An integrated approach to the taxonomy of the genus *Verticillium*

YAN JUN,¹† PAUL D. BRIDGE¹ and HARRY C. EVANS²*

¹International Mycological Institute, Ferry Lane, Kew, Surrey TW9 3AF, UK

²International Institute of Biological Control, Silwood Park, Ascot, Berkshire SL5 7TA, UK

(Received 22 October 1990; revised 4 February 1991; accepted 13 February 1991)

The taxonomic relationship among 64 isolates of the genus *Verticillium* was investigated using a range of morphological, physiological and biochemical characters with particular reference to *V. lecanii*. Forty-five isolates pertaining to the *V. lecanii* complex were compared, including isolates from arthropods (entomopathogens), rust fungi (hyperparasites) and 'saprophytic' sources. Other *Verticillium* species from invertebrate, fungal and plant hosts were included in the study to explore relationships within the genus. By analysing 41 characters, similarities were calculated between isolates using Gower's coefficient, and dendrograms were derived by average linkage clustering. Six cluster groups were delimited: *V. lecanii* isolates (7), mostly from homopteran insects; hyperparasitic isolates (3), including one of *V. psalliotae*, from rusts; *V. lecanii* isolates (18) mainly from insects of UK origin; *V. lecanii* isolates (7) from coffee rust; *V. lecanii* isolates (11) from scale insects, predominantly tropical; and two isolates of *V. albo-atrum*. The remaining 16 isolates, mostly from *Cordyceps* and *Torrubiella* infections of tropical forest arthropods, did not fit into any cluster group and all probably represent undescribed anamorphs. A similar pattern was obtained when the electrophoretic properties of catalase isoenzymes were compared. Twelve catalase bands were identified. The taxonomic significance of these findings is discussed.

Introduction

The genus *Verticillium* Nees (Hyphomycetes, Deuteromycotina) is a large, heterogeneous assemblage of taxa grouped according to relatively simple and ill-defined characters. A monographic study of the genus 'is overdue but will present a task requiring a period of some years' (Gams & Van Zaayen, 1982). Earlier, Gams (1971) re-described the genus *Verticillium* and proposed the new section *Prostrata* to accommodate taxa without well-defined, erect conidiophores. These comprised mainly invertebrate pathogens, originally placed in the genus *Cephalosporium*, with teleomorphs in the Clavicipitaceae (Evans & Samson, 1982). Balazy (1973) disagreed with these 'too far-reaching generalisations' and conserved the species with verticillate phialides in *Cephalosporium*. This classification has not been generally accepted (Carmichael *et al.*, 1980) and subsequently, Gams & Van Zaayen (1982) proposed two new sections, *Nigrescentia* and *Albo-erecta*, accommodating the plant-pathogenic

and fungicolous species, respectively. Nevertheless, *Verticillium* continues to be a difficult and controversial genus (Evans & Samson, 1986; Gams, 1988) and one of more than academic interest, since it contains parasitic species of considerable economic importance. Any name changes could have commercial repercussions, for example in the registration of new products. *Verticillium lecanii* (Zimm.) Viegas has long been recognized as a potential biocontrol agent of arthropod pests (Petch, 1925; Samson *et al.*, 1988) and is currently being commercially re-evaluated (Quinlan, 1988; van der Schaaf *et al.*, 1990). Moreover, its ability to hyperparasitize Uredinales has led to a number of studies on its possible use for the biological control of rust diseases (Spencer, 1980; Blakeman & Fokkema, 1982; Srivastava *et al.*, 1985; Uma & Taylor, 1987). A wide range of isolates attributable to this species is held in the culture collection of the International Mycological Institute (IMI) and such is the variation in the species complex that eight synonymous taxa were described by the same author over a 17 year period (Gams, 1971). The main objective of the present study was to test the validity of the *V. lecanii* species concept, as defined by Gams (1971), by comparing a representative selection of IMI isolates

† Present address: Experiment Centre of Integrated Biological Control on Northern Forest Pests and Diseases of China, Huanghe St, Shenyang, China.

using the multidisciplinary technique adopted by Bridge *et al.* (1989) and Mugnai *et al.* (1989) to resolve similar problems in the genera *Penicillium* and *Beauveria*, respectively. Biochemical and physiological, as well as morphological characters are employed in this integrated approach to distinguish between taxa. Other clavicipitaceous fungi with *Verticillium*-like anamorphs, isolated from tropical forest arthropods, together with several common fungicolous and plant pathogenic species, were included to test both species and generic concepts.

Methods

Isolates examined. The 64 isolates studied are listed in Table 1 under their IMI accession code and designated species name, together with details of host or substrate and geographical origin. A total of 66 isolates appears in the list but two of these were found subsequently to be duplicates (41 = 25 and 56 = 40).

Inoculation of test media. All inoculations were by means of agar plugs (5 mm diameter) containing hyphal tips from 10 d cultures grown on tap water agar (TWA) (Smith & Onions, 1983).

Morphological characterization. For macromorphological studies, isolates were grown both in the dark (at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and light (supplemented with UV, at $30^{\circ}\text{C} \pm 3^{\circ}\text{C}$) on potato dextrose agar (PDA), potato carrot agar (PCA) and malt agar (MA). Culture characteristics were assessed after 2 and 4 weeks. For micromorphological studies, isolates were grown using the slide culture technique (Booth, 1971) and examined as described previously (Mugnai *et al.*, 1989). In addition, 3- to 5-week-old PCA and MA cultures were screened for the presence of secondary structures (chlamydospores or resting mycelium). Morphological characters were coded by the 'all present' system (Mugnai *et al.*, 1989; Bridge & Sackin, 1991) to account for the effect of exclusive characters.

Physiological characterization. The hydrolysis of aesculin, Tween 80, RNA and gelatin, and growth on citric and lactic acids as sole carbon sources, were assessed according to Bridge (1985). Urease activity was measured using the method of Mugnai *et al.* (1989). The ability to grow at reduced water activity on G₂₅N medium (Pitt, 1980) and at varying

Table 1. *Verticillium* isolates studied

Isolate no. and accession code	Species designation	Host or substrate	Country and date of isolation
1. IMI 21167	<i>Verticillium lecanii</i> (Zimm.) Viegas	<i>Icerya purchasi</i> (Coccidae: Homoptera)	Sri Lanka 1924
3. IMI 68689a	* <i>V. lecanii</i>	<i>Trialeurodes vaporariorum</i> (Aleyrodidae: Homoptera)	UK 1972
4. IMI 68689b			
5. IMI 68689c			
6. IMI 79606	<i>V. lecanii</i>	<i>Pulvinaria floccifera</i> (Coccidae: Homoptera)	Turkey 1960
7. IMI 115197	<i>V. lecanii</i>	<i>Myzus persicae</i> (Aphididae: Homoptera)	UK 1965
8. IMI 176057	<i>V. lecanii</i>	Contact lens	UK 1973
9. IMI 176391	<i>V. lecanii</i>	<i>Scolytus scolytus</i> (Curculionidae: Coleoptera)	UK 1973
10. IMI 179172	<i>V. lecanii</i>	<i>Macrosiphoniella sanborni</i> (Aphididae: Homoptera)	UK 1973
11. IMI 179173	<i>V. lecanii</i>	<i>Brachycaudus helichrysi</i> (Aphididae: Homoptera)	UK 1973
12. IMI 235048	<i>V. lecanii</i>	<i>Cecidophyopsis ribis</i> (Acarinae: Arachnida)	UK 1979
13. IMI 255033	<i>V. lecanii</i>	<i>Coccus viridis</i> (Coccidae: Homoptera)	Sri Lanka (Lunuwila) 1981
14. IMI 255034	<i>V. lecanii</i>	<i>Coccus viridis</i>	Sri Lanka (Monergala) 1981
15. IMI 268316	<i>V. lecanii</i>	<i>Thrips tabaci</i> (Thripidae, Thysanoptera)	UK 1982
16. IMI 268317	<i>V. lecanii</i>	<i>Thrips tabaci</i>	UK 1982
17. IMI 270376	<i>V. lecanii</i>	Contaminated pesticide solution	UK 1982
18. IMI 272211	<i>V. lecanii</i>	<i>Pteridium aquilinum</i> (Pteridophyta)	UK 1982
19. IMI 282532	<i>V. lecanii</i>	<i>Cossus cossus</i> (Cossidae: Lepidoptera)	Italy 1984
20. IMI 288986	<i>V. lecanii</i>	Scale insect (Coccidae: Homoptera)	USA 1984
21. IMI 293226	<i>V. lecanii</i>	Laboratory solution	UK 1985
22. IMI 293227	<i>V. lecanii</i>	Soil	UK 1985
23. IMI 293228	<i>V. lecanii</i>	Leaf litter	UK 1985
24. IMI 303103	<i>V. lecanii</i>	<i>Hemileia vastatrix</i> (Coffee rust)	Colombia 1986
25. IMI 304815	<i>Verticillium</i> state of <i>Torrubiella rubra</i> Pat. & Lagerh.	Scale insect (Coccidae: Homoptera)	Dominica 1986
26. IMI 304817	<i>V. lecanii</i> state of <i>Torrubiella confragosa</i> Mains	Scale insect (Coccidae: Homoptera)	Dominica 1986
27. IMI 317424	<i>V. lecanii</i>	<i>Hemileia vastatrix</i>	Jamaica 1987
28. IMI 317425	<i>V. lecanii</i>	Coffee scale (Coccidae: Homoptera)	Jamaica 1987
29. IMI 317441	<i>V. lecanii</i>	Coffee scale (Coccidae: Homoptera)	Jamaica 1987
30. IMI 317442	<i>V. lecanii</i> state of <i>T. confragosa</i>	<i>Hemileia vastatrix</i>	Jamaica 1987
31. IMI 317444	<i>V. lecanii</i> state of <i>T. confragosa</i>	<i>Hemileia vastatrix</i>	Jamaica 1987
32. IMI 317451	<i>V. lecanii</i> state of <i>T. confragosa</i>	<i>Hemileia vastatrix</i>	Jamaica 1987
33. IMI 321293	<i>V. lecanii</i>	<i>Hemileia vastatrix</i>	Colombia 1986
35. IMI 329758	<i>V. lecanii</i>	<i>Hemileia vastatrix</i>	Mexico 1988
36. IMI 329759	<i>V. lecanii</i>	<i>Hemileia vastatrix</i>	Mexico 1988
37. IMI 331542	<i>V. lecanii</i> state of <i>T. confragosa</i>	<i>Penthocrates styx</i> (Limacodidae: Lepidoptera)	Philippines 1985
38. IMI 331550	<i>V. lecanii</i>	Coffee scale (Coccidae: Homoptera)	Indonesia 1985
39. IMI 331588	<i>V. lecanii</i> state of <i>T. confragosa</i>	Forest scale (Coccidae: Homoptera)	Dominica 1986
40. IMI 304807	<i>V. lecanii</i> state of <i>T. confragosa</i>	Coffee scale (Coccidae: Homoptera)	Dominica 1986

Table 1—continued

Isolate no. and accession code	Species designation	Host or substrate	Country and date of isolation
41. IMI 304815	<i>Verticillium</i> state of <i>T. rubra</i>	Scale insect (Coccidae: Homoptera)	Dominica 1986
42. IMI 338013	<i>V. lecanii</i>	<i>Lawana conspersa</i> (Flatidae: Homoptera)	Malaysia 1988
43. IMI 338014	<i>V. lecanii</i>	Scale insect (Coccidae: Homoptera)	Mexico 1988
44. IMI 338015	<i>V. lecanii</i>	<i>Hemileia vastatrix</i>	Uganda 1988
45. IMI 338016	<i>V. lecanii</i> state of <i>T. confragosa</i>	<i>Saissetia</i> scale (Coccidae: Homoptera)	Ecuador (Galapagos) 1976
46. IMI 331547	<i>V. chlamydosporium</i> Goddard	Slug eggs (Mollusca)	Brazil 1985
49. IMI 317438	<i>V. lecanii</i>	Scale insect (Coccidae: Homoptera)	Jamaica 1987
50. IMI 317439	<i>V. lecanii</i>	Citrus scale (Coccidae: Homoptera)	Jamaica 1987
51. IMI 317440	<i>V. lecanii</i>	Coffee scale (Coccidae: Homoptera)	Jamaica 1987
53. IMI 304801	<i>V. lecanii</i>	Scale insect (Coccidae: Homoptera)	Dominica 1986
54. IMI 304805	<i>Verticillium</i> sp.	Bamboo scale	Dominica 1986
55. IMI 304806	<i>V. lecanii</i>	Citrus scale (Coccidae: Homoptera)	Dominica 1986
56. IMI 304807	<i>V. lecanii</i> state of <i>T. confragosa</i>	Coffee scale (Coccidae: Homoptera)	Dominica 1986
57. IMI 304809	<i>V. lecanii</i>	Homopteran	Dominica 1986
58. IMI 331569	<i>Verticillium</i> sp.	Armoured scale (Diaspididae: Homoptera)	Kenya 1986
59. IMI 331593	<i>V. lecanii</i>	Forest aphid (Aphididae: Homoptera)	Ecuador 1976
61. IMI 331563	<i>V. hemipterigenum</i> Petch	Bamboo scale (Coccidae: Homoptera)	Trinidad 1985
62. IMI 62464	<i>V. alboarum</i> Reinke & Berthold	<i>Humulus lupulus</i> (Hops, Cannabidaceae)	UK 1955
63. IMI 172738	<i>V. alboarum</i>	<i>Lycopersicon esculentum</i> (Tomato, Solanaceae)	UK 1971
64. IMI 90246	<i>V. psalliotae</i> Treschow	Unidentified rust	India 1961
65. IMI 331549	<i>Verticillium</i> sp. state of <i>Torrubiella</i> sp.	Moth pupa (Lepidoptera)	Indonesia 1985
67. IMI 338017	<i>Verticillium</i> sp. state of <i>Cordyceps</i> sp.	Beetle larva (Coleoptera)	Ecuador 1974
68. IMI 331576	<i>Verticillium</i> sp. state of <i>Cordyceps</i> sp.	Bagworm larva (Psychidae: Lepidoptera)	Ecuador 1975
69. IMI 338018	<i>Verticillium</i> sp. state of <i>Cordyceps caloceroides</i> Berk. & Curt.	Spider (Araneida: Arachnida)	Brazil 1978
70. IMI 331582	<i>Verticillium</i> sp. state of <i>Cordyceps</i> sp.	Beetle larva (Elateridae: Coleoptera)	Brazil 1978
71. IMI 331591	<i>Verticillium</i> sp.	Spider (Araneida: Arachnida)	Ecuador 1974
72. IMI 338019	<i>Verticillium</i> sp. state of <i>Cordyceps</i>	Spider (Araneida: Arachnida)	Brazil 1979
73. IMI 331580	<i>Verticillium</i> sp. state of <i>Cordyceps</i> sp.	Bagworm larva (Psychidae: Lepidoptera)	Brazil 1978

* Isolates separated on basis of secondary metabolites produced.

temperatures (15, 20, 25, 30 and 37 °C) on MA (Smith & Onions, 1983) was also tested. Growth with sodium nitrite was assessed on nitrite/sucrose agar (Frisvad, 1981). Growth and pigmentation on media containing ammonium oxalate and mannitol were evaluated as previously described (Bridge *et al.*, 1986; Brayford & Bridge, 1989). The ability to grow in the presence of crystal violet (0.04 and 0.2 g l⁻¹) and CuSO₄ (0.5 g l⁻¹) was tested on Czapek agar (CZ). The inhibitors were added to the autoclaved medium through a sterilization filter (0.45 µm pore size). Production of the extracellular enzymes β-glucosidase, β-galactosidase, N-acetyl-β-D-glucosaminidase and chitinobiosidase was determined in culture fluid from 9-d-old cultures grown on glucose-yeast extract medium (GYM) (Mugnai *et al.*, 1989), with 4-methylumbelliferone-substituted substrates (Barth & Bridge, 1989).

Catalase isoenzymes were detected by electrophoresis from cell-free extracts of 55 representative isolates, prepared as described by Mugnai *et al.* (1989). Electrophoresis was carried out on samples containing 50 µg protein in 1 mm thick, 80 × 90 mm, vertical, 8.2% (w/v) acrylamide gels with a Tris-citrate/Tris-glycine buffer system (Mugnai *et al.*, 1989). Catalase activity was visualized by soaking gels in hydrogen peroxide and staining in a ferric chloride/potassium ferricyanide mixture (Woodbury *et al.*, 1971).

Data analysis. All characterization tests were performed in duplicate; further tests were undertaken in the case of discrepant results. A basic three-point coding system of 0 for negative, 2 for positive and 1 for weak results (see footnote, Table 2) was used for most characters. Tween 80 hydrolysis results, recorded at 7 and 14 d, growth and pigmentation on nitrite agar, and the two concentrations of crystal violet were each combined to give five-point quantitative scales. Similarities between isolates were calculated using Gower's coefficient,

negative cross-matches being excluded, and dendrograms were derived by unweighted pair group method analysis (UPGMA), average linkage-clustering (Sneath & Sokal, 1973). All computations were carried out with the MINIPAC numerical taxonomy package on an Amstrad PCW8512 microcomputer (Mugnai *et al.*, 1989).

Results

The clusters obtained by analysing all the morphological and physiological data (41 characters) in dendrogram form are shown as groups in Tables 2 and 3. All isolates were able to hydrolyse aesculin and Tween 80, and were able to grow on mannitol, lactic and citric acids; none grew at 37 °C. These characters were therefore excluded from the analysis. Similarly, growth data at 20 °C and 25 °C showed no significant differences and were not included.

Six cluster groups can be delimited from the dendrogram (Fig. 1) based on 21 morphological and 20 physiological characters (Table 2): group I, *V. lecanii* isolates (7) from insects, mostly Aphididae; group II, rust hyperparasites (3), including one isolate of *V. psalliotae*; group III, isolates (18) of *V. lecanii* mainly from insects and mainly from the UK; group IV, *V. lecanii* isolates (7)

Table 2. Numbers of isolates positive for each attribute in cluster groups

Character	No. of isolates positive*					
	I (7)	II (3)	Cluster group (and no. of isolates)		V (11)	VI (2)
			III (18)	IV (7)		
Conidia subglobose	0	0	0	7	0	0
Conidia ellipsoidal	3	3	18	7	11	2
Conidia cylindrical-clavate	7	3	18	7	11	2
Conidia fusiform-acerose	0	0	0	0	0	0
Conidia curved	0	3	0	0	0	0
Conidia formed in heads	7	3	18	7	11	2
Dark resting mycelium	0	0	0	0	0	2
Chlamydospores	0	0	0	0	0	0
<i>Cordyceps</i> teleomorph	0	0	0	0	0	0
<i>Torrubiella</i> teleomorph	1	0	0	5	5	0
Colony cottony, white	6	3	18	7	0	2
Colony cream-yellow	2	0	1	0	11	0
Colony vinaceous	0	0	0	0	0	0
Reverse pigmented	2	2	2	3	8	2
Colony height† (A, B, C)	6 (B)	3 (C)	17 (B)	5 (C)	11 (A)	2 (C)
Conidia width small (<1.4 µm)	0	0	18	7	11	0
Conidia width medium (1.4–2 µm)	7	3	0	0	0	2
Conidia width large (>2 µm)	0	0	0	0	0	0
Conidia length small (<3 µm)	0	0	0	6	0	0
Conidia length medium (3–5 µm)	5	2	18	7	11	2
Conidia length large (>5 µm)	5	1	0	0	0	0
Urease	6	3	18	7	8	2
Growth on nitrite agar	7	3	18	7	10	2
Gelatinase	0	3	4	6	1	0
Yellow pigment on CZNH ₄ agar	0	0	5	2	11	0
Dark pigment on CZNH ₄ agar	0	1	0	1	0	0
Growth on G ₂₅ N agar	1	1	7	2	1	0
Tolerance of crystal violet‡	6	2	17	1	2	0
Tolerance of copper sulphate	7	3	11	7	3	0
Yellow pigment on mannitol agar	2	1	0	3	11	0
Pink pigment on mannitol agar	0	0	0	3	0	0
β-Glucosidase	1	0	0	0	2	0
β-Galactosidase	1	0	0	0	0	0
N-Acetyl-β-D-glucosaminidase	7	3	18	7	11	0
Chitobiosidase	7	2	18	0	11	2
RNAase	7	3	18	7	9	2
Colonies§ small (<15 mm) at 15 °C	0	0	0	0	2	0
Colonies§ medium (15–30 mm) at 15 °C	7	3	16	7	10	2
Colonies§ large (>30 mm) at 15 °C	0	0	2	0	0	2
Colonies§ small (<22.5 mm) at 30 °C	7	3	17	6	11	2
Colonies§ large (>22.5 mm) at 30 °C	0	1	1	1	0	0

* Results of 1 (weakly positive) included as positive results.

† Colony height graded according to dominant type: A, low, appressed; B, not appressed, weakly floccose; C, strongly floccose.

‡ Five-point scale based on both concentrations (see Methods).

§ On malt agar.

from coffee rust; group V, *V. lecanii* isolates (11) from insects, predominantly tropical scale insects; and group VI, two isolates of *V. alboatrum*. The plant-pathogenic isolates of group VI can be separated from other isolates by the absence of *N*-acetyl-β-D-glucosaminidase, β-glucosidase, β-galactosidase and gelatinase activity. The failure of group VI isolates to grow on G₂₅N, and to tolerate crystal violet and CuSO₄ also indicates that *V. alboatrum* is physiologically distinct from the other *Verticillium* species examined. Morphologically, this

species can readily be distinguished from the other isolates on the basis of the dark resting mycelium produced in culture. Mycelial form and colour also distinguish *V. lecanii* isolates of group V (ex tropical Coccidae) from those of groups III (ex mainly European insects) and IV (ex coffee rust): colonies of groups III and IV isolates are white and cottony; colonies of group V isolates are citron yellow, compact and appressed. Significantly, none of the isolates from coffee rust had chitobiosidase activity in contrast to the majority of

Table 3. Characteristics of ungrouped isolates

Character	Isolate no.															
	54	14	72	45	46	42	67	71	25	61	65	68	73	69	70	58
Conidia subglobose	—	+	—	—	+	—	+	—	—	—	—	—	—	—	+	—
Conidia ellipsoidal	+	+	—	+	+	+	—	+	—	—	—	—	—	—	—	+
Conidia cylindrical-clavate	+	+	+	+	—	+	—	—	+	—	+	+	+	+	+	+
Conidia fusiform-acerose	+	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+
Conidia curved	+	—	—	—	—	—	—	—	+	+	+	—	—	—	—	+
Conidia formed in heads	+	+	—	+	+	+	—	+	+	—	+	—	—	—	+	+
Dark resting mycelium	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chlamydospores	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—
Cordyceps teleomorph	—	—	+	—	+	—	+	+	—	—	—	+	+	+	+	—
Torrubiella teleomorph	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	+
Colony cottony, white	+	+	+	+	+	+	+	+	—	+	+	+	+	—	+	—
Colony cream-yellow	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Colony vinaceous	—	—	—	—	—	—	—	—	+	—	—	+	+	—	—	—
Reverse pigmented	—	—	—	—	+	—	—	—	+	—	—	+	+	+	+	+
Colony height† (A, B, C)	B	B	B	C	C	B	B	B	A	B	C	C	C	C	A	A
Conidia width small (<1.4 µm)	+	+	+	+	+	+	—	—	—	—	+	—	—	+	—	+
Conidia width medium (1.4–2 µm)	—	—	—	—	—	—	+	+	+	+	—	+	+	—	—	—
Conidia width large (>2 µm)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
Conidia length small (<3 µm)	—	+	—	—	+	—	+	+	—	—	—	—	—	—	+	—
Conidia length medium (3–5 µm)	+	+	+	—	+	+	—	—	—	—	—	—	—	—	—	+
Conidia length large (>5 µm)	—	—	—	+	—	—	—	—	+	+	+	+	+	+	—	—
Urease	—	+	+	—	+	+	+	+	+	+	+	—	—	+	+	—
Growth on nitrite agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase	—	+	+	—	—	+	—	+	—	—	—	—	—	—	—	—
Yellow pigment CZNH4 agar	—	—	+	—	—	—	—	—	—	—	—	—	—	+	—	+
Dark pigment on CZNH4 agar	—	—	—	—	—	—	+	—	+	+	+	+	+	—	—	—
Growth on G ₂₅ N agar	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Tolerance of crystal violet†	—	—	+	+	—	—	+	+	—	—	—	+	+	—	—	+
Tolerance of copper sulphate	+	+	+	+	+	—	+	+	—	—	—	—	—	—	—	—
Yellow pigment on mannitol agar	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+
Pink pigment on mannitol agar	—	—	—	+	+	—	+	—	+	—	+	+	+	—	—	—
β-Glucosidase	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—
β-Galactosidase	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+
N-Acetyl-β-D-glucosaminidase	+	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+
Chitinobiosidase	+	+	+	+	—	—	—	+	+	—	—	+	+	+	+	—
RNAase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Colonies§ small (<15 mm) at 15 °C	—	—	—	—	—	—	—	—	—	+	—	+	+	—	+	—
Colonies§ medium (15–30 mm) at 15 °C	+	—	—	+	+	+	+	+	+	—	+	—	—	+	—	+
Colonies§ large (>30 mm) at 15 °C	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Colonies§ small (<22.5 mm) at 30 °C	+	+	+	+	—	—	+	—	+	+	+	+	+	+	+	+
Colonies§ large (>22.5 mm) at 30 °C	—	—	—	—	+	+	—	+	—	—	—	—	—	—	—	—

Symbols refer to footnotes in Table 2.

isolates from insects (groups I, III, V). Sixteen of the isolates could not be grouped and their combinations of characters are shown in Table 3. Most of these isolates are undescribed but three isolates received as *V. lecanii* (14, 42, 45) are included in this category. A similar grouping pattern was obtained when catalase activity was analysed and twelve different bands were distinguished (Table 4). The electrophoretic banding patterns are represented schematically in Fig. 2 and there is a consistent distinction between *V. lecanii* isolates from insects and those from coffee rust, with the exception of isolate 33 from coffee rust in Colombia. This may be explained if isolate 33 originated from an endemic insect host and had adapted to the rust following its recent

arrival in the country (Fernandez *et al.*, 1986). Similarly, the two *V. alboatrum* isolates (62, 63) were distinctive in banding pattern, having a single band not shared by any other isolate. The ungrouped isolates listed in Table 3 can also be separated on the basis of their catalase activity. There are, however, some anomalous results, for example several isolates designated as *V. lecanii* (14, 42, 45) which do not fit into any of the relevant groups, as shown in the analysis of morphological and physiological characters. In addition, two other *V. lecanii* isolates (35, 44) fall within the *V. psalliotae* group and recent SEM studies of conidiogenesis have confirmed that they do not belong in the *V. lecanii* complex (H. C. Evans & Y. Jun, unpublished results).

Fig. 1. UPGMA dendrogram based on 41 morphological and physiological characters. Similarities derived from Gower's coefficient discounting matching negative results, showing grouped and ungrouped isolates.

Table 4. Numbers of isolates giving catalase isoenzyme bands

Cluster group*	Isolates included	Catalase bands											
		1	2	3	4	5	6	7	8	9	10	11	12
I	1, 7, 10, 17, 57, 59	2	0	0	0	6	0	0	0	0	6	0	0
II	35, 44, 64	3	0	0	0	3	0	0	0	3	0	0	0
III	3, 4, 5, 6, 12, 15, 16, 18, 19, 22, 23, 33, 38	7	0	0	0	13	0	0	0	0	13	0	0
IV	24, 27, 30, 31, 32, 36, 37	0	0	0	0	6	0	0	0	0	0	7	0
V	13, 20, 26, 28, 29, 33, 51, 53, 55	3	0	0	0	9	0	0	0	0	9	0	0
VI	62, 63	0	0	0	0	0	0	0	0	0	0	0	2
Ungrouped isolates	58	0	0	0	0	0	0	0	1	0	0	0	0
	54	0	1	0	0	0	0	0	0	1	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	1	0
	72	0	0	0	0	0	0	0	1	0	0	0	0
	45	0	0	0	0	1	0	0	0	0	1	0	0
	46	0	0	0	0	0	0	0	1	0	0	0	0
	42	0	0	0	0	0	1	0	0	0	0	0	0
	67	0	0	0	0	0	0	0	1	0	0	0	0
	71	0	0	0	0	0	0	1	0	0	0	0	0
	25	1	0	1	0	0	0	0	1	0	0	0	0
	61	0	0	1	0	0	0	0	1	0	0	0	0
	65	0	0	0	0	1	0	0	1	0	0	0	0

* Cluster groups as designated in Fig. 1.

Discussion

The results of the present study show that there are highly significant physiological as well as morphological differences between *V. alboatrum* and *Verticillium* species pertaining to the section *Prostrata*, which includes both entomopathogenic and fungicolous species. There is evidence that *V. alboatrum* may behave as a mycoparasite (Barron & Fletcher, 1970), having the ability to penetrate and grow within the conidiophores of other fungi. However, the type of mycoparasitism ascribed to *V. alboatrum* appears to differ from the lytic mechanism

Fig. 2. Schematic representation of electrophoretic data based on catalase activity of 55 representative strains of *Verticillium*.

exhibited by strains of *V. lecanii* hyperparasitic on rusts (Garcia Acha *et al.*, 1965). Moreover, fungal-lysing enzymes were absent from the plant-pathogenic *Verticillium* species tested, including *V. albo-atrum*. The data presented here demonstrate clear differences in enzyme composition and patterns between taxa of the section *Prostrata* and *V. albo-atrum*.

Isolates of groups I, III, IV and V all fall within the morphological concept of the *V. lecanii* complex. However, there are sufficient physiological and biochemical characters included in the study to demonstrate clear distinctions within this complex which correlate with host and geographical origin. Similar results were obtained when the physiological and biochemical characters of a range of isolates of *Beauveria bassiana* (Bals.) Vuill. were subjected to principal components analysis (Mugnai *et al.*, 1989). *B. bassiana* isolates from lepidopteran hosts, and to a lesser extent from hymenopteran hosts, clustered together whilst isolates on other hosts from the same collecting locality, as well as from other geographical regions, showed no grouping. The *V. lecanii* isolates from Coccidae (group V) are morphologically distinct, having short or appressed, yellow mycelia and compact phialides, whilst isolates belonging to groups I, III and IV produce abundant white cotton-like, aerial mycelium. The group II hyperparasites are also readily distinguished from the group IV coffee rust hyperparasites on enzyme characters, and these isolates are closer to *V. psalliotae*. This species has previously been reported as a mycoparasite of coffee rust (Lim & Nik, 1983) and the association of two *Verticillium* species with this rust has undoubtedly led to misidentifications in the past, as in the case of isolates 35 and 44. Whether or not these *V. lecanii* groups should be assigned separate taxonomic status, at the varietal or sub-species level, is open to debate but the grouping shown here clearly demonstrates the existence of subgroups within nomen species *V. lecanii* and probably explains why so many synonyms have appeared in the literature (Gams, 1971; Balazy, 1973; Brady, 1979).

The miscellaneous *Verticillium* isolates, including some designated as *V. lecanii*, predominantly from tropical arthropod hosts, proved to be ungrouped and all may represent separate taxa, the majority of which remain to be described (H. C. Evans & Y. Jun, unpublished results).

Isolates 25, 41 and 68, 73 do, in fact, cluster together (see Fig. 1), but the former pair are duplicates of the same isolate and the latter pair represent different geographical isolates from the same lepidopteran host. These results confirm the validity of the integrated or multidisciplinary approach adopted here and provide a reliable and reproducible method for identification of fungal taxa. Whilst Gams (1988) recognized that it would be

'highly desirable' to split the genus *Verticillium*, more physiological and biochemical characters, in particular enzyme patterns and DNA composition, will be required to support the case for generic separation of the entomopathogenic, fungicolous and plant-pathogenic sections.

The senior author would like to thank the Gesellschaft für Technische Zusammenarbeit (GTZ, Germany) for the provision of a training scholarship, and the Director of the International Mycological Institute for laboratory facilities.

References

- BALAZY, S. (1973). A review of entomopathogenic species of the genus *Cephalosporium* Corda (Mycota, Hyphomycetales). *Bulletin de la Société des Amis des Sciences et des Lettres de Poznan* **14**, 101–137.
- BARRON, G. L. & FLETCHER, J. T. (1970). *Verticillium albo-atrum* and *V. dahliae* as mycoparasites. *Canadian Journal of Botany* **48**, 1137–1139.
- BARTH, M. G. M. & BRIDGE, P. D. (1989). 4-Methylumbelliferyl substituted compounds as fluorogenic substrates for fungal extracellular enzymes. *Letters in Applied Microbiology* **9**, 177–179.
- BLAKEMAN, J. P. & FOKKEMA, N. J. (1982). Potential for biological control of plant diseases on the phylloplane. *Annual Review of Phytopathology* **20**, 167–192.
- BOOTH, C. (1971). Introduction to general methods. *Methods in Microbiology* **4**, 1–47.
- BRADY, B. H. K. (1979). *Verticillium lecanii*. *CMI Descriptions of Pathogenic Fungi and Bacteria* no. 610. Kew: Commonwealth Mycological Institute.
- BRAYFORD, D. & BRIDGE, P. D. (1989). Differentiation of *Fusarium oxysporum* from *Fusarium solani* by growth and pigmentation on media containing sugar alcohols. *Letters in Applied Microbiology* **9**, 9–12.
- BRIDGE, P. D. (1985). An evaluation of some physiological and biochemical methods as an aid to the characterization of species of *Penicillium* subsection *Fasciculata*. *Journal of General Microbiology* **131**, 1887–1895.
- BRIDGE, P. D. & SACKIN, M. J. (1991). Stability of classification of filamentous fungi under changes in character coding strategy. *Mycopathologia* (in the Press).
- BRIDGE, P. D., HAWKSWORTH, D. L., KOZAKIEWICZ, Z., ONIONS, A. H. S. & PATERSON, R. R. M. (1986). Morphological and biochemical variation in single isolates of *Penicillium*. *Transactions of the British Mycological Society* **87**, 389–396.
- BRIDGE, P. D., HAWKSWORTH, D. L., KOZAKIEWICZ, Z., ONIONS, A. H. S., PATERSON, R. R. M., SACKIN, M. J. & SNEATH, P. H. A. (1989). A reappraisal of the terverticillate penicillia using biochemical, physiological and morphological features. I. Numerical taxonomy. *Journal of General Microbiology* **135**, 2941–2966.
- CARMICHAEL, J. W., KENDRICK, W. B., CONNERS, I. L. & SINGER, L. (1980). *Genera of Hyphomycetes*. Edmonton: University of Alberta Press.
- EVANS, H. C. & SAMSON, R. A. (1982). Entomogenous fungi from the Galapagos Islands. *Canadian Journal of Botany* **60**, 2325–2333.
- EVANS, H. C. & SAMSON, R. A. (1986). The genus *Verticillium*: taxonomic problems in species with invertebrate hosts. In *Fundamental and Applied Aspects of Invertebrate Pathology*, pp. 186–189. Edited by R. A. Samson, J. M. Vlak and D. Peters. Wageningen, Netherlands: Foundation of the Fourth International Colloquium of Invertebrate Pathology.
- FERNANDEZ, B., WALLER, J. M. & COOKMAN, G. P. (1986). Coffee leaf rust (*Hemileia vastatrix* Berk. and Br.) in Colombia. In *Proceedings of the British Crop Protection Conference 1986*, pp. 377–383. Thornton Heath, Surrey: British Crop Protection Council Publications.
- FRISVAD, J. C. (1981). Physiological criteria and mycotoxin production as aids in identification of common asymmetric penicillia. *Applied and Environmental Microbiology* **41**, 568–579.

- GAMS, W. (1971). *Cephalosporium-artige Schimmelpilze (Hyphomycetes)*. Stuttgart: G. Fischer.
- GAMS, W. (1988). A contribution to the knowledge of nematophagous species of *Verticillium*. *Netherlands Journal of Plant Pathology* **94**, 123–148.
- GAMS, W. & VAN ZAAYEN, A. (1982). Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. *Netherlands Journal of Plant Pathology* **88**, 57–78.
- GARCIA ACHA, I., LEAL, J. A. & VILLANEUVA, J. R. (1965). Lysis of uredospore germ tubes of rusts by species of *Verticillium*. *Phytopathology* **55**, 40–42.
- LIM, T. K. & NIK, W. Z. (1983). Mycoparasitism of the coffee rust pathogen, *Hemileia vastatrix*, by *Verticillium psalliotae* in Malaysia. *Pertanika* **6**, 23–25.
- MUGNAI, L., BRIDGE, P. D. & EVANS, H. C. (1989). A chemotaxonomic evaluation of the genus *Beauveria*. *Mycological Research* **92**, 199–209.
- PETCH, T. (1925). Entomogenous fungi and their use in controlling insect pests. *Bulletin of the Department of Agriculture, Ceylon* no. 71. Colombo: Government Printer.
- PITT, J. I. (1980). *The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces*. London: Academic Press.
- QUINLAN, R. J. (1988). Use of fungi to control insects in glasshouses. In *Fungi in Biological Control Systems*, pp. 19–36. Edited by M. N. Burge. Manchester: Manchester University Press.
- SAMSON, R. A., EVANS, H. C. & LATGE, J. P. (1988). *Atlas of Entomopathogenic Fungi*. Berlin: Springer-Verlag.
- VAN DER SCHAAF, D. A., MALAIS, M. & RAVENSBERG, W. J. (1990). The use of *Verticillium lecanii* against whitefly and thrips in glasshouse vegetables in the Netherlands. In *Proceedings and Abstracts of the Sixth International Colloquium on Invertebrate Pathology and Microbial Control*, p. 391. Adelaide, Australia: Society for Invertebrate Pathology.
- SMITH, D. & ONIONS, A. H. S. (1983). *The Preservation and Maintenance of Living Fungi*. Kew: Commonwealth Mycological Institute.
- SNEATH, P. H. A. & SOKAL, R. R. (1973). *Numerical Taxonomy*. San Francisco: Freeman.
- SPENCER, D. M. (1980). Parasitism of carnation rust (*Uromyces dianthi*) by *Verticillium lecanii*. *Transactions of the British Mycological Society* **74**, 191–194.
- SRIVASTAVA, A. K., DEFAGO, G. & KERN, H. (1985). Hyperparasitism of *Puccinia horiana* and other microcyclic rusts. *Phytopathologische Zeitschrift* **114**, 73–78.
- UMA, N. U. & TAYLOR, G. S. (1987). Parasitism of leek rust urediniospores by four fungi. *Transactions of the British Mycological Society* **88**, 335–340.
- WOODBURY, W., SPENCER, A. K. & STAHMANN, M. A. (1971). An improved procedure using ferricyanide for detecting catalase isozymes. *Analytical Biochemistry* **44**, 301–305.