Relationship of Potato Leaf Sterols to Development of Potato Late Blight Caused by *Phytophthora infestans* on U.S. Potato Clones and Breeding Lines

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ABSTRACT

Hazel, W. J., Bean, G. A., and Goth, R. W. 1988. Relationship of potato leaf sterols to development of potato late blight caused by *Phytophthora infestans* on U.S. potato clones and breeding lines. Plant Disease 72:203-205.

Detached leaves of *Solanum tuberosum* numbered clonal selections B6026-WV-5, B6039-WV-9, and B6086-WV-21 and the cultivars Irish Cobbler and Sebago inoculated with zoospore suspensions of a race 1,2,3,4 isolate of *Phytophthora infestans* had sporangial production at the infection sites that was correlated to their respective field resistances. However, the sterol content of the leaves did not relate to either field resistance or sporulation. The sterols sitosterol and cycloartenol were most prevalent, but cholesterol, campesterol, stigmasterol, and 24-methylene cycloartenol were present in varying concentrations in all the foliar tissues assayed. When sterol extracts from foliar tissue were added to Elliott's defined medium at rates comparable to those present in foliar tissue, sporulation of *P. infestans* was increased relative to medium without sterol extracts.

Elliott et al (1), Haskins et al (5), Hendrix (6), and Leal et al (8) reported that various *Phytophthora* and *Pythium* spp. require sterols for zoospore production. It was also reported that addition of sterols to media increased growth rate and stimulated sporangial production by *Phytophthora infestans* (Mont.) de Bary. Langcake (7) noted that sporangial production and growth rate, measured by mycelial dry weight and colony diameter, were influenced by sterol type, and sporangial production was greatest on media containing sitosterol and lowest on media containing lanosterol.

In 1969, Elliott and Knights (3), using *P. cactorum* as a model, suggested that the ratio of cycloartenol to sitosterol found in potato leaves may influence the susceptibility of potato clones to infection by *P. infestans*. They hypothesized that resistance reactions would occur when a high level of the sitosterol precursor cycloartenol, which interferes with growth and sporulation of *P. infestans*, was present. However, Langcake in an early study reported that two susceptible Scottish potato cultivars, Majestic with minor r gene resistance and Ulster Chieftain with no resistance, contained more cycloartenol relative to sitosterol than the resistant cultivar Pentland Dell with R1,2,3 major gene resistance (10). Furthermore, when sterol extracts from Majestic and Pentland Dell were added to media at adjusted levels of sitosterol comparable to that found in leaf tissue, growth of *P. infestans* was the same although the cycloartenol levels were different (7).

The objective of this investigation was to use a U.S. isolate of *P. infestans* to study the importance of sterols on disease development in U.S. potato clones and numbered clonal selections by: 1) analyzing the sterol contents of three U.S. numbered clonal selections and two potato cultivars that differ in field and major gene resistance to *P. infestans*, 2) correlating sterol content with the number of sporangia produced on inoculated detached leaves of numbered clonal selections and cultivars with different resistance levels, and 3) incorporating sterols extracted from leaves of the clonal selections and

Maryland Agricultural Experiment Station Scientific Article A-2475, Contribution 5505.

Accepted for publication 23 September 1987.

cultivars into defined media to determine their influence on sporulation of *P. infestans* in vitro.

MATERIALS AND METHODS

Growth of pathogen and potato tissue. *P. infestans* race 1,2,3,4 used in this study was maintained at 18 C in the dark on Elliott's defined medium (1). Throughout this study, cultures were routinely transferred to Elliott's defined medium at intervals of 3–4 wk.

The *Solanum tuberosum* L. numbered clonal selections and cultivars used were B6026-WV-5 (R genes 1,3 and good field resistance), B6086-WV-21 (R genes 1,2,3 and fair field resistance), B6039-WV-9 (R genes 1,2,3,4 and medium to low field resistance), Sebago (no R genes and medium to low field resistance), and Irish Cobbler (no R genes and susceptible under field conditions). Plants were grown in the greenhouse under a 12-hr light-dark regime or continuous light provided by high-pressure sodium lights with intensity of 26.2–32.2 $\mu E/m^2/sec$. The ambient temperature ranged from 15 to 22 C. Compound leaves at the sixth and eighth nodes from base were detached for inoculation studies and sterol extractions.

Detached leaf inoculation and sporangial counts. Ten leaves, two from each of five plants (collected at the sixth and eighth nodes from the base), were removed from each numbered clonal selection and cultivar. The method of Goth (4) was used for inoculating detached leaves. Petioles of the detached leaves were inserted into moist vermiculite in a plastic humidity chamber with clear plastic covers. Pathogenicity tests were made on comparable detached and attached leaves of the test plants. All inoculated leaves and controls were maintained for 6 days at 15 C in humidity chambers with 100% relative humidity and continuous light (6.1 $\mu E/m^2/sec$).

The *P. infestans* isolate was grown in plastic petri dishes 100 × 15 mm containing 15 ml of Elliott's defined media (1) at 15 C in the dark. The inoculum was prepared by washing 21-day-old cultures of *P. infestans* with 5 ml of chilled (5 C) distilled water. The resultant sporangial suspension was placed in a sterile plastic petri dish 100 × 15 mm and placed in an incubator at 15 C until 50% of the sporangia had released zoospores (4). A Spencer Brite Line hemacytometer was used to count the sporangia and zoospores. Pathogenicity tests were made using a Pasteur pipet to apply 200 μl of inoculum or 1.5×10^3 or 2.5×10^5 zoospores per milliliter to a 5-mm Whatman No. 40 filter paper disk that was placed on the adaxial surface of the leaf similar to the method described by Goth (4).

Six days after inoculation, the amount of infection was determined by counting the sporangia that developed in the sporulating annulus that surrounded each infection site. Counts were made by removing three 0.8-cm disks from the sporulating annulus and placing them in test tubes 8 × 1 cm containing 1 ml of distilled water. The test tube was shaken vigorously with a vortex stirrer for 30 sec. The number of sporangia in the resultant suspension was determined with a Spencer Brite Line hemacytometer. The resultant sporangial count was divided by three to give a mean count, and the total sporulation per infection site was calculated by extrapolation.

Sterol analysis of potato leaves. Opposite compound leaves, similar in age to those inoculated, were removed, one each from the sixth and eighth node from the bases of the respective plants. Five plants of each clonal selection and cultivar were sampled as follows: All leaves collected from a specific node of a given cultivar or selection were combined and dried at 110 C for 48 hr. Dried leaves were ground into a powder and a dry weight taken. Lipids were extracted and weighed, and sterols, including free and esterified sterols and terpenoids, were isolated and purified by the procedure developed by Patterson (9). Sterols were identified by gas liquid chromatography on a Chromalab-310 gas chromatograph (Glowall Corporation).

Sporangial production on media containing potato leaf sterols. The sterols extracted from two plants from each of the numbered clonal selections and cultivars were incorporated into Elliott's medium at concentrations equivalent to those found in leaf tissue on a wet weight basis. A 10-mm disk from 2-wk-old cultures of *P. infestans* growing on sterol-free Elliott's medium was placed in the center of each 9-cm petri dish containing 15.0 ml of Elliott's medium plus the sterols. Plates were incubated at 18 C in the dark for 21 days, and disks 6–7 mm in diameter were removed at random from the plate and suspended in 1.0 ml of distilled water. Sporangial counts were taken, the sporangia were washed, and zoospore production was evaluated using the method of Goth (4). Growth rates were also determined by measuring colony diameters after 12 days.

RESULTS

Sterol analyses. Degree of resistance, total lipids, and total sterols of the numbered clonal selections and cultivars are compared in Table 1. The plants in experiment A received 24 hr of light, whereas the same number of plants in experiments B and C received an alternating 12-hr light-dark regime. The total lipid in the most resistant numbered clonal selection, B6026-WV-5 (110 mg/g dry wt), was twice that of the most susceptible cultivar, Irish Cobbler (55 mg/g dry wt), whereas the other numbered clonal selections and cultivar had intermediate levels of lipids and had intermediate infections. The total sterol content was essentially the same (1.6–2.5 μg/g) in all test lines, whereas percent sterol per dry weight of plant tissue was highly variable between samples in experiments A, B, and C.

The percentages of the individual sterols in the leaf tissue of each numbered clonal selection and cultivar are summarized in Table 2. In all test lines, the sterols found in highest concentrations were sitosterol and cycloartenol. The

Table 1. Lipid content of leaves of numbered clonal selections and potato cultivars with different degrees of field resistance to *Phytophthora infestans*

Clone or cultivar	Degree of field resistance	Total lipid/g dry wt[a]	Total sterol/g total lipid[a]	Total sterol/g dry wt (g)			
				A	B	C	Mean
B6026-WV-5	Good	110	1.6	29	156	305	163
B6086-WV-21	Fair	73	1.8	12	189	158	120
B6039-WV-9	Medium–low	56	1.9	46	63	152	87
Sebago	Medium–low	21	2.5	208	24	133	122
Irish Cobbler	None	55	1.9	60	108	117	95

[a]Data are means of three experiments. Each experiment used two leaves from each of five plants.

Table 2. Sterol profiles of numbered clonal selections and potato cultivars with field resistance to *Phytophthora infestans*[a]

Clone or cultivar	Degree of field resistance	Percentage of total sterol[b]						
		A	B	C	D	E	F	G
B6026-WV-5	Good	5	11	1	4	30	45	4
B6086-WV-21	Fair	7	12	15	2	45	17	2
B6039-WV-9	Medium–low	6	11	7	5	46	23	2
Sebago	Medium–low	5	3	1	11	56	20	4
Irish Cobbler	None	4	17	1	6	28	40	4

[a]Data are means of three experiments, two leaves from each of five plants for each experiment.
[b]Codes for sterols: A = cholesterol, B = unknown sterol, C = campesterol, D = stigmasterol, E = sitosterol, F = cycloartenol, and G = 24-methylene cycloartenol.

percentage of the sitosterol component of the total sterols ranged from 56% in the cultivar Sebago to 28% in cultivar Irish Cobbler, and the cycloartenol content ranged from 45% in numbered clonal selection B6026-WV-5 to 17% in numbered clonal selection B6086-WV-21. The other sterols generally occurred in much lower concentrations, but in no instance was it possible to relate the level of a particular sterol to the degree of field resistance. An unidentified sterol (B in Table 2) with a relative retention time (RRT) of 1.09 on 3% SE-30 was found in all numbered clones and cultivars. Its retention time suggests it is a 4-dimethyl sterol, possibly desmosterol or ^{5,7}-cholestadienol, which have the same RRT as brassicasterol (RRT = 1.12) (9). The ratio of cycloartenol to sitosterol was the same (1.5) for the most resistant numbered clonal selection (B6026-WV-5) and the most susceptible cultivar (Irish Cobbler). Our results, using the indigenous race 1,2,3,4 isolate of *P. infestans* to inoculate numbered clonal selections with specific genes for resistance and the cultivars Sebago and Irish Cobbler, with no specific R gene resistance, agree with those of Langcake (7), who used the Scottish cultivars Majestic (with minor r gene resistance) and Pentland Dell (with R1,2,3 major gene resistance).

Detached leaf inoculation. Two days after inoculating leaves of the most resistant numbered clonal selections (B6026-WV-5 and B6086-WV-21) with race 1,3, necrotic flecks (1–2 mm diameter) were observed at each inoculation site. The susceptible cultivars, Sebago and Irish Cobbler, had confluent necrotic areas ranging from 5 to 10 mm in diameter at each inoculation site. These symptoms are characteristic field resistant and susceptible responses.

Sporangia production on detached leaves of the numbered clonal selections and cultivars is summarized in Table 3. There was a relationship between field resistance of a test line and the number of sporangia that occurred on its inoculated leaf. For example, on Irish Cobbler leaves (no field resistance), 2.9×10^5 sporangia were produced, whereas on the field resistant numbered clonal selection B6026-WV-5, 1.1×10^5 sporangia were produced. The remaining numbered clonal selections and cultivar produced intermediate amounts of sporangia. Zoospore production by sporangia from all sources was similar.

Sporangial production on defined media. The amount of sporangial production that occurred on Elliott's medium containing sterol extracts adjusted to levels comparable to those present in leaves from the numbered clonal selections and cultivars is also shown in Table 3. The growth rate of *P. infestans* was not affected by these levels of sterols in the medium. After 12 days of incubation, the colony diameters were the same for all treatments (8 cm). However, media without sterol and media with Irish Cobbler sterol extracts produced less sporangia than any of the treatments (Table 3). In contrast, the highest sporangial counts occurred on media containing sterol extracts from the most field resistant numbered clonal selections, B6026-WV-5 and B6086-WV-21. The sterol concentrations did not affect zoospore production of these sporangia.

Table 3. Sporangial production by *Phytophthora infestans* on detached leaves of numbered clonal selections and potato cultivars and on defined medium containing sterol concentrations adjusted to levels found in leaf tissue[x]

Clone or cultivar	Degree of field resistance	Total sterol/g dry weight	Sporangia[y] on detached leaves $\times 10^5$ (no.)	Sporangia[z] on defined medium $\times 10^5$ (no.)
B6026-WV-5	Good	164	1.1 a	9.0 a
B6086-WV-21	Fair	120	2.2 b	9.2 a
B6039-WV-9	Medium–low	87	2.2 b	8.0 ab
Sebago	Medium–low	122	2.4 b	8.3 ab
Irish Cobbler	None	95	2.9 c	7.0 bc
Check				5.8 c

[x] Data are means of three experiments; each experiment had two leaves from five plants. Numbers followed by the same letter are not significantly different from each other according to Duncan's multiple range test ($P = 0.05$).

[y] Data represent the number of sporangia on a 1-cm-diameter disk cut from the sporulating annulus.

[z] Data represent the number of sporangia on a 0.7-cm-diameter disk cut from the surface 3-wk-old cultures grown on Elliott's defined media (1) and respective sterol. Check consisted of Elliott's unamended medium.

DISCUSSION

The effects of sterols on the growth and development, especially sexual reproduction of *Phytophthora* spp., are well documented (1,2). However, their role in asexual reproduction, virulence, host resistance, and concomitant disease development caused by *P. infestans* infection of *S. tuberosum* germ plasm is not well understood. The results of this study suggest that total lipid content of leaf tissue may be related to field resistance, but their effect is not on sporangial production or lesion size, which are criteria used to explain field resistance. For example, leaves of the most resistant numbered clonal selection used in this study (B6026-WV-5) contained twice as much total lipids (110 mg/g dry wt) as did the most susceptible entry cultivar Irish Cobbler (55 mg/g dry wt). However, both attached and detached leaves of numbered clonal selection B6026-WV-5 with good field resistance and the highest sterol content (164 μg/g dry wt) produced the fewest sporangia (1.1×10^5/1-cm disk cut from sporulating annulus), whereas leaves on cultivar Irish Cobbler with sterol contents of 95 μg/g dry wt with no field resistance produced the most sporangia (2.9×10^5/1-cm disk cut from sporulating annulus).

Although earlier workers Elliott and Knights (3) suggested that tissue with a high cycloartenol/sitosterol ratio would be the most susceptible, our results confirmed the results of Langcake (7), who found that growth of *P. infestans* in vitro was not affected by the cycloartenol/sitosterol ratio. We also conclude that the cycloartenol/sitosterol ratio did not affect the degree of sporulation of *P. infestans* on both detached and attached leaves in vivo.

These results suggest that lipids are a contributing but not the sole factor in a complex of factors involved in the field resistance phenomenon of *S. tuberosum* to infection by *P. infestans*.

ACKNOWLEDGMENTS

We thank R. J. Young, West Virginia University, for numbered clones B6026-WV-5, B6039-WV-9, and B6086-WV-21, developed by USDA-ARS Potato Breeding Program, Beltsville, MD, and selected at West Virginia University. We also thank K. L. Deahl, Vegetable Laboratory, HSI, USDA, Beltsville, MD, for the race 1,2,3,4 isolate of *P. infestans*.

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