Aggressiveness of *Conidiobolus obscurus* against the Pea Aphid: Influence of Cuticular Extracts on Ballistospore Germination of Aggressive and Non-aggressive Strains

By JEAN-PAUL LATGÉ,*† LAURA SAMPEDRO, PAUL BREY
AND MICHEL DIAQUIN

Unité de Lutte biologique contre les Insectes, Institut Pasteur, 25 rue du Dr Roux,
75724 Paris Cedex 15, France

(Received 23 January 1987)

Cuticular extracts of *Acyrtosiphon pisum*, the pea aphid, stimulated the formation of germ-tubes of ballistospores of aggressive strains of *Conidiobolus obscurus*, but showed no influence on those of non-aggressive strains. Aggressive strains were separated into two groups: (a) those stimulated by both (i) water- or alcohol-soluble cuticular extracts originating from honeydew and (ii) lipidic extracts of the cuticle, and (b) those stimulated by (ii) only. The hydrocarbon and polar fractions of the cuticular lipids were the most active. These results obtained with *C. obscurus* are compared to the effect of cuticular compounds on spore germination in other entomopathogenic fungi.

INTRODUCTION

One of the first critical events in the establishment of a fungal infection in arthropods is the germination of the spore on the host cuticle. Spore germination is influenced by the environmental conditions found on the cuticular surface. Temperature and humidity have been generally considered as the most important factors for controlling the germination of fungal spores (Gottlieb, 1978). However, in fungi pathogenic to plants and animals, nutrients present on the surface of the host also influence the development of the fungal propagules and are consequently a key factor in the establishment of the pathogenicity of a fungal strain (Cole & Nozawa, 1981; Wynn, 1981). Nutrients present on the host surface may be endogenous to the cuticle or exogenously deposited by leachates from aerial surfaces, host exudates or arthropod faecal and regurgitated material.

Ballistospores of the entomogenous Entomophthorales which make contact with an insect can interact in one of three ways: they may form germ-tubes able to infect the insect; they can resporulate and form secondary spores which will be forcibly discharged; they may not germinate and their survival will depend on the environmental conditions and their own nutritive resources. The aggressiveness of *Conidiobolus obscurus* (Zygomycetes: Entomophthorales) has been correlated to the germination capabilities of this fungus: the aggressive (A⁺) strains have more spores producing germ-tubes on the cuticle of the pea aphid than the non-aggressive ones (A⁻) (Latgé *et al.*, 1982). However, the germination of these strains *in vitro* was variable, with some of the A⁺ strains being unable to form germ-tubes under the different experimental conditions tested (Sampedro *et al.*, 1984). These results suggested that some aphid cuticular components may have a specific effect on conidial germination of *C. obscurus*. The aim of this study was to examine the influence of the nutritional conditions of the aphid cuticle on conidial germination.

† Present address: Unité de Mycologie, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France.

METHODS

Fungal and aphid strains. The origin of aggressive (542⁺ or 722⁺, 637⁺, 879⁺ and 940⁺) and non-aggressive (280⁻, 313⁻ and 455⁻) strains of *C. obscurus* towards the pea aphid has been previously reported (Latgé & Boucias, 1984). The isolates were cultured on an egg-yolk agar medium (Sampedro *et al.*, 1984). Pea aphids, *Acyrtosiphon pisum* of clone B as defined by Papierok & Wilding (1979), were reared on bean plants at 18 °C under constant light.

Isolation and fractionation of cuticular extracts. Laboratory-reared aphids of all developmental stages were collected in plastic boxes and inactivated by storage at -20 °C for 5–10 min. Some aphid batches were stored at -20 °C. Aphids (20 g fresh weight samples) were submerged for 30 s to 5 min at 10 °C into 500–1000 ml of various solutions: water, 50 % (v/v) ethanol, chloroform/methanol (2:1 v/v). The crude extracts were recovered by filtration and concentrated at 40 °C under vacuum in a rotary evaporator.

The desiccated chloroform/methanol extract was dissolved in 150–200 ml of a mixture of chloroform/methanol/water (8:4:3 by vol.). The upper phase with the polar material and the lower phase containing total lipids were concentrated in a rotary evaporator and stored respectively in water and hexane at -20 °C. The lipid extract was fractionated in a silicic acid column (10 g silicic acid pre-heated at 110 °C for 12 h for 10–15 mg lipid extract poured as a hexane solution). The lipid fractions were eluted with 50 ml of the following solvents: hexane (fraction I), 50% (v/v) diethyl ether/hexane (fraction II), 2% (v/v) methanol/diethyl ether (fraction III), 2% (v/v) glacial acetic acid/diethyl ether (fraction IV) (Baker *et al.*, 1979). Quantitative extraction of residual lipids on the column was done for 24 h successively with 100 ml chloroform (fraction V) and 100 ml methanol (fraction VI). All these fractions were concentrated in a rotary evaporator and their composition was checked by TLC on silica gel (Merck 60) plates developed in hexane/diethyl ether/acetic acid (80:20:1 by vol.) and charred with 70% (v/v) perchloric acid for 10 min at 100 °C. Further purification of fractions II–V was done using preparative silica gel TLC with the same solvent system. The bands corresponding to these components were scraped into flasks and extracted overnight with the corresponding solvents used in the silicic acid column chromatography. All extracts were stored at -20 °C.

The methanolic extract (5 mg ml⁻¹) was fractionated using an ultrafiltration cell (Diaflo-Amicon) at 3 atm (304 kPa) under a nitrogen flow and constant agitation. Five fractions were separated according to their molecular weight (*M_r*): (1) >10000, (2) 5000–10000, (3) 1000–5000, (4) 500–1000, (5) ≤500.

Recovery of water-soluble non-structural components of the cuticle. Anal secretions (honeydew) of the aphid, composed mainly of free sugars and amino acids, often contaminated the cuticle of these insects. Honeydew was recovered as previously described (Brey *et al.*, 1985) and stored as an aqueous solution at -20 °C.

Assay of conidial germination. Microscope slides were covered with 3.5 ml 1 or 2% (w/v) agar (Difco) containing 20 mM-sodium-potassium phosphate buffer, pH 6.2 or 8.0. These two pH values were chosen because 6.2 was the pH of the aphid cuticular surface and induced mainly secondary conidia *in vitro*; in contrast, basic pH (8.0) promoted germ-tube formation (Sampedro *et al.*, 1984). Alcoholic and aqueous cuticular extracts and the honeydew fractions were directly incorporated into the agar at concentrations ranging from 0.5 to 25.0 mg per ml agar medium. One or several successive 25 µl drops of the lipidic fractions at concentrations ranging from 2.5 to 50 mg ml⁻¹ (corresponding to a final range of lipid concentration of 0.12–1.0 mg cm⁻²) were deposited as a spot on the surface of the agar. Germination was tested after evaporation of the solvents (15 min at 20 °C).

Ballistospores produced from scraped mycelial thalli (Sampedro *et al.*, 1984) were projected onto the test slides and incubated at 20 °C in darkness. Depending on the strain, spores germinated 2–4 h after being discharged on the agar. They produced germ-tubes (GT) or secondary spores (C2) as previously defined (Sampedro *et al.*, 1984). Two ratios were estimated: the total percentage of germinating spores, [(GT + C2)/(GT + C2 + UG)] × 100, and the percentage of spores forming germ-tubes, [GT/(GT + C2 + UG)] × 100, where UG = ungerminated spores. To avoid any confusion between a true germ-tube and a young conidiophore which had not yet formed a secondary spore, counts for estimating the percentage of germ-tubes were made after 6–10 h of incubation (depending on the strain). One hundred conidia were counted on each slide, with four to eight replicates per experiment. The germ-tube length was measured on 20 conidia per slide. Results were analysed statistically after an angular transformation of the percentage of germination (*p*) by a one- or two-way variance analysis. They are expressed as $\arcsin \sqrt{p} \pm \text{SD}$.

RESULTS

Influence of polar extracts on total germination

Water-soluble cuticular extracts at a concentration of 0.5–5.0 mg ml⁻¹ stimulated the total germination of strains 542⁺ and 879⁺ but showed no influence on strains 637⁺, 940⁺, 313⁻ and 455⁻. A dose of 25 mg ml⁻¹ inhibited the germination of all strains tested. Comparable results were obtained for all these strains at pH 6.2 and 8.0 (Fig. 1). The 50% (v/v) ethanol extract, the methanolic fraction of the chloroform/methanol cuticular extract, and an aqueous solution of

Fig. 1. Influence of polar aphid cuticular components extracted by water on total germination of strains 542⁺ (○), 940⁺ (□) and 455⁻ (●) at pH 6.2 (full line) and at pH 8.0 (dotted line). x , Initial concentration of the cuticular extract (mg ml⁻¹).

the honeydew stimulated the germination of strains 542⁺ and 879⁺, at pH 6.2, but did not influence strains 940⁺, 637⁺, 313⁻ and 280⁻ (Table 1).

In the methanolic extract, the fraction containing the low- M_r components (≤ 500) was the only one active on strain 542 (Fig. 2).

Influence of polar extracts on germ-tube formation

Strains 637⁺, 940⁺, 313⁻ and 455⁻ rarely produced germ-tubes in the presence of the four different polar extracts tested at concentrations up to 25 mg ml⁻¹ at pH 6.2 and 8.0. However, germ-tube formation never attained 1%. Conversely, at pH 6.2, the formation and growth of germ-tubes of strains 542 and 879 was stimulated by the polar cuticular extracts and the honeydew (Table 2). Between 0.5 and 5.0 mg ml⁻¹, the concentration of the extract did not influence germ-tube formation and growth (Table 2); however, a concentration of 25 mg ml⁻¹ strongly reduced the formation of germ-tubes. The method of extraction of the polar extract did not influence the effect on germ-tube production (Table 2). Extraction time did not influence either of the results, similar data being obtained with fractions collected after 0.5, 5 and 30 min of extraction. The low- M_r fraction was the only one able to stimulate the formation of germ-tubes (Fig. 2).

The ease of extraction of the fractions and the positive effect of the low- M_r components and of the honeydew strongly suggest that the components extracted by water and alcohol are probably non-structural metabolites originating mainly from the honeydew.

Influence of lipidic extracts on total germination

Cuticular lipid extracts did not influence the percentage germination of strains 542⁺, 722⁺ and 879⁺ but inhibited the total percentage of germination of strains 940⁺, 637⁺, 313⁻ and 455⁻ (Table 3). However, the lipid extracts only delayed the germination of these strains, since the same percentage of germination was observed 8–10 h after discharge in the control and treated samples (data not shown).

Fig. 2. Influence of fractions of different M_r of the methanolic fraction of the chloroform/methanol extract on the total germination and germ-tube formation of strain 542⁺ of *C. obscurus*. The results are expressed as the mean percentage, p . The statistical analysis is made on an angular transformation of p expressed as $\arcsin \sqrt{p} \pm \text{SD}$. Percentages followed by the same letter are not significantly different using Duncan's multiple range test ($P=0.05$).

Influence of lipidic extracts on germ-tube formation

Cuticular lipids promoted the formation of germ-tubes of strains 940⁺ and 637⁺ (which were not stimulated by polar extracts) at pH 6.2 and 8.0: 14–90% of spores produced germ-tubes on the slides covered by the cuticular lipid extracts while no germ-tubes were produced in the control. These lipidic extracts also stimulated the formation of germ-tubes of strain 879⁺, which was stimulated by the polar extracts (Table 4). Lipid extracts were also able to counter-influence the effect of physical factors on the mode of germination. For example, at pH 6.2, all spores of strain 722⁺ produced secondary spores. In the presence of lipids, at least 25% of these spores formed germ-tubes (data not shown). In contrast, the cuticular lipids were not able to provoke the formation of germ-tubes of the A⁻ strains tested (280⁻, 313⁻, 455⁻) at pH 6.2 or 8.0. A basic pH (8.0) and a high concentration of lipidic extract (5.0 mg cm⁻²) were the most favourable conditions for inducing the formation of germ-tubes of strains 637⁺ and 940⁺ (Table 4). A concentration less than or equal to 0.1 mg cm⁻² did not stimulate the formation of germ-tubes (data not shown).

Table 1. Influence of different polar extracts on the total germination of strains 542⁺ 940⁺ and 313⁻ at pH 6.2 (4 ± 1 h after discharge)

All the results are expressed as mean percentage, *p*. In parenthesis is given $\arcsin \sqrt{p} \pm \text{SD}$ (at least four replicates of 100 conidia per replicate). Means followed by the same letter are not significantly different using Duncan's multiple range test ($P = 0.05$). Results for each strain and each type of extract were analysed separately. ND, Not done. CM, chloroform/methanol.

Extract concn (mg ml ⁻¹) ...	Total germination									
	Strain 542 ⁺					Strain 940 ⁺				
	0	0.5	0.5	5.0	0	0.5	0.5	5.0	0	5.0
Water extract	42 ^a (40 ± 24)	89 ^b (71 ± 16)	98 ^b (82 ± 6)	98 ^b (82 ± 6)	62 ^a (52 ± 28)	54 ^a (47 ± 10)	62 ^a (52 ± 10)	62 ^a (52 ± 10)	45 ^a (42 ± 22)	50 ^a (46 ± 18)
Ethanol (50% v/v) extract	49 ^a (45 ± 4)	ND	94 ^b (76 ± 5)	94 ^b (76 ± 5)	54 ^a (47 ± 10)	ND	49 ^a (45 ± 1)	49 ^a (45 ± 1)	ND	ND
Methanolic fraction of the CM extract	25 ^a (30 ± 12)	81 ^b (69 ± 15)	85 ^b (67 ± 18)	85 ^b (67 ± 18)	54 ^a (47 ± 12)	ND	67 ^a (55 ± 12)	67 ^a (55 ± 12)	60 ^a (50 ± 3)	66 ^a (54 ± 4)
Honeydew	36 ^a (37 ± 26)	81 ^{ab} (64 ± 28)	99 ^c (83 ± 7)	99 ^c (83 ± 7)	62 ^a (52 ± 12)	55 ^a (49 ± 20)	75 ^a (60 ± 18)	75 ^a (60 ± 18)	80 ^a (63 ± 4)	86 ^a (68 ± 1)

Table 2. Influence of polar cuticular extracts on the germ-tube formation (10 h after discharge) and growth of spores of strains 542⁺ and 879⁺

The results are expressed as in Table 1. Values in square brackets represent germ-tube length (μm).

Extract concn (mg ml ⁻¹) ...	Germ-tube formation									
	Strain 542 ⁺					Strain 879 ⁺				
	0	0.5	0.5	5.0	25.0	0	0.5	0.5	5.0	5.0
Water extract	12 ^a (20 ± 20) [102 ± 48] ^a	70 ^b (57 ± 27) [206 ± 144] ^b	84 ^b (66 ± 19) [244 ± 93] ^b	84 ^b (66 ± 19) [244 ± 93] ^b	8 ^a (26 ± 13) [ND]	18 ^a (25 ± 14) [92 ± 51] ^a	81 ^b (64 ± 15) [144 ± 75] ^b	81 ^b (64 ± 15) [144 ± 75] ^b	59 ^b (50 ± 10) [144 ± 96] ^b	59 ^b (50 ± 10) [144 ± 96] ^b
Ethanol (50% v/v) extract	0 ^a	ND	97 ^c (80 ± 1)	97 ^c (80 ± 1)	14 ^b (31 ± 3)	ND	ND	ND	ND	ND
Methanolic fraction of the CM extract	3 ^a (11 ± 15)	78 ^b (62 ± 19)	68 ^b (56 ± 23)	68 ^b (56 ± 23)	20 ^a (26 ± 16)	0 ^a (0)	56 ^b (48 ± 2)	56 ^b (48 ± 2)	53 ^b (47 ± 1)	53 ^b (47 ± 1)
Honeydew	3 ^a (7 ± 10)	30 ^{ab} (33 ± 32)	75 ^c (60 ± 24)	75 ^c (60 ± 24)	ND	ND	ND	ND	ND	ND

Table 3. *Influence of different concentrations of the lipidic extract (chloroform fraction of the chloroform/methanol extract) of the aphid cuticle on the total germination of C. obscurus primary spores at pH 6.2 (3 ± 1 h after discharge, depending on the strain)*

The results are expressed as in Table 1.

Strain	Extract concn (mg cm ⁻²) . . .	Total germination	
		0	5
722 ⁺		48 ^a	66 ^a
		(29 ± 6)	(43 ± 14)
879 ⁺		57 ^a	64 ^a
		(35 ± 7)	(40 ± 6)
637 ⁺		36 ^a	75 ^b
		(22 ± 17)	(51 ± 17)
940 ⁺		26 ^a	80 ^b
		(15 ± 8)	(54 ± 11)
313 ⁻		9 ^a	51 ^b
		(10 ± 15)	(35 ± 14)
455 ⁻		17 ^a	68 ^b
		(10 ± 14)	(46 ± 12)

Table 4. *Influence of different concentrations of the lipidic extract (chloroform fraction of the chloroform/methanol extract) on the formation of germ-tubes of C. obscurus primary spores (10 ± 2 h after discharge)*

The results are expressed as in Table 1. All spores of strain 879⁺ produced germ-tubes at pH 8.0.

Strain	Extract concn (mg cm ⁻²)	Germ-tube formation at pH:	
		6.2	8.0
879 ⁺	0	41 ^a	
		(25 ± 16)	ND
	5.0	86 ^b	
		(64 ± 19)	ND
637 ⁺	0	0 ^c	0 ^c
	0.5	14 ^b	22 ^b
		(22 ± 12)	(28 ± 5)
	5.0	65 ^a	82 ^a
		(53 ± 7)	(65 ± 7)
940 ⁺	0	0 ^b	0 ^c
	0.5	61 ^a	31 ^b
		(51 ± 14)	(34 ± 11)
	5.0	47 ^a	92 ^a
		(43 ± 6)	(73 ± 3)

Of the six lipidic fractions recovered from preparative TLC only fraction I (hydrocarbon, R_f 0.95) and fraction VI (a very polar material, R_f 0) gave one spot on the thin-layer chromatograms. Fraction II–V indicated four major components, corresponding to alkyl esters, fatty acid methyl esters, free fatty acids and alcohols according to Brey *et al.* (1985). The use of preparative TLC allowed partial purification of the fractions in the corresponding lipid class but these fractions remained contaminated with one or two other lipid classes. No further purification was undertaken. Lipid fractions were deposited on the agar slides at a dose identical to the concentration of each fraction in a 5 mg cm⁻² parent extract stimulatory for germination. Amongst the lipid fractions, the hydrocarbons (fraction I) and polar fraction (fraction VI) induced the formation of germ-tubes of strain 940⁺ at rates similar to those obtained with the unfractionated extract (Table 5). The stimulation of germ-tube formation by these two fractions

Table 5. Influence of the different classes of the chloroform extract of the aphid cuticle on the germination of primary spores of strain 940⁺ of *C. obscurus* at pH 6.2

The results are expressed as in Table 1.

Fraction*	Total germination	Germ-tube formation
I	47 ^a (43 ± 25)	15 ^{ab} (23 ± 15)
II	5 ^b (13 ± 21)	0 ^c (1 ± 4)
III	1 ^b (7 ± 13)	0 ^c (1 ± 3)
IV	2 ^b (8 ± 16)	0 ^c (0 ± 0)
V	0 ^b (5 ± 10)	0 ^c (1 ± 2)
VI	55 ^a (48 ± 21)	9 ^b (17 ± 11)
(I + II + III + IV + V + VI) = VII	9 ^b (17 ± 19)	0 ^c (3 ± 7)
Unfractionated extract	81 ^a (64 ± 23)	21 ^a (27 ± 12)

* Concn equal to that of each fraction in a 5 mg cm⁻² parent extract (unfractionated extract).

appeared to be specific since they did not modify the total percentage of germination. Fractions II–V strongly inhibited the total germination (Table 5). Subsequent experiments with non-inhibitory concentrations (10⁻¹ dilution) of fractions II–V confirmed that they were not able to provoke the formation of germ-tubes of 940⁺ spores (data not shown). An intriguing result was the failure of the pooled fractions to stimulate germination, including fractions I and VI at concentrations promoting the formation of germ-tubes (Table 5).

Stimulation of germination by aphid cuticular extracts appeared specific since strains 940⁺ and 637⁺ did not produce germ-tubes on slides covered by various commercial lipids at 1.0 or 5.0 mg cm⁻² (pH 6.2). The lipids tested were fatty acids (myristic, stearic, palmitic and oleic acid), triacylglycerols (trimyristin, tripalmitin, tristearin), alcohols (arachidyl, behenyl, cetyl alcohol and triacontanol), hydrocarbons (heptacosane, tetracosane, triacontane, heneicosane and octacosane), sterols (cholesterol and sitosterol) and complex lipids (lanoline, lanolyl alcohol and sunflower oil).

DISCUSSION

The results of our studies of the germination of *C. obscurus* spores *in vivo* (Latgé *et al.*, 1982) and *in vitro* are summarized in Fig. 3. Several conclusions can be drawn from this figure. (1) The proportion of spores forming germ-tubes was higher *in vitro* than *in vivo*; a similar conclusion was observed concerning the total germination percentage (Latgé *et al.*, 1982; Sampedro *et al.*, 1984). (2) A few germ-tubes were formed by A⁻ strains *in vivo* while none were observed *in vitro*. (3) A⁺ strains can be separated into two groups: strains 542⁺ or 722⁺ (isolated from aphids infected with strain 542⁺) and 879⁺ can be stimulated by water-soluble extracts present on the surface of the aphid cuticle (group A⁺1); strains 940⁺ and 637⁺ produce germ-tubes only in the presence of cuticular lipids (group A⁺2). The difference in the *in vitro* behaviour of these two groups has been previously reported (Sampedro *et al.*, 1984). Germ-tube formation in strains 542⁺ or 722⁺ and 879⁺ could be induced or stimulated by the addition of salts, sugars and protein hydrolysates to the germination media. It appeared therefore, that low-*M*_r molecules present on the surface of the aphid cuticle stimulated the germination of these strains. These components probably originate from the honeydew which is rich in free sugars and amino acids (Brey *et al.*, 1985),

Fig. 3. Schematic histogram representing germ-tube formation by spores of the A⁺1, A⁺2 and A⁻ groups of strains of *C. obscurus*: (1) *in vivo* on the aphid cuticle; (2) *in vitro* with no cuticular extract; (3) *in vitro* in the presence of total polar water- or alcohol-soluble extracts, or in the presence of low-*M_r* (500) components of the polar extracts; (4) *in vitro* in the presence of honeydew; (5) *in vitro* in the presence of total lipid cuticular extracts or in the presence of hydrocarbons of the polar fraction of the lipidic extract.

which stimulated germ-tube formation, but could also come from leaf leachates, cornicular secretions or moulting residues. In contrast, germ-tube production was not induced *in vitro* with strains of group A⁺2, 940⁺ and 637⁺ when water-soluble nutrients were added to the germination medium (Sampedro *et al.*, 1984). This separation between the two groups of A⁺ strains has also been made on the basis of biological characters (resting spore production) or pathological characters (incubation time of the disease, sporulation capacity of the cadavers) (Latgé *et al.*, 1979; Papierok & Wilding, 1981). Electrophoretic and serological tests have confirmed the clustering of the A⁺ strains in two groups (Latgé & Boucias, 1984; Latgé *et al.*, 1986).

Lipids play an important role in the morphological and physiological development of the Entomophthorales. Growth and sporulation of these fungi are stimulated non-specifically by several plant and animal fats (Latgé *et al.*, 1978). The results of the current study demonstrated that the spore development of A⁺ strains of *C. obscurus* could be specifically regulated by the aphid cuticular lipids, particularly by the hydrocarbon and polar fractions. Kerwin (1982, 1984) was able to demonstrate that the limitation of *Erynia variabilis* (Thaxter) Remaudière & Hennebert to the adult dipteran *Fannia canicularis* host could be partly due to the cuticular lipids of this insect. Unlike *C. obscurus*, the active fraction in the determination of the specificity of *E. variabilis* for adult dipterans seemed to be the free fatty acid fraction, the presence of sufficient oleic acid inducing the vegetative germination of this species and high levels of palmitoleic acid promoting mycelial growth after germination.

The influence of cuticular lipids varies according to the insect-entomopathogenic fungus system considered. Boucias & Pendland (1984) reported that conidial germination of *Nomuraea rileyi* was stimulated by extracts from *Anticarsia gemmatilis*, with the sterol and diacylglycerol fractions being the most active. Cuticular extracts from *Oryctes rhinoceros* and *Cetonia aurata*, however, did not influence the germination of *Metarhizium anisopliae* (Fargues, 1981).

Several reports have been made of an antagonistic effect of the cuticular lipids on fungal infection of the insects (Koidsumi, 1957; Evlakhova & Chekourina, 1962; Smith & Grula, 1982). This inhibitory effect was attributed in Lepidoptera to short-chain fatty acids such as capric and caprylic acids (Koidsumi, 1957; Smith & Grula, 1982; Saito & Aoki, 1984); however, the fatty acids were not tested at concentrations comparable to that present in the epicuticle (Latgé, 1972). Furthermore, short-chain fatty acids are not present in all insect orders and have not been detected in significant amounts in the cuticle of the different Coleoptera and Homoptera studied (J.-P. Latgé, unpublished; Champlin *et al.*, 1981; Brey *et al.*, 1985). In the *C. obscurus* system, the inhibitory effect of the fatty acid fraction is not due to short-chain fatty acids since Brey *et al.* (1985) demonstrated that the cuticular lipids of the aphids do not contain short-chain fatty acids. The inhibitory role of these short-chain fatty acids in the resistance of the insects to entomogenous fungi has probably been overestimated.

In *C. obscurus*, the role of the epicuticular lipids appeared quite complex. By pooling all the fractions or by removing the hydrocarbon fraction, the extract became antagonistic. Such intriguing results could indicate that substances both stimulatory and inhibitory to germ-tube formation were present in the same extract. Slight variations in the proportions of the amalgamated fractions may alter germination significantly. Light microscope observations indicated that the morphology of the lipid surface of the pooled fractions was different from the original extract. Fractions II-V displayed the same hairy-crystalline aspect as the pooled fractions whereas fractions I, VI and the crude extract looked more homogeneous (unpublished). Slight modification in the topography of the lipid constituents could affect the water and/or nutrient availability to the spores and consequently influence the germination behaviour of *C. obscurus* spores. Several parasitic fungi are known to be very sensitive to minor differences in host surface topography (Wynn, 1981; Staples *et al.*, 1983).

As for *C. obscurus*, spores of various entomogenous hyphomycetes need water-soluble nutrients on the cuticle of the insects for germination (Fargues, 1981; Boucias & Pendland, 1984; Woods & Grula, 1984). Amino acids and glucosamine seemed the most effective polar components (Kerwin, 1982; Boucias & Pendland, 1984; Sampedro *et al.*, 1984; Woods & Grula, 1984). Water-soluble extracts can also have a negative effect on the germination of entomogenous fungi. Ethanolic extracts from a pink clone of *A. pisum* resistant to *C. obscurus* infection inhibited the germination of A⁺ strains of this species (J.-P. Latgé, unpublished). Water-soluble cuticular phenols inhibit the mycelial growth of *Aphanomyces astaci* (Ajaxon & Söderhäll, 1982) and conidial development of *Cordyceps militaris* (J.-P. Latgé, unpublished). Phytopathogenic fungi can be similarly stimulated or inhibited by water leachates from plants (Godfrey, 1976; Wildman & Parkinson, 1981; Kaminskyj & Day, 1984). As in plant pathogens (Matta, 1982), the saprophytic microflora associated with free nutrients present on the cuticular surface of the insect could also influence the pathogenesis of entomogenous fungi. The presence of fungal and bacterial contaminants on the beetle *Hylobius pales* inhibited the germination of the conidia of *M. anisopliae* (Schabel, 1978). The microflora of the pea aphid (about 3·10⁶ bacteria per aphid, mainly Gram-positive cocci), when tested *in vitro*, was ineffective against the 722⁺ strain group but could inhibit the germination of the 940⁺ type strain (A. Uziel & J.-P. Latgé, unpublished).

The influence of the cuticular extracts is highly dependent on their concentration. The concentration of the lipid extract has been roughly estimated as 0·2–0·5 mg per cm² of aphid cuticle (10–20 mg lipid per g of fresh aphids, corresponding to a volume of aphids of 5 cm³). Lipid concentrations less than the cuticular doses will not induce germ-tube formation. Doses 5–10 times greater than the cuticular concentration still induced the formation of germ-tubes. Similarly, the concentration of polar components was about 4–8 µg cm⁻² µm⁻¹ (5–10 mg extract per 5 cm³ of aphids, calculation based on the 50 µm outer-layer). The doses tested which induced

the formation of the germ-tubes were between 1 and 20 $\mu\text{g cm}^{-2} \mu\text{m}^{-1}$. A concentration of 0.1 $\mu\text{g cm}^{-2} \mu\text{m}^{-1}$ failed to enhance the formation of germ-tubes (data not shown). These results stress the necessity of using a concentration of cuticular extracts similar to those encountered in the cuticle in *in vitro* germination tests of entomopathogenic fungi.

The influence of the cuticular components on the behaviour of entomogenous fungi has always been tested with fractions extracted from non-infected whole insects however, components secreted by the insect in response to the recognition of the fungal pathogen and arriving at the surface of the cuticle through the pore canals could also influence the germination of the spores. Such components, induced in plants by phytopathogenic fungi (Roberts & Aist, 1984), have not been identified in entomopathogenic fungal systems. A role of cuticular monomers released by exocellular enzymes has been suggested in the pathogenesis of *C. obscurus* and *Beauveria bassiana* (Latgé *et al.*, 1984; Woods & Grula, 1984).

The difference in the germination behaviour of the two groups of A⁺ strains could have an ecological or epidemiological significance. In low-density aphid populations, aphids are rarely in contact and their cuticles would rarely be contaminated with honeydew. This would result in very limited development of the saprophytic microflora. In these conditions, strains of the 940⁺ group, stimulated by the cuticular lipids, have the highest chances of infecting an insect. The persistence of these strains in low-density aphid populations would be favoured by their high sporulating capacity (Papierok & Wilding, 1981), increasing the chance of contact between the discharged conidia and the scarce aphids. In heavy insect populations, aphids are constantly in close contact and their cuticles would be readily covered by the honeydew (stimulating the 722⁺ group) and its associated saprophytic flora (inhibiting the 940⁺ group). In consequence, strains of the 722⁺ group would preferentially infect the aphids. The complete destruction of the aphid population would be accelerated by the fast killing action of this group of *C. obscurus* strains.

In addition, these strains have the highest capacity for resting spore formation in the aphid body under various environmental conditions (Papierok & Latgé, 1980). The eradication of the aphid population would be then accompanied by the presence of numerous cadavers filled with resting spores that would facilitate the persistence of the fungus in the absence of aphids until the subsequent breeding season (Latgé *et al.*, 1979).

We are indebted to D. Boucias, J. L. Kerwin and N. Wilding for providing valuable suggestions and English corrections.

REFERENCES

- AJAXON, R. & SÖDERHÄLL, K. (1982). Effect of quinones and melanin on mycelial growth of *Aphanomyces* spp. and extracellular protease of *Aphanomyces astaci* a parasite on crayfish. *Journal of Invertebrate Pathology* **39**, 105–109.
- BAKER, J. E., SUKKESTAD, D. R., NELSON, D. R. & FATLAND, C. L. (1979). Cuticular lipids of larvae and adults of the cigarette beetle, *Lasioderma serricornis*. *Insect Biochemistry* **9**, 603–611.
- BOUCIAS, D. G. & PENDLAND, J. C. (1984). Nutritional requirements for conidial germination of several host range pathotypes of the entomopathogenic fungus *Nomuraea rileyi*. *Journal of Invertebrate Pathology* **43**, 288–292.
- BREY, P. T., OHAYON, H., LESOURD, M., CASTEX, H., ROUCACHE, J. & LATGÉ, J. P. (1985). Ultrastructure and chemical composition of the outer layers of the cuticle of the pea aphid *Acyrtosiphon pisum* (Harris). *Comparative Biochemistry and Physiology* **A82**, 401–411.
- CHAMPLIN, F. R., CHEUNG, P. Y. K., PEKRUL, S., SMITH, R. J., BURTON, R. L. & GRULA, E. A. (1981). Virulence of *Beauveria bassiana* mutants of the pecan weevil. *Journal of Economic Entomology* **74**, 617–621.
- COLE, G. T. & NOZAWA, Y. (1981). Dimorphism. In *Biology of Conidial Fungi*, vol. 1, pp. 97–133. Edited by G. T. Cole & B. Kendrick. New York: Academic Press.
- EVLA KHOVA, A. A. & CHEKOURINA, T. A. (1962). L'activité de défense de la cuticule de la punaise des céréales (*Eurygaster integriceps* Put.) contre les microorganismes végétaux. Colloque international de pathologie des insectes, *Entomophaga. Mémoires hors série* **12**, 137–141.
- FARGUES, J. (1981). *Spécificité des hyphomycètes entomopathogènes et résistance interspécifique des larves d'insectes*. Thèse de Doctorat d'Etat, Université de Paris VI.
- GODFREY, B. E. S. (1976). Leachates from aerial parts of plants and their relation to plant surface microbial populations. In *Microbiology of Aerial Plant Surfaces*, pp. 433–439. Edited by C. H. Dickinson & T. F. Preece. London & New York: Academic Press.
- GOTTLIEB, D. (1978). *The Germination of Fungus Spores*. Durham: Meadowfield Press.
- KAMINSKY, S. G. W. & DAY, A. W. (1984). Chemical induction of infection structures in rust fungi. I. Sugars and complex media. *Experimental Mycology* **8**, 63–72.

- KERWIN, J. L. (1982). Chemical control of the germination of asexual spores of *Entomophthora culicis*, a fungus parasitic on dipterans. *Journal of General Microbiology* **128**, 2179–2186.
- KERWIN, J. L. (1984). Fatty acid regulation of the germination of *Erynia variabilis* conidia on adults and puparia of the lesser housefly, *Fannia canicularis*. *Canadian Journal of Microbiology* **30**, 158–161.
- KOIDSUMI, K. (1957). Antifungal action of cuticular lipids in insects. *Journal of Insect Physiology* **1**, 40–51.
- LATGÉ, J.-P. (1972). Contribution à l'étude du *Cordyceps militaris* (Fr.) Link. Systématique, biologie, physiologie. Doctorat de spécialité, Université de Toulouse.
- LATGÉ, J.-P. & BOUCIAS, D. (1984). Intraspecific variability in *Conidiobolus obscurus*. *Journal of General and Applied Microbiology* **30**, 135–150.
- LATGÉ, J.-P., REMAUDIERE, G. & DIAQUIN, M. (1978). Un nouveau milieu pour la croissance des champignons entomophthorales pathogènes d'aphides. *Annales de Microbiologie* **B129**, 463–476.
- LATGÉ, J.-P., PERRY, D. F., PAPIEROK, B., COREMANS-PELSENEER, J., REMAUDIERE, G. & REISINGER, O. (1979). Induction de la formation des spores de résistance d'*Entomophthora obscura* Hall & Dunn. *Comptes rendus hebdomadaires de séances de l'Académie des Sciences* **D288**, 599–602.
- LATGÉ, J.-P., PAPIEROK, B. & SAMPEDRO, L. (1982). Aggressivité de *Conidiobolus obscurus* vis-à-vis du puceron du pois. I. Comportement des conidies sur la cuticule avant la pénétration du tube germinatif dans l'insecte. *Entomophaga* **27**, 323–330.
- LATGÉ, J.-P., SAMPEDRO, L. & HALL, R. (1984). Aggressivité de *Conidiobolus obscurus* vis-à-vis du puceron du pois. III. Activités enzymatiques exocellulaires. *Entomophaga* **29**, 185–201.
- LATGÉ, J.-P., SAMPEDRO, L. & BOUCIAS, D. (1986). Aggressiveness of *Conidiobolus obscurus* against the pea aphid. IV. Electrophoretic and immunoelectrophoretic characterization of aggressive strains. *Journal of Invertebrate Pathology* **48**, 159–166.
- MATTA, A. (1982). Mechanisms in non-host resistance. In *Active Defense Mechanisms in Plants*, pp. 119–141. Edited by R. K. S. Wood. New York: Plenum Press.
- PAPIEROK, B. & LATGÉ, J.-P. (1980). Considérations sur le pouvoir pathogène de *Entomophthora obscura* Hall & Dunn à l'égard des pucerons des céréales. *Bulletin OILB/SROP* **III**, 4, 27–29.
- PAPIEROK, B. & WILDING, N. (1979). Mise en évidence d'une différence de sensibilité entre 2 clones du puceron du pois *Acyrtosiphon pisum* Harr. (Homoptère, Aphididae) exposés à 2 souches du champignon Phycomycète *Entomophthora obscura* Hall & Dunn. *Comptes rendus hebdomadaires de l'Académie des Sciences* **288D**, 93–95.
- PAPIEROK, B. & WILDING, N. (1981). Etude du comportement de plusieurs souches de *Conidiobolus obscurus* (Zygomycètes *Entomophthoraceae*) vis-à-vis des pucerons *Acyrtosiphon pisum* et *Sitobion avenae* (Hom.: Aphididae). *Entomophaga* **26**, 241–249.
- ROBERTS, D. W. & AIST, J. R. (editors) (1984). *Infection Processes of Fungi (a Bellagio conference in March 1983)*. New York: The Rockefeller Foundation.
- SAITO, T. & AOKI, J. (1984). Toxicity of free fatty acids on the larval surface of two lepidopterous insects towards *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces famoso-roseus* (Wize) Brown et Smith (Deuteromycetes: Moniliales). *Applied Entomology and Zoology* **18**, 225–233.
- SAMPEDRO, L., UZIEL, A. & LATGÉ, J.-P. (1984). Aggressivité de *Conidiobolus obscurus* vis-à-vis du puceron du pois. II. Mode de germination *in vitro* des conidies primaires de souches d'agressivité différente. *Mycopathologia* **86**, 3–19.
- SCHABEL, H. G. (1978). Percutaneous infection of *Hylobius pales* by *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* **31**, 180–187.
- SMITH, R. J. & GRULA, E. A. (1982). Toxic components on the larval surface of the corn earworm (*Heliothis zea*) and their effects on germination of *Beauveria bassiana*. *Journal of Invertebrate Pathology* **39**, 15–22.
- STAPLES, R. C., GRAMBOW, H. J. & HOCH, H. C. (1983). Potassium ion induces rust fungi to develop infection structures. *Experimental Mycology* **7**, 40–46.
- WILDMAN, H. G. & PARKINSON, D. (1981). Comparison of germination of *Cladosporium herbarum* and *Botrytis cinerea* conidia *in vitro* in relation to nutrient conditions on leaf surfaces. *Canadian Journal of Botany* **59**, 854–861.
- WOODS, S. P. & GRULA, E. A. (1984). Utilizable nutrients on *Heliothis zea* available for growth of *Beauveria bassiana*. *Journal of Invertebrate Pathology* **43**, 259–269.
- WYNN, W. K. (1981). Tropic and toxic responses of pathogens to plants. *Annual Review of Phytopathology* **19**, 237–255.