

インドにおいてマメ科植物のpurple leaf spotなどをおこすProtomyces 菌の生存

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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発行元	日本植物病理學會
巻/号	37巻4号
巻号補足	
掲載ページ	p. 242-248
発行年月	1971年9月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



Perpetuation of *Protomyces* Species Causing Purple Leaf Spot of Legumes in India

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M. P. HAWARE and M. S. PAVGI: インドにおいてマメ科植物の purple leaf spot
などをおこす *Protomyces* 菌について

Abstract

Protomyces patelii Pavgi and Thirumalachar causing angular black spot of *Phaseolus mungo* L. and *P. radiatus* L., and *Protomyces thirumalacharii* Pavgi causing purple leaf spot of *Sesbania grandiflora* Pers. are perpetuated from one season to another through desiccated but viable, heat-resistant chlamydospores in the host residue and those developed *in vitro* in the soil. The heat-resistant mycelium is later consumed in forming the resting chlamydospores in the soil, thus adding another dimension to the initial soil-borne inoculum potential of the disease.

(Received January 7, 1971)

Introduction

Angular black spot of black gram (*Phaseolus mungo* L.) and green gram (*Phaseolus radiatus* L.) caused by *Protomyces patelii* Pavgi and Thirumalachar⁵⁾ and purple leaf spot of 'Agasta' (*Sesbania grandiflora* Pers.) caused by *Protomyces thirumalacharii* Pavgi⁴⁾ are well distributed in the plains of India. The disease has been recurrently observed in severe form in the intensively cultivated fields, appearing in early August and is at the peak of its development during August-September. Its outbreak in Varanasi is associated with muggy, humid weather and medium high temperature (25 to 30°C). A monoculture of beans with an abundance of diseased residue from the previous crop maintains large quantities of initial inoculum, thereby causing increase in incidence of disease in Varanasi, Uttar Pradesh. Cultural practices like minimum tillage leave considerable debris in the surface soil exposed during early part of the crop season. Possibility of survival of chlamydospores released from the diseased crop debris and or developing on the saprobic mycelium in the soil through the high summer temperature (35 to 40°C) during May-June would provide an additional source of primary inoculum for the pathogen. Annual addition to the crop debris and free chlamydospores through intensive cultivation and the severity of incidence in the crop led to ascertain the mode of perpetuation of the 2 pathogenic species.

Materials and methods

Members of the genus *Protomyces* Magnus develop reddish brown, thick-walled, resistant chlamydospores on the intercellular mycelium within the host leaves. Infected leaves of *Phaseolus mungo* and *Sesbania grandiflora* were air-dried during October and stored in lots at 10 to 12°C, room temperature (24 to 26°C) and buried in the soil out of doors in February; chlamydospores

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drawn from these lots were tested for viability at regular intervals. Air-dried, infected leaf pieces bearing viable, mature chlamydospores of the 2 pathogens were buried separately at 1, 2 and 3 inch depths in fine sandy loam, potted soil from the infested fields and the pots were exposed out of doors. The chlamydospores from the 3 depths were separately sampled at monthly intervals from March onward and tested for germination by the method suggested by Thirumalachar *et al.*^{7,8)}, and percent germination was determined for each lot.

Surface soil samples from intensively cultivated fields with severe disease incidence were collected at random upto half inch depth and examined for presence and viability of the chlamydospores of both the species. Soils under the trees of *S. grandiflora* from 2 different locations were similarly screened for the presence of chlamydospores. Ten g soil was suspended in 50 ml distil. water, supernatant centrifuged and the sediment was examined microscopically; the chlamydospores fixed in smears were tested for viability by germinating them under condensed moisture at room temperature (24 to 26°C).

Protomyces patelii and *P. thirumalacharii* were cultured on potato dextrose agar (with 0.1% yeast extr.) by transferring vesicular contents of single germinating chlamydospores. Cultures of both the species contained mycelial growth. Films of mycelial smears on sterile cover glasses were desiccated over CaCl_2 under cover for 24 hr and exposed separately to increasing temperatures, cooled overnight, inverted over plated PDA medium and examined after 3 to 4 days for mycelial growth of the pathogen along the margin of the cover glass. Portions of a week old wet cultures similarly exposed to increasing temperatures were transferred onto fresh PDA slants for viability test.

The infection spots embedding mature, viable chlamydospores stored at room temperature were desiccated over CaCl_2 for 24 hr and exposed to the increasing temperatures for 10 min. Chlamydospores in wet condition (infection spots dipped in 2 ml distil. water in thin-walled tubes) were also exposed similarly. The chlamydospores from the 2 treated sets were teased out onto microslides and induced to germinate at room temperature^{7,8)}. The percent spore germination was recorded after 7 days incubation.

The organisms were also recovered in culture isolations from the field soil during July-November, where the disease was observed in severe form. Ten g air-dried soil from the surface layer to 1 inch depth was thoroughly mixed with 100 ml sterile distil. water, vigorously shaken for 2 min, serially diluted, plated over rose bengal agar (PDA+0.01% rose bengal) and incubated at 25°C. Colonies identical to earlier isolates from germinated chlamydospores were transferred to PDA and tested for pathogenicity on the respective host plants raised in isolation.

Observations

Onset of disease in the field

Surveys from mid-July through September for the time of appearance of initial symptoms of angular black spot of black and green grams and purple leaf spot of 'Agasta' (*Sesbania*) in the field indicated that 5% of bean plants had disease symptoms in the second week of August. The plants had only 1 to 3 lesions on the lower leaves; a few of the seedlings had single lesions on the upper leaflets, but none in the crown foliage. The symptoms on 'Agasta' appeared in early August on the lowermost leaves of seedlings. Such fields had a large amount of undecomposed debris on the soil, until 7 to 8 weeks before planting of host crops. Although most of the host debris was ploughed in the soil prior to planting, substantial amounts remained on the ground surface until early July. Most of the residue was decomposed at the time of seedling

emergence; the emerging seedlings came in contact with this soil-borne inoculum. Primary symptoms on the lowermost leaflets in contact with such soils indicated that the infested soil was the source of initial inoculum.

Oversummering and longevity of chlamydospores in soil

A low percent germination of chlamydospores was observed for 4 to 5 months from the infected leaves stored at room temperature (24 to 26°C), which were not exposed to any weathering conditions as in the field. There was a linear increase in the germination percentage in both the species, as the storage time advanced, upto 90% germination count after storage for 10 months. It seemed that the dormant chlamydospores required a rest period before attaining the highest germination potential. The percent germination gradually declined about a year after their development in the host tissues, but remained potential enough for the next year (2 years after development), should it have missed the crop host in the following season. Their viability continued even beyond 2 years, when observations on the germination count were discontinued. The continuous high germination count and a belated peak in percent germination of the chlamydospores are probably due to the protection given by the embedding host tissues against the weathering effects of environment. The chlamydospores stored at low temperature (10 to 12°C) remained viable for over 2 years and germinated similarly as those stored at room temperature (Fig. 1).

The chlamydospores embedded in the host tissues were buried in the potted soil at various depths simulating conditions in the field infested with infected host debris ploughed after harvest, as the infected host tissues usually remain partially buried in the ploughed soil. No chlamydospores were seen released from the host tissues in the soil, until about 8 months, although the tissues showed signs of disintegration in the same year. The percent germination count in these chlamydospores improved with passage of time until 8 months after burial, reaching the peak in germination during August-September. The differential rate of decomposition of the host tissues at varying depths appeared conditioned by O_2/CO_2 variations at these depths as well as effects of other chemical and biological factors. It declined steadily after 8 months to

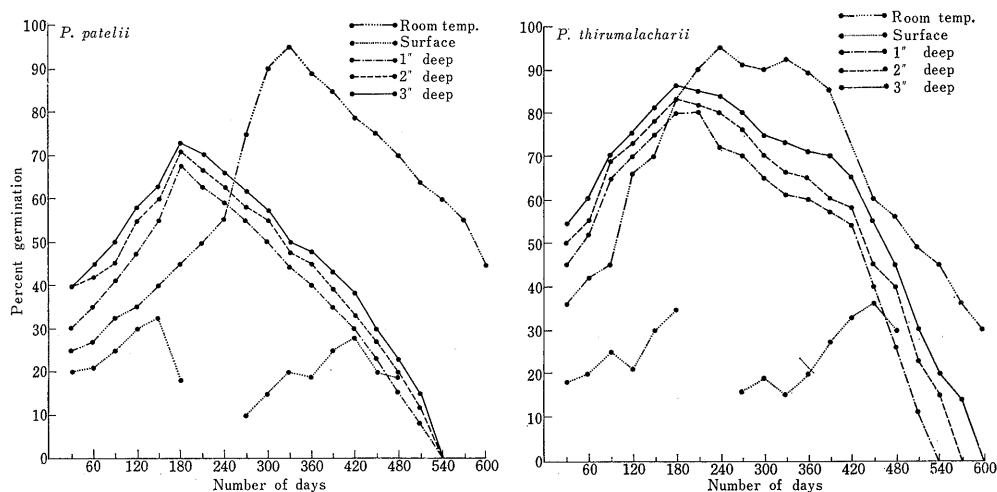


Fig. 1. Germination of chlamydospores of *Protomyces patelii* and *Protomyces thirumalacharii* (percent germination curves) stored at room temperature, from surface soil and at 1, 2 and 3" depths.

complete incapacitation of the chlamydospores to germinate further. A rest period appeared necessary before germination of the chlamydospores, but it did not interfere with the quantum of inoculum potential necessary to infect the host plants in the field. Depth of soil had no effect on retention of viability of the chlamydospores in both the species of *Protomyces*, although a slight numerical variation in the percent germination was observed at various depths (Fig. 1).

The diseased crop debris exposed to weathering in the field becomes desiccated and crisped into fragments, while the chlamydospores embedded in the host tissues remain partially protected from direct adverse effects. Dry and undecomposed infected host tissues were in abundance in the field till late July. Periodic examination of such a field soil heavily infested through intensive cultivation of the host crops showed presence of viable chlamydospores until through July (Fig. 1). None were observed during August-September, although empty exospores were seen in most of the soil samples. Their absence during these months indicated that viable chlamydospores on the soil surface germinated during the monsoon period living saprobially as mycelial colonies in the soil. Although the chlamydospores were spent out, the inoculum was actively present in the soil in the form of mycelial colonies. Recovery of chlamydospores from the soil though less again became possible from October onward, until a fresh charge of chlamydospores was added to the soil through the renewal of host debris. This will account for 2 sources of

Table 1. Heat resistance of mycelium in artificial culture of *Protomyces patelii*

Temp. °C	Dry					Wet				
	1	12	24	48	72 hr	1	12	24	48	72 hr
30-50	+	+	+	+	+	+	+	+	+	+
55	+	+	+	+	+	+	+	+	+	—
60	+	+	+	+	—	+	+	+	—	—
65	+	+	+	—	—	+	+	—	—	—
70	+	+	—	—	—	+	—	—	—	—
75	+	+	—	—	—	—	—	—	—	—
80	+	—	—	—	—	—	—	—	—	—
85	—	—	—	—	—	—	—	—	—	—

+ Viable; — Not viable.

Table 2. Heat resistance of mycelium in artificial culture of *Protomyces thirumalacharii*

Temp. °C	Dry					Wet				
	1	12	24	48	72 hr	1	12	24	48	72 hr
30-50	+	+	+	+	+	+	+	+	+	+
55	+	+	+	+	+	+	+	+	—	—
60	+	+	+	+	—	+	+	—	—	—
65	+	+	+	—	—	+	+	—	—	—
70	+	+	—	—	—	+	—	—	—	—
75	+	—	—	—	—	+	—	—	—	—
80	+	—	—	—	—	—	—	—	—	—
85	+	—	—	—	—	—	—	—	—	—
90	—	—	—	—	—	—	—	—	—	—

+ Viable; — Not viable.

primary inoculum, viz. a) the chlamydospores released from the remnant debris, and b) chlamydospores freshly developed *in vitro* by the mycelium in the soil.

Heat resistance of artificial cultures and chlamydospores

The vegetative mycelium of both the species of *Protomyces* remained resistant to desiccation and heat at 50°C for 72 hr and progressively lost the resistance with rise in temperature and fall in the period of exposure (Table 1, 2). Both the species showed less resistance to heat in wet cultures. They could not survive for long at higher temperatures beyond 70 to 75°C, but survived an exposure to 65°C for 12 hr. The heat resistance of vegetative hyphae and their isolation in culture during July–September from the infested field soil indicated possibility of their survival in the soil through viable vegetative hyphae desiccated during the summer months. The hyphal cells grow in the rainy season and come in contact with the respective hosts.

Development of conidia by *Protomyces thirumalacharii* in artificial culture ascertained their formation in the soil during the growth season. The conidia, developed on the soil surface, are shot off in air to infect the host leaflets. *In vitro* development of chlamydospores on vegetative mycelium in soil cultures has been observed in *P. thirumalacharii*⁶⁾. The vegetative mycelium while multiplying in soil during the rainy season, probably develops the chlamydospores similar to those developed *in vitro*, in the absence of favorable environment in the field, thus adding another dimension to the source of primary inoculum. Similar thick-walled, resistant bodies resembling the chlamydospores developed in the cultures of *P. patelii*, but they failed to mature further into functional (germinable), pigmented chlamydospores. In view of this, heat resistance of the chlamydospores developed in the leaflets was similarly tested by exposure to increasing temperatures.

The chlamydospores of *P. patelii* became devitalised at 94 and 92°C and those of *P. thirumalacharii* at 96 and 94°C under dry and wet conditions respectively. Survival of the chlamydospores was adversely affected by heat in the presence of moisture. They died much more rapidly in wet heat, showing a steep fall in germinability above 70°C in both the species (Fig. 2). The chlamydospores developed *in vitro* in artificial soil cultures also showed a similar trend of response to exposure to rising temperatures.

Recovery of fungous cultures from field soil

Protomyces patelii and *P. thirumalacharii* could be successfully isolated in culture from the field soil and from below the 'Agasta' (*Sesbania*) tree respectively during July–November. Obviously chlamydospore germination was initiated in soil with the onset of monsoon rains in

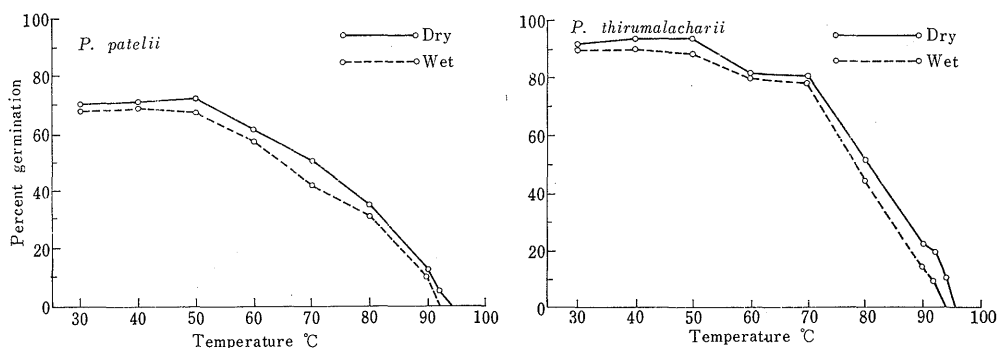


Fig. 2. Germination of chlamydospores of *Protomyces patelii* and *Protomyces thirumalacharii* (percent germination curves) heated for 10 min at increasing temperatures under dry and wet conditions.

July, which continued through September. Although colonies available during July were relatively few, they were available in abundance during August-September. Their continued recovery through November might be due to ability of the mycelium for resistance to desiccation and temperature of the environment. The mycelium was apparently consumed later for *in vitro* development of the chlamydospores and no isolates became available from December onward. The culture isolates were tested for pathogenicity from time to time on the seedlings of respective hosts raised in isolation; spray inoculations on the host seedlings (30 to 40 days old) yielded positive results in each case.

Discussion

The 2 pathogenic species of *Protomyces* causing angular black spot and purple leaf spot of *Phaseolus mungo* and *Sesbania grandiflora* are perpetuated through a variety of sources serving as the primary inoculum for the diseases. The primary inoculum provided is 3-dimensional, viz. 1) the chlamydospores obtained through the host debris from the previous season, 2) the vegetative saprobic mycelium remaining dormant in the soil, and 3) the chlamydospores developed *in vitro* in soil by the mycelium. All these stages are resistant to desiccation and high temperature of soil, prevailing in the northern plains of India in the tropical belt. The chlamydospores of both the species can survive direct exposure to high temperature for varying periods of time resembling conditions prevalent in Varanasi, Uttar Pradesh.

The inoculum potential is kept at a high level through annual additions of the infected debris in intensively cultivated fields. The thick-walled, dark pigmented resting chlamydospores remain protected in the embedding host tissues over a longer period, if not exposed directly to weathering effects of the environment. They show a maximum survival of 2 years, covering 2 off-season with potential pathogenicity on the host and saprobic multiplication in the soil. They, however, lose viability in a soil depth of 2 inch or more beyond the 2-year period. If retained undisturbed, they are likely to be surfaced again through reploughing, when they germinate normally for fresh infection. A 3-year crop rotation plan avoiding the host pulse crop may help better in the control in view of the limited host range or host specificity of *Protomyces* and devitalization of chlamydospores beyond 2 years under soil depth.

The endowed capacity to resist the effects of the desiccation and exposure to high temperature appears a common feature in these pathogenic fungi in the order Taphrinales. Earlier reports have demonstrated similar capacities for resistance by *Protomyces macrosporus* Unger (fam. Protomycetaceae)³⁾, *Taphrina deformans* (Berk.) Tul., *T. mirabilis* Atkins, and *T. maculans* Butler (fam. Taphrinaceae)^{1,2,9)}, all belonging to the order Taphrinales. The genus *Protomyces* allied to the genus *Protomyces* belongs to Protomycetaceae. Most of the species cited above are tropical or subtropical in distribution and appear well adapted to the high temperature conditions during the summer months in the tropics. A similar capacity for resistance in *T. deformans* is interesting in view of its wider distribution from the tropical to temperate regions.

Acknowledgement

The first author expresses his gratitude to the Council of Scientific and Industrial Research, New Delhi for the award of a Junior Research Fellowship.

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和文摘要

インドにおいてマメ科植物の purple leaf spot などをおこす *Protomycopsis* 菌の生存

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ケツルアズキ (*Phaseolus mungo* L.) およびブンドウ (*P. radiatus* L.) の angular black spot をおこす *Protomycopsis patelii* Pavgi and Thirumalachar と シロゴチャウ (*Sesbania grandiflora* Pers.) の purple leaf spot をおこす *Protomycopsis thirumalacharii* Pavgi は、寄主植物組織の残がいの中で乾燥した耐熱厚膜胞子および土の中で形成された厚膜胞子によって、次の作期まで生き残る。耐熱性の菌糸体はのちに土中で休眠厚膜胞子の形成のために使い尽くされる。