Sprinkling Irrigation Enhances Production of Oospores of *Phytophthora Infestans* in Field-Grown Crops of Potato

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ABSTRACT

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Two field experiments were conducted to study the effect of overhead sprinkling irrigation on oospore formation by the late blight fungus *Phytophthora infestans* in potato. Total rain (natural + sprinkling) accumulated in treatments of experiment 1 (winter 1997 to 1998) were 765, 287, and 219 mm and treatments of experiment 2 (winter 1999 to 2000) were 641, 193, and 129 mm. Sporangia from 11 isolates of *P. infestans* were combined in eight pairs, seven of A1 and A2 and one of A2 and A2 mating type, and were sprayed on field-grown potato crops (42 plants per plot at 7 m² each) and examined for their ability to form oospores in the host tissues. In experiment 1, oospores were recorded in a total of 132 of 1,680 leaflets (7.9%), 24 of 105 stems, and 2 of 90 tubers. In experiment 2, oospores were recorded in 40 of 519 leaflets (7.7%), but not in any of the 90 stems or the 45 tubers examined. Both the proportion of leaflets

containing oospores and the number of oospores per leaflet increased with time after inoculation and were dependent on the rain regime, the position of leaves on the plant, and the isolate pair combination. In both field trials, increasing the rainfall significantly enhanced oospore production in leaves. Leaf samples collected from the soil surface had significantly more oospores than those collected from the midcanopy. Two pairs in experiment 1 were more fertile than the others, whereas the pair used in experiment 2 was the least fertile. The total number of oospores per leaflet usually ranged from 10 to 100 in experiment 1, but only from 2 to 10 in experiment 2. Maximal oospore counts in the field were 200 and 50 in experiments 1 and 2, respectively, but ranged from ≈2,000 to 12,000 oospores per leaflet in detached leaves in the laboratory. We concluded that *P. infestans* can produce oospores in the foliage of field-grown potato crops, especially when kept wet by regular overhead sprinkling irrigation, but production was far below that in the laboratory.

Additional keyword: tomato.

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a major threat to potatoes and tomatoes worldwide (8,10,25). Vegetative reproduction of *P. infestans* via mycelia and sporangia were the only routes for fungal dissemination and infection in the western hemisphere until recently, when new genotypes of the A1 and A2 mating types migrated from Mexico, displaced the old genotypes, and provided the opportunity for sexual reproduction wherever both mating types occurred (10–12,16–19). Although the production of oospores of *P. infestans* in vitro has been extensively studied (4–6,9,13,14,21,24,27), only a limited number of experimental studies have been conducted on the sexual reproduction of the organism in the host tissue of potato and tomato in the field. In the late 1950s, scientists discovered that oospores were produced by Mexican isolates in artificially inoculated leaves of potato (13,14,27). Niederhauser reported on oospores in naturally infected potato leaves in Toluca Valley in Central Mexico (25). Frinking et al. (9) and Mosa et al. (24) showed oospore formation in intact potato plants in growth chambers. Andrivon stated that there is much experimental information to indicate that oospores could be an important source of inoculum, but there is a paucity of data from the field (1). Drenth et al. (5,6) demonstrated that oospores of *P. infestans* were produced in field-grown potato after inoculation with mixed A1 + A2 isolates. Such oospores survived the winter in the soil in the Netherlands and caused late blight on potato leaves. Zwankhuizen (29) provided genetic evidence that oospores may serve as an inoculum source for late blight in potato and tomato in the Netherlands. Stromberg

et al. (28) showed that oospores can infect underground stems and tubers of potato in soil. Medina et al. (23) showed that oospores can survive –20 and 50°C for 90 days and can overwinter in soil under Canadian conditions.

The infrequent occurrence of oospores in the field outside Mexico (1,2,8,10–12,19,20) stimulated us to study the factors affecting their production in potato and tomato leaves in growth chambers. We found that sexual reproduction in detached leaves is abundant when such leaves are floating on water (4). The ratio between the A1 and A2 sporangia coinoculated on these detached leaves had little effect on the abundance of the oospores formed. Under these conditions, oospores were produced over a wide range of temperatures (8 to 23°C) and their concentration could reach 100 oospores per mm² of leaf tissue (4).

The fact that both A1 and A2 mating types now frequently occur in nature, sometimes in the same location (15), suggests that mating may take place in the field. Drenth et al. (5,6) observed oospores in leaves and stems of field-grown potato and tomato plants ≈2 weeks after inoculation with mixed sporangia of A1 and A2 isolates. Hanson and Shattock (21) similarly inoculated potato crops in plastic tunnels and in the field. Oospores were found in most cultivars grown in tunnels but in only six leaflets taken from the field. DNA fingerprinting analysis conducted by Drenth et al. (7) showed a field isolate recovered 1 year later was a recombinant offspring of the two parents they had used.

However, in spite of the ample evidence on the likelihood of sexual reproduction in nature, little experimental data is available on the biotic and environmental factors influencing oospore formation.

The primary objective of this study was to investigate the role of water supply on oospore production by *P. infestans* in field-grown potato plants. We assumed that excessive water supply was critical for oospore formation (4). Because the bottom leaves of

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the crop stay wet longer, we analyzed bottom and middle leaves separately. We used several A1 + A2 isolate pairs for inoculation to verify that water supply is essential for oospore formation in as many isolate pairs as possible. To achieve these objectives, we artificially inoculated potato crops with mixed sporangia of A1 and A2, exposed the crops to increasing amounts of rain, and periodically examined oospore production in bottom and middle leaves. The data clearly showed that sexual reproduction in the field is primarily dependent on the availability of free moisture and leaf position. A preliminary report on some of the data presented here has been published elsewhere (3).

MATERIALS AND METHODS

Two field trials were conducted during two winter seasons, both at the Agricultural Farm of Bar-Ilan University, Ramat-Gan, Israel.

Experimental design of experiment 1 (winter 1997 to 1998). Twenty-one field plots of 42 certified potato seed tubers of the cv. Alpha were planted on 5 November 1997. The plots (7 m² each) were aligned in three rows designated A, B, and C, with plots numbered 1 to 7 in each row. Rows and plots were separated by fallow land (4 m between rows and 2 m between plots). Six weeks after sowing (16 December), when plants reached a height of ≈40 cm, they were spray-inoculated (described below) with sporangia of *P. infestans*. Ten days after inoculation, when first disease symptoms were visible, sprinkling irrigation was applied to the field for the following 32 days in the following manner: (i) row A, plots 1 to 7, 15 min of sprinkling per h 24 times a day; (ii) row B, plots 1 to 7, 5 min of sprinkling every 90 min 16 times a day; and (iii) row C, plots 1 to 7, 5 min of sprinkling every 3 h 8 times a day. Sprinkling was applied with a Na'an sprinkler (Na'an Company, Na'an, Israel) with a capacity of 3.2 mm of rain per h located at the center of each plot at a height of 1 m above ground. During the 42 days of the experiment (16 December 1997 to 27 January 1998), 16 natural rain events (>1 mm) occurred, totaling 151 mm of rain. With the supplemented sprinkling, the total rain, as measured with a rain gauge placed in each row, was 765, 287, and 219 mm in rows A, B, and C, respectively. A thermohygrograph was located in the middle of the field 30 cm above ground.

Fungal isolates. Table 1 describes the isolates used for inoculation. Isolates 1 to 10 were used in pair combinations 1 to 7 (Table 2). Pairs 1 to 6 each included one A1 and one A2 isolate, whereas pair 7 consisted of two A2 isolates, one sensitive (minimal inhibitory concentration [MIC] = 0.1 ppm) and the other resistant to metalaxyl (MIC > 100 ppm; [22]). The rationale for choosing a sensitive and a resistant isolate in each pair combination was to identify recombinant metalaxyl-intermediate isolates in the next growing season. Fungal isolates were permanently maintained on potato tuber slices (cv. Alpha) with repeated inoculations at 14°C in the dark.

Inoculation. One-week-old tuber slice cultures of the organism were used. Sporangia were removed from tuber slices by mo-

mentary dipping in distilled water at 4°C. Inoculum concentration was adjusted with the aid of a hemacytometer to 1,000 sporangia per ml. Pair combining (Table 2) was done by mixing 1 liter of sporangial suspension of one isolate with 1 liter of sporangial suspension of the other isolate. Each mixed isolate suspension (2 liters) was sprayed (at 1500 h) on three potato plots growing in the field. Pair number 1 was inoculated on plots A1, B1, and C1, and pair number 2 on plots A2, B2, and C2. (Table 2).

The formation of oospores in vitro from the same mixtures of sporangia was measured as described previously (4). Briefly, an aliquot of each sporangial mixture was applied to 10 detached leaflets (≈15 cm² each) of potato (cv. Alpha) with 10 droplets (10 µl each) per leaflet. Leaflets were floated on water in petri dishes and incubated for 10 days at 15°C with a 12-h light photoperiod. Oospore counts are given in Table 2.

Disease assessment. Disease development was monitored as previously described (26). A visual estimate of the percentage of leaf area blighted in each plot was taken periodically. The mean severity over the seven plots of each row (rain regime) was plotted against time and used to compute areas under the disease progress curves (AUDPC).

Mating type determination. Infected potato leaflets, each harboring a single lesion of late blight (≈5-mm diameter), were collected from the field. Each leaflet was placed in a moistened plastic bag and incubated at 15°C in the dark for ≈18 h to allow for fungal sporulation. The produced sporangia were collected in 2 ml of cold distilled water and used for mating type determination by coinoculation with two tester isolates (314 [A1] and 317 [A2]) in the following manner: one potato (cv. Alpha) leaflet, laid on moistened filter paper in a petri dish, was inoculated with 6 droplets (10 µl each) of the tested isolate, a second leaflet was inoculated with 3 droplets of the tested isolate on one side of the midvein and with 3 droplets of isolate 314 (A1) on the opposite side of the midvein, and a third leaflet was inoculated with 3 droplets of the tested isolate and 3 droplets of isolate 317 (A2). Leaflets similarly inoculated with 314 + 317 were used as positive controls. Dishes were incubated for 10 days at 15°C (12 h of light per day) and examined for oospore production as described previously (4). When the tested isolate formed oospores in a leaflet coinoculated with isolate 314 it was considered to be A2, with isolate 317 it was considered to be A1, and with both 314 and 317 it was considered to be a mixture of A1 and A2.

Sampling. First leaf samples were collected from the field on 4 January 1998 (19 days after inoculation, ≈2 weeks after initial appearance of disease symptoms). Other leaf samples were taken on 7, 11, and 14 January. On 23 January, stem samples were collected. On 26 January the canopy of all treatments was totally destroyed by blight. Tubers were harvested on 27 January, stored in separate paper bags for each plot at 20°C for 2 weeks, and examined for oospores.

At each sampling time two samples of 10 leaves each were collected. One sample was removed from the soil surface (detached, completely blighted leaves were either stripped off the soil or taken with a thin layer of soil) and the other sample was removed from the plant (attached, completely blighted leaves hanging on the plant at a height of ≈20 cm). Each sample contained leaves representing the various parts of the plot. A leaf sample was placed in a beaker, washed thoroughly with running water over a screen to remove soil particles, and clarified in boiling ethanol (50%) for 10 min. Individual leaflets were randomly taken from the beaker and mounted on microscope slides in 50% glycerol for microscopical examination (4). A total of 20 leaflets per plot were examined microscopically at each sampling date, 10 from the soil surface and 10 from 20 cm above ground.

Five stems were removed from each plot from ≈15 cm height and a 5-cm segment was taken from each stem. Stem segments were longitudinally cut to fine slices, boiled as described above, mounted in glycerol, covered with 6-cm cover slip, gently

TABLE 1. Field isolates of *Phytophthora infestans* used in this study

Isolate	Isolate code	Year	Mating type	Metalaxyl ²
1	EH	1994	A2	Resistant
2	EHF	1995	A2	Sensitive
3	GE	1997	A1	Resistant
4	KS	1994	A1	Sensitive
5	RE	1993	A2	Resistant
6	YAS	1995	A2	Sensitive
7	NE	1994	A1	Resistant
8	TH	1995	A1	Resistant
9	314	1987	A1	Sensitive
10	317	1987	A2	Resistant
11	T0497	1997	A1	Sensitive

² Based on the tuber-disk bioassay of Kadish and Cohen (22). Minimal inhibitory concentration (MIC) for sensitive isolates at 0.1 µg a.i./ml metalaxyl and for resistant isolates >100 µg/ml.

squashed, and examined microscopically. The number of oospores in a leaflet was determined by a dissecting microscope at $\times 40$ magnification (or, when required, at $\times 100$).

Oospores in blighted tubers. Only a limited search for oospores in tubers was conducted. After 2 weeks of storage at 20°C, 10 blighted tubers were randomly taken from plots inoculated with pairs 1, 5, and 6. Each tuber was cut into two halves and 10 pieces of $\approx 1 \times 1 \times 0.1$ cm were sliced off from areas showing necrosis. Tissue was boiled in 1% HCl for 30 min to dissolve starch grains, mounted in 50% glycerol, and examined microscopically for the presence of oospores. Preliminary observations indicated that boiling in 1% HCl does not destroy oospores.

Data analysis. Two parameters were used for analysis: (i) the proportion of leaflets in a sample containing oospores (= frequency of oospore-bearing leaflets) and (ii) the mean number of oospores per leaflet in a sample.

Three variables were analyzed for their effect on each of the two parameters: (i) total rain (219, 287, or 765 mm during the experiment), (ii) position of the leaflets on the plant's canopy (0 to 20 cm above soil surface), and (iii) pair combination (six pairs of A1 + A2 isolates). Pair combination number 7, which was composed of A2 + A2 isolates and showed a single leaflet with oospores (described below), was omitted from all analyses. Because only one replicate plot was used for each pair combination in each rain regime, no variance analysis could be employed. Therefore, we have presented means and grand means of pooled data.

Experiment 2 (winter 1999 to 2000). Nine plots (7 m² each) were planted with 42 potato tubers cv. Mondial on 19 October 1999. Plots were separated by 3 m of rye crops to the south and north and 4 m of fallow land to the east and west. A single, overhead sprinkler (with a capacity of 3.2 mm rain per h), was placed in the center of each plot (0.8 m high). Five weeks after planting (24 November) plots were spray-inoculated (at 1700 h) with mixed sporangial suspension at a ratio of 1:1 of pair number 8 (Table 2). Ten days after inoculation, when disease symptoms were visible, sprinkling irrigation was applied to the field for the following 30 days. Plots were divided into three groups with three plots in a group in a complete-randomized block design. Plots in group A were sprinkle-irrigated for 15 min/h, plots in group B were sprinkle-irrigated for 5 min every 90 min, and plots in group C were sprinkle-irrigated for 5 min every 3 h, in a similar program as in experiment 1. All three replicate plots in a group were connected to the same irrigation-control electronic device. A thermohygrometer was stationed in the middle of the field at 0.5 m above ground.

During the experimental period only four natural rain events occurred (between 14 to 25 December) totaling 65 mm rain. With the supplemented sprinkling the total rain in plots of groups A, B, and C amounted to 641, 193, and 129 mm, respectively.

Leaf samples were collected from each plot separately, on 12 December (18 days after inoculation), 21 December (26 days), and 3 January (40 days). Stem samples were collected on 3 January, and tubers were harvested on 6 January and stored in paper bags for 3 weeks at 15°C. At each date, fully blighted leaves were

collected from the ground level (0 cm) and from the midcanopy (20 cm above ground) as in 1998. Leaf and stem samples were processed for microscopical examination as described above.

Statistical analysis. Duncan's multiple range test was applied to examine the effect of rain level on oospore leaf frequency and the number of oospores per leaflet.

RESULTS

Weather conditions, disease progress, and mating type frequency in experiment 1. Minimal night temperatures ranged between 4 and 11°C, and maximal day temperatures ranged between 15 and 27°C. These temperatures were favorable for oospore formation under laboratory conditions (4). Disease progressed almost equally fast in all 21 plots. No differences in disease progress were recorded between the plots (various isolate-pair combinations) in each row (data not shown). Mean AUDPC values for rows A, B, and C were 1,894, 1,818, and 1,665, respectively. At 23 days we sampled 10 leaflets at random from each plot (plots 1 to 6 from each of the three rows totaling 180 leaflets) for mating type analysis. At this time, $\approx 40\%$ of the foliage area was blighted. Care was taken that each leaflet harbored a single small (≈ 5 mm) lesion to achieve a better resolution of the behavior of the various isolates. We found that about half of the leaflets were colonized with both mating types, one quarter with A1 and one quarter with A2 mating type, indicating an equal frequency of colonization by each mating type. Grouping the mating type distribution according to the rain regime showed no correlation.

Oospore production in the foliage. Nineteen days after inoculation, only 2 of the 420 leaflets examined had oospores. Both leaflets were collected from the soil surface of row A (heavily irrigated). On day 22, eight leaflets showed oospores, all collected from the soil surface. A major increase in the number of oospore-bearing leaflets was noticed on day 26. Thirty, eight, and one leaflet collected from the soil surface of rows A, B, and C, respectively, showed oospores, versus 10, 2, and 1 in leaflets collected from the midcanopy. Similar results were found on day 29, indicating that leaflets lying on the ground supported better sexual reproduction of the fungus than leaves not in soil contact. Out of 1,680 leaflets examined during the season, a total of 132 (7.86%) leaflets bear oospores (Table 3). Only one leaflet with oospores was found in plots inoculated with pair 7 (A2 + A2). Classifying the distribution of the oospore-bearing leaflets (a total of 132) according to rain regime and leaf position showed 50.8, 18.9, and 4.5% of them were detected on the soil surface in rows A, B, and C, respectively (total of 74.2%), whereas 18.9, 6.1, and 0.76% of them were detected on the midcanopy in rows A, B, and C, respectively (total of 25.8%). Thus, the occurrence of oospore-bearing leaflets was about three times higher under heavy rain than under moderate rain and three times higher under moderate rain than under light rain. It was also about three times higher at ground level than at 20 cm above soil (Table 3).

TABLE 2. Combined isolate mixtures of *Phytophthora infestans* for inoculating potato crops in the field and their capacity to form oospores in detached leaves in petri dishes

Experiment	Pair no.	Paired isolates ^x	Inoculated to plots ^y	Oospores per leaflet ^z (in vitro)
1 (winter 1997 to 1998)	1	GE (A1, R) + EHF (A2, S)	A1, B1, C1	9,750 $\pm$ 2,250
	2	KS (A1, S) + EH (A2, R)	A2, B2, C2	9,500 $\pm$ 1,050
	3	KS (A1, S) + RE (A2, R)	A3, B3, C3	11,750 $\pm$ 3,250
	4	NE (A1, R) + YAS (A2, S)	A4, B4, C4	8,000 $\pm$ 1,100
	5	314 (A1, S) + 317 (A2, R)	A5, B5, C5	9,250 $\pm$ 750
	6	TH (A1, R) + YAS (A2, S)	A6, B6, C6	6,250 $\pm$ 250
	7	EHF (A2, S) + RE (A2, R)	A7, B7, C7	0
2 (winter 1999 to 2000)	8	T0497 (A1, S) + 317 (A2, R)	A1, A2, A3 B1, B2, B3 C1, C2, C3	2,400 $\pm$ 280

^x Isolate code, mating type, and sensitivity to metalaxyl: S = sensitive and R = resistant to metalaxyl.

^y A = heavy, B = moderate, and C = light rain regime.

^z Mean $\pm$ SD from 10 leaflets. Leaflets were floated on water in petri dishes at 15°C (4). Pairs 1 to 7 were tested on potato cv. Alpha and pair 8 on cv. Mondial.

Of the 35 stems taken from each of rows A, B, and C on 23 January (38 days), 16, 4, and 4, respectively, carried oospores (Table 3). One of the sixteen oospore-bearing stems in row A was found with pair 7 (A2 + A2).

Table 4 presents the mean number of oospores counted per leaflet at each sampling date for all six pair combinations. Mean oospore density per leaflet increased with time more rapidly as rain was heavier, and was greater in leaves collected from ground level than in leaves collected from 20 cm above ground. At all sampling dates oospore density was higher under heavy rain than under moderate rain and higher under moderate rain than under light rain. The grand mean data (Table 4) shows a significant difference in oospore counts between row A and row C at ground level but not at 20 cm above ground, with about two to four times more oospores produced in leaves lying on the soil surface than in those higher on the canopy. Oospore counts per leaflet usually ranged between 10 to 100 and never exceeded 200. The maximal mean number of oospores per leaflet was 43. This figure is far below the counts (several thousand) obtained in growth chambers (Table 2).

Pooled data for leaflets in each pair in all rows (irrigation regimes) and sampling dates showed that the various A1 + A2 paired isolates did not differ in either the frequency of oosporic leaflets or the density of oospores per leaflet in samples taken from the midcanopy (20 cm). However, in samples collected from the soil surface (0 cm), pair 5 was more fertile than any other pair and produced 25.8% oospore-bearing leaflets compared with 8.3 to 16.7% in the other pairs, and 22 oospores per leaflet compared with 5 to 16.5 in the other pairs. Pair 5, however, was not the most fertile in growth chamber studies (Table 2).

Oospores in tubers. Because of the severe blight on the foliage, tuber yield was low. Only 128 to 235 tubers were harvested per plot (data not shown) at the end of the season. Mean percentage of blighted tubers in rows A, B, and C (for all seven plots in a row) was 36.1 ± 10.9 , 55.0 ± 18.1 , and $33.1 \pm 11.1\%$, respectively. Mean percentage of blighted tubers in plots inoculated with pairs 1 to 7 ranged between 37 and 49%. Ten blighted tubers per plot from plots inoculated with pairs 1, 5, and 6 in rows A, B, and C (totaling 90 tubers) were microscopically examined for the pres-

ence of oospores. Ten sections were scored per tuber. Oospores were found in only 2 of the 90, both from row A and inoculated with pair 1. In one tuber, 1 of the 10 sections had 20 oospores and in the other tuber one section had 40 oospores. These data are consistent with results of our in vitro experiments (A. Levin and Y. Cohen, *unpublished data*) which showed that very few oospores are formed within tubers of cv. Alpha inoculated with a mixture of A1 + A2 isolates.

Experiment 2. Minimal night temperature ranged between 2 to 19°C (mean $\pm$ SD = 9.07 ± 3.59) and maximal day temperatures between 17 to 26°C (21.9 ± 2.4).

Initial disease symptoms were observed on 1 December 1999, 7 days after inoculation. Disease progressed quite rapidly in all plots causing blight in 97.0 ± 0.2 , 96.5 ± 1 , and $93.3 \pm 4\%$ of the foliage at day 43 in plots A, B, and C, respectively. Disease progressed somewhat faster in plot A than B, and faster in plot B than C. AUDPC accumulated to 2,022, 1,904, and 1,805 units in the A, B, and C plots, respectively. Mean AUDPC in the A plots was significantly (at 5% level) higher than in the C plots but not higher than the B plots. AUDPC values in the B and C plots were not significantly different.

On 1 December 1999, five infected leaflets were randomly collected from each plot, totaling 45 leaflets, and placed on wet filter paper in petri dishes at 15°C to induce the formation of oospores (4). After 10 days oospores were found in all leaflets indicating the establishment of both mating types in the field. On 3 January 2000 (40 days) 22 leaflets, each bearing a single lesion of ≈ 0.5 -cm diameter were collected from the field. Sporangia were collected from each leaflet separately and examined for mating type by coinoculating healthy potato leaves with each of the parent isolates with the two tester isolates. Of the five samples collected from plot A, three were A1 and two were A2; of nine samples from plot B, five were A1, three were A2 and one consisted of both A1 and A2; and of eight samples from plot C, five were A1 and three were A2. The results indicate that both mating types survived until the end of the epidemic (59% A1 and 36% A2).

The occurrence of oospores in the field was monitored in leaflets collected at 18, 26, and 40 days after inoculation when disease

TABLE 3. The number of blighted potato leaflets (out of 10) with oospores at 0 or 20 cm above soil surface and stems (out of five) collected from field plots of experiment 1 (winter 1997 to 1998) at various times after inoculation with six pairs of A1 and A2 and one pair of A2 + A2 *Phytophthora infestans* isolates

Row irrigation regime	Plot/pair	19 days		22 days		26 days		29 days		Total		38 days
		0 cm	20 cm	0 cm	20 cm	0 cm	20 cm	0 cm	20 cm	0 cm	20 cm	Stems
A (high)	1	0	0	0	0	4	0	2	3	6	3	2
	2	1	0	2	0	5	0	2	2	10	2	2
	3	0	0	0	0	4	1	8	6	12	7	3
	4	1	0	4	0	2	3	2	2	9	5	4
	5	0	0	2	0	7	5	8	2	17	7	1
	6	0	0	0	0	7	1	4	0	12	1	3
	7	0	0	0	0	1	0	0	0	1	0	1
Subtotal per row		2	0	8	0	30	10	26	15	67	25	16
B (moderate)	1	0	0	0	0	1	1	2	1	3	2	3
	2	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	1	0	0	0	0	0	1	0	0
	4	0	0	0	0	1	0	2	1	3	1	1
	5	0	0	1	0	3	1	6	2	10	3	0
	6	0	0	1	0	3	0	4	2	8	2	0
	7	0	0	0	0	0	0	0	0	0	0	0
Subtotal per row		0	0	3	0	8	2	14	6	25	8	4
C (low)	1	0	0	0	0	0	1	1	0	0	1	0
	2	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	1
	4	0	0	1	0	0	0	1	0	2	0	0
	5	0	0	0	0	1	0	2	0	3	0	2
	6	0	0	0	0	0	0	1	0	1	0	1
	7	0	0	0	0	0	0	0	0	0	0	0
Subtotal per row		0	0	1	0	1	1	5	0	6	1	4
Grand total per date		2	0	12	0	39	13	45	21	98	34	24

reached a level of ≈ 40 , ≈ 60 , and $\approx 80\%$, respectively. Overall, 40 of the 519 leaflets examined had oospores (7.7%); all were collected from the soil surface. Data presented in Table 5 indicates a significant effect of rain on oospore formation. On days 18 and 26, oospores were only detected in the heavily sprinkled plots (641 mm). At 40 days, oospores were found in all three rain regimes with a significantly higher frequency and density measured in plots of regime A as compared to plots of regimes B and C. Unexpectedly, no oospores were found in leaves taken from 20 cm above soil level nor in any of the 45 infected stem segments collected on 3 January (40 days) from the midcanopy.

The mean frequency of oospore-bearing leaflets increased with time in plots of regime A. Thus, at 18, 26, and 40 days, a mean of 3.7, 21.7, and 81.7% of the leaflets contained oospores, respectively. However, the mean number of oospores per leaflet was quite low and reached a maximal mean of 8.06 oospores per leaflet at day 26. At 40 days, 81.7, 11, and 7.3% of the leaflets in the A, B, and C plots, respectively, harbored oospores, with an extremely low density of 5.1, 0.22, and 0.15 oospores per leaflet, respectively. Oospore counts usually ranged between 2 to 10 and never exceeded 50. Artificial coinoculation of potato leaflets (cv. Mondial) with the same pair of isolates, 317 + T0497, under laboratory conditions at 15°C yielded 2400 ± 280 oospores per leaflet ($\approx 15 \text{ cm}^2$). This pair was the least fertile among the eight pairs used in this study (Table 2).

At harvest no blight symptoms were seen on the tubers. After 3 weeks of incubation at 15°C in the dark, blight symptoms were detected in 13.6 ± 2.2 , 16.9 ± 5.8 , and $15.5 \pm 0.9\%$ of the tubers collected from the plots exposed to heavy (A), moderate (B), and light (C) rain regimes, respectively. Mean number of tubers harvested per plot was 161 ± 6.5 , 171 ± 2.2 , and 161 ± 4.3 for regimes A, B, and C, respectively. At 4 weeks after harvest, five blighted tubers per plot (total of 45 tubers) were sectioned and observed for presence of oospores. No oospores were detected in these tubers.

DISCUSSION

It is commonly accepted that sexual reproduction of *P. infestans* poses a threat to the potato and tomato industries worldwide (8,10,25). Not only would oospores provide a long-lasting inocu-

lum source (1), but also more damaging recombinant genotypes could be produced (10,11,16,19). It was not the purpose of the present study to address these important questions of recombination and persistence of oospores of *P. infestans*, but rather to study the effect of free moisture on oospore formation in potato tissue under field conditions.

Our previous data (4) showed that one, if not the most, important environmental factor affecting oospore production in leaves of tomato and potato is the availability of free water. Detached leaves floating on water in ≈ 10 days produced abundant (up to 100 per mm^2) oospores after inoculation with mixed A1 + A2 sporangia, whereas attached leaves in whole plants kept under water-saturated atmosphere produced only a few. This is why we set up our present experiments in field-grown potato crops treated with various doses of overhead sprinkling irrigation.

The data presented show that oospore production in the field was poor compared to the oospore production of detached leaves in growth chambers. Both the proportion of leaves in which oospores were found and the number of oospores produced per leaflet were low. In experiment 1, of the 1,440 blighted leaflets collected from 18 plots inoculated with six pairs of A1 + A2 sporangia only 131 harbored oospores (9.1%). The number of oospores per leaflet was usually between 10 to 100 and never exceeded 200. Similar leaflets ($\approx 15 \text{ cm}^2$) produced as many as 6,000 to 12,000 oospores per leaflet when floated on water at 15°C in a growth chamber (Table 2). In experiment 2, only 40 of the 519 leaflets collected (7.7%) contained oospores. The number of oospores was usually between 2 and 10, with one maximal sample containing 50. This pair was the least fertile in the detached leaflet assay, among the eight pairs used (Table 2). Leaf position on the potato canopy was another major factor influencing oospore formation. Leaves lying on the soil surface allowed for earlier, more frequent, and denser oospore production than intact leaves at 20 cm above soil surface. While no oospores were detected in leaves 20 cm above soil level in experiment 2, a several-fold increase (2.6 to 7.3 times in frequency and 2.8 to 4.3 times in density) in oospore production was observed in bottom versus upper leaves in experiment 1 (Table 3). Leaves lying on the soil surface were wet for longer periods than leaves located at 20 cm above ground on the stem. This may provide one possible explanation why such leaves had consistently higher proportions

TABLE 4. The influence of sprinkling irrigation and leaf position on the mean number of oospores formed per leaflet by six pairs of A1 + A2 isolates of *Phytophthora infestans* in potato crops under field conditions of experiment 1 at 19, 22, 26, and 29 days after inoculation^y

Row	Total rain (mm)	19 days		22 days		26 days		29 days		Grand mean ^z	
		0 cm	20 cm	0 cm	20 cm	0 cm	20 cm	0 cm	20 cm	0 cm	20 cm
A	765	2.0	0	18.0	0	43.0	16.0	32.0	18.5	23.8 $\pm$ 17.4 b	8.6 $\pm$ 10.0 a
B	287	0	0	2.5	0	11.5	1.5	21.5	13.5	8.9 $\pm$ 9.8 ab	3.8 $\pm$ 6.5 a
C	219	0	0	0.4	0	0.4	0.4	4.0	0	1.2 $\pm$ 1.9 a	0.1 $\pm$ 0.2 a

^y Pooled data for six pair combinations (Table 2).

^z Values within the same column followed by different letters are significantly different according to Duncan's multiple range test ($P > 0.05$; $n = 4$).

TABLE 5. The influence of sprinkling irrigation and leaf position on the mean percentage of leaflets with oospores and the mean number of oospores per leaflet by one pair of A1 + A2 isolates of *Phytophthora infestans* in potato crops under field conditions in experiment 2 at 18, 26, and 40 days after inoculation^y

		18 days (12 Dec. 1999)		26 days (21 Dec. 1999)		40 days (3 Jan. 2000)		Grand mean	
Plot	Total rain (mm)	0 cm	20 cm	0 cm	20 cm	0 cm	20 cm	0 cm	20 cm
% Leaflets with oospores ^z									
A	641	3.7 a	0	21.7 b	0	81.7 b	0	35.7 b	0
B	193	0 a	0	0 a	0	11.0 a	0	3.7 a	0
C	129	0 a	0	0 a	0	7.3 a	0	2.4 a	0
No. of oospores per leaflet									
A	641	0.37 a	0	8.06 b	0	5.10 b	0	4.70 b	0
B	193	0 a	0	0 a	0	0.22 a	0	0.07 a	0
C	129	0 a	0	0 a	0	0.15 a	0	0.05 a	0

^y Values within a column followed by different letters are significantly different according to Duncan's multiple range test ($P > 0.05$).

^z Sample size per plot ($n = 3$) per leaf position consisted of nine leaflets, except for day 26 at 0 cm in plot A when 20 leaflets consisted of a sample. Total number of leaflets sampled was 519, of which 40 had oospores (7.7%).

of oospore leaflets and higher oospore counts than leaves taken from the midcanopy. Another explanation may be related to leaf age. Our laboratory-detached leaflet assay indicated that bottom leaves of 3-month-potted potato plants support higher ($1.3\times$ to $1.5\times$) oospore production than middle leaves (data not shown). Our data stand in accordance with the results obtained by Hanson and Shattock (21). They counted up to 80 oospores per leaflet in potato crops growing in plastic tunnels, whereas up to $\approx 20,000$ oospores per cm^2 were produced in leaf disks in petri dishes. The fact that these workers found only six oospore leaflets among the same cultivars growing in the open field emphasizes the importance of free water on oospore formation.

Although we provide good evidence on the influence of wetness and leaf position on oospore formation, it is still puzzling why oospore production was so low. It is obvious that the two mating types were well established in the field in both experiments. However, our experimental evidence indicates the occurrence of both mating types in only half of the leaflets examined. This may partially explain the low proportion of oospore-bearing leaflets found but not the low number of oospores produced in a leaflet. It is also clear that ambient temperatures were favorable to oospore production in both seasons. Also, the time period was long enough to enable oospore production in the field. In the laboratory, 9 to 10 days postinoculation sufficed for abundant oospore formation (4). Drenth et al. (6) observed abundant oospores (numbers not specified) in field-grown potato 13 to 17 days after inoculation. In the present study, leaf samples were collected from 29 to 40 days postinoculation, until the canopy was almost or fully blighted, and still oospore production was low. In experiment 1 no direct relationship was observed between the fertility of a pair-isolate combination in a growth chamber as compared with the field. Pair 5 was somewhat more fertile in the field but not necessarily in growth chambers. In experiment 2, the poor fertility of pair 8 in the laboratory reflected poor oospore yields in the field, but still laboratory counts were far higher than those measured in the field.

We therefore, suggest that the relatively low oospore yields in the field resulted from lack of sufficient moisture. The high yields in detached leaves floating on water in growth chambers is artificial and cannot be mimicked under normal growth conditions of potato crops in the field.

Surprisingly, tubers, although heavily blighted, did not support oospore production. Only 20 and 40 oospores were found in 2 of the 90 tubers examined in experiment 1, and no oospores were found in experiment 2. This is diametrically opposed to the abundant oospores (several thousand per gram tissue) formed in tomato fruits upon coinoculation with A1 + A2 isolates (Y. Cohen, unpublished data). It seems that oospores are formed more abundantly in water-rich tomato fruits than in comparatively drier tubers. Our previous study (20) also showed low oospore occurrence in tubers collected from the field. The reasons for this poor oosporeogenesis in tubers is not understood. Perhaps the lack of free water is a factor.

Although oospores were observed in many stems of experiment 1, no oospores were detected in any stems in experiment 2. This lack of oospores in stems corresponds to the lack of oospores in leaves at 20 cm above ground level, and to the general, very low oospore yield observed in leaves lying on the soil surface. The overall low sexual productivity of the fungus in experiment 2 may result from the intrinsic low fertility of the pair combination used.

In conclusion, the evidence provided in this study shows that oospore production by *P. infestans* in potato foliage in the field was far lower than that observed in detached leaves in growth chambers. Under the favorable temperature conditions that prevailed, moisture environment and leaf age had prominent influence on oospore formation. Heavy rain supported earlier and higher oospore production. Bottom (wetter and older) leaves touching the soil surface enabled oospore production more frequently and abundantly than leaves on the midcanopy.

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