aggressiveness on MN313, which was only displayed by American isolates (Ae109 and Ae173 in type III, Ae110 and Ae142 in type I). These results suggest that the genotype MN313 carries a resistance factor which is highly efficient against a part of the US populations, but not against any of the French *A. euteiches* populations. Conversely, the virulence types VII, VIII and IX and XI were only found in France. However, we lack *A. euteiches* samples from the different other countries to assert with certainty that these types are specific of the French populations.

This study showed also a wide range of aggressiveness within type I, and underlined the higher aggressiveness of the French isolates compared to the foreign isolates. This point was also observed during experiments in the USA (JM Kraft, pers. comm., 1999). Are the French populations differentiated, in particular from the American populations, for virulence and aggressiveness? Additional data and experiments are required to confirm this and to find explanations.

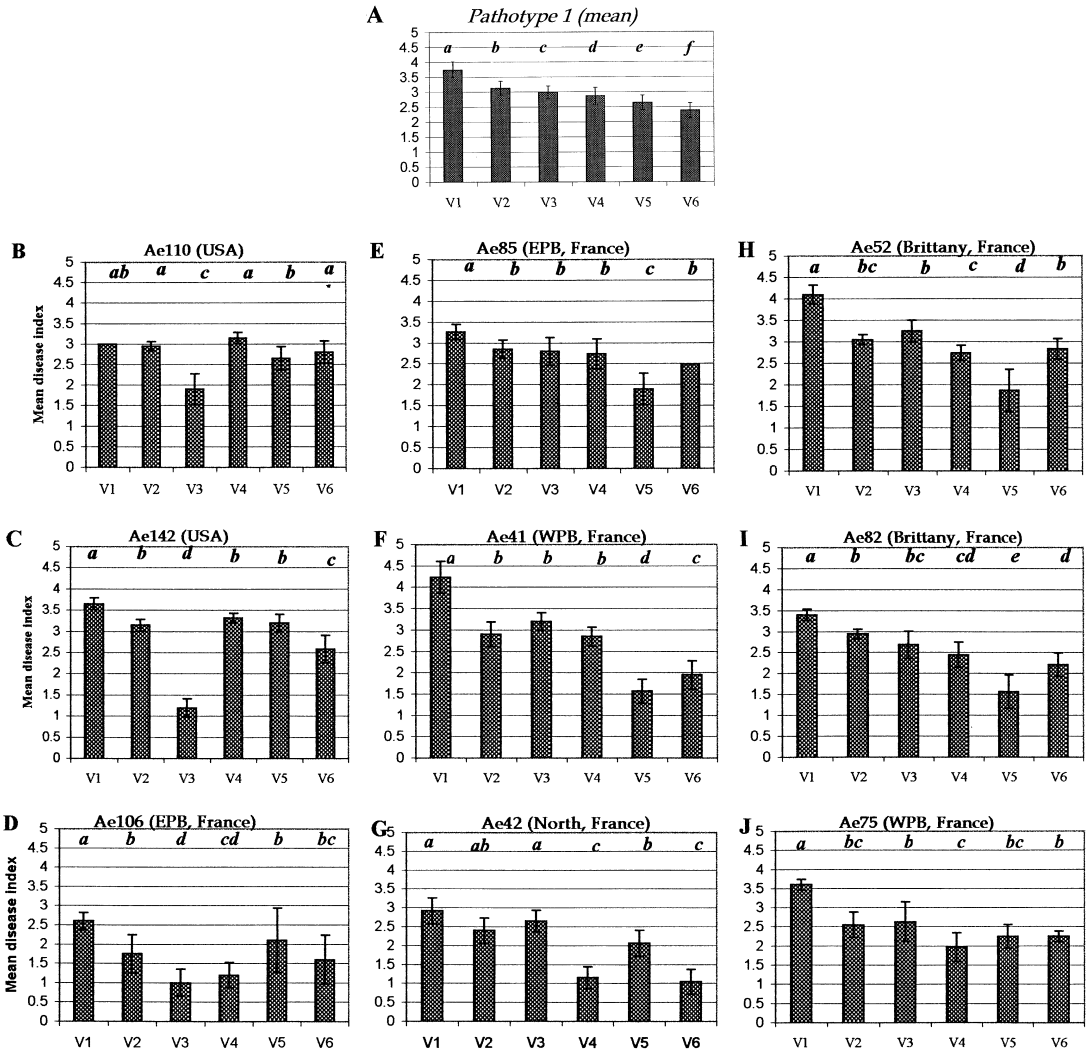

Figure 1. Aggressiveness patterns of *A. euteiches* isolates within the virulence type I: mean pattern of 82 isolates from France and foreign countries, and particular patterns (V1 = Baccara, V2 = Capella, V3 = MN313, V4 = 902131, V5 = 552, V6 = PI180693). The bars marked by the same letter within the same graph are not significantly different (Newman–Keuls test, $P = 0.05$).

In this study, the virulence types were defined by the use of a criterium dealing with the early stages of the plant–pathogen interaction, when the pathogen tries to penetrate the root tissues, and to invade the whole root system: the reaction was considered incompatible if the fungus did not succeed in producing active mycelium within the roots. But the results also showed that some isolates of virulence type I were less aggressive on MN313 or 552, whereas the majority of the isolates were least aggressive on PI180693. There may be other resistance factors, expressing after the successful penetration of the fungus, that were possibly not taken into account with this methodology. Kraft and Boge (1996) showed that resistance of pea to *A. euteiches* was expressed through different quantitative components, that were pre- and post-infection events: lowering the zoospore germination in the exudates, the progression of the symptoms in the roots. In the pathosystem subterranean clover/*Phytophthora clandestina*, evidence exists that race-specific resistance could express after the penetration of the fungus in the roots, by limiting the degree of root colonisation (Purwantara et al., 1995;

1998). Additional studies should be done to characterise the expression of resistance of pea to *A. euteiches* in the course of time, as well as the race-specificity or non-specificity of the components of this resistance.

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