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Production and viability of *Peronospora parasitica* in radish

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Abstract. In *Peronospora parasitica* the inoculum load is found in the form of oospores in the leaf and seed tissues of radish. Out of 400 seeds tested, 10% showed the presence of oospores in the pericarp and 0·1% in the embryo. The 2,3,5-triphenyltetrazolium chloride test is a quick method of determining the viability of the oospores. Viability of oospores based on infection capacity after storage, though a long process, is effective and reliable. Results of *in vitro* and *in vivo* experiments show that the oospores need natural weathering, under field conditions, for a period of one year for maximum infection in radish and those stored for two years under the same conditions, has an adverse effect on their infection capacity. Infection capacity was higher among oospores exposed to weathering than those retained in laboratory.

Keywords. *Raphanus sativus*; *Peronospora parasitica*; oospores production; viability.

1. Introduction

Peronospora parasitica (Pers. ex. Fr.) Fr. causes downy mildew disease in *Raphanus sativus* L. (Baudys 1928). The inoculum occurs as mycelium in the host tissues (Baudys 1928; Ramsey *et al* 1954). However, the inoculum as oospores has not been documented in radish. The present study aims at unravelling details on the production of oospores and their viability.

2. Materials and methods

2.1 *Production of oospores*

Susceptible cultivar of radish (Japanese white) was sown in downy mildew nursery at Mysore. Infector rows were sown 2 weeks earlier to testar cultivars. When downy mildew disease appeared, oospores production was estimated at different stages viz., leaf, flowering and seed setting stages.

Maceration technique (Shetty *et al* 1978) was followed to detect the oospores in leaf tissues from the first pair to the tenth pair. The same technique was used to detect internally borne oospores in seeds (400 seeds were used for each treatment).

2.2 *Viability of oospores by triphenyltetrazolium chloride test*

Seeds and leaf tissues containing oospores were soaked in water for 12 h and then kept in different tubes containing 1% triphenyltetrazolium chloride (TTC) solution of pH 7. Such treated tissues were incubated at 30°C for 48 h in darkness and observed under the microscope. Based on the colour reaction, the oospores were

judged as viable or non-viable. Two susceptible cultivars were used to provide the oosporic materials at leaf, flowering and pod stages.

2.3 *Viability of oospores based on their age and percentage of infection after storage*

Dried powdered leaf tissues containing oospores were mixed with sterile garden soil and kept in dry small bags made of cheese cloth. Fifteen such bags were prepared, 5 of them retained in the laboratory, 5 of them kept in Downy Mildew Research Laboratory field on the surface of soil and the rest placed 15 cm below the soil level and covered by the same scooped soil. These bags were left as such for 1 and 2 years after which they were tested for viability.

Susceptible radish seeds were sown and number of seedlings grown and infected with such treated oospores were counted. Newly formed oospores mixed with garden soil served as control.

To determine the infectivity of oospores in soil, radish seeds were sown in pots containing sufficient oosporic materials mixed with garden soil and kept in green house. One month later, after recording the number of systemically infected seedlings, plants were pulled out before oospore formation and fresh lots of seeds were sown. After each month one crop was raised in each pot in which oospore inoculum was added once.

3. Results

3.1 *Production of oospores*

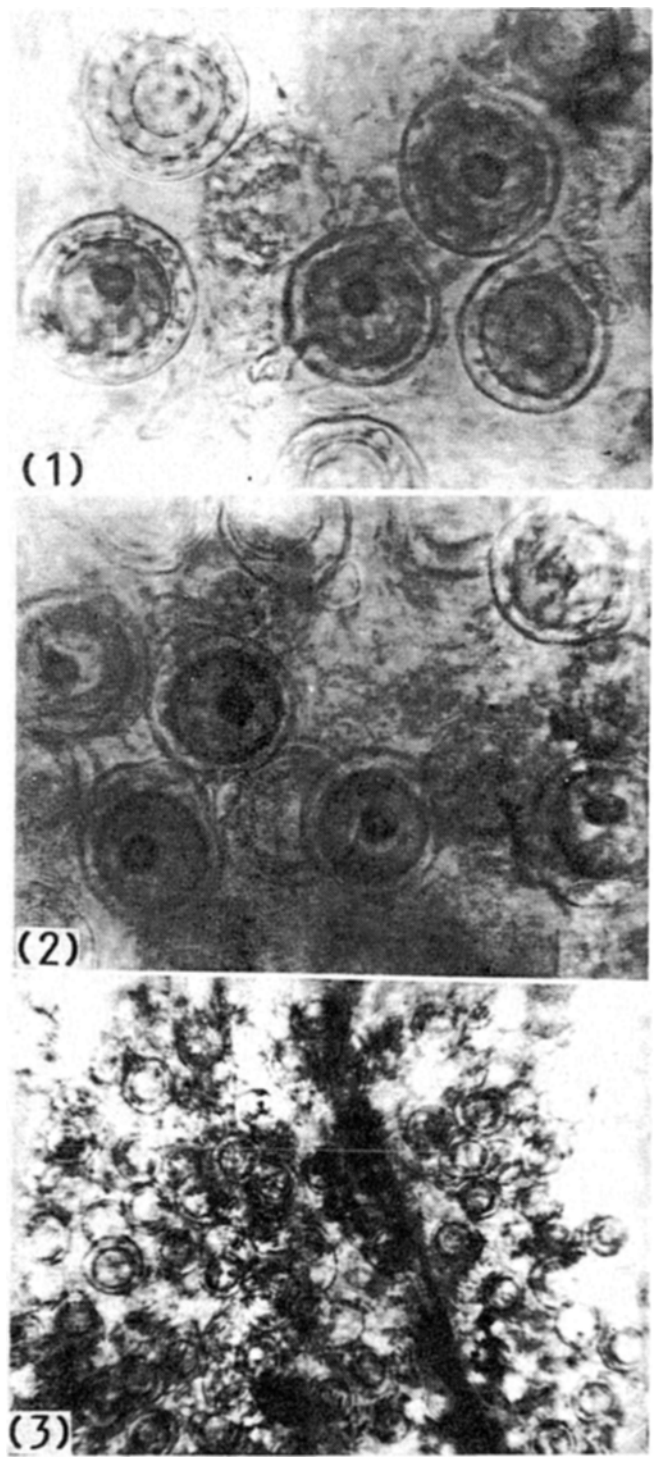
Oospores were formed in leaf tissues (figure 1) and were also detected in the pericarp (figure 2) and embryo (figure 3). Oospores production in leaves increased from fourth (2%) to the tenth pair (15·9%). At the flowering and pod stage, the leaves showed higher percentage of oospores production (16·7 and 50% respectively). Mature seeds (10·1%) showed the presence of oospores in the pericarp, 0·1% in embryo region and no oospores were located in the endosperm.

3.2 *Viability of oospores by TTC test*

In the tetrazolium test oospores showing red colour in the cytoplasmic region were considered viable and non-viable oospores did not take any stain. Percentage of viable oospores was more in Japanese white than the other susceptible cultivar at all the stages (table 1).

3.3 *Viability of oospores based on their age and percentage of infection after storage*

Oospores viability was seen to be related to their age, since oospores stored for 1 and 2 years, respectively, showed variation in the percentage of infection (table 2). Maximum infection percentage was obtained with 1 year old oospores. There was a gradual reduction in infection percentage from first to second year. Oospores were very highly infective in the first year, thereafter their infection capacity decreases.



Figures 1–3. Oospores of *P. parasitica* in radish. **1.** Leaf tissues (× 1000). **2.** Pericarp region (× 1000). **3.** Embryo (× 200).

Table 1. Viable oospores of *P. parasitica* as tested by TTC.

Cultivars	Growth stages	Viable oospores (%) (based on 400 oospores count) Leaf	Seed
Arka nishant	Flowering stage with immature seeds	2·5	2·2
Japanese white	-do-	3·9	4·0
Arka nishant	Flowering stage with mature seeds	10·5	14·5
Japanese white	-do-	15·1	16·2
Arka nishant	Pod stage with mature seeds	16·0	16·8
Japanese white	-do-	20·2	21·1

Table 2. Effect of age on oospore viability of *P. parasitica*.

	Age in years	
	1	2
Laboratory[a]		
Seedlings infected/grown	105/400	40/400
Infected plants (%)	30·75	15·50
Exposed to weathering[a] on the level of soil		
Seedlings infected/grown	410/450	205/460
Infected plants (%)	85·90	47·10
Below soil (15 cm)		
Seedlings infected/grown	440/455	210/460
Infected plants (%)	90·50	59·20
Control[b]		
Seedlings infected/grown	250/410	255/415
Infected plants (%)	55·90	30·50

[a]Replicated thrice; [b]newly formed oospores.

However, infection was higher among oospores exposed to weathering than those retained in laboratory conditions.

Crops raised in pots under green house conditions became infected due to oospores inoculum added to soil before sowing (table 3).

4. Discussion

Oospores production is an important process in the sexual stages of many plant pathogens and these oospores in most of the cases, form the primary source of inoculum. The present investigation reveals oospores formation in infected plants from fourth pair of true leaves and agrees with that of McMeekin (1960), who observed abundant oospores in necrotic leaves of *Brassica oleracea*. Oospores were not produced at cotyledons stage of radish seedlings which is contradictory to the observations of McMeekin (1960). Production of abundant oospores are noticed at the pod stage in the leaf tissues. Sansome and Sansome (1974) suggested that since both *Albugo candida* and *P. parasitica* commonly occur together in some Crucifers,

Table 3. Per cent infected plants in soil infested with oospores of *P. parasitica.*

Date of[a] sowing	Date of observation	Infected plants (%)
1st May 1983	20th May 1983	82·5
10th June 1983	24th June 1983	95·2
5th July 1983	28th July 1983	85·0
1st Aug. 1983	20th Aug. 1983	83·1
9th Sept. 1983	27th Sept. 1983	79·5
10th Oct. 1983	24th Oct. 1983	78·1
5th Nov. 1983	28th Nov. 1983	89·2
1st Dec. 1983	20th Dec. 1983	89·2
9th Jan. 1984	27th Jan. 1984	50·9
3rd Feb. 1984	26th Feb. 1984	15·1
11th March 1984	30th March 1984	03·0
2nd April 1984	22nd April 1984	05·0

[a]Oospores inoculum added only once before sowing; 400 seedlings raised in each crop.

cross stimulation of sexual reproduction may be possible. Further research work on 'interspecific stimulation' which may lead to abundant oospores formation of *P. parasitica* in radish is suggestive.

Oospores of *P. parasitica* in radish seeds could be an important source of primary inoculum particularly in the downy mildew free area. Such seeds should be given immediate importance since, the pathogen can move from an infested area to an uninfested one. The presence of oospores in radish seeds as revealed by our study, necessitates stringent seed testing before transporting seed materials to different places. Detection of oospores of the pathogen in seed tissue is also significant from epidemiological point of view of the disease in radish crops. Hence, control measure for spread of the disease among commercially grown radish crops, through seeds, should be given an immediate thought.

The tetrazolium test is found to be a quick method of testing the viability of *P. parasitica* oospores in radish in comparison with the viability test based on age of oospores and their infection capacity. Several workers have successfully tried the TTC method (Pathak *et al* 1978; Shetty *et al* 1978; Rao *et al* 1984).

Age of the oospores is seen to be an important factor which accounts for the viability of the oospores of *P. parasitica.* Percentage of infected plants decreased as age of the oospores increased from first to second year. The present findings are in confirmity with the observations made by Bhander and Rao (1967), who observed that oospores of *Sclerospora graminicola* were very highly infective in the first 3 years of storage and infectivity suddenly decreased during the fourth year. This is also true of *S. sorghi* (Kaveriappa 1973) and *S. graminicola* (Safeeulla 1976). Present study reveals that infection percentage by *P. parasitica* in radish is dependent on the age of the oospores, an important factor which decide their viability.

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