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ISOLATE DIFFERENCES IN *OLPIDIUM BRASSICAE*

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(With Plate 17 and 1 Text-figure)

Eight *Olpidium* isolates from lettuce and cabbage roots were examined for differences in morphology and pathogenicity. Although zoospore diameter, flagellum length and pathogenicity were relatively constant features of each isolate, they could not be used to group isolates into *species* or *formae speciales*. It was concluded that the fungi studied were strains of the same species, *O. brassicae*. Studies on multiflagellate zoospores showed that resting spores may arise by non-sexual processes.

The scant information supplied in Woronin's (1878) original report on *Olpidium* (*Chytridium*) *brassicae* (Wor.) Dang. led to the description of many isolates as separate species. Thus, forms which had stellate resting spores and zoosporangia which rarely produced exit-tubes were described as *Asterocystis radicis* De Wilde. and *O. radicicolum* De Wilde. (De Wildeman, 1893; Marchal, 1900), whereas forms which produced large numbers of exit-tubes were recently described by Sahtiyanci (1961) as *Pleotrachelus brassicae* (Wor.) Saht. and *P. virulentus* Saht. Sampson (1939) studied four isolates, from *Agrostis*, cabbage, cauliflower and swede. She reported wide variation in the size and frequency of exit-tubes and further showed that the shape of the resting spore depended on the nature of the mounting medium. For these reasons, she concluded that *O. brassicae*, *O. radicicolum* and *A. radicis* were synonyms. The original inadequate description of *Pleotrachelus* by Zopf (1884), and the variation in exit-tube frequency between the species of that genus, led Tomlinson & Garrett (1964) to the conclusion that *P. brassicae* and *P. virulentus* strains were of one variable species, *O. brassicae*.

It has been shown that *Olpidium* isolates can transmit the viruses causing tobacco necrosis (Teakle, 1960), lettuce big-vein (Tomlinson & Garrett, 1962) and tobacco stunt (Hiruki, 1965). The isolates used by the various workers were referred to the species *O. brassicae*, although it was noted by Tomlinson & Smith (1960) that the lettuce fungus would not infect cabbage. The variations between eight *Olpidium* isolates from lettuce and cabbage were studied, therefore, in order to decide whether any of them should be classified separately.

MATERIALS AND METHODS

The isolates studied were obtained from the roots of lettuce (at Heswall, Cheshire; Lower Langford, Somerset; Over Peover, Cheshire; and Wellesbourne, Warwick) and cabbage (at Birmingham and Welles-

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bourne). The Heswall lettuce isolate has been described previously (Tomlinson & Garrett, 1964).

Studies on the morphology of the isolates in roots were made using rhizodermal strips. Infected roots were incubated for 2 h at 30 °C in 0·05 M phthalate buffer (pH 4) containing 0·2 % pectinase. The rhizodermis (which contained the fungus) and the hypodermis separated from the cells of the cortex during incubation and were then mounted in water (for phase-contrast microscopy) or in lactophenol containing 0·1 % cotton blue.

Zoospores were examined after discharge into tap water and their nuclei and flagella were stained by Ledingham's method (McLean & Cook, 1952). Zoospores, killed by the addition of a trace of osmic acid to the suspension, were washed in distilled water and a drop of the suspension was dried on a clean microscope slide. The film was covered with a few drops of a stain containing crystal violet (1 part in 20,000) and basic fuchsin (1 part in 100,000) in water, allowed to dry slowly and then rinsed with distilled water to remove excess stain. The film was dried and examined by ordinary microscopy, or by phase-contrast microscopy using red light.

Zoospore diameters were determined by measuring recently killed zoospores mounted in water, whereas flagella were measured on phase-contrast photomicrographs of unstained zoospore films.

Table 1. *Dimensions* (μ) *of the structures formed by the isolates studied compared with those of* Olpidium brassicae *described by Sampson* (1939)

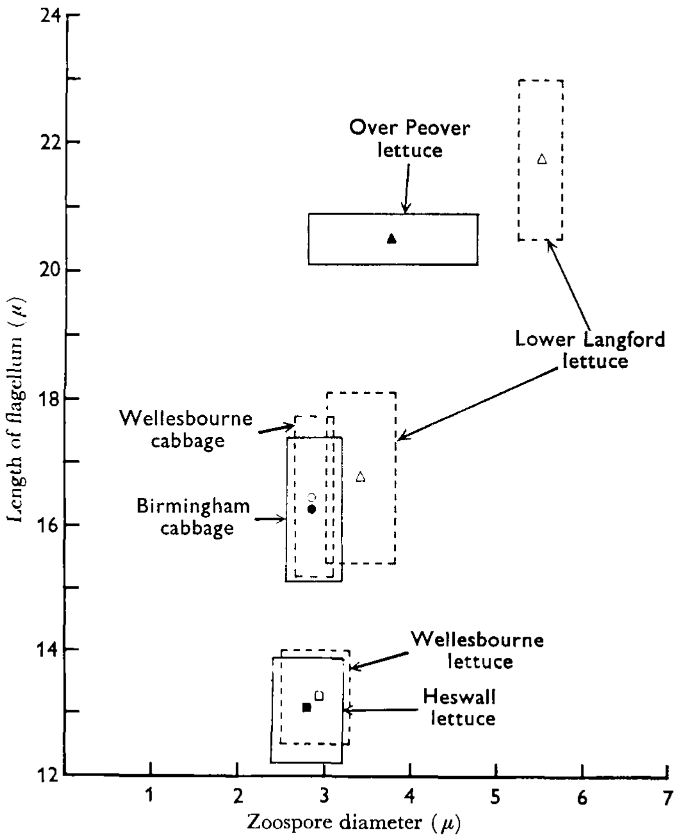
	Zoospores			Resting spores	
Isolate	Diameter	Length of flagellum	Zoosporangia and exit-tube frequency	Mean diam.	Range
Heswall (lettuce)	2·8 ±0·4*	13·1 ±0·9*	16×16 to 27×92, exit-tubes uncommon	17·1	13–24
Wellesbourne (lettuce)	2·9 ±0·5	13·2 ±0·8	10×10 to 30×88, exit-tubes uncommon	16·9	12–31
Lower Langford (lettuce A)	3·5 ±0·4	16·8 ±1·3	16×16 to 20×83, exit-tubes uncommon	18·0	10–29
(lettuce B)	5·6 ±0·5	21·8 ±1·2	15×15 to 24×98, exit-tubes uncommon	22·6	15–34
Over Peover (lettuce)	3·7 ±0·7	20·5 ±0·4	14×14 to 25×134, exit-tubes common	17·7	10–23
Birmingham (cabbage)	2·8 ±0·4	16·2 ±1·1	14×14 to 25×124, exit-tubes common	16·1	10–29
Wellesbourne (cabbage)	2·8 ±0·2	16·3 ±1·3	10×10 to 20×87, exit-tubes uncommon	18·4	12–28
O. brassicae	3	17	12×12 to 45×220, exit-tubes common, usually 1–3 per zoosporangium	17	8–30

* Standard error.

MORPHOLOGY

There were only small differences in morphology between the isolates studied (Table 1).

Because Sahtiyanci's isolates possessed numerous exit-tubes she identified them as *Pleotrachelus* spp. In the present study numerous mature zoosporangia of each isolate were observed (i.e. those which had discharged their zoosporangia or were about to do so). Exit-tubes were found on only



Text-fig. 1. The mean zoospore diameters of four lettuce and two cabbage isolates of *Olpidium* plotted against their mean flagellum lengths. Standard errors of shaded symbols, —; of unshaded symbols, - - - .

approximately 10 % of the zoosporangia of the Over Peover and Birmingham isolates and never on more than 1 % of the zoosporangia of the other isolates. They usually occurred singly, but a few zoosporangia had two or occasionally three exit-tubes. The great majority of zoosporangia discharged their zoospores through a small pore in the zoosporangium wall.

The most distinctive character of each isolate was the size of the zoospore and the length of its flagellum. When these two measurements were plotted against each other (Text-fig. 1) it was found that the isolates could be separated into groups. Lettuce isolates from Heswall and Wellesbourne formed a group with small zoospores and short flagella, while those from Over Peover had larger zoospores with longer flagella. The Lower Langford isolate, however, produced zoospores of two sizes which had different flagellum lengths. By inoculating lettuce seedlings with a suspension containing low numbers of zoospores, some seedlings became infected by zoospores of one type only. Thus, two isolates were separated which produced zoospores of different sizes and flagellum lengths.

OCCURRENCE OF MULTIFLAGELLATE ZOOSPORES

The majority of the *Olpidium* zoospores seen in the present work possessed one flagellum which was attached to the nucleus by a thin cytoplasmic thread (Pl. 17, fig. 1). A small proportion of the zoospores (0·1 % or less) were biflagellate and contained two nuclei, each similarly connected to one of the flagella (Pl. 17, figs. 2, 3). Some biflagellate zoospores, however, had only one nucleus which was slightly larger than those of the uniflagellate zoospores and which was joined to both flagella (Pl. 17, figs. 4, 5). A few triflagellate zoospores were seen (Pl. 17, fig. 6) and these contained three nuclei each connected to one of the flagella.

No evidence was obtained that the multiflagellate zoospores represented sexual phases in the life cycles of the isolates studied. The proportion of such zoospores did not increase during the swimming time of the zoospores (1–2 h) or when this was artificially increased up to 20 h by the addition of 0·5 M proline (Teakle & Gold, 1964) to the suspension. Only once was fusion of two swimming zoospores observed, while on another occasion two zoospores were seen swimming together for $1\frac{1}{2}$ h, apparently united by a thread in a manner similar to that reported by Kole (1954) for the zoospores of *Spongospora subterranea* (Wallr.) Lagerh. Furthermore, there was no relationship between the proportion of multiflagellate zoospores in suspensions and the proportion of plasmodia (2–5 %) which developed into resting spores in recently infected roots.

PATHOGENICITY

The lettuce isolates infected the roots of eighteen of the thirty-six plant species tested, representing a wide range of wild and cultivated plants, but only one difference in pathogenicity was found between them. Thus, although the isolates from Over Peover and Lower Langford infected *Chrysanthemum* spp. (*C. coronarium* L., *C. morifolium* Ramot. and *C. segetum* L.) those from Heswall and Wellesbourne did not. The host range of the Heswall isolate has been described by Tomlinson & Garrett (1964).

All the isolates from lettuce failed to infect the roots of brussels sprout, cabbage and turnip. Conversely, the two cabbage isolates infected the roots of these plants but failed to infect lettuce. No other differences in pathogenicity between the lettuce and cabbage isolates were found and hosts common to all isolates included *Hypochaeris radicata* L., *Nicotiana glutinosa* L., *N. tabacum* L. (White Burley), *Pisum sativum* L. (Meteor), *Spinacea oleracea* L. (Elsom's 23) and *Tripleurospermum maritum* (L.) Koch. ssp. *inodorum* Hyl. ex Vaar. The results of some pathogencity studies are shown in Table 2 and are compared with those reported by other authors with different isolates.

Table 2. *The pathogenicity and zoospore size* (μ) *of* Olpidium *isolates*

		Pathogenicity to				Zoospores	
Original host	Isolate	Lettuce	Cabbage	Spinach	Chrysanthemum	Mean diam.	Flagellum length
Lettuce	Heswall, Cheshire	+	–	+	–	2·8	13
	Wellesbourne, Warwick	+	–	+	–	2·8	13
	Lower Langford—isolate A	+	–	+	+	3·5	17
	Lower Langford—isolate B	+	–	+	+	5·6	22
	Over Peover, Cheshire	+	–	+	+	3·7	20
	Sahtiyanci (1961)	+	(1)	+	.	4	21
	Mowat (1963)	+	–	+	.	.	.
	Rich (1960)	+	.	–	.	.	.
	Marchal (1900)	+	–	.	.	.	.
Cabbage	Birmingham	–	+	+	–	2·8	16
	Wellesbourne	–	+	+	–	2·8	16
	Sahtiyanci (1961)	–	+	+	.	4	21
	Mowat (1963)	–	+	+	.	.	.
	Jacobsen (1943)	+	+	.	.	3–4	18
	Grogan *et al* (1958)	(2)	+	.	.	.	.
	Marchal (1900)	+	+	.	.	.	.

(1) Sahtiyanci (1961) described an *Olpidium* isolate which reproduced abundantly in lettuce but only weakly on cabbage.
(2) The inoculum used was broccoli roots from a lettuce-growing region. The possibility that the roots were externally contaminated by lettuce *Olpidium* resting spores cannot therefore be excluded.

All lettuce isolates used in the present study were either carrying lettuce big-vein virus when found or were able to transmit the virus but, because a host common to both the virus and the cabage isolates is not known, it was not possible to test cabbage isolates as vectors of the virus.

DISCUSSION

The *Olpidium* isolates studied closely resembled *O. brassicae*, as redescribed by Sampson (1939), but some differences in pathogenicity and morphology were found between them.

The main differences in pathogenicity are the failure of lettuce isolates to infect cabbage and the failure of cabbage isolates to infect lettuce. The inadvisability of separating isolates into *formae speciales* based on this character has already been discussed (Tomlinson & Garrett, 1964). Marchal (1900) and Jacobsen (1943) reported cabbage isolates which infected lettuce roots, and Sahtiyanci (1961) described a lettuce isolate which infected but failed to survive in the roots of cabbage. Furthermore, the classification of isolates according to their ability to infect and multiply in the roots of lettuce or cabbage would involve the grouping together of isolates which differed greatly in zoospore morphology (Table 2).

As shown in Text-fig. 1, it was possible to separate isolates according to their zoospore diameters and flagellum lengths. It is not yet known, however, if this character can be used to group *Olpidium* isolates, since the number studied was small and there was no evidence that zoospore morphology could be correlated with other morphological characters. There was, however, some evidence that pathogenicity and zoospore morphology could be combined to characterize particular isolates. Thus, the lettuce isolates known to infect spinach and *Chrysanthemum* had zoospore diameters and flagellum lengths significantly greater than those of lettuce isolates which did not infect *Chrysanthemum.* Furthermore, with the exception of the Lower Langford isolate with small zoospores, lettuce isolates differed from cabbage isolates in zoospore morphology. As shown in Table 2, however, Sahtiyanci and Jacobsen each described cabbage isolates with large zoospores.

Thus, although the isolates have certain distinctive features, no evidence has been found to justify their separation into distinct *species* or *formae speciales* and they are best regarded, therefore, as different strains of *O. brassicae.*

The studies on multiflagellate zoospores suggested that the development of resting spores in these isolates was not necessarily dependent on the prior fusion of swimming zoospores as gametes. Although the process of sexual reproduction of most chytrids is as yet unknown, it has occasionally been reported that resting spores developed from thalli formed by the fusion of two zoospores (Kusano, 1912; Sparrow, 1960). Sparrow also reports, however, that in some chytrid species resting spores, indistinguishable from those formed sexually, may arise by non-sexual processes.

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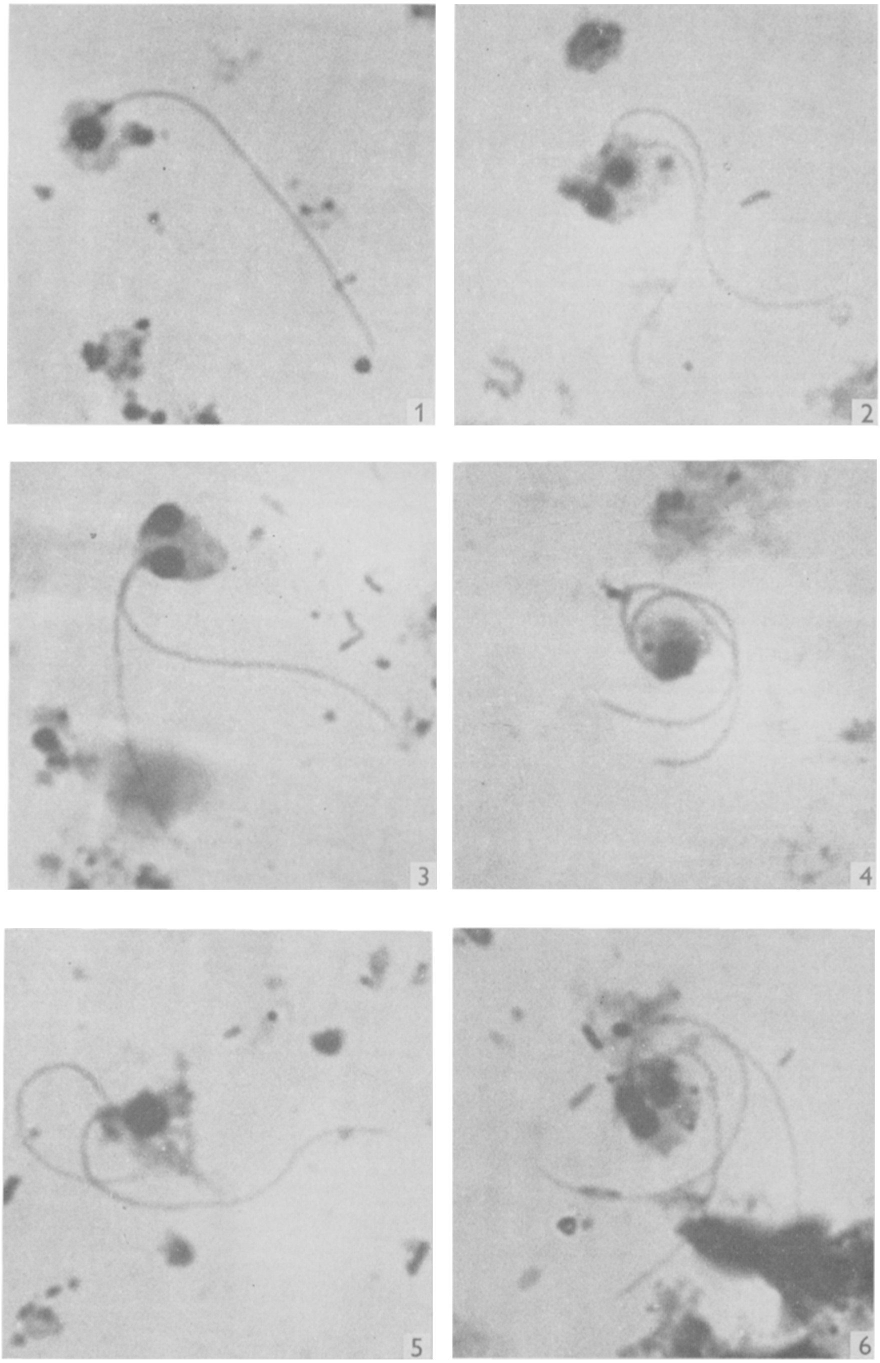

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EXPLANATION OF PLATE 17

Multiflagellate zoospores of *Olpidium brassicae* from Heswall stained by Ledingham's method. Phase-contrast photomicrographs made using red light (×4,300)

Fig. 1. A uniflagellate zoospore with one nucleus.

Figs. 2, 3. Biflagellate zoospores each with two nuclei.

Figs. 4, 5. Biflagellate zoospores each with one nucleus.

Fig. 6. A triflagellate zoospore with three nuclei.

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