A Procedure for Isolation and Maintenance of *Peronospora destructor* on Onion

A. A. Abd-Elrazik and J. W. Lorbeer

Former visiting associate professor and professor, respectively, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853. Present address of senior author: Department of Plant Pathology, Assiut University, Assiut, Egypt.

Accepted for publication 29 January 1980.

ABSTRACT

ABD-ELRAZIK, A. A., and J. W. LORBEER. 1980. A procedure for isolation and maintenance of *Peronospora destructor* on onion. Phytopathology 70:780-782.

Procedures described by previous workers were not satisfactory for the isolation and subsequent maintenance of *Peronospora destructor* on onion plants in the greenhouse. High levels of infection and subsequent sporulation were obtained when leaves of onion plants grown from bulbs in a greenhouse were wiped once with a dry pad of cotton immediately prior to inoculation. Sporangia collected from naturally infected onion plants in commercial onion fields in New York (fields sprayed weekly with chlorothalonil and/or mancozeb) were washed three or four times, suspended in tap water, and used as inoculum. Inoculated plants were incubated in a moist chamber at 14 C for 24 hr in the dark, placed in a growth chamber (21,500 lux = 2,000 ft-c) at 14 C for 4 days, and then placed on a greenhouse bench with supplemental fluorescent light (16 hr) at 18 C for 8 days. Sporulation was induced overnight by placing the infected plants in a moist chamber at 14 C in the dark commencing at 1600–1700 hours. Once isolated, the pathogen was maintained by transfering 1- to 2-day-old unwashed sporangia to healthy plants every 3 wk and using the cotton wipe technique and the inoculation and incubation procedures described. All inoculated plants became infected and abundant sporulation was obtained.

Additional key words: downy mildew of onion, *Allium cepa.*

Downy mildew of onion (*Allium cepa* L.), caused by *Peronospora destructor* (Berk.) Caspary, has become economically important in recent years in New York State. It affects onion yields where environmental conditions favorable for the disease are found (2,4,5,8). Epidemiological factors determining the severity of this disease under field conditions are inoculum level, temperature, free moisture, relative humidity, and wind (8). Successful inoculation with the fungus in the greenhouse depends on the method of inoculation, period of incubation, the stage of development of the host, and the environmental factors affecting the severity of the disease in the field (1,2,7,8). The most important factors affecting sporulation of the fungus on infected plants under controlled conditions are light, humidity, and temperature (5,6,8).

Procedures described previously by other workers (2,7) for isolation and maintenance of *P. destructor* on its host were not satisfactory for maintenance of the fungus; they resulted in a low percentage of infection and subsequently a low level of sporulation.

We sought to develop a reliable technique for obtaining a high level of infection and abundant sporulation by studying the roles of several factors affecting viability of the inoculum and levels of infection and sporulation.

MATERIALS AND METHODS

Sporangia of *P. destructor* used in most of the experiments in this study were obtained at different times during August and September 1978 from infected onion plants in commercial onion fields at Prattsburg, NY. These fields were sprayed with chlorothalonil and/or mancozeb, generally on a weekly basis. Infected leaves with freshly produced sporangia were placed individually in covered cylindrical glass jars and transferred to Ithaca during the same day in a shaded carton box. Sporangia were collected from the surfaces of the infected leaves with a camel's-hair brush and suspended in distilled water for utilization in subsequent experiments. Sporangia used in several of the experiments in this study were obtained from artificially inoculated plants grown in a greenhouse at Ithaca.

Onion plants utilized for artificial inoculation with sporangia of *P. destructor* were grown in a greenhouse from bulbs. Generally they were 3 wk old when inoculated. All leaves on all plants were inoculated in all experiments conducted. In one series of experiments, onion seedlings were utilized.

Studies were conducted to measure the effect of washing sporangia of *P. destructor* collected from the leaves of onion plants growing in commercial fields on their subsequent germination. Sporangia were washed one to four times with distilled water by centrifugation in an International Clinical Centrifuge (International Equipment Co., Boston, MA 02194) for 3 min at 2,500 rpm (3). The sporangia then were spread onto plates of 1% water agar. After 24-hr of incubation at 10 C, 500 sporangia were examined and the percentage germination was determined (Table 1). Unwashed sporangia were used as controls.

To study the effect of incubation time and temperature on infection by *P. destructor*, 21-day-old onion plants grown in the greenhouse from bulbs were inoculated with a suspension of sporangia washed four times. These sporangia were collected from leaves of onion plants growing in commercial onion fields. Spore suspensions were applied with a Preval sprayer (Precision Valve Corporation, P.O. Box 309, Yonkers, NY 10702). Inoculated plants

TABLE 1. The effect of washing on germination of sporangia of *Peronospora destructor* obtained from leaves of infected onion plants in commercial onion fields sprayed with chlorothalonil and/or mancozeb

Washings[a] (no.)	Germination[b,c] (%)
0 (not washed)	8 A
1	17 B
2	22 B
3	45 C
4	46 C

[a] Each washing involved centrifugation in distilled water for 3 min at 2,500 rpm.

[b] Incubation on 1% water agar for 24 hr at 10 C. Five hundred sporangia were examined and the percentage of those germinated was determined.

[c] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

0031-949X/80/08078003/$03.00/0

were incubated in a moist chamber at 14 C for 24 hr in darkness and then grown under a series of different temperature regimes for a 12-day period (Table 2). After the 12-day growth periods, sporulation was induced by placing the infected plants overnight in a moist chamber at 14 C in darkness starting at 1600–1700 hours. The percentage of plants infected and the amount of sporulation on the following morning were recorded. Sporulation was rated from 0 to 4 (0 represented no sporulation and 4 represented abundant sporulation).

To study the effect of aging on the viability of sporangia and their subsequent infectivity, plants were inoculated with spore suspensions prepared with 1- to 4-day-old sporangia obtained from artificially infected onion plants grown in a greenhouse at Ithaca. The sporangia were not washed prior to inoculation. The percentage of plants infected was recorded after 12 days of incubation (4 days at 14 C and 8 days at 18 C). Viability of the same sporangia was measured by germination on water agar in simultaneous experiments (Table 3).

The effect of wiping leaves with a cotton pad immediately before inoculation was studied utilizing onion plants grown in a greenhouse from sprouted bulbs or from seeds. All of the leaves on these plants were wiped. Leaves of separate plants that were not wiped with a cotton pad were used as controls. Field-collected sporangia were utilized in these experiments and were washed four times prior to inoculation (Table 4). Inoculations were made at 0, 2, 4, and 7 days after wiping the leaves with a cotton pad to study the effect of time after wiping and inoculation on subsequent infection levels. All of the leaves on these plants were wiped with a cotton pad, unwiped plants were used in controls. Sporangia collected from artifically inoculated onion plants grown in a greenhouse were utilized in these experiments and were not washed prior to inoculation (Table 5). In both series of experiments, the percent of infection and the sporulation levels were recorded for each treatment after 12 days of incubation. Sporulation was induced by incubation at 14 C as described previously.

TABLE 2. Effect of incubation time and temperature on infection of onion by sporangia of *Peronospora destructor* obtained from leaves of infected onion plants in commercial onion fields sprayed with chlorothalonil and/or mancozeb[a]

Incubation time and temperature[b]	Infection[c,d] (%)	Sporulation[e]
12 days at 18 C	40 C	0.63
2 days at 14 C + 10 days at 18 C	40 C	1.40
4 days at 14 C + 8 days at 18 C	70 A	1.48
6 days at 14 C + 6 days at 18 C	60 B	1.23
8 days at 14 C + 4 days at 18 C	60 B	0.86
10 days at 14 C + 2 days at 18 C	20 D	0.34
12 days at 14 C	10 E	0.25

[a] Sporangia washed four times in distilled water and then suspended in tap water prior to inoculation.
[b] The plants were maintained in a growth chamber at 14 C (21,500 lux = 2,000 ft-c). The plants were maintained on a greenhouse bench at 18 C with supplemental fluorescent light (16 hr/day).
[c] Percent infection represents the number of plants infected of 10 plants inoculated.
[d] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[e] Sporulation is rated on a scale of 0–4; 0 represents no sporulation and 4 represents abundant sporulation.

TABLE 3. Effect of age of *Peronospora destructor* sporangia collected from artificially inoculated plants grown in the greenhouse on germination and infectivity on onion leaves[a]

Age (days)	Germination[b,c] (%)	Infection[c,d] (%)
1	80 A	70 A
2	74 A	70 A
3	55 B	40 B
4	27 C	20 C

[a] Sporangia not washed prior to inoculation.
[b] Incubation on 1% water agar for 24 hr at 10 C. Five hundred sporangia were examined and the percentage of those germinated was determined.
[c] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[d] Percent infection represents the number of plants infected of 10 plants inoculated.

RESULTS AND DISCUSSION

Effect of washing field-collected sporangia of *P. destructor* on their subsequent germination. The germination of *P. destructor* sporangia collected from naturally infected onion plants grown in commercial fields sprayed with chlorothalonil and/or mancozeb was affected by prior washing (Table 1). The percentage of germination increased with the number of washings. The highest percent of germination was observed for sporangia washed three or four times. The increased germination of sporangia after washing may have been due to the removal of compounds affecting the sporangia. The fungicides chlorothalonil and/or mancozeb are used for chemical control of onion foliage diseases and they may have functioned as inhibitors to germination of the field-collected sporangia. However, self inhibitors in the sporangia also could be involved; this point needs additional study.

Effect of incubation time and temperature on infection by *P. destructor*. The percentage of infection and the sporulation levels of *P. destructor* on infected plants were affected greatly by the time and temperature of incubation (Table 2). Exposure of inoculated plants to 14 C for 4 days in a growth chamber and then to 18 C for 8 days in a greenhouse resulted in the highest percentage of infection and the most abundant sporulation. The lowest percentage of plants infected and the lowest level of sporulation occurred on plants incubated at a continuous 14 C for 12 days in a growth

TABLE 4. Effect of wiping the leaves of greenhouse-grown onion plants with a cotton pad immediately prior to inoculation with *Peronospora destructor*[a]

Treatment	Infection[b,c] (%)	Sporulation[d]
Wiped plants	100 A	3.54
Unwiped plants	70 B	1.46
Wiped seedlings	10 C	0.25
Unwiped seedlings	0 D	0.00

[a] Sporangia were collected from leaves of onion plants growing in commercial onion fields sprayed with chlorothalonil and/or mancozeb. Sporangia washed four times in distilled water and then suspended in tap water prior to inoculation.
[b] Percent infection represents the number of plants infected of 10 plants inoculated.
[c] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[d] Sporulation is rated on a scale from 0–4; 0 represents no sporulation and 4 represents abundant sporulation.

TABLE 5. Effect of the time period between wiping the leaves of greenhouse-grown onion plants with a cotton pad and inoculation on subsequent infection and sporulation by *Peronospora destructor*[a]

Period (days)	Infection[b,c] (%)	Sporulation[d]
0	100 A	3.16
2	100 A	1.24
4	60 B	0.40
7	60 B	0.36

[a] Sporangia obtained from artificially inoculated plants grown in the greenhouse. The sporangia were not washed prior to inoculation.
[b] Percent infection represents the number of plants infected of 10 plants inoculated.
[c] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[d] Sporulation is rated on a scale from 0–4; 0 represents no sporulation and 4 represents abundant sporulation.

chamber.

Effect of age of *P. destructor* sporangia on their viability and infectivity. The age of sporangia affected both their viability and infectivity (Table 3). Germination of sporangia and the percentage of infection decreased as the age of sporangia increased (Table 3). Sporangia 1–2 days old exhibited a higher percentage of germination and infectivity than did those of other ages tested and confirms the observations of Yarwood (8).

Effect of preinoculation wiping of the leaves of greenhouse-grown onion plants with a cotton pad on infection and sporulation by *P. destructor*. Plant surface wax is one of the factors which affects the establishment of infection by several foliage pathogens (1,3). Macroscopic observations of leaves of onion plants grown in a greenhouse for inoculation purposes demonstrated a thick layer of surface wax as compared with plants grown under field conditions. Wiping the leaves of greenhouse-grown plants (whether grown from sprouted bulbs of from seed) with a cotton pad immediately prior to inoculation with *P. destructor* increased both the percentage of plants infected and the subsequent sporulation levels (Table 4). These results could be explained by either increased wettability and susceptibility of the wiped leaves (because surface wax removal might result in increased germination of sporangia and subsequent penetration) or the removal of some germination inhibitors (waxes, phenols, etc.) from the surface of the leaves.

Both the highest percentage of plants infected and most abundant sporulation were observed on plants inoculated immediately after the leaves were wiped (Table 5). Leaves on plants inoculated 2 days after wiping showed a high percentage of plants infected, but the sporulation level was lower. When plants were inoculated 4 or 7 days after being wiped, a much lower percentage of the plants were infected and much lower levels of sporulation occurred (Table 5).

Based on the results of the present investigation and the observations of Yarwood (8), the following procedure was developed for the isolation and maintenance of *P. destructor* on onion plants in a greenhouse.

Leaves of onion plants grown from bulbs in the greenhouse are wiped once with a dry pad of cotton immediately before inoculation. Sporangia collected from the leaves of naturally infected onion plants in commercial onion fields sprayed with fungicides (chlorothalonil and/or mancozeb) are washed three or four times with distilled water (each washing is followed by centrifugation in an International Clinical Centrifuge for 3 min at 2,500 rpm), suspended in tap water, and sprayed onto the leaves of onion plants wiped once with a cotton pad immediately before inoculation. Inoculated plants are incubated in a moist chamber at 14 C for 24 hr in darkness, placed in a growth chamber (21,500 lux = 2,000 ft-c) at 14 C for 4 days, and then placed on a greenhouse bench with supplemental fluorescent light (16 hr) at 18 C for 8 days. Sporulation is induced by placing the infected plants overnight in a moist chamber at 14 C in darkness starting at 1600–1700 hours. Once isolated, the pathogen is maintained by transferring 1- to 2-day-old sporangia to healthy plants every 3 wk.

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