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Production of *Phytophthora infestans* oospores *in planta* and inoculum potential of *in vitro* produced oospores under temperate highlands and sub-tropical plains of India

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Summary

High moisture content of the host tissue (≤ 88%) and low ambient r.h. (50-54%) favoured oospore formation under controlled environments. It took 14-16 days for oospores to develop; thereafter the number of oospores increased with time and decreased with moisture content of host tissue. High ambient r.h. (> 80%) did not favour oospore formation under field or controlled conditions. Oospore formation was detected in inoculated plants grown in the field when the ambient r.h. declined to ≤ 74% and moisture content of host tissue decreased to 83.7-85.6%. It took 8 days (cv. Kufri Chandramukhi) to 13 days (cv. Kufri Jyoti and Kufri Badshah) for oospores to develop. Cultivars also differed in their response to oospore production, cv. Kufri Chandramukhi being more responsive (4800 oospores g^{-1} f wt) than cv. Kufri Jyoti and Kufri Badshah (1320 and 390 oospores g^{-1} f wt respectively). Oospores produced *in vitro* remained viable when buried in soil in the temperate highlands of Himachal Pradesh and sub-tropical plains of Uttar Pradesh, India for more than 150 days, i.e. beginning of the next crop season. The oospores germinated and initiated late blight infection at the base of the stems after 21-30 days of incubation of the potato plants raised in oospore-infested soil. It took 2 days for newly formed oospores to germinate and this delay time increased to 75-77 days after 180-days burial. It took 15 days for their germination (47%) in soil extract as compared to 50 days in sterilised distilled water.

Key words: *Phytophthora infestans*, potato, oospore, infection potential

Introduction

As a result of recent germplasm exchanges, the A_2 mating type of *Phytophthora infestans* has spread from Mexico to most potato growing regions (Fry *et al.*, 1993; Goodwin & Fry, 1991). In India, the A_2 mating type was reported from the Shimla Hills and the North-eastern hill region in 1990 (Singh *et al.*, 1994) and since then it has been recorded throughout the country (Gupta *et al.*, 2001). The frequency of the A_2 type has gradually increased, although the rate of increase varied from region-to-region (Gupta *et al.*, 2001). Currently, the population of the two mating types is almost in equal proportion in the sub-tropical plains (Anon., 1998), whereas in the temperate highlands the A_2 mating type predominates (Anon., 1999).

It is now well known that the two mating types when inoculated either in equal proportion or in proportions ranging up to 19:1 on host tissue produce oospores in abundance (Frinking *et al.*, 1987; Mosa *et al.*, 1991; Drenth *et al.*, 1995; Cohen *et al.*, 1997; Turkensteen *et al.*, 2000). Recent investigations have shown that *P. infestans* produces oospores naturally in host tissue where the two mating types are present (Flier & Turkensteen, 1999; Medina & Platt, 1999; Gavino *et al.*, 2000). The present paper describes (i) the role of ambient r.h. and moisture content of host tissue on oospore production, (ii) oospore survival and germination under natural conditions, and (iii) the role of oospores as primary inoculum for late blight in the temperate highlands and sub-tropical plains of India.

Materials and Methods

Phytophthora infestans *isolates*

One isolate each of the A_1 and A_2 mating types of *P. infestans* were used throughout the study. A_1 isolate PAT-A was collected from Patna (Bihar State) whereas A_2 isolate SH-9 was obtained from Shillong Hills during 1993. Both of these isolates were maintained on Rye B agar medium up to 1997, and afterwards, in liquid N_2 using 10% glycerol (w/w) as cryoprotectant (Forbes, 1997). The isolates were re-activated by warming to 37°C for 2 min and then they were transferred to fresh Rye A agar medium. Thereafter, the isolates were transferred to tuber slices for maintenance and multiplication.

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Oospore production in host tissue

Production of oospores under a controlled environment

Potato plants of cv. Kufri Chandramukhi were raised in 23-cm earthen pots in a glasshouse. Thirty days after planting of tubers, the plants were inoculated with a mixed zoospore suspension of A_1 and A_2 mating type isolates in equal proportion. The two isolates were multiplied on potato tuber slices. The sporangia of both isolates were washed separately in distilled water, calibrated to 15 000 sporangia ml^{-1} and incubated at 12°C for 90 min to induce zoospore release. The zoospore suspensions from both mating type cultures were mixed in equal proportion and sprayed on plants until run-off stage in the first week of May using a glass atomiser. In addition, 3 mm diameter filter paper disks dipped in zoospore suspension were placed on the leaf axils to facilitate stem and petiole infection. The inoculated plants were incubated in a late blight screening chamber in darkness at 18 ± 1°C at r.h. 100% for 48 h and thereafter for 4 days in light (18 ± 1°C, r.h. > 80%). Plants that developed late blight stem lesions were subsequently incubated under three sets of environment *viz.*, i) open air (19-22°C, r.h. 40-50%), ii) growth chamber (18°C, r.h. 50-55%), iii) growth chamber (18°C, r.h. 82-90%). Differential relative humidities in the two chambers were created by an automatic fogging system fitted with a humidity sensor and r.h. was monitored daily by hygrograph. Eight plants were kept under each of the three environments. Late blight-infected stem portions (10 mm long) were cut from each of two plants after 7, 14, 16 and 18 days incubation and the epidermis was stripped using a pair of tweezers. One epidermal peeling measuring 3 mm × 10 mm was obtained from each stem portion and cleared for 24 h in a 3:1 (v/v) mixture of 96% ethanol and glacial acetic acid (*c.* 100%) at room temperature (Frinking *et al.*, 1987). The peelings were examined under a microscope (10×) and number of oospores in each strip counted. The remaining stem portions were weighed and dried at 80°C for 3 days for determination of moisture content.

Production of oospores under field conditions

Crops of cvs Kufri Chandramukhi (susceptible), Kufri Jyoti (moderately resistant) and Kufri Badshah (resistant) were raised in 1 m^2 plots (single row of 10 tubers) at the Central Potato Research Institute farm, Shimla. The crop was sprayed with a zoospore mixture of A_1 and A_2 mating types in equal proportion (6×10^4 zoospores ml^{-1}) immediately after the conditions for late blight appearance were met (Bhattacharyya *et al.*, 1982). Lesions were allowed to develop until the natural occurrence of late blight. Stem pieces carrying late blight lesions were collected 5, 8, 13, 16, 24 and 30 days after inoculation and oospore numbers counted. Epidermal peelings (1 g) were crushed with an equal volume of water using pestle and mortar. The volume was made to 5 ml and the oospores counted using a haemocytometer. The ambient r.h. and moisture content of the stems were also measured.

In another set, 10 potted plants (30-days-old) of each of three cultivars raised in the glasshouse were transferred to the field in the first week of September when rains had receded and the ambient r.h. had dropped below 80%. The plants were inoculated with an A_1 and A_2 mating type mixture using 6×10^4 zoospores ml^{-1} and covered with a polyethylene sheet. High relative humidities were maintained by spraying the plants with water, two-three times daily until the appearance of lesions, and later, the plants were kept at ambient r.h. and temperature. Epidermal peelings (0.5 g) were collected from two infected plants at defined intervals and examined for oospore numbers as described above.

Monitoring of oospores in the field

The occurrence of oospores in the host tissue under temperate highland conditions (Kufri, Himachal Pradesh) was monitored by collecting infected stems throughout the season from 13 *S. tuberosum* germplasm accessions carrying varying levels of late blight resistance *viz.*, KS-93-205, KS-93-4, KS-93-259, JTH/C-109, KS-93-142, KS-92-264, KS-93-242, KS-93-180, KS-93-407, KS-93-7, KS-195-45, KS-195-49 and KS-195-53. Samples were collected in September when rains had receded and ambient r.h. was below 75%. The peelings were stripped, processed as described earlier and observed for oospore presence under a microscope (20×). The moisture content of stems was also determined.

Oospore production in vitro

Five mm diameter disks were cut from the growing edge of the 7-day-old cultures of the two mating types and placed 3-4 cm apart on the Rye B agar medium in 9 cm diameter Petri dishes (Mosa *et al.*, 1991). The dishes containing the paired isolates were incubated at 18 ± 1°C for 10 days in darkness (Shattock *et al.*, 1986; Singh *et al.*, 1994), and then stored at 8°C until oospores were extracted.

Oospores formed at the interface of interacting A_1 and A_2 colonies were extracted after 20 days by modifying the method of Förster *et al.* (1983). The mycelium at the interface of the paired isolates was extracted by scraping with a spatula; the scrapings containing oospores were ground in distilled water for 2 min using an electric grinder. The resultant suspension was sonicated (Branson Sonifier 450, USA) at 20% duty cycle for 10 min to break up and separate mycelium from oospores and then centrifuged at 12 170 ***g*** for 10 min. The supernatant was discarded and the pellets containing oospores

were wrapped in nylon cloth (pore size 20 µm, M/S Millipore India Ltd) and pouches measuring 1.5 cm × 3 cm were prepared (Pittis & Shattock, 1994).

Survival of in vitro*-derived oospores in natural soils*

Oospore survival was studied under two sets of conditions: (i) temperate highlands of Himachal Pradesh (Shimla): ambient temp. -5 to 24°C, r.h. 20.6 to 77.2%, rainfall 37.9 mm, and (ii) sub-tropical plains of Uttar Pradesh (Meerut): ambient temperature 14 to 44°C, r.h. 25 to 99.8%, rainfall 38.7 to 71.3 mm during the study period. The nylon pouches each containing 150-200 *P. infestans* oospores were buried 5 cm deep in natural soil kept in a wooden tray measuring 42 cm × 30 cm × 15 cm. The soil was subjected to natural weathering by placing the trays in open air. Survival of oospores was assessed by determining their viability at monthly intervals starting from the end of the potato crop season (October in temperate highlands and April in sub-tropical plains) to the beginning of the next crop season (March in temperate highlands and October in sub-tropical plains). The experiment was repeated once in the sub-tropical plains. Oospores were recovered from nylon pouches by scraping with a spatula, and soaked in sterilised distilled water for 1-2 h and triturated with the help of mortar and pestle. The resultant oospores in temperate highlands were tested for their viability using the MTT test (Sutherland & Cohen, 1983) and plasmolysis tests using 4 M NaCl and 3 M sucrose (Jiang & Erwin, 1990), whereas in the sub-tropical plains only the latter test was used. Viable oospores developed a red/blue colour, whereas non-viable spores were unstained. Oospores that plasmolysed were considered to be viable.

Oospore germination

An oospore suspension (1 ml) as prepared above was added to 1 ml distilled water (1:1 ratio) in 3.5 cm diameter Petri dishes and incubated at 18 ± 1°C in darkness (Förster *et al.*, 1983). They were observed daily for germination under the microscope to a maximum of 48 and 77 days in temperate highlands and sub-tropical plains, respectively.

Effect of root exudates, extracts and soil extract on oospore germination

Root extracts and exudates of five potato cvs Kufri Chandramukhi, Kufri Jyoti, Kufri Badshah, Kufri Megha and Kufri Jawahar, along with soil extract prepared as per details described by Banihashemi & Mitchell (1976), were evaluated for their effect on 6-month-old oospores. One ml aliquots each of root extracts, root exudates, soil extracts and sterilised distilled water were added to 3.5-cm diameter Petri dishes individually. An equal volume of oospore suspension was added to each dish and incubated at 18 ± 1°C (Jiang & Erwin, 1990). Oospores were observed daily for 50 days for presence/absence of germ tubes.

Infection potential of germinated oospores

Tuber slices (1-cm thick) of susceptible cv. Kufri Chandramukhi were cut with a sterilised knife and 100 µl suspension of germinating oospores was added to each slice. The inoculated tuber slices were kept in Petri dishes placed in airtight plastic boxes lined with moist foam sheet incubated at 18 ± 1°C for 12 days. High humidity was maintained by adding 100 µl sterilised water daily on each slice. The slices were observed daily for lesion development and formation of sporangia after 6 days incubation.

Oospores as primary source of late blight

The experiments were conducted under two sets of conditions as described above for soils using field soils of the two regions (Shimla soils- Patchumults, pH 6, organic C > 2%; and Meerut soils- Ustochrepts, pH 7, organic C < 0.5%). Soil (4.5 kg) was inoculated with a sonicated 600-ml oospore suspension containing 3400 oospore ml^{-1} and mixed thoroughly and 375 g (containing 170 000 oospores) placed in each of 12 plastic pots. Another set of pots filled with uninfested field soil served as control. The pots were filled and kept in open air for natural weathering (150 and 180 day at Shimla and Meerut, respectively) in the third week of October in temperate highlands and last week of March in sub-tropical plains. At the beginning of next crop season, i.e. first week of March in temperate highlands and in first week of October in sub-tropical plains, respectively, each pot was planted with one tuber eye of susceptible cv. Kufri Chandramukhi and the pots were watered daily to initiate sprouting of buds. The pots were transferred to the late blight screening chamber 20 days after planting where temperature, r.h. and light were maintained at 18 ± 1°C, > 98% and at 120-140 µmoles $m^{-2}s^{-1}$ for 8 h daily, respectively. The plants were observed visually for late blight infection.

Statistical analyses

The statistical analyses of the data was performed using MSTAT-C (version 1.42). A two-factorial completely randomised design (CRD) was carried out for the data on: (i) production of oospores in stem peelings under three environments (factor: environment × duration), (ii) oospore production in the field under artificial inoculation (factor: days × cultivar), and (iii) survival of oospores in temperate highlands (factor: days × tests). Analysis of variance for one-way comparison of means was carried out for the data on oospore production in stem peelings

under three sets of conditions and oospore survival and germination in temperate highlands and sub-tropical plains. The least significant difference (LSD) was calculated at 0.05 probability.

Results

Oospore production in host tissue

Production of oospores in potato stems under controlled environment was dependent on ambient r.h. and moisture content of the host tissue. Moisture content ≤ 88% was required for formation of oospores. Plants kept in open air and in growth chamber under low r.h. developed oospores within 14 and 16 days of inoculation, respectively. The number of oospores increased with time after inoculation and decreased in moisture content of the host tissue under both environmental conditions (mean number of oospores/30 mm^2 2.33 and 12.33 at 14 and 18 days of inoculation, respectively under open air; SE ± 1.12, $LSD_{(0.05)}$ 4.5, df 20 as compared to 0.0 and 10.17, respectively under low r. h.; SE ± 0.89, $LSD_{(0.05)}$ 2.85, df 20; Fig. 1a,b,c).

No oospores developed in inoculated plants under field conditions at Shimla in July when r.h. was high (> 80%). Few oospores were detected in peelings of cv. Kufri Badshah in September when ambient r.h. had fallen below 80%, whereas stalks of cvs Kufri Jyoti and Kufri Chandramukhi had rotted completely by that time. Plants of all three cultivars inoculated in second week of September under low r.h. (60.8-80%) developed oospores. They were first detected 8 days after inoculation in peelings from cv. Kufri Chandramukhi (susceptible), whereas it took 13 days in case of cvs Kufri Jyoti (moderately resistant) and Kufri Badshah (resistant). Moisture content of the host tissue was < 90% when oospores were first detected. More oospores formed (4800 g^{-1} f wt) in cv. Kufri Chandramukhi, followed by Kufri Jyoti and Kufri Badshah (mean oospore number 1320 and 390 g^{-1} f wt, respectively; SE ± 0.11, $LSD_{(0.05)}$ 0.32, df 24; Fig. 2a,b,c). Oospore numbers increased with time after inoculation and decreased in moisture content of the host tissue (430, 1060 and 6180 oospore/g f wt at 8, 13 and 16 days after inoculation, respectively; SE ± 0.13, $LSD_{(0.05)}$ 0.37, df 24).

Few oospores were detected in peelings of late blight resistant genotypes, KS-93-7, KS-195-45, KS-195-49 and KS-195-53 (1-3 oospores per 50 random microscopic fields). By 15 September, stalks of most of the genotypes, except late blight resistant ones, rotted in the field and therefore they could not be screened for presence of oospores. Oospore formation in nature was conditioned by low ambient r.h. (52.5-70%) and moisture content of the host tissue (82-86.2%).

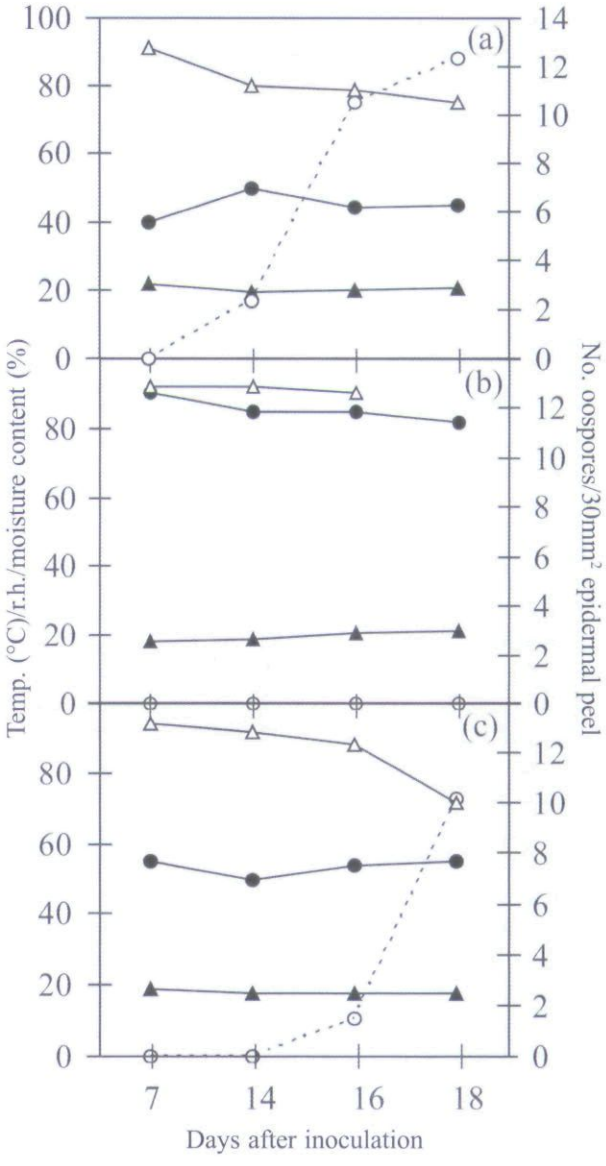

Fig. 1. Oospore production in stem peelings of cv. Kufri Chandramukhi under three sets of environment: (a) open air, $LSD_{(0.05)}$ 4.5, df 20; (b) High r.h. (c) Low r.h., $LSD_{(0.05)}$ 2.85, df 20: △ , moisture content (%); ●, ambient r.h. (%); ▲, temperature (°C); ○, oospore production.

Survival and germination of oospores

Temperate highlands

All three viability tests were equally effective in determining the oospore survival (mean oospore viability 68.8%, 65.5%, 64.9% in MTT, NaCl and sucrose plasmolysis tests respectively; SE ± 1.95, $LSD_{(0.05)}$ Non significant, df 18). Viability of oospores decreased gradually (except at 60 days burial) with the increase in burial period (Fig. 3). The MTT test, however, showed false positives of viability since aborted oospores also developed blue/red stain ranging from 0-11%. These false positives were not included when calculating oospore viability in MTT test. There was no statistical difference in the viability of oospores up to 60 days burial. A sudden decrease in their viability was observed at 90th day

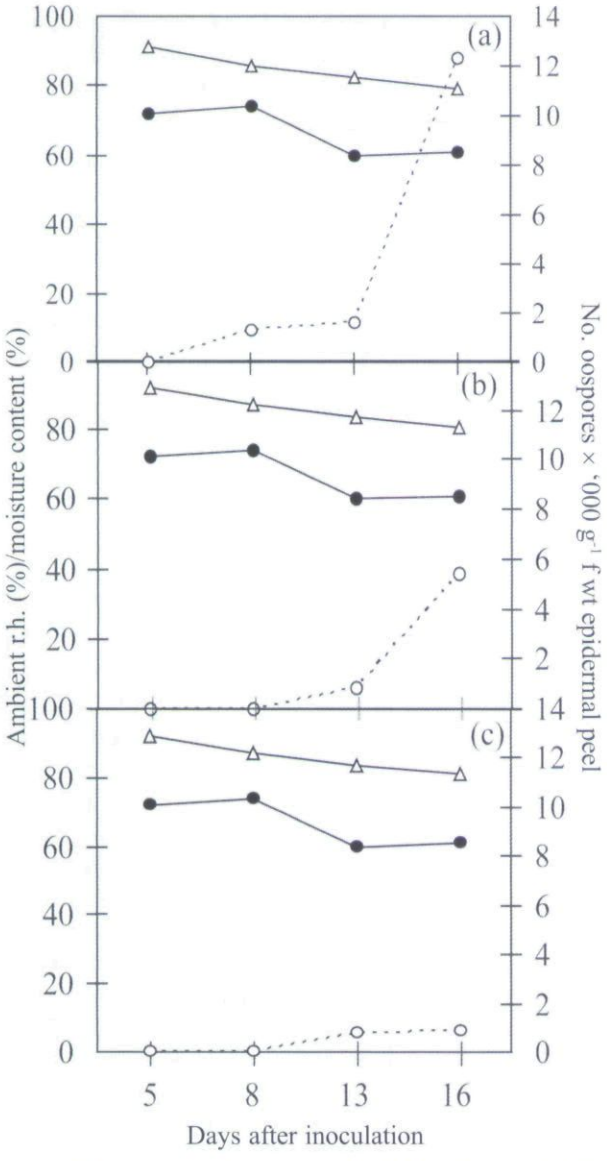

Fig. 2. Oospore production in the field under artificial inoculation: (a) Kufri Chandramukhi (b) Kufri Jyoti (c) Kufri Badshah. Days after inoculation, $LSD_{(0.05)}$ 0.38, df 24; Cultivar, $LSD_{(0.05)}$ 0.32, df 24; Days × cultivars, $LSD_{(0.05)}$ 0.65, df 24: △, moisture content (%); ●, ambient r.h. (%); ○, oospore production.

after burial (mean oospore viability 88.75% and 52% at 60 and 90 days burial, respectively; SE ± 2.76, $LSD_{(0.05)}$ 10.56, df 18). Thereafter, decrease in viability continued with increase in burial period. At 150 days, i.e., the start of the next crop season, the oospore viability decreased to 40.3%.

Oospores germinated readily in distilled water almost entirely through the antheridia. Highest germination was recorded in newly formed oospores (81.3%), followed by oospores buried for 30 days. A drastic reduction in their germination was recorded at 60 days burial (mean oospore germination 79.8% and 45.6% at 30 and 60 days burial, respectively; SE ± 4.95, $LSD_{(0.05)}$ 14.72, df 18, Fig. 4). Any further decrease in germination was not significant. After 150 days burial, 32.4% of oospores were able to

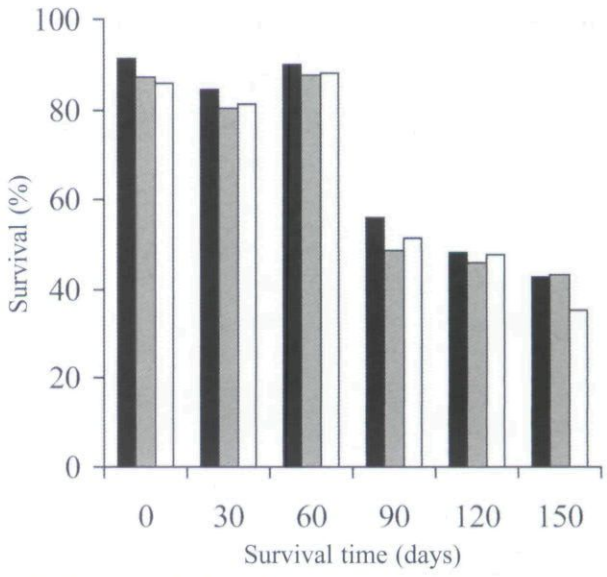

Fig. 3. Survival of *P. infestans* oospore under temperate highlands of Himachal Pradesh. MTT test, $LSD_{(0.05)}$ 13.57, df 18; NaCl plasmolysis test, $LSD_{(0.05)}$ 12.77 df 18; Sucrose plasmolysis test, $LSD_{(0.05)}$ 14.35, df 18; Burial time, $LSD_{(0.05)}$ 10.56, df 54; Test, $LSD_{(0.05)}$ NS;Time × Test, $LSD_{(0.05)}$ NS. Black bars = MTT test; grey bars = NaCl test; unshaded bars = sucrose test.

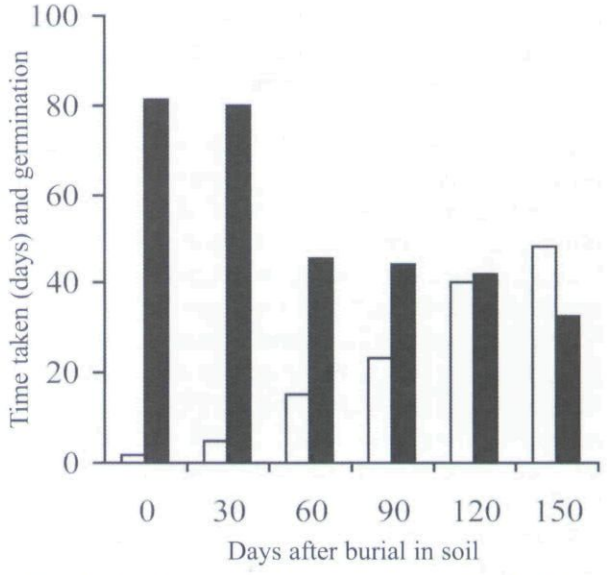

Fig. 4. *P. infestans* oospore germination (in distilled water) in relation to burial period under temperate highlands of Himachal Pradesh. $LSD_{(0.05)}$ 14.72, df 18. Unshaded bars = time taken for germination (days); black bars = % germination.

germinate. The time required for germination of oospores varied with burial time. It took 2 days for the newly formed oospores to germinate, but this delay time increased to 48 days after 150 days burial.

Sub-tropical plains

The year and year × burial period effects were significant, both for oospore viability as well as germination and therefore, annual data are shown (Table 1). In the year 2001 there was no significant

Table 1. *Effect of burial time on viability and germination of* P. infestans *oospores in sub-tropical plains during 2001-2002*

	Viability (%)		Germination (%)	
Days after burial	2001	2002	2001	2002
0	87.3	90.7	87.6	85.0
30	91.3	84.0	84.2	80.0
60	90.8	73.0	83.0	69.0
90	90.0	66.6	80.0	62.6
120	55.2	53.6	34.4	30.4
150	34.3	37.9	20.0	17.0
180	29.4	24.5	17.4	14.6
SE ±	4.03	1.35	1.80	0.67
$LSD_{(0.05)}$ df = 21	13.5	5.3	8.2	2.6

difference up to 90 days burial. Thereafter, oospore viability decreased significantly up to 150 days burial. At 180 days burial a slight decrease in viability was recorded, but it was non-significant (mean oospore viability 34.3 and 29.4 at 150 and 180 days burial, respectively; SE ± 4.03, $LSD_{(0.05)}$ 13.47, df 21). Oospore viability did not follow the above trend during 2002 where significant decreases in oospore viability were recorded at each burial period. After 180 days burial oospore viability was 24.5% as compared to 90.7% in newly formed oospores.

There was no significant difference in oospore germination up to 90 days burial during 2001 (mean oospore germination 87.6% and 88.0% in newly formed and at 90 days burial, respectively, SE ± 1.80, $LSD_{(0.05)}$ 8.20, df 21). Thereafter, oospore germination decreased drastically up to 150 days burial. A slight decrease in oospore germination was recorded after 150 days, but this difference was non significant (20.0% and 17.4% at 150 and 180 days burial, respectively; SE ± 1.80, $LSD_{(0.05)}$ 8.20, df 21). During 2002 the trend in oospore germination was different from that of 2001 in the sense that significant decreases in oospore germination were recorded at each burial time (Table 1). It took 2 days for newly formed oospores to germinate, and this delay time increased to 75-77 days after 180 days burial.

Root exudates and extracts of all the five potato cultivars studied failed to induce oospore germination, even up to 50 days incubation period. However, the soil extract had a stimulatory effect. It took 15 days for their germination (47%) in soil extract as compared to 50 days in sterilised distilled water (28%).

Oospores as primary sources of late blight

Potato plants raised in oospore-infested soil showed late blight infection at their bases after 21-30 days incubation and within 60 days, all plants were infected and collapsed. No such symptoms appeared on any plant raised in un-infested soil. Further observations on infected plants revealed that the infection was first evident on the tuber eyes and later spread to the stem base. The role of oospores in disease initiation was further confirmed by inoculating the tuber slices separately with germinated oospores in soil extract and soil infested with oospores. In both the cases, oospore-derived infections were produced within 7-10 days.

Discussion

A series of experiments on production of oospores by *P. infestans* in the host tissue revealed that they were formed only under drought stress conditions which supports the earlier findings of Frinking *et al.* (1987) but appears to contradict other studies showing oospore production under high humidity (Cohen *et al.*, 2000). However, the oospore response to moisture stress varied between cultivars, cv. Kufri Chandramukhi being more responsive than others. In North-eastern Hill region and in Shimla Hills, from the early tuberisation stage there are continuous rains and the crop is never subjected to water stress after late blight attacks. Therefore, it seems unlikely that *P. infestans* could form oospores naturally in these regions. This hypothesis has been tested and oospores were not detected in these regions during the test years. However, oospores were formed in stems of some late blight resistant genotypes, which remained turgid and green even up to September, when ambient r.h. had touched 70% and stem moisture content had decreased to less than 90%. These observations were confirmed through artificial inoculation studies. The decrease in moisture content of host tissue under low humidity might have induced sexual reproduction in established disease lesions. The rarity of oospore formation in nature under Shimla conditions could also be attributed to an imbalanced proportion of A_1 and A_2 mating types (1:4; Gupta *et al.*, 2001), although oospore formation even up to a ratio of 19:1 for A_1 and A_2 mating types has been reported (Cohen *et al.*, 1997).

Oospore biology in *P. infestans* has been the subject of intense study ever since the classical work of Smoot *et al.* (1958) and Gallegly & Galindo (1958) who first reported that *P. infestans* produced thick-walled oospores when the two opposite mating types were paired *in vitro* and in host tissue. Numerous studies in Europe and America have since confirmed the formation of oospores *in vitro* (Frinking *et al.*, 1987; Pittis & Shattock, 1994; Drenth *et al.*, 1995). Recent reports have indicated that *P. infestans* produces thick-walled oospores in host tissue that initiated natural late blight infection (Pittis & Shattock, 1994; Stromberg *et al.*, 1999; Flier *et al.*, 2001). However, oospore production by *P. infestans* in potato leaves/stems under natural

conditions is a still rarely reported phenomenon. The present studies attempted to identify the factor(s) which induced oospore formation in host tissue and their likely role in late blight infection under Indian conditions. Viability was highest in the newly formed, as compared to the buried, oospores declining over time after burial. Pittis & Shattock (1994), Drenth *et al.* (1995), Zarzycka & Sobkowiak (1997), Medina & Platt (1999) and Mayton *et al.* (2000) also recorded adverse effects of burial period on oospore viability. In reported studies, the extent of viability varied from region to region, being 7.8% after 10-months burial in UK (Pittis & Shattock, 1994) and 5-15% after 12 months in Prince Edward Island, Canada (Medina & Platt, 1999). However, in the present work high percentages of oospore viability (i.e., 81.3-89.8%) were recorded in the newly formed oospores which decreased to 32.5% after 150 days burial under temperate highlands and 26.9% after 180 days burial under sub-tropical plains. These observations are similar to the data (40% survival) recorded by Medina & Platt (1999) after 7-months burial. The differences in overall oospore viability under different regions/countries might be due to variations in weather conditions, especially the temperature which has a significant effect on oospore viability (Pittis & Shattock, 1994; Drenth *et al.*, 1995). In the present study, the MTT tests proved slightly better than plasmolysis tests as reported by Medina & Platt (1999), but the MTT test is not entirely reliable because of the high proportion of false positives when applied to this material (0.1-12%).

Like oospore viability, the germination was also highest in the newly formed oospores (81.3-86.3%) which decreased gradually with increase in burial period. Smoot *et al.* (1958), Pittis & Shattock (1994) and Medina & Platt (1999) have also recorded a decrease in oospore germination with increasing burial period, but the extent of germination was much higher under the present study. There are now reports that *P. infestans* produces oospores naturally (Gotz, 1988; Flier & Turkensteen, 1999) and these overwinter in soil and serve as initial source of inoculum of the disease (Pittis & Shattock, 1994; Medina & Platt, 1999). Present findings revealed that *P. infestans* oospores can overwinter in the temperate highlands of Himachal Pradesh (temperature –5°C to 24°C, r.h. 20.6-77.2%, rainfall 37.9 mm during the burial period) and survive summer under sub-tropical plains (temperature 14°C to 44°C, r.h. 25-99.8%, rainfall 38.7-71.3 mm during the burial period) to initiate the disease by infecting plants at the stem base. The oospores took 21-30 days to germinate and infect the plants. Stromberg *et al.* (1999) also observed brown discolouration on the underground stem and tuber after a 1-month incubation. The role of oospores in disease initiation was further confirmed by oospore baiting experiments using tuber slices. Late blight symptoms on tuber slices developed within 7-10 days of inoculation. These results are similar to those obtained by Flier & Turkensteen (1999), except that they observed symptoms after only 4 days of incubation. The differences in incubation time in the two studies could have been due to the use of different baits, or different experimental conditions.

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