Leaf and Sprout Infection of Potato by Verticillium albo-atrum

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Michigan Agricultural Experiment Station Journal Article No. 4656. Supported in part by grants from the Michigan Potato Industry Council and the Michigan Department of Economic Expansion. Editorial comments of M. L. Lacy and G. W. Ayers are gratefully acknowledged.

Accepted for publication 13 August 1969.

ABSTRACT

Evidence was obtained histologically that hyphae of *Verticillium albo-atrum* penetrated potato leaves directly into the epidermis. Small, bronze-colored areas surrounded darkened centers. Later, the leaf became generally chlorotic. Hyphae first intracellular soon became intercellular also. Mesophyll tissue was quickly invaded sometimes within 24 hr. From inoculated leaves, invasion of the vascular elements of petioles was demonstrated histologically and by isolation. Further systemic spread of the fungus from the leaf lamina to the stem and to the tuber was shown by isolation.

Direct penetration through epidermal cells of young sprouts preceded granulation and death of the protoplast. Necrosis of apical and leaf primordial meristems extended close to the vascular elements. Phytopathology 60:196-203.

Verticillium albo-atrum was first described in 1879 as a potato parasite by Reinke & Berthold (20). Brown flecks on leaves of *Verticillium*-infected plants were sparse and not constant (20). This symptom, not usually considered typical of the disease as currently understood, suggests leaf infection. That potato leaves could be primary infection sites has received relatively little attention.

Infection of aerial parts by *V. albo-atrum* was demonstrated in 1959. Clover leaves were infected, and seedlings died after spraying leaves with spore suspensions (24). Leaf infection of tomato and eggplant occurred in the field (19), and in greenhouse inoculations the disease syndrome was similar to that resulting from root infection. Tomato leaves were infected with *V. tricorpus* in the presence of nutrients (8).

Subterranean potato stems may be infected by *V. albo-atrum* from the internally infected tuber (1, 16, 21) or from infected roots (2, 23). Inoculum borne on the seed tuber surface may be a more important source of infection for the developing plant than inoculum borne within the seed tuber (21, 22, 26), suggesting that a study of direct infection of the sprout is appropriate. Reinke & Berthold (20) illustrated brown patches on subterranean stems of *Verticillium*-infected potato, but they did not report isolation of *Verticillium* from such lesions. Dale (4), described "blindness" as infection of potato sprouts and eyes by *V. albo-atrum*. "Coiled sprout" of potato followed infection of subterranean sprouts by *V. nubilum* Pethybr. (17). Lesions on subterranean tomato stems (23) may have resulted from infection through the stem surface.

MATERIALS AND METHODS.—"Microsclerotial" (MS) and "dark mycelial" (DM) isolates of *V. albo-atrum* from potato were prepared as two types of inocula. Type 1, a suspension of mycelial fragments and conidia, approximately $1 - 1.2 \times 10^5$ conidia/ml, was prepared by scraping the surface of colonies from potato-dextrose agar (PDA) cultures. Type 2, a suspension of mycelial fragments and conidia from cornmeal-Perlite culture adjusted to the same spore concentration as 1, also had abundant microsclerotial or dark mycelial resting bodies and nutrients from the cornmeal. The inoculum grown on cornmeal-Perlite (400 g and 250 g, respectively, per liter of water) was crumbled and suspended in water.

Sprouts of potato tubers were exposed to type-2 inoculum from an MS isolate of the fungus. Inoculations were either made on detached sprouts growing in a moist chamber, or surface-infested tubers were planted in damp Sphagnum moss infested with inoculum.

Plant material used for isolation was surface-sterilized for 1 min with 1% sodium hypochlorite. Isolations from potato petioles and stems were made on PDA adjusted to approximately pH 5 with 25% lactic acid.

Sections for histological study were cut from fresh plant material with a microtome (11). Photomicrographs of fresh, unfixed plant material are shown.

RESULTS.—*Symptoms of leaf infection.*—Similar symptoms developed in laboratory, greenhouse, and field inoculations (Fig. 1-A,B,C) after the dipping of leaves in inoculum suspension or the spraying of inoculum on the foliage. In the field, typical symptoms followed inoculation beneath the leaf canopy. Certain aspects of the field trials are described elsewhere (26).

Small, bronze-colored spots developed on leaf lamina 48 hr after inoculation. These enlarged during the next 2-3 days, and the center of the lesion became darker brown to black and somewhat water-soaked. The entire leaf became bronze to yellow 4-6 days after inoculation. Chlorosis was usually most prominent in the interveinal areas, with the larger veins maintaining the green color longer. By the 5th day after inoculation, some leaflets were dead, and infection had occasionally involved the entire leaf. After 15-20 days, most inoculated leaves were dead.

Vascular discoloration of the petiole followed leaf infection, and *V. albo-atrum* was readily isolated from discolored vascular tissue. The fungus was also isolated from the nondiscolored vascular tissue of healthy-appearing petioles of inoculated leaves. Vascular discoloration of stems and tubers followed leaf infection.

Macroscopically evident sporulation on leaf surfaces occurred only on wounded detached leaves in low light

intensity at high relative humidity in the laboratory and greenhouse. This was not observed in the field, possibly because dead leaves were soon invaded by saprophytic fungi, especially species of *Botrytis* or *Alternaria,* which made identification of *Verticillium*-infected leaves difficult. Abundant microsclerotia formed on dead leaves if the leaves were kept for 1 month or more under moist chamber conditions. Microsclerotia within or on dead leaf lamina were not found in the field, but intensive search for these was not made.

Infection of detached leaves.—Inoculum was placed near the tip, but not at the edge, of each leaflet in a factorial experiment involving inoculum type, light intensity, relative humidity, and influence of wounding the leaf surface (Table 1). Petioles of leaves with 5-7 leaflets were placed in small glass vials containing tap water. Care was taken during and following leaflet inoculation to avoid contamination of the water. For each treatment, 3-7 leaves were used with three replications. A bronze-colored spot developed in the inoculated area 48-72 hr after inoculation.

A disease index was developed by placing diseased leaves into classes ranging from 0 = healthy to 5 = dead, and expressing this on a percentage basis. Significant differences were determined by the Tukey test (9). The leaf was considered systemically invaded when the fungus was isolated from the petiole. At this time, inoculated leaflets were dead or almost dead, and non-inoculated basal leaflets and the petiole still appeared in good health.

More leaves developed symptoms, symptoms were more severe, and infectivity was higher with Type-2 inoculum than with Type-1 inoculum. Leaf survival with Type-1 inoculum after 30 days was similar to that of controls. In contrast, leaf survival with Type-2 inoculum was low. Systemic infection of petioles after 10 or 30 days was high with Type-2 inoculum, and was low with Type 1.

Disease was more severe in low (20-25 ft-c) than in moderate (100 ft-c) light intensity. Leaf symptoms after 10 days were less severe, and systemic infection of petioles was less frequent in low relative humidity than in high relative humidity. Wound inoculation did not appreciably increase severity of infection. Interaction of the above factors was significant. Most severe infection was obtained with Type-2 inoculum in low light, and in high relative humidity.

In all these trials, younger leaves were more readily infected, while older leaves were more quickly killed.

Infection of attached leaves.—Attached potato leaflets of the Russet Arenac variety were readily infected in the greenhouse by *V. albo-atrum.* A drop of inoculum was placed on one apical leaflet of a plant, and these plants were covered by plastic bags for 5 days and then placed on the greenhouse bench.

Three to 4 days after inoculation, inoculated areas discolored bronze. No symptoms were evident in control plants. Symptoms developed on the inoculated leaflet of 52 plants in a population of 60.

To determine frequency of systemic invasion of stems following leaf infection, unwounded leaves of

TABLE 1. Influence of inoculum type, light intensity, humidity, and wounding on infection of detached inoculated potato leaves by *Verticillium albo-atrum*

Factors compared	Leaves tested	Leaves with symptoms after 10 days		Disease index	Leaves surviving after inoculation		*V. albo-atrum* in petioles after	
					10 Days	30 Days	10 Days	30 Days
	no.	*no.*	%[a]	%[a]	%	%[a]	%	%[a]
Inoculum type[b]								
Control	117	0	0	0	92	87*b*	0	0
Type 1	84	14	17*b*	5*b*	97	94*b*	3	3*b*
Type 2	90	51	57*a*	35*a*	84	22*a*	41	83*a*
Light intensity[c]								
Low, control	81	0	0	0	93	84*b*	0	0
inoculated	96	54	56*a*	36*a*	85	54*a*	40	43*a*
Moderate, control	36	0	0	0	100	97	0	0
inoculated	78	11	14*b*	5*b*	94	79*b*	7	43*a*
Relative humidity								
In moist chamber, control	81	0	0	0	96	90*b*	0	0
inoculated	96	51	53*a*	31*a*	87	64*a*	35	45*a*
No moist chamber, control	36	0	0	0	92	84*b*	0	0
inoculated	78	14	18*b*	10*b*	89	68*a*	12	42*a*
Inoculation method								
Wounded, control	60	0	0	0	95	88*b*	0	0
inoculated	90	40	44*a*	24*a*	86	48*a*	24	45*a*
Not wounded, control	57	0	0	0	95	88*b*	0	0
inoculated	84	25	30*a*	17*a*	87	51*a*	24	45*a*

[a] Similar letters, *a* or *b,* indicate results not differing significantly from each other at the 1% level of probability. Interaction of four factors was significant at the 1% level of probability.

[b] Inoculum Type 1 = mycelial fragments and conidia; Type 2 = mycelial fragments, conidia, and resting bodies.

[c] 12 hrs light/24 hr; low = 20-25 ft-c; moderate = 100 ft-c.

TABLE 2. Infection of attached potato leaves and petioles following inoculation of leaf lamina with *Verticillium albo-atrum*

Variety and light intensity[a]	Leaves inoculated	Inoculated leaves with symptoms after 3 days	Leaves with *V. albo-atrum* in petioles after 15 days	
	no.	*no.*	*no.*	%
Sebago				
Low light	37	35	28	76
High light	25	8	7	28
Kennebec				
Low light	32	32	23	72
High light	52	18	17	33
Cherokee				
Low light	11	9	5	45
High light	9	0	0	0

[a] Light intensity: low = 10-15 ft-c; high = 1,000-5,000 ft-c.

Sebago, Kennebec, and Cherokee varieties were exposed to Type-2 inoculum of either DM or MS culture types. Every other leaf of each plant was inoculated by dipping into an inoculum suspension, and plants were placed in plastic bags for 3 days. Since the DM and MS cultures incited essentially similar types and frequencies of infection, the data were combined (Table 2). Half the plants (low light) were placed under the greenhouse bench with 10-50 ft-c light intensity during the day (14 hr light), while the other plants (high light) were kept in natural daylight but not in direct sunlight at 1,000-5,000 ft-c.

V. albo-atrum was not originally present in the plants because isolations made from randomly distributed petioles of each plant before leaf inoculation were uniformly negative. Symptoms first visible 2 days after inoculation became clearly evident by the 3rd day. *V. albo-atrum* was isolated from petioles in limited trials on the 3rd day, and by the 15th day was isolated from 56 of 80 petioles at low light intensity and from 24 of 86 petioles at high light intensity (Table 2).

Evidence of petiole infection obtained by isolations was supported by histological examination. Hyphae similar in size to those of rapidly growing hyphae in culture were present within xylem elements in sections of leaf petioles from inoculated leaves. Such filaments were absent in petioles of noninoculated controls. Granular material which either plugged or partially plugged the vessels was present in diseased petioles and absent in control petioles.

Systemic invasion of Sebago and Russet Arenac stems followed inoculation (Table 3) by dipping the upper two-thirds of the plant into Type-2 inoculum of an MS-type isolate. To avoid soil contamination, pots were covered with an aluminum foil sheet tied around the plant stem, and plants were watered by subirrigation. Leaves of noninoculated controls were similarly dipped into tap water. Plants were kept under conditions favorable for infection, i.e., high relative humidity and low light intensity, for 2.5 days.

Isolations (Table 3) were attempted from five sections of each stem at intervals after inoculation. *V. albo-atrum* was present in the vascular region of at least one node in 48 of 51 inoculated plants of Sebago and Kennebec within 22 days of inoculation. Frequency of positive isolations was lower 60 days after inoculation.

Infected leaves tended to abscise and plants defoliated early following severe leaf infection. *Verticillium* isolations attempted from petioles of dead, abscised leaves were almost always negative.

Soil infestation was attempted to determine if inadvertent soil contamination could account for the presence of *V. albo-atrum* in petioles. Inoculum was poured over soil in pots containing growing plants, and isolations, attempted 10 days later from stems of these plants, were uniformly negative. This is additional evidence that the fungus penetrated through leaves during the 7- and 10-day periods, and did not follow uncontrolled root infection.

Penetration and subsequent progress of the fungus.— Leaves inoculated with water suspensions of conidia were sectioned. After 24 hr, *V. albo-atrum* had penetrated directly into epidermal cells (Fig. 1-D), becoming intracellular and promptly spreading into the mesophyll. Penetration through stomata was not observed, and it did not seem probable that infection took place between cells of the epidermis. Epidermal cells, even those over heavily invaded mesophyll, were relatively free from mycelium. Gumlike substances similar to those in xylem vessels were occasionally present in infected epidermal cells.

Relatively large infected areas were evident as soon as 26 hr after inoculation. In these, the fungus was

TABLE 3. Systemic invasion of potato stems following leaf inoculation with *Verticillium albo-atrum*

Variety and treatment	No. plants inoculated	Plants with *V. albo-atrum* in the stem after[a]				
		7 days	10 days	15 days	22 days	60 days
Sebago						
Control	20			0/4	0/8	0/8
Inoculated	35	2/3	2/3	4/4	9/9	6/16
Kennebec						
Control	18			0/7	0/11	
Inoculated	32	7/7	6/7	7/7	11/11	
Russet Arenac						
Control	8					0/8
Inoculated	16					5/16

[a] Fractions indicate number of positive isolations from the stem per number of plants exposed to infection.

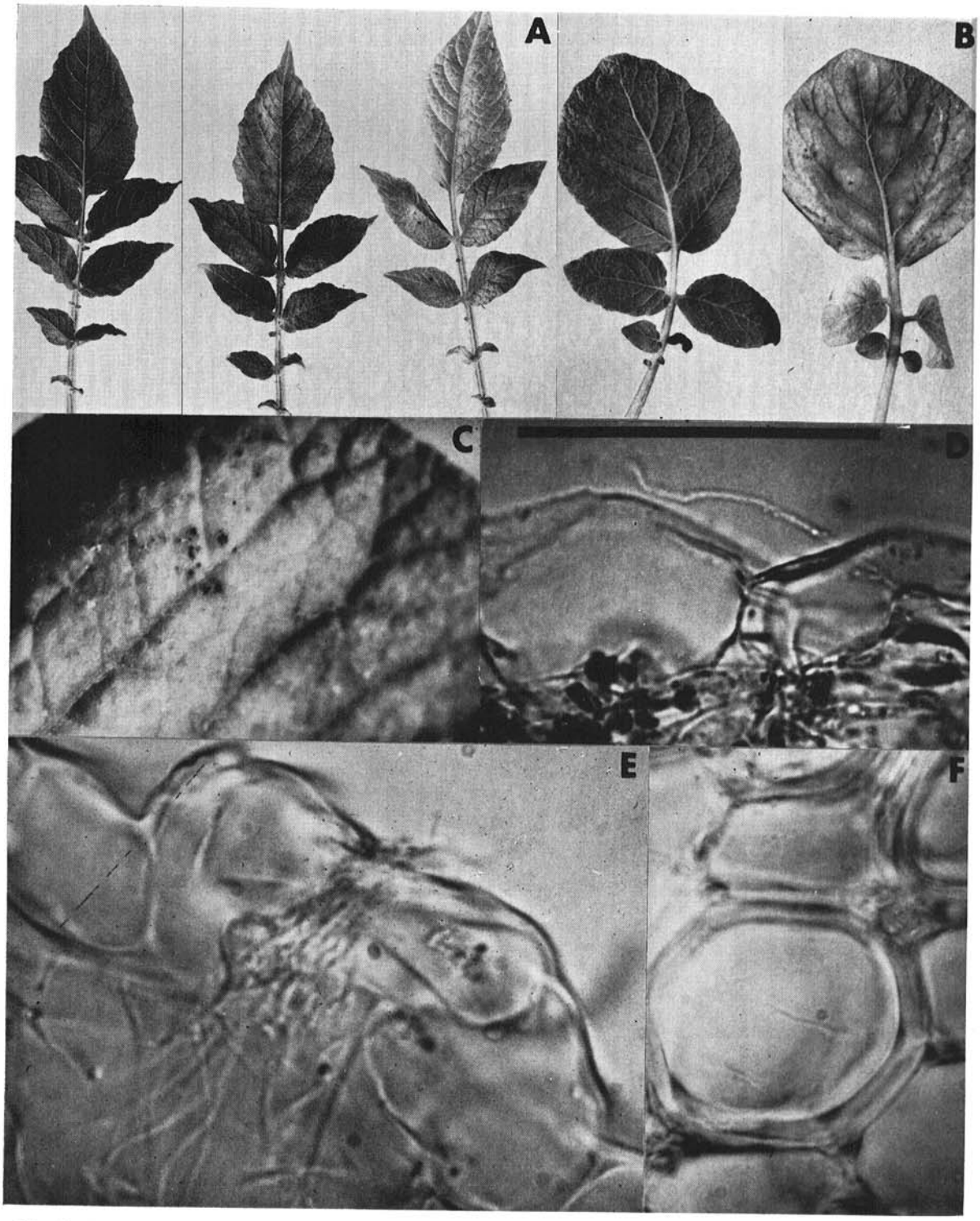

Fig. 1. Potato leaves infected by *Verticillium albo-atrum*. Bronzing symptoms on leaves 3 days after greenhouse inoculation. **A)** Sebago, noninoculated, left; inoculated, center and right. **B)** Kennebec, noninoculated, left; inoculated, right. **C)** Enlarged portion of inoculated Kennebec leaf. **D)** Penetration directly into epidermis of Sebago leaves 1 day after inoculation. **E)** Mycelium in substomatal chamber and in stoma of leaf. **F)** Hyphae in vascular element of leaf 4 days after inoculation. Reference line indicates 170 μ for D, 146 μ for E, and 100 μ for F. Photographs A and B by P. G. Coleman.

abundant in the mesophyll, being mostly intracellular but also intercellular. Necrosis promptly followed infection.

By the 4th day, a mass of hyphae was present in the substomatal chamber (Fig. 1-E). Although hyphae emerged through stomata, sporulation was delayed until later.

Fungus hyphae approximately 1-1.2 μ in diam were present in xylem and xylem parenchyma of leaf lamina 4 days after inoculation (Fig. 1-F). In control plants, similar hyphae were absent in vascular tissue of leaves and petioles. Isolations from petioles were negative.

Infection of tuber sprouts.—Sebago, Kennebec, and Russet Burbank tubers with sprouts approximately 0.5 cm long were planted in damp Sphagnum moss mixed with cornmeal-Perlite inoculum. Inoculum was also placed on the sprouted tubers. In all cases, sprouts from inoculated tubers were less vigorous, and in some cases, the number of sprouts per tuber and the length were reduced as compared to the controls. Sprout tips present at the time of inoculation became necrotic (Fig. 2-A, B) and those developing after inoculation grew almost exclusively as lateral or secondary branches from the original sprout. On a few tubers, sprouts did not form. In several tests, tuber germination was reduced by approximately 20%.

The length and fresh wt of roots from sprouted tubers were noticeably reduced. In contrast, when tubers were dormant at inoculation, root growth was unimpaired.

Apical meristems of detached sprouts became necrotic after tips were dipped into Type-2 inoculum, and the basal portion was inserted in water agar. Lateral buds were usually killed, but when they were not, they germinated, producing secondary sprouts. Results with Type-1 inoculum were essentially similar but less severe. Controls grew normally, and sprouts had vigorous green tips.

The following sequence of events developed in sprouts grown on water agar. *Verticillium albo-atrum* hyphae were capable of penetrating epidermal cells, apparently at any location (Fig. 2-C). Incomplete evidence suggested infection also through hair cells. Soon after penetration, cell walls on the sprout surface became brown, and protoplasm of penetrated cells (Fig. 2-D) became granulated, turned brown, and died. Necrosis spread to neighboring cells. The fungus progressed intracellularly (Fig. 2-E). Apical meristems and leaf primordia were killed (Fig. 2-F), limiting further growth of the sprout.

Often necrosis was limited to the surface. Necrotic tissue sometimes extended into close proximity to spiral xylem elements, suggesting that systemic invasion by *V. albo-atrum* could follow (Fig. 2-G). Of 20 isolation attempts from the vascular tissue of sprouts, two were positive as early as 7 days after inoculation. Soon after superficial tissue had been killed, conidiophores (Fig. 2-H) produced conidia.

DISCUSSION.—Leaf infection of potato by *V. albo-atrum* may be of greater importance in natural incidence of disease than was heretofore suspected. In our trials, conidia were produced on both potato sprouts and leaves following laboratory inoculation, but they were not observed in the field. Others have presented considerable evidence that airborne inoculum is present under natural conditions. Conidia form in the field on dead and dying plant parts of potato (2, 15), tomato (3), hop (13), lucerne (5, 12), red clover (24), and cotton (6), and following inoculation of potato (22), red clover (24), and tomato and eggplant (19). Conidiophores also grow from epidermal hair cells of potato stems (20) and *Dahlia* leaf (14). Aerial dispersal of resting body inoculum is important in certain field situations (6, 29, 31), and viable inoculum has been demonstrated in the air above fields of wilted lucerne (5, 12) and from the air within lucerne drying sheds (5). Hollomon (10) found spores of *Verticillium* sp. on potato leaf surfaces in the field. Although pathogenicity was not demonstrated, the possibility that leaf infection could follow is not precluded.

Direct penetration of epidermal cells of potato leaves and sprouts was demonstrated in our trials within 24 hr following inoculation. Potato leaf mesophyll cells apparently presented little resistance to progression of mycelium, and appressoriumlike structures were lacking. Evidence suggesting breakdown of middle lamella was not observed, but cells of affected mesophyll became soft and quickly disorganized. Unusual structures similar to the safranin staining protuberances described by Van der Meer (28) were lacking in potato leaves and sprouts. Van den Ende (27) also did not observe such protuberances. Griffiths & Isaac (8) describe, in tomato leaves, lignification of epidermal cell walls before penetration, and cellulose protuberances in walls of mesophyll cells at points of contact with penetrating hyphae. Reinke & Berthold (20) and Klebahn (14) illustrate penetration of cell walls of potato and dahlia, respectively, in which the mycelium was noticeably constricted as it passed through the cell wall. Garber & Houston (7) describe similar constrictions as appressoriumlike in cells of cotton. Griffith & Isaac (8) report resting structures of *V. tricorpus* in tomato leaves which may have appressoriumlike functions.

We are not certain that infection of potato did not take place between epidermal cells, but such penetration was not observed. Direct penetration of epidermal cells of roots and penetration between epidermal cells has been reported for *V. albo-atrum* (7, 27) and for *V. tricorpus* infecting tomato leaves (8).

Infection of potato leaves and sprouts was independent of wounds as infection sites. This is in agreement with infection of tomato leaves by *V. tricorpus* (8) which is also independent of wounds. Infection by *V. albo-atrum* conidia through freshly cut lucerne stems is known (12). Provvidenti & Schroeder (19) report that airborne *V. albo-atrum* inoculum entered leaves of tomato and eggplant through hydathodes. They postulate another avenue of entrance, perhaps wounds, because chlorotic areas also developed in low frequency independent of the vein endings. Instead of wound invasion, direct penetration may have been involved. Invasion of the vascular system of the leaf was prompt (19), with abscission being premature and the fungus

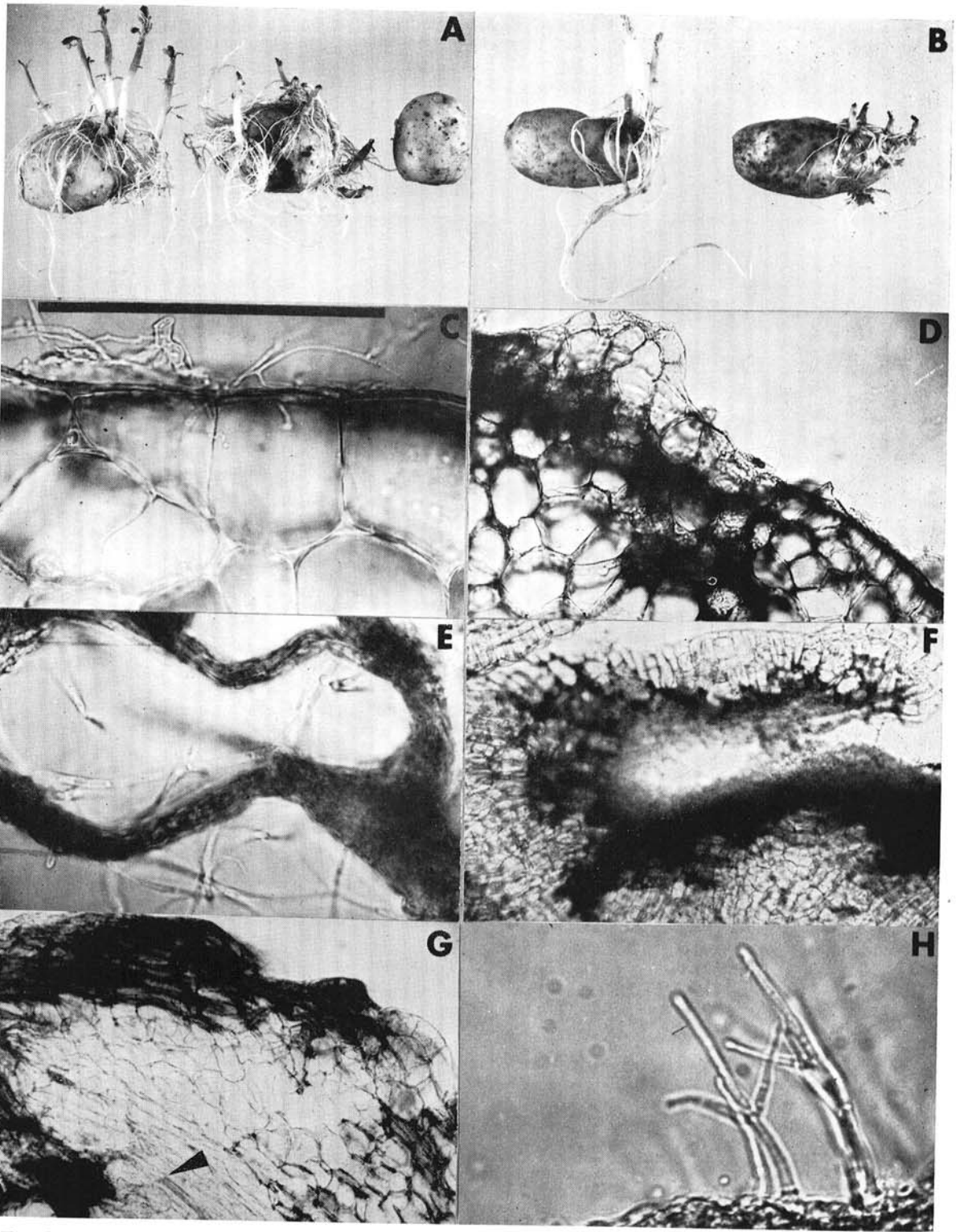

Fig. 2. Potato tuber sprouts infected by *Verticillium albo-atrum*. Necrosis of sprout tips and reduced sprout growth (noninoculated control at left), **A)** Sebago. **B)** Kennebec. **C)** Penetration through epidermis of sprout. **D)** Hyphae of *V. albo-atrum* within cells of sprout. Note granulation of protoplasts. **E)** Hyphae within sprout tissue. **F)** Apical meristem of a sprout following infection. **G)** Necrotic peripheral cells of sprout apical meristem. Note proximity of necrotic tissue to spiral vascular elements (arrow). **H)** Conidiophores of *V. albo-atrum* on necrotic potato sprout. Reference line indicates 100 μ for C and E, 400 μ for D, F, and G, and 146 μ for H. Photographs A and B by P. G. Coleman.

seldom becoming systemic. We obtained no evidence with potato that hydathodes served as sites for leaf infection. Infection was initiated most frequently away from leaf margins.

Evidence was not obtained that potato leaf hairs served as infection sites. Infection through epidermal hairs of potato sprouts was suggested in limited but inconclusive observations.

In inoculated potato leaves, abundant mycelia were present near the infection point as early as 24 hr and as infection progressed cells were packed with mycelium. In contrast, Provvidenti & Schroeder (19) observed, following hydathode infection of tomato leaves, that mycelium was confined to the tracheal tissues of leaves with primary symptoms. Garber & Houston (7) observed only infrequent invasion of parenchyma of cotton leaves following root infection. Furthermore, in leaves systemically invaded following root inoculation, some vessels were packed with mycelium and others were filled with gumlike substances. Vessels of inoculated potato leaves were only sparsely filled with mycelium. Brown, gumlike materials were rare in diseased mesophyll and epidermal cells of leaves, but fairly common in diseased cells of sprouts.

Thick-walled resting structures were not observed in vessels of potato petioles when isolations were made to determine systemic spread. Diameters of thin walled hyphae, 1-1.2 μ, were in reasonable agreement with 1-1.5 μ of rapidly growing hyphae of these isolates in PDA culture, with 1.6 μ reported (22) in culture, and with 1.3 μ reported in cotton (7). The thin-walled hyphae in petioles were apparently short-lived, and isolation attempts from dead petioles were negative in contrast to successful isolations from living petioles. Wilhelm (30) describes conidia and thin-walled hyphae as being short lived.

Potato plants frequently became systemically infected following leaf inoculations. This is in contrast to the relatively low frequency of systemic infection of tomato following leaf inoculation with *V. albo-atrum* (19) and the lack of systemic infection with *V. tricorpus* (8). Possibly a larger number of infection sites is present on potato leaves than on tomato. In potato, leaf abscission may have been relatively slower than that in tomato, permitting infection of the stem.

Spread of *V. albo-atrum* from the inoculated potato leaf downward into the petiole was at least twice the growth rate when compared with increase in colony diam in culture. This may have been due to a more rapid mycelial growth rate in potato vessels than on agar, or conidia may have been produced promptly and transported within the vessels. Provvidenti & Schroeder (19) also report downward movement, apparently against the flow of the sap stream within tomato and eggplant leaves. Conidia move rapidly upward (7, 18, 25) in xylem vessels of cotton and hop plants. Following leaf inoculation of potato, postulating transport of conidia against the direction of flow in the sap stream is difficult to justify. Perhaps invasion of petioles and, ultimately, the stem may be best understood by postulating unusually rapid vegetative growth within the vessels. This is compatible with our observations of hyphae within vessels of leaves and petioles.

Verticillium infection of potato in Michigan is usually characterized by relatively mild symptoms, with little stunting and very mild chlorosis. Late-season field symptoms frequently include early maturity with flagging and death of leaves progressing up the plant. Similar symptoms followed leaf inoculations in the greenhouse. At least a portion of the symptoms present on potatoes late in the season may have resulted from leaf infection in the field.

Heavily shaded foliage under the leaf canopy in the field may be easily infected. Leaves in reduced light were more susceptible than those exposed to high light intensity. In our trials, old leaves collapsed more quickly than did young leaves.

Routine fungicide applications for foliage disease control such as currently are used in potato culture may play a major role in protecting plants from leaf infection by *V. albo-atrum*. Early maturity of potato caused by early blight and early killing of vines by late blight present difficult problems in maintaining adequately controlled field experiments for evaluating this aspect of the *Verticillium* disease.

V. nubilum (17) infects the outer four to five layers of cortical cells of potato sprouts becoming both intercellular and intracellular. Further penetration of sprouts is impeded by formation of a suberized layer. In our trials, sprout infection by *V. albo-atrum* was rapid, and no evidence of a protective reaction was observed. Necrosis and fungus hyphae penetrated in such close proximity to the vascular tissue that systemic infection from cortical invasion is probable. Robinson & Ayers (21) increased incidence of *Verticillium* wilt by surface-infesting seed tubers, but they do not report stand reduction. Incidence of wilt (26) was increased and stand was somewhat reduced after surface infestation of seed pieces. Additional evidence is herein presented suggesting that surface-borne inoculum on tubers is capable of infecting sprouts directly, resulting in systemic invasion.

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