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Survival of endoconidia of Thielaviopsis basicola in soil

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Abstract

Lysis and viability of endoconidia of *Thielaviopsis basicola* artificially introduced into clay loam and sandy soil were microscopically examined at intervals. Most of the endoconidia remained morphologically unchanged in moist clay loam for more than one year, while in moist sandy soil all had disappeared by lysis within 4 months. One percent of the endoconidia that persisted for 15 months in clay loam germinated when tested with V–8 juice. Endoconidia transformed into chlamydospore-like structures when incubated in phosphate buffer solutions supplemented with glucose and asparagine but not in those without these compounds. The significance of these phenomena for the survival of the pathogen in soil is discussed.

Introduction

Chlamydospores of *Thielaviopsis basicola* (Berk. et Br.) Ferr. have for a long time been considered as the main structures with which this soil-borne pathogen survives in natural soil (Tsao and Bricker, 1966). Until recently the conidia were supposed to originate secondary infections soon after their formation but to have little importance in perpetuating *T. basicola* from year to year (Mathre and Ravenscroft, 1966). In 1967 Linderman and Toussoun found that some endoconidia of *T. basicola* isolated from diseased cotton and tobacco remained viable in natural soil for at least 7 months. Papavizas (1968) noticed that the great majority of endoconidia of *G. basicola* causing root rot of bean perished in soil within 1–4 weeks, but that a small number remained viable and morphologically unchanged for as long as 10 months. This paper presents data on lysis and viability of endoconidia of *T. basicola* isolated from diseased *Lathyrus odoratus* L. incubated in non-sterilized soils and on their behaviour in liquid media in relation to survival of the fungus in natural soils.

Materials and methods

The strain of *T. basicola* used, was isolated from roots of diseased *Lathyrus odoratus* and cultured on potato dextrose agar (PDA). Pure endoconidia were obtained from 7-day-old cultures before chlamydospores had formed. The endoconidia were washed from the agar slants and centrifuged twice at 10.000 g for 10 min. in distilled water. The soils used were a clay loam (pH 7.9–8.2) from the North East Polder and a sandy soil (pH 7.2) from the horticultural gardens "Canton Park" in Baarn. For preparing "endoconidial soil", washed endoconidia were thoroughly mixed through 100 g of clay loam or 100 g of sandy soil. The water contents of these soils was then lowered

by evaporation to the original water contents of approximately 20% determined by oven-drying samples at 105 °C and weighing. Five to ten endoconidia could usually be found in thin smears of these soils at one high power field × 450. Lysis and germination of endoconidia were studied by microscopic examination of soil smears stained with 0,1% acid fuchsin in 85% lactophenol. The percentages of endoconidia that lysed during incubation in soils were roughly estimated at intervals by relating the numbers of morphologically unchanged endoconidia present in smears of the soil per high power field to the numbers of endoconidia originally present per high power field at the first day of incubation. The percentages germinative endoconidia were estimated by examining 500 endoconidia in soil smears 24 h after 0.2 ml of carrot root extract (Mathre and Ravenscroft, 1966) or 0.2 ml of 30% V-8 juice had been added to 0.5 g samples of the soil on oven-dry-weight basis.

Experiments and results

Lysis and viability of endoconidia in non-sterilized soils

Approximately 50 g of clay loam and 50 g of sandy soil, both artifically infested with endoconidia were allowed to dry in the air, while two other 50 g samples of each "endoconidia soil" were kept at a 20% water contents with tap-water. The soil samples were incubated in glass vials at 20 °C. The percentage lysed and germinative endoconidia in these soils was estimated at intervals (Table 1). In moist and dry clay loam and in

Table 1. Lysis and germination of endoconidia of *T. basicola* after various periods of incubation in moist clay loam and moist sandy soil and in clay loam and sandy soil that were allowed to dry in the air. The figures represent the percentages lysed endoconidia, while those between brackets represent the percentages endoconidia that had germinated 24 h after carrot root extract or V–8 juice* had been added to small samples of the soils.

Incubation period in days	Soil type							
	air-dry				moist			
	clay loam		sandy soil		clay loam		sandy soil	
6	0	(3)	0	(6)	0	(3)	0	(7)
14	2	(4)	3	(7)	4	(4)	13	(4)
19	3	(2)	2	(1)	6	(5)	8	(1)
26	4	(2)	4	(0)	9	(2)	48	(0)
60	10	(1)	10	(2)	10	(3)	75	(1)
95	10	(1)	20	(1)	20	(1)	90	(0)
125	20	(1)	30	(1)	20	(1)	100	(0)
185	20	(0)	30	(0)	30	(0)	100	(0)
305	20	(0)	30	(0)*	30	(8)*	100	(0)*
457	–	–	–	–	90	(1)*	–	–

Tabel 1. Lysis en kieming van endoconidiën van T. basicola op verschillende tijdstippen gedurende de incubatie in vochtige klei en vochtige zandgrond en in klei en zandgrond welke aan de lucht kon drogen. De cijfers geven de percentages gelyseerde endoconidiën weer, terwijl die tussen haakjes de percentages van de gekiemde endoconidiën weergeven 24 uur nadat extract van peenwortel of van V–8 sap aan monstertjes van de grond was toegediend.*

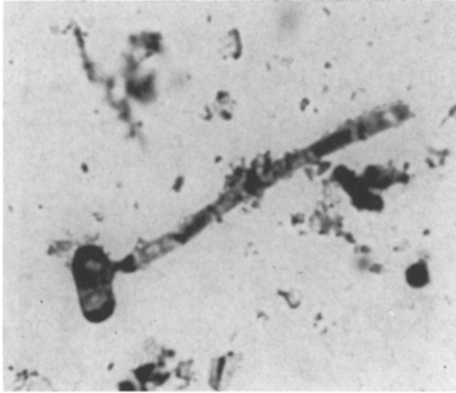

Fig. 1. Germinated endoconidium of *T. basicola* in non-sterile soil.

Fig. 1. Gekiemd endoconidium van T. basicola in niet-steriele grond.

air-dry sandy soil the percentages lysed endoconidia slowly increased but were still less than 30% after 305 days of incubation. More than 90% of the endoconidia had been lysed after 457 days of incubation. In moist sandy soil, however, nearly 50% of the endoconidia were lysed within 26 days, while after 125 days no endoconidia could be found anymore in this soil. From these data it may be concluded that lysis of endoconidia in non-sterilized soil highly depends on soil type and soil moisture contents. Endoconidia may stay morphologically unchanged in non-sterilized soil for at least 15 months. The percentage of endoconidia that germinated in soil when tested with carrot root extract varied between 3 and 7% in both moist and air-dry soils after 5 days of incubation (Fig. 1). It then dropped progessively to 1–3% within 60 days of incubation. Until 125 days, germinating endoconidia could be found in moist clay loam, and in air-dry clay loam and air-dry sandy soil though less than 1%. Although more than 70% of the original endoconidia remained morphologically unchanged in the soils, except moist sandy soil, not one germinated when tested with carrot root extract after 185 days of incubation. When tested with V-8 juice, however, 8% of the endoconidia germinated in moist clay loam, none in the other soils. After 457 days of incubation in moist clay loam still 1% of the endoconidia germinated when tested with V-8 juice. Similar results were obtained with a second identical experiment.

To test the persistence of endoconidia to low temperature in the field during the winter season, 100 g of sandy soil artificially infested with endoconidia were packed in nylon cloth and kept buried at 15–20 cm depth in soil in a field from February 12th, to April 1st. The temperature of the endoconidial soil in the field varied between –2°C to +5°C during this period. One percent of the non-lysed endoconidia germinated when tested with carrot root extract at the end of the incubation period in the field.

Transformation of endoconidia into chlamydospore-like structures

Stover (1950) reported the formation of thick-walled chlamydospore-like structures (secondary chlamydospores) from endoconidia in cultures of *T. basicola* on agar media. It is difficult to trace the occurrence of such structures in natural soil because factors controlling their formation are unknown. Their appearance on agar media suggests that nutrients have to be available. The need of nutrients for the formation of

chlamydospores-like structures therefore was studied by microscopical examination of endoconidia incubated in phosphate buffer solutions or in non-sterile soils, amended and non-amended with glucose and asparagine. Washed endoconidia were suspended at a concentration of 4×10^5 endoconidia per ml in phosphate buffer solutions pH 5 and pH 7 (0.03 m NaH_2PO_4, 0.03 m Na_2HPO_4) and in these buffer solutions supplemented with 0.01 m glucose and 0.01 m asparagine. Twenty-five ml of each solution with endoconidia were shaken in each of two Erlenmeyer flasks at 23 °C. After 25 days the shake cultures were examined. In a second and third experiment the cultures were examined after 10 and 25 days. In the third experiment the endoconidia were also incubated in phosphate buffer pH 7 supplemented with 0.001 m glucose and 0.001 m asparagine.

Endononidia that had transformed into globose structures with double cell-walls were found in phosphate solutions pH 5 and pH 7 supplemented with glucose and asparagine in all three experiments, but had not been formed in phosphate buffer solutions without these compounds. Data on the formation of these chlamydospore-like structures are given in Table 2, experiment I, II, III, but only for solutions buffered at pH 7.

The percentage of endoconidia that had turned into chlamydospore-like structures increased during the incubation period to a maximum of 54% after 25 days in exp. I. The increase was much less in exp. II and III and in buffer solutions amended with 0.001 m glucose and 0.001 m asparagine.

The formation of chlamydospore-like structures in phosphate buffer solutions pH 5 amended with glucose and asparagine was much less than in those of pH 7 and amounted at maximum to 6%, 1%, and 11% in exp. I, II and III, respectively after 25 days of incubation. The percentage germinating endoconidia varied in amended and non-amended buffer solutions (Table 2). The chlamydospore-like structures seem to be formed by the development of a new cell-wall around the somewhat enlarged cell contents inside the endoconidial cell wall (Fig. 2).

Table 2. Transformation of endoconidia of *T. basicola* into chlamydospore-like structures in phosphate buffer solutions pH 7, with and without glucose and asparagine. The figures represent the percentage of 500 endoconidia that transformed into chlamydospore-like structures, while those between brackets represent the percentage germinated endoconidia.

Buffer solution supplemented with:	Incubation period in days	Percentage chlamydospore-like structures					
		exp. I		exp. II		exp. III	
–	10	–	(–)	0	(13.5)	00	(23)
–	25	0	(0.5)	0	(20)	0	(19.5)
0.01 m glucose +	10	–	(–)	5	(0)	11	(5)
0.01 m asparagine		54	(0.5)	38	(0)	20.5	(14.5)
0.001 m glucose +	10	–	–	–	–	2	(14.5)
0.001 m asparagine		–	–	–	–	5.5	(26)

Tabel 2. Omvorming van endoconidiën van T. basicola in chlamydospore-achtige structuren in fosfaat bufferoplossingen pH 7, met en zonder glucose en asparagine. De cijfers geven de percentages weer van 500 onderzochte endoconidiën die zich tot chlamydospore-achtige structuren hebben omgevormd, terwijl die tussen haakjes de percentages gekiemde endoconidiën weergeven.

Fig. 2. Transformation of endoconidia of *T. basicola* into chlamydospore-like structures.

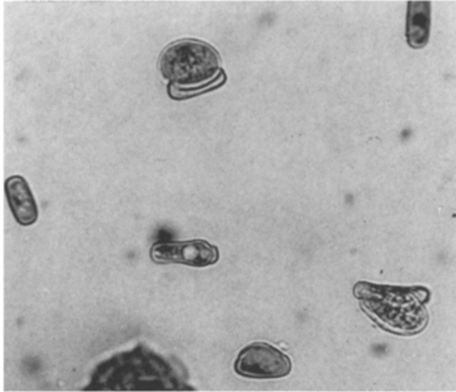

Fig. 2. Omvorming van endoconidia van T. basicola in chlamydospore-achtige structuren.

Fig. 3. Germinated chlamydospore-like structure formed from an endoconidium. The endoconidial cell wall remained attached to this structure.

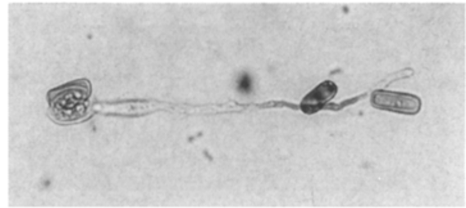

Fig. 3. Gekiemde chlamydospore-achtige structuur welke werd gevormd uit een endoconidium. De celwand van het endoconidium bleef met deze structuur verbonden.

They resemble the "secondary chlamydospores" as observed by Stover (1950) in cultures of *T. basicola* on agar media. Some of the thickwalled cells were found germinating (Fig. 3).

When sandy soil or clay loam artificially infested with endoconidia and non-infested sandy soil or clay loam were treated with 0.1 ml of a solution of 1 % glucose and 1 % asparagine in water per 0.5 g of soil on a dry weight basis at two-day intervals, a notable increase in numbers of chlamydospores took place in all soils within 12 days. The increase in non-infested soils which in most cases was much less than in infested soils, has to be ascribed to other chlamydospore-producing soil-fungi. Though most chlamydospores in the infested soils had probably been formed also by other chlamydospore-producing fungi, it should not be excluded that some originated from endoconidia of *T. basicola*.

Discussion

The differential decrease in numbers of endoconidia microscopically observed in moist and air-dry sandy soil and clay loam during an incubation period of 15 months, suggests that survival of endoconidia of *T. basicola* in soil depends on soil type and soil moisture contents.

One percent of the endoconidia that stayed morphologically unchanged for 15 months in moist clay loam at 20 °C, germinated when V-8 juice had been added to soil. Also 1 % of the endoconidia that persisted in sandy soil incubated in the field for two winter months germinated when tested with V-8 juice. These observations do presume that endoconidia may contribute to the survival of *T. basicola* in natural soil and in perpetuating this pathogen from one year to another. In nature, however, the chance for endoconidia to survive lytic activity may be much less, because lytic activity in the rhizosphere, the site where endoconidia are borne, is in general more intensive than in non-rhizosphere soil.

Endoconidia were transformed into viable globose thick-walled cells when incubated

in phosphate buffer amended with glucose and asparagine, but not in this buffer solution without these compounds. What chemical or physical stimulants activated the endoconidia to transformation is not yet clear. If the availability of a carbon and nitrogen source is an important prerequisite to the transformation of endoconidia into chlamydospore-like structures, one might expect such a transformation in the rhizosphere, the site where the endoconidia are formed in nature and where carbohydrates and nitrogen compounds are continuously released by roots. However, the formation of globose cells may very well be of osmotic origin.

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Samenvatting

Overleving van endoconidiën van Thielaviopsis basicola in grond

Kiemkracht en lysis van endoconidia van *Thielaviopsis basicola* gedurende een incubatieperiode van maximaal 15 maanden in luchtdroge en vochtige klei- en zandgrond werden microscopisch onderzocht. Het merendeel der endoconidiën in luchtdroge en vochtige kleigrond en in luchtdroge zandgrond onderging geen morfologische verandering gedurende de 10 maanden van incubatie, terwijl in vochtige zandgrond alle endoconidiën reeds binnen 4 maanden door lysis waren verdwenen. Eén procent van de endoconidiën bleek na een 15 maanden durende incubatie in vochtige kleigrond nog te kunnen kiemen. Van de endoconidiën die gedurende een verblijf van twee wintermaanden in zandgrond in het veld geen morfologische verandering ondergingen was eveneens nog 1 % kiemkrachtig. Deze waarnemingen doen vermoeden, dat endoconidiën mede verantwoordelijk kunnen zijn voor de overleving van *T. basicola* in natuurlijke grond. Omvorming van endoconidiën in chlamydospore-achtige structuren werd waargenomen in fosfaatbuffers waaraan glucose en asparagine was toegevoegd, echter niet in fosfaatbuffers zonder deze verbindingen. De vorming van deze structuren in natuurlijke grond kon niet worden aangetoond.

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