PHYSIOLOGICAL STUDY OF THE PARASITISM OF PYTHIUM DEBARYANUM HESSE ON THE POTATO TUBER

By LON A. HAWKINS and RODNEY B. HARVEY

Plant Physiologists, Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The physiology of parasitism and the relations existing between the host and parasite have been the subject of numerous investigations, many of which have taken up the method by which the fungus obtains entrance into the plant or passes from cell to cell within the tissues of its host.

There are, of course, several possible ways by which a parasitic plant may obtain entrance into the cells of its host plant. It may enter through an opening already made; if it makes the opening itself, it may push its way through mechanically, or it may soften or digest the cell walls. It is possible, also, that the fungus hyphae might so stimulate the cells of the host plant that enzyms secreted by the host itself would break down its own cell walls and allow the fungus to enter. A combination of these methods is, of course, possible—for example, a fungus might penetrate the cell wall by a small opening and then enlarge this opening either mechanically or by a solution of a portion of the cell wall. Some of the investigations on this subject will be considered here.

In 1886 De Bary (3)[1] showed that *Sclerotinia libertiania* secreted a toxic substance which killed the cells ahead of the growth of the fungus. He concluded that the breaking down of the cell walls was due to an enzym secreted by the fungus.

Ward (27) concludes, in his study of Botrytis on lily, that the tip of the fungus hypha "excretes relatively large quantities of ferment substance and dissolves its way into the cell wall." Nordhausen (21) considers that *Botrytis cinerea* dissolves its way through the cell walls of its host plant. Büsgen (9) considers that this fungus does not make its way through cell walls or even cuticle by mechanical means alone. Miyoshi (20) showed that *Botrytis cinerea* could force its way through membranes of collodion, paper, and other substances, and details some experiments in which Penicillium pushed its way through gold leaf. Peirce (23) has shown that the haustoria of Cuscuta will puncture tinfoil 0.2 mm. in thickness.

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[1] Reference is made by number (italic) to "Literature cited," p. 295-297.

Brown (*6*) in a recent study of parasitism in Botrytis has shown that this fungus secretes an enzym which breaks down the middle lamellae of tissues which it invades. He demonstrated that the enzym secretion was more powerful from freshly germinated spores than from old cultures. A toxin, which is apparently closely associated with the enzym, is also secreted. This toxin is not oxalic acid or an oxalate. Blackman and Welsford (*5*), in the second of this series of papers, have shown that this fungus apparently penetrates the cuticle of the broad bean leaf by pushing its way through mechanically. These writers are in agreement with Brown that an enzym secreted by the fungus breaks down the tissues in the interior of the leaves. Brown (*7*) later showed that the infecting germ tubes were unable to affect chemically the cuticle of the host plant and that the toxic substance could not pass through the cuticle. He concludes that penetration of the cuticle must take place in a purely mechanical way. In the fourth paper of this series (*8*) he contrasts thick and thin sowings of spores and finds that thick sowings 2 to 4 days old yield the most active preparations of enzym. He considers that the cytase is much more active at the tip of the hypha.

From this review, which of course covers only part of the literature on this subject, it is apparent that there is good evidence that some parasitic plants make their way into their host plants by breaking through the tissues mechanically; but there is no doubt that some fungi secrete enzyms which break down the cell walls of certain plants and are thus able to make their way through the tissues of their hosts.

The parasitism of *Pythium debaryanum* Hesse on some of its numerous hosts has been investigated, but how it gains entrance into the host plant seems not to have received any considerable attention. This fungus has recently been shown to be the cause (*15*) of a tuber-rot of potatoes which is of considerable commercial importance in the San Joaquin Valley of California. A method of controlling this disease under commercial conditions has been worked out and described (*17*). In the present study the effect of the fungus on the sugars, pentosans, and starch of the potato tuber was determined, and the rate of growth of the fungus in three different varieties of potatoes was measured. An attempt was made to correlate certain physical and chemical characteristics of the potatoes with their susceptibility or resistance to this disease, and the growth of the fungus in the potato tissue was observed and studied. Some information on the mode of entrance of this fungus into the cells of the potato was obtained, and a possible explanation was found as to why some varieties of potatoes are much more susceptible to this disease than others.

EXPERIMENTAL WORK

The methods followed in the study of the effect of the fungus on the starch, sugars, and pentosans of the potato tuber were essentially those described for the work with the Fusarium tuber-rots (*16*). They will not be discussed here. The results of the analyses of the sound and rotted quarters are shown in Table I.

TABLE I.—*Starch, sugar, and pentosan content of the sound and rotted quarters of potatoes rotted with Pythium debaryanum*

[Expressed as percentage of wet weight]

								Sugar.		
Pentosans.			Starch.					Sucrose as dextrose.	Reducing sugar as dextrose.	
Tuber No.	Sound quarter.	Rotted quarter.	Tuber No.	Sound quarter.	Rotted quarter.	Tuber No.	Sound quarter.	Rotted quarter.	Sound quarter.	Rotted quarter.
1.........	0. 548	0. 44	5....	16. 07	14. 64	9...	0. 98	0. 030	0. 220	0. 003
2.........	. 39	. 32	6....	16. 61	16. 34	6...	. 63	. 009	. 252	. 002
3.........	. 33	. 27	7....	16. 73	13. 04	10...	. 11	. 008	. 392	. 012
4.........	. 35	. 31	8....	15. 65	11. 42	11...	. 46	. 020	. 440	. 001

In Table I it is noticeable that the sugars, both sucrose and reducing sugars, had almost disappeared in the rotted portions of the tuber, while appreciable amounts were present in all the uninfected quarters. The fungus is evidently able to utilize the sugars of its host. In this its action is similar to that of *Fusarium oxysporium*, *F. radicicola*, and *F. coeruleum* (*13*) on potatoes, *Sclerotinia fructigenia* on apples (*4*) and peaches (*14*), *Sphaeropsis malorum* on apples (*12*), and *Rhizopus nigricans* (*25*) on strawberries, all of which cause a decrease in the sugar content of the host plant or part of the host invaded. The results with these several fungi seem to justify the conclusion that rot-producing fungi are usually able to break down and utilize the sugars of the host. The starch content of the potato also decreases when rotted by *Pythium debaryanum*, as is shown in columns 5 and 6 of Table I. Starch grains were frequently found corroded; and an extract of the fungus mycelium, which had been grown on either potato plugs or mashed potatoes which had been sterilized, was able to pit potato starch grains as well as to digest gelatinized potato starch. In this respect the rot produced by this fungus is different from that produced by any of the three species of Fusarium mentioned above. With the Fusarium-rots no corrosion of the starch grains was noticeable, and the starch content of the rotted portions was not lower than that of the corresponding sound quarter. An extract of the mycelium of any of these three fungi was apparently incapable of corroding grains of potato starch even

after a long period, though gelatinized potato starch and soluble starch were readily digested by the extracts. The results obtained with *P. debaryanum* on potato starch are not in accord with the findings of Ward (*26*), who concluded that this fungus did not attack the starch of the potato. His conclusions were based entirely on microscopical observations. The pentosan content of portions of the tuber-rots by *P. debaryanum* is somewhat lower than that of the corresponding sound quarters, and from this it may be concluded that the fungus is able to digest the pentosans. This conclusion is supported by the fact that in potatoes rotted by this organism the middle lamellae of the cells are broken down and the cells may be readily teased apart on a slide. Extracts of the mycelium also digest the middle lamellae, and a slice of potato ½ mm. in thickness disintegrates in 12 hours when immersed in it. The middle lamellae, however, seem to be the only portion of the cell wall affected, for when a bit of rotted tuber is placed on a slide and teased out the cells float free, while only in exceptional cases are broken cells seen. The fungus penetrates the tissue in all directions but seems most frequently to pass directly through the cell wall.

In inoculation experiments with this fungus it was found that Bliss Triumph and Green Mountain potatoes were very susceptible to this disease, while the White McCormick potatoes were not. In the experiments all the Bliss Triumph and Green Mountain tubers rotted eventually—90 per cent as a result of the first inoculation—while with the McCormicks only about 30 per cent of the potatoes seemed to be susceptible to the disease even when inoculated three different times. When a McCormick tuber did become infected, the rot usually developed very slowly. This variety, while not immune to the disease, seemed to be highly resistant.

Measurements of the rate of growth of the fungus were made in tubers of the three different varieties mentioned. The method followed was to cut cylinders of the potato tubers about 12 mm. in diameter and 30 mm. long. These cylinders were placed on end in a small moist chamber and inoculated on the upper end from stock cultures of the fungus. After incubating for 24 hours the cylinders were sliced transversely into sections 1 mm. in thickness. These slices were numbered in order and placed in a moist chamber. The slices in which the rot developed were noted, and it was thus possible to determine within a millimeter the distance the fungus had progressed in 24 hours. This method is somewhat similar to the method followed by Jones, Giddings, and Lutman (*18*) in their study of the resistance of potatoes to *Phytophthora infestans*. The rapid rate of growth of the fungus used in the present study, however, made it possible to simplify the method considerably. The results of these experiments are shown in Table II.

TABLE II.—*Average rate of growth of Pythium debaryanum in tissue of Bliss Triumph, Green Mountain, and McCormick potatoes*

Experiment No.	Green Mountain.		Bliss Triumph.		McCormick.	
	Number of cylinders.	Average growth per hour.	Number of cylinders.	Average growth per hour.	Number of cylinders.	Average growth per hour.
		Mm.		*Mm.*		*Mm.*
1.............................	6	0.354	7	0.430	7	0.071
2.............................	9	.458	9	.425	8	.187
3.............................	7	.290	8	.453	8	.049
Average..........	....	.366	....	.436	....	.102

It is noticeable in Table II that the rate of growth of the fungus in Bliss Triumph and Green Mountain tubers is from three to four times as rapid as in McCormick under the conditions of the experiment. In some cases the fungus was apparently unable to affect the cylinders from the McCormick and had not, at the end of the experiment, penetrated into the first millimeter of tissue. Other cylinders from this variety were much more susceptible, however, so that the average rate of growth of the fungus, as shown in the table, is fairly high.

In order to relate this rate of growth to the number of cells traversed, measurements of the cells in the cortex and central portions of tubers of these three varieties were made. About 1,500 measurements were made with each variety. The averages for the cortex and central portions of the three varieties are given below:

TABLE III.—*Average size of cells in the three varieties of potatoes from 1,500 measurements on each variety*

	Bliss Triumph.	Green Mountain.	McCormick.
Cortex................................	269μ × 303μ	294μ × 311μ	269μ × 303μ
Central portion......................	318μ	318μ	347μ

If it is considered, then, that the same rate of growth holds for the cortex and interior of the tuber, the average length of time required for the fungus to pass through an average cell in the interior would be 43, 50, and 204 minutes, respectively, for the three varieties, Bliss Triumph, Green Mountain,[2] and McCormick. The fact that the cells are so nearly of the same size in the three varieties would eliminate the possibility that the relatively slow rate of growth of the fungus in the McCormick tubers was due to the small size of the cells and the consequently larger number

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[2] In a former paper (*17*) one of the writers gives the size of the cell in a Green Mountain tuber as 138.7μ, which is erroneous. The tuber mentioned was of the Burbank variety. The size of the cells and rate of growth given in the present paper are correct for this variety.

of cell walls for the fungus to penetrate in traveling a given distance. It may, however, be due to some resistant quality of the cell wall.

That the fungus secretes a toxic substance which kills potato cells was demonstrated experimentally by a method somewhat similar to that followed by Brown (*6, 7, 8*) in his work with *Botrytis cinerea*. Cultures of the fungus were grown for two weeks on sterilized potato mush, and potato plugs and the mycelium were removed in such a way that none of the culture medium adhered to the mat of mycelium. The mycelium was then ground in a mortar with sand, extracted with distilled water, and filtered. Cylinders about 1 cm. in diameter were cut from potato tubers and sliced into disks 0.5 mm. in thickness. Some of these disks were placed in the extract of mycelium and some in distilled water and examined at intervals. After three hours the disks in the fungus extract had lost their turgidity so that when grasped by the edge with a pair of forceps and held in a horizontal position they collapsed limply. The disks from the distilled water preparation remained turgid for 12 hours or more. Disks from the preparation of fungus extract did not resume their normal turgidity when washed and placed in distilled water. The cells of the potato are apparently killed by some substance extracted from the ground mycelium. The loss of turgidity can not be accounted for by a loss of water from the potato cells caused by a higher osmotic pressure in the extract, because tests showed that the lowering of the freezing point of the extract used was only about one-fifth that of the juice from the potato tuber. All three varieties of potatoes used in these experiments behaved in the same way. From these experiments it seems hardly probable that resistance to fungus attack can be due directly to the living protoplasm.

The macerating effect of this extract on the potato tissue has been mentioned earlier in this paper. The properties of the toxic substance secreted by the fungus were not determined, though the problem is well worthy of investigation.

It has been shown in some cases that resistance to certain fungus diseases was correlated with higher acidity of the host plant tissues. Thus Averna-Sacca (*2*) has shown that the resistance to diseases of grapes caused by Oidium and Peronospora was correlated with a relatively high acidity. Comes (*11*) has demonstrated that a variety of wheat (Rieti), resistant to rust, has an acidity considerably higher than the varieties in the same locality which are susceptible to this disease. Further, this writer has shown that when this resistant variety is grown in other localities where the environmental conditions tend to produce a plant of lower acidity, the plant is susceptible to the disease. These researches indicate that acidity may play a very important rôle in the resistance of a plant to disease.

There are, of course, many other factors that tend to influence the resistance or susceptibility of a plant to disease. The literature on this

subject has been ably reviewed in the papers of Ward (*28*), Appel (*1*), Orton (*22*), and Butler (*10*) and will not be considered in this paper except as it relates directly to the problem.

Inasmuch as *Pythium debaryanum* is rather susceptible to acids, it was considered worth while to test the acidity of two of the varieties of potatoes used in these experiments—Bliss Triumph, which is very susceptible to the disease, and McCormick, the variety which had proved rather resistant. Determinations of hydrogen-ion concentration were made on the expressed juice of tubers of these two varieties by the potentiometric method, and it was found that juice from the McCormick potatoes had a C_H 8.67 $\times$ 10^{-8} while that from Bliss Triumph had a C_H 8.63 $\times$ 10^{-7}. The McCormick had a hydrogen-ion concentration of about 10 times that of Bliss Triumph. To obtain further evidence on this point the fungus was grown in a series of potato-juice cultures made up to known hydrogen-ion concentration with $N^1/100$ sodium phosphate buffer mixture. The results of these experiments are shown in Table IV.

TABLE IV.—*Growth of Pythium debaryanum in potato juice of various hydrogen-ion concentrations*

C_H of culture medium.	Behavior of fungus.
3.936 $\times$ 10^{-9}	No growth in 3 days.
5.035 $\times$ 10^{-8}	1 grew in 3 days.
1.738 $\times$ 10^{-7}	2 grew well in 3 days.
1.660 $\times$ 10^{-6}	Growth covered plates in 3 days.
9.84 $\times$ 10^{-6}	Do.
2.535 $\times$ 10^{-5}	Do.
5.585 $\times$ 10^{-5}	Do.
3.741 $\times$ 10^{-4}	Do.
8.69 $\times$ 10^{-4}	5 mm. growth in 3 days.

According to Table IV the fungus grows well and fruits in a C_H 3.741 $\times$ 10^{-4}, which is considerably higher than that of the McCormick potato. The resistance of the McCormick potato to this disease, then, is not due to its high acidity. This is in accordance with the conclusions of Jones, Giddings, and Lutman (*18*) in regard to resistance of potatoes to *Phytophthora infestans.*

The experiments described in the foregoing pages seemed to indicate that the resistance to the progress of the fungus in McCormick potatoes might be due to some property of the cell wall—that is, it is possible that the fungus makes the opening in the cell wall through which it passes mechanically. If this is true, cell walls of potatoes resistant to the disease should show a higher resistance to puncture by mechanical means than the cell walls of susceptible varieties. This hypothesis seemed worth testing out, so an apparatus for measuring the pressure necessary to puncture the tissue of a potato was arranged.

This apparatus (Pl. 35)[3] consisted of a modified Joly balance, accurately graduated, and with a vernier for close reading. The lower end of the spring was attached to a metal rod which passed through a short glass tube fixed to the stand of the instrument. Hair lines on both the tube and rod made it possible to determine accurately the point at which the tension on the spring balanced any given weight. Tension was applied to the spring by means of a rack and pinion adjustment. It was possible with this balance to weigh to a milligram, which was well within the limits of experimental error in these determinations. A glass rod, the weight of which was less than the capacity of the balance, was suspended from the pan of the balance, and a small glass needle with a rounded end was attached to the lower end of the glass rod. In operating this apparatus a slice of potato was placed on the stage of the instrument, which was so adjusted that the tip of the needle just touched the surface of the potato when the hair line of the indicator on the spring coincided with the hair line of the fixed indicator of the balance. The tension on the spring was then slowly released by means of the rack and pinion adjustment until a sudden drop of the needle indicated that the tip of the needle had penetrated the tissue. The position of the column that supported the spring was then noted on the graduated scale. The weight required to balance the pull of the spring at this point was determined and subtracted from the weight of the needle. The result was the weight required to push the needle into the potato tissue.

Inasmuch as the needles used in these experiments were always from one-sixth to one-fourth the average diameter of potato cells, it is evident that in most cases at least the needle was pushed through the cell wall and that the weights obtained were a close approximation of the pressure necessary to break through the cell wall. The needles were drawn from small glass tubing over a micro burner and were drawn out in such a way as to leave a relatively heavy shoulder so that the slender portion which was thrust into the potato was not more than a millimeter in length. It was found that long needles of the small size necessary in this work were very easily broken. They were rounded and slightly larger at the end so that friction against the sides of the puncture would be reduced to a minimum. The needles used in these experiments were from 58.3 to 71 microns in diameter. In practice 20 determinations were made on each tuber, 10 in the cortex and 10 in the central portion within the ring of bundles. The weights obtained were averaged for each region; and, since the diameter of the needle was known, the weight required to break through tissue per square millimeter of surface was readily calculated. It was shown in this work by using different needles on the same potato that the weights required to puncture potato tissue were about propor-

tional to the area of the cross section of the needles. The determinations made with different needles are thus comparable.

In the experiments, the results of which are shown in Tables V to VII, inclusive, the potatoes were inoculated first in the cortex. This was done by removing a piece of the potato skin about 1 mm. in thickness and 5 to 10 mm. in diameter. A Van Tieghm cell was cemented to the surface of the potato around this wound with vaseline, and a drop or two of sterilized distilled water was placed within the ring and inoculated with mycelium of the fungus. The top of the cell was then closed with a cover glass and vaseline. The inoculated tubers were then placed in an incubator maintained at 30° C. and examined daily. If the tubers became infected, they were removed before they were more than half rotted. They were sliced through the point of inoculation, the distance the rot had progressed was measured, and the weight necessary to puncture the tissue in the two different regions of the sound portions of the tubers was determined. If the inoculation was unsuccessful, a second inoculation was made in the cortex in the same way as the first; and if this inoculation did not result in an infection the potato was inoculated beneath the cortex in the central part. If the results from this third inoculation were negative, as they usually were with McCormick potatoes, the tuber was considered to be immune, and the weight necessary to puncture the tissue was determined as described above. It is worthy of note that Bliss Triumph and Green Mountain potatoes usually rotted as a result of the first inoculation.

The tables in which the results of these experiments are given show the diameter of the needle in microns and the pressure required in grams per square centimeter to penetrate the tissue of the freshly cut potato in the cortex and central portion, respectively. In every case the numbers given as the pressure required to penetrate the tissue are averages of 10 determinations. The same needle was always used for both the cortex and the central portion. The number of inoculations made on each tuber, the region in which they were made, and the result after the length of time indicated are also given. In Table VII, under "results of inoculation," the term "slight rot" appears. This was used to characterize the results of inoculations when there was a browning and slight softening of the tissue immediately around the point of inoculation which seemed to indicate that infection had occurred. The rot had not progressed a measureable distance, however, and the tuber thus affected was apparently practically immune.

TABLE V.—*Pressure in grams per square centimeter required to puncture tissue of freshly cut surface of Green Mountain potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture cell wall.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
27................................	71	51,556. 4	31,938. 2	1	Cortex..	40 mm. rot in 3 days.
28................................	71	48,248. 3	35,648. 8	1	...do.....	30 mm. rot in 3 days.
29................................	71	37,747. 1	32,072. 7	1	...do.....	27 mm. rot in 3 days.
30................................	71	39,390. 7	28,500. 0	1	...do.....	40 mm. rot in 3 days.
31................................	71	57,571. 5	47,774. 0	1	...do.....	16 mm. rot in 3 days.
65................................	67	42,812. 7	33,344. 0	1	...do.....	30 mm. rot in 4 days.
66................................	67	47,566. 3	42,667. 0	1	...do.....	19 mm. rot in 3 days.
67................................	67	31,132. 0	30,160. 4	1	...do.....	34 mm. rot in 4 days.
68................................	67	34,439. 6	34,749. 0	1	...do.....	29 mm. rot in 4 days.
69................................	67	33,540. 3	33,902. 0	1	...do.....	49 mm. rot in 4 days.
70................................	67	41,344. 0	25,736. 6	1	...do.....	25 mm. rot in 4 days.
72................................	67	32,372. 4	29,457. 6	1	...do.....	25 mm. rot in 5 days.
73................................	67	42,965. 1	39,525. 0	1	...do.....	30 mm. rot in 5 days.
74................................	67	39,008. 1	26,367. 1	1	...do.....	33 mm. rot in 5 days.
75................................	67	31,276. 4	22,843. 6	1	...do.....	34 mm. rot in 5 days.
80................................	67	60,103. 9	41,953. 8	3	Deep....	12 mm. rot in 10 days.
61................................	67	75,525. 1	28,351. 7	3	...do.....	18 mm. rot in 4 days.
55................................	68. 3	47,897. 0	39,152. 8	3	...do.....	18 mm. rot in 2 days.
Average...................	....	48,682. 0	31,325. 0			
Average for tubers which when inoculated in cortex rotted.................		40,731. 3				
Average for tubers which when inoculated in cortex did not rot...........		61,175. 3				

TABLE VI.—*Pressure in grams per square centimeter required to puncture tissue of freshly cut surface of Bliss Triumph potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture cell wall.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
32................................	71	40,548. 1	30,345. 4	1	Cortex..	32 mm. rot in 3 days.
33................................	71	51,457. 7	27,923. 0	1	...do.....	31 mm. rot in 3 days.
34................................	71	42,662. 5	27,824. 6	1	...do.....	Do.
35................................	71	39,380. 2	30,656. 5	1	...do.....	24 mm. rot in 3 days.
36................................	71	53,028. 7	35,401. 0	1	...do.....	19 mm. rot in 5 days.
39................................	71	45,953. 9	34,946. 0	1	...do.....	68 mm. rot in 5 days.
40................................	71	51,726. 4	34,264. 0	1	...do.....	42 mm. rot in 5 days.
41................................	71	40,332. 1	36,610. 2	2	...do.....	28 mm. rot in 4 days.
42................................	71	40,579. 1	29,034. 0	2	...do.....	46 mm. rot in 4 days.
43................................	71	37,798. 6	29,892. 0	2	...do.....	32 mm. rot in 4 days.
62................................	67	41,447. 3	29,365. 0	1	...do.....	18 mm. rot in 4 days.
63................................	67	34,501. 5	22,504. 4	1	...do.....	23 mm. rot in 4 days.
64................................	67	33,282. 0	29,819. 4	1	...do.....	24 mm. rot in 4 days.
71................................	67	33,282. 0	22,677. 2	1	...do.....	28 mm. rot in 4 days.
56................................	68. 3	45,705. 8	41,529. 0	3	Deep....	5 mm. rot in 2 days.
57................................	68. 3	40,000. 3	30,343. 4	3	...do.....	Do.
Average...................	....	41,980. 0	38,209. 0			
Average for tubers which when inoculated in cortex rotted.................		38,998. 0				
Average for tubers which when inoculated in cortex did not rot...........		42,852. 9				

TABLE VII.—*Pressure in grams per square centimeter required to puncture the tissue of freshly cut surface of McCormick potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture tissue.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
41	71	75,057.3	61,375.2	3	Deep....	Slight rot in 10 days.
46	71	86,357.1	62,615.5	3	...do....	Slight rot in 5 days.
47	71	105,644.3	82,429.2	3	...do....	Do.
48	58.3	112,803.3	67,111.7	3	...do....	Slight rot in 7 days.
49	58.3	77,189.2	55,835.1	3	...do....	Slight rot in 5 days.
50	58.3	100,362.2	62,284.9	3	...do....	No rot in 7 days.
51	58.3	138,760.5	113,923.0	3	...do....	Do.
53	58.3	67,566.4	51,101.6	3	...do....	10 mm. rot in 7 days.
54	58.3	78,119.5	54,243.3	3	...do....	12 mm. rot in 10 days.
83	67	51,824.7	48,083.2	1	Cortex.	35 mm. rot in 8 days.
Average		89,368.4	65,900.2			
Average for tubers which when inoculated in cortex did not rot		93,539.9				
Average for tubers which when inoculated in central part did not rot			72,224.9			
Average for tubers which when inoculated in central part rotted			52,672.4			

Tables V to VII show that there is considerable difference in the pressure required to puncture the tissue of the different regions of tubers of the three varieties used. If the pressures required to puncture the tissues of similar regions in the different varieties are compared, it is evident that the pressure is considerably higher for McCormick than for the two susceptible varieties, Bliss Triumph and Green Mountain, while the averages for the last two mentioned are in much closer agreement.

In regard to susceptibility to infection by the fungus, only 1 McCor-mick tuber out of 10 became infected when inoculated in the cortex, and the pressure required for puncturing the cortex of this tuber was much below the average required for the central portion of this variety. Two other McCormick potatoes became infected when inoculated in the central part, and these tubers were also lower in their resistance to puncture in this region than the others.

Three tubers of Green Mountain potatoes did not become infected even when inoculated twice in the cortex. The average pressure required for the cortex of these three tubers is 61,175.3 gm. per square centimeter, or considerably more than the average, 40,731.3 gm., required for the cortex of the tubers which rotted when inoculated in that region. All the tubers of the Green Mountain variety rotted. All the Bliss Triumph tubers, except two, became infected from cortical inoculations. These two required a somewhat higher pressure to puncture the tissue of the cortex than the average for this region, but the difference was not great. There is evidently a correlation between the resistance of the tuber to puncture and resistance to infection by the fungus.

The difference in resistance to puncture by mechanical means in these three varieties of potatoes is very marked; and it was considered of interest to see if there was correlated with it some variation in the chemical composition of the tubers, especially in the constituents of the cell wall. Accordingly, determinations of the pentosan and crude fiber content of the two regions, cortex and central portion, of potatoes of each of the three varieties were made.

In preparing the samples for analysis the potato was cut into slices about 8 mm. in thickness, a thin peeling removed with a sharp knife, and the cortex sliced away. This was dried, ground, and analyzed. The central portion of the potato after the ring of bundles had been peeled off was treated in like manner. Pentosan and crude-fiber analyses were made and were calculated to dry weight, the dry weights being obtained in the usual way by drying to constant weight in a vacuum oven. Duplicate determinations were made. The data obtained from the analyses are given in Tables VIII and IX.

TABLE VIII.—*Pentosan content of cortex and interior tissue of potatoes*

[Expressed as percentage of dry weight]

Location of tissue.	McCormick.	Green Mountain.	Bliss Triumph.
Cortex...	2. 00 2. 23	1. 60 1. 70	1. 86 1. 74
Interior...	1. 40 1. 32	1. 60 1. 70	1. 71 1. 65

TABLE IX.—*Crude-fiber content of cortex and interior tissue of McCormick, Bliss Triumph, and Green Mountain potatoes*

[Expressed as percentage of dry weight]

Location of tissue.	McCormick.	Green Mountain.	Bliss Triumph.
Cortex...	3. 42 3. 12	2. 01 1. 93	1. 98 1. 95
Interior...	2. 12 2. 18	1. 96 1. 83	1. 88 1. 92

From Table VIII it is evident that the pentosan content of the cortex of McCormick potatoes is somewhat higher than that for the other two varieties. The pentosan content of the central portion, however, seems to be somewhat lower in this variety. In Table IX is shown the crude-fiber content of the three varieties. McCormicks are higher in crude-fiber content than either of the other varieties. In the cortex of the McCormicks there is over 50 per cent more crude fiber than in the same region of the other two varieties. The interior also of the McCormick tubers

is higher in crude-fiber content than the cortex of Green Mountain or of Bliss Triumph. There is evidently correlated with resistance to mechanical puncture and resistance to infection by *Pythium debaryanum* a higher crude-fiber content.

Further evidence that the resistance of potatoes to infection was correlated with resistance of the tissue to mechanical puncture was obtained from experiments in which Bliss Triumph and Green Mountain tubers were prepared for inoculation by scooping out small plugs of tissue from the cortex of the tubers and allowing the wounds to dry for a given length of time. The plugs were removed by means of a small curved knife, leaving a cavity in the cortex of the tuber about 4 mm. in diameter and of the same depth, without sharp corners or rough surfaces. Part of these potatoes were inoculated as controls, and all of them were placed in the incubator at 30° C. At 3-hour intervals for 12 hours a number of the uninoculated tubers were removed from the incubator, inoculated in the cavities made at the beginning of the experiment, and replaced in the incubator. The inoculations were made in the usual way by placing a bit of mycelium in a drop or two of sterilized water in the cavity, which was then inclosed in a covered Van Tieghm cell. When at the end of 4 days the tubers were removed from the incubator and examined, it was found that only the controls inoculated when the experiment was set up had rotted. None of the wounded potatoes inoculated 3 hours or more after they had been placed in the incubator were rotting. Apparently exposure to the air at 30° C. for 3 hours was sufficient time for the formation of a layer over the wound resistant to fungus attack.

It is commonly considered by the potato growers of the San Joaquin Valley, Calif., that wounds which have had opportunity to cork over will not become infected. This has been shown to be true in these studies by many unsuccessful attempts to inoculate tubers in old wounds. From the experiments described in this paper it is evident that the protective covering is formed very quickly under the conditions of the experiment. Appel(1) claims that the tissue of some varieties of potatoes begins to cork over in 6 hours. That a protective covering is formed in 3 hours under the conditions of the experiment is evident. There is, however, no evidence that it is a true suberization.

The pressure necessary to puncture the tissue of these Green Mountain and Bliss Triumph potatoes was determined on freshly cut slices of the tubers and on slices which had remained in the incubator for 3 hours. The results are shown in Table X.

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TABLE X.—*Pressure in grams per square centimeter required to puncture tissue in slices of potatoes freshly cut and after drying for 3 hours at 30° C.*

GREEN MOUNTAIN

Potato No.	Cortex.			Interior.		
	Fresh surface.	Dried 3 hours.	Average increase in pressure required for puncturing dried tissue.	Fresh surface.	Dried 3 hours.	Average increase in pressure required for puncturing dried tissue.
1................................	40,852.1	59,225.1	....	30,554.8	58,388	....
2................................	47,738.9	72,409.8	....	30,554.3	52,737.8	....
3................................	39,553.2	68,851.9	....	22,528.2	57,551	....
4................................	43,111	76,385.9	....	31,810	70,344.8	....
5................................	27,833.7	74,711.7	....	32,019.2	48,552	....
Average...................	39,017.7	70,316.8	31,299.1	29,493.3	57,514.7	28,021.4

BLISS TRIUMPH

Potato No.	Cortex.			Interior.		
1................................	42,528.3	76,176.8	....	29,994.8	48,133.6	....
2................................	47,715	79,315.9	....	27,624.4	46,250.1	....
3................................	41,018.1	64,456	....	22,601.9	78,068	....
4................................	38,448.3	57,132.6	....	33,065.6	49,644.2	....
5................................	35,577	60,690.1	....	26,368.8	57,715	....
Average...................	41,057.3	67,554.2	26,496.9	27,931.1	55,962.1	28,031.0

From the results shown in Table X it is evident that the resistance of the wounded surface of the potato to puncture is appreciably increased in every instance by exposure to the air for 3 hours. In the five Green Mountain potatoes the average increase in pressure required to puncture the cortical tissue was 79 per cent, and the average increase for the central tissue was 95 per cent. With the same number of Bliss Triumph tubers the results were 64 per cent and 100 per cent for the cortex and central portions, respectively.

There is then correlated with the resistance to infection shown by wounds after 3 hours' drying a very marked resistance to mechanical puncture. If the fungus penetrates the tissue mechanically, it is quite possible this increase in resistance, due to drying, would be sufficient to prevent its entrance. It is noticeable that the pressure required to puncture the dried cortex, the region in which the inoculations were made in these experiments, most closely approaches the averages for the inner portion of McCormick tubers which did not become infected.

Another point which is of interest in this connection is the fact that no cases of natural infection through the potato skin have been observed, and repeated attempts in the laboratory to inoculate tubers on the surface have yielded negative results. Correlated with this resistance to infection is a very marked resistance to mechanical puncture. It was exceedingly difficult to puncture the skin of the potato with the round-

tipped glass needles, and the pressure required was considerably more than that required for any portion of the cut surface of tubers tested.

No direct evidence was obtained that the fungus could exert sufficient pressure upon the cell walls of susceptible potato tubers to puncture them. However, some indirect measurements were made of the pressure the fungus might be capable of exerting under certain conditions. When fungus filaments were plasmolyzed in cane sugar solution it was found that it required a solution capable of exerting about 54 atmospheres, or 55,773.3 gm., per square centimeter to plasmolyze them. If, then, the protoplasm of the fungus is not permeable to cane sugar, the filaments are capable of withstanding nearly 55,773.3 gm. pressure per square centimeter; or, stated in another way, the filaments are capable of exerting that much pressure. This is considerably more pressure than is required to puncture the tissue of the central parts of Bliss Triumph and Green Mountain potatoes. It is sufficient pressure to puncture the cell walls of the cortex of all tubers of these varieties which rotted when inoculated in that region except one. This exception is tuber 31 in Table V, a Green Mountain tuber which required 57,571.5 gm. per square centimeter, or 1,798.2 gm. more than the osmotic pressure of the fungus filament as found in this study. It is also sufficient to puncture the tissue of the two McCormick tubers which rotted when inoculated in the central portion and the one which rotted when inoculated in the cortex.

The pressure would not be sufficient to puncture the cell walls of McCormick tubers when they were resistant to infection, and it is lower than that required for the cortex of two of the three Green Mountain tubers that did not rot when inoculated in that region. The third Green Mountain and the two Bliss Triumph tubers that did not rot when inoculated in the cortex required pressures considerably below the osmotic pressure of the fungus filament to puncture the cell walls. Just why these three potatoes did not rot is not apparent. It is, of course, possible that the 10 determinations of the pressure required to puncture cells of the cortex were made on less resistant cells than those upon which the inoculations were made. Another possibility is that a weak culture of the fungus was used. These 3 potatoes, however, were exceptions to the rule.

The experiments for the determination of the pressure required to puncture the tissue of the potatoes on the fresh surface and when dried at 30° C., as detailed in Table X, show that the pressures required for the cortex of the dried tubers, which were resistant to infection, were considerably higher than the osmotic pressure of the fungus filament. This is in agreement with the evidence just brought out from data in Tables V to VII. It would seem from this work that the mechanical pressure of the fungus filament against the cell wall of the potato is an important factor in the penetration of the potato tissue by the fungus.

One consideration detracts from the value of this indirect method for the determination of the pressure the fungus filament is able to exert against the cell walls. In the osmotic pressure determinations, an attempt was made to determine the total pressures within the filaments of the fungus, and these may or may not be the pressure the fungus is able to exert against the cell wall of its host plant. The cell walls of the fungus filament are apparently able under ordinary conditions to withstand the pressure within the filament, except at the growing points. The pressure exerted on the cell wall of the potato under the most ideal conditions would be the pressure that the contents of the filaments
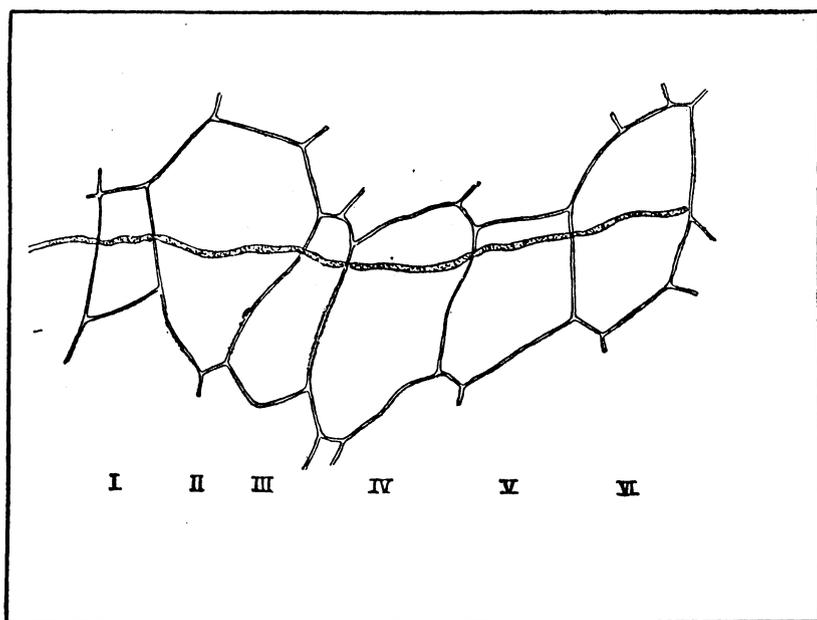
FIG. 1.—Drawing to illustrate growth of a Pythium hypha in potato tissue. Note the constriction of the hypha where it penetrates the wall.

were capable of exerting minus the pressure necessary to push out the wall or rudimentary wall of the tip of the fungus filament.

Further evidence on the method by which the fungus penetrated the cells of the potato was furnished by direct observations of the hyphae of the fungus within the tissue of the potato. In these experiments sections of raw potato were prepared as nearly sterile as possible and inoculated with the fungus. When kept overnight in hanging drop cells at 30° C. a good growth of hyphae was usually obtained. Numerous instances of cell-wall penetration were observed, and the method of penetration was followed both by serial drawings and by motion photo-micrographs (Pl. 36, 37). The part of the section selected for observa-

tion was usually two or three cells thick, since if the section is much thinner the hyphae are liable to grow over the surface.

The hypha shown in figure 1 was watched continuously for three hours. During this time it grew 1,976 μ at a room temperature of about 70° F. The time required to penetrate the wall was about 5 minutes. The distance traversed and the time required for each cell were as follows:

> The hypha in cell I, traversed 200.3 microns in 25 minutes.
> The hypha in cell II, traversed 493.9 microns in 35 minutes.
> The hypha in cell III, traversed 186.9 microns in 20 minutes.
> The hypha in cell IV, traversed 387.2 microns in 20 minutes.
> The hypha in cell V, traversed 333.7 microns in 20 minutes.
> The hypha in cell VI, traversed 373.8 microns in 30 minutes.
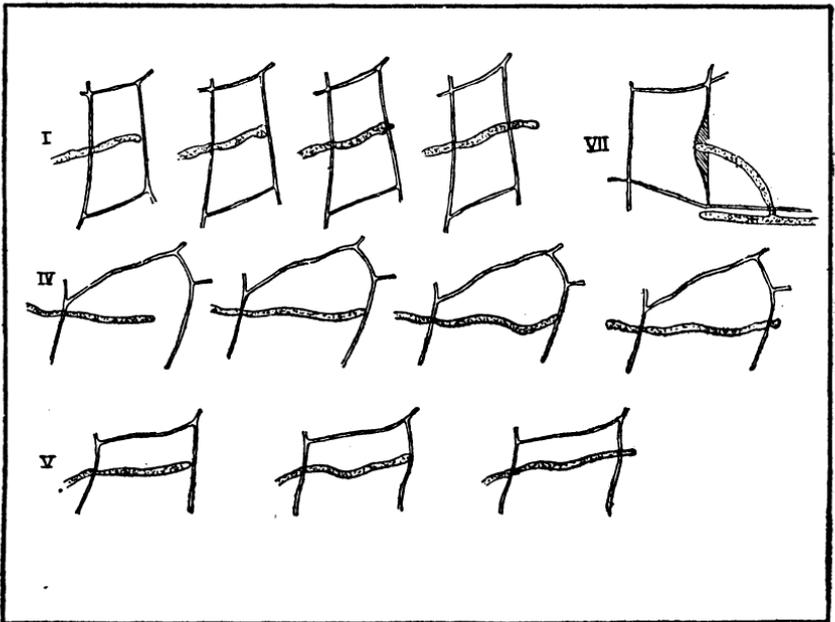
FIG. 2.—Drawing to illustrate method of cell-wall penetration in cells I, IV, and V. For explanation see text.

The drawings of figure 2, made at the time of observation to show the relative positions assumed, give the characteristic methods of cell-wall penetration as observed in this study. In passing through the cell wall between cells I and II the hypha approached the cell wall nearly at right angles; it formed a swelling at the end, bent slightly, and penetrated the wall by a small tube. After passing through the cell wall into the next cell the hypha expanded to its usual diameter. Considerably more bending of the hypha is shown in cells IV and V. It is noticeable that the wall in cell V bends outward under the pressure of the hypha and that the hypha straightens after the wall is penetrated.

There is quite clear evidence of the exertion of mechanical pressure. In cell VII another hypha was observed growing toward the potato cell wall. At first the hypha was straight; then as growth pressed it against the wall it bent upward, at the same time making a dent in the wall near the center of the wall face. This hypha did not penetrate the wall, for when it had reached the position given in the figure, rapid growth was begun by the growing tip just below it, and the pressure of the upper hypha seemed to be insufficient to break the wall.

Where the hypha approaches the cell wall at right angles it usually passes through as shown in cell I; but when the tip strikes the wall obliquely it does not usually penetrate but pushes along the wall and may go entirely around the cell, forming a coil within it. In traveling around the cell the tip may reach a corner, in which case the wall is frequently penetrated and the hypha grows between the cells. This is characteristic, and hyphae are frequently found following the middle lamellae. When the growth of the young tip is stopped for a few moments as by strong light, a cell wall is apparently formed over the tip; and on the resumption of growth this wall is broken at its weakest point by the pressure developed within the hypha. The formation of a strong hyphal wall requires about two minutes under the conditions of these experiments. The swelling of the tip shown in cell I took place in about two minutes—that is, the cell wall of the hypha seemed to become strong enough in that length of time to withstand the pressure within. There must, then, be a rapid transformation of the fluid protoplasmic material to form this wall. This transformation may be of the nature of a precipitation at the boundary between the hyphal sap and the potato cell sap. It is possible that the precipitation of substances to form the strong hyphal wall occurs only in contact with the cell sap of the potato, at least occurs more rapidly in contact with the potato cell sap than in contact with the cell wall. If this is true the hypha would form a tube of plastic materials against the cell wall and the growth pressure of the fungus filament would be applied directly to the cell wall of the potato. This might be the explanation of the mechanics of cell-wall penetration by the fungus. Evidence that the hyphae are sometimes cemented fast to the cell wall was secured during observations of hyphae that strike the cell wall obliquely, slide along it for a short distance, and then stop and penetrate the wall.

DISCUSSION OF RESULTS

In a consideration of the results brought out in the foregoing pages it is apparent that there is much evidence that the fungus makes its way through the cell walls of the potato mechanically. It is, of course, impossible to prove in work of this kind that some enzym is not secreted at the tip of the hypha which softens or destroys the portion of the cell wall with which it is directly in contact. If, then, no evidence of the

existence of such an enzym was brought out in this study, the fact that the fungus requires such a short time (about five minutes) to pass completely through a cell wall seems to indicate that the main factor, at least in the breaking through the cell wall, is mechanical pressure, for in enzym action it would be necessary to have a diffusion of the enzym from the tip of the hypha into the cell wall and at least a softening, if not a dissolving, of a portion of the cell wall at that point. Whether a substance, such as an enzym, with a relatively high molecular weight and consequently low rate of diffusion could diffuse into and through the cell wall with sufficient rapidity to soften or dissolve this tissue in the time required for the fungus to pass through the cell wall is doubtful. Another point in support of the hypothesis that the opening in the cell wall is made mechanically is that there is apparently no considerable increase in the size of the opening after the tip of the hypha passes through. If the fungus secretes an enzym which acts on the cell wall, it would seem probable that this enzym action would continue after the tip of the hypha passed through and the opening would be larger than the hypha. Hasselbring (*13*) has figured the breaking down of the host tissue around the fungus hypha. This phenomenon is common where a fungus breaks down the cell walls of its host enzymically. With *Pythium debaryanum* on potato, however, the opening in the cell wall is never larger than the mean diameter of the hypha in the lumen of the cells and is usually considerably smaller.

Another point which supports the hypothesis of the mechanical puncture of the cell walls of the host by the fungus is the fact that apparently only the middle lamella of the potato cell wall is affected. The fungus seems not to break down the secondary thickening of the cell walls, and when a piece of well-rotted potato is teased out on a slide the cells full of starch grains float free.

If, then, as seems probable, the fungus makes its way through the cell walls by mechanically puncturing them, a potato with cell walls strong enough to withstand the pressure exerted by the fungus would be immune to the disease. If we consider the osmotic pressure within the fungus filament—as determined in this study—as the pressure the fungus is able to exert against the wall of its host plant, then the resistance of potatoes in all cases in which they did not become infected would be explained, with the three exceptions mentioned earlier. This would also account for the infection of all potatoes which became infected in either the central portion of the tuber or the cortex, with the exception of one Green Mountain tuber. Another point which should be noted in this connection is that correlated with this resistance is a higher crude-fiber content in the McCormick. This is probably due to more secondary thickening in the cell walls. It is quite possible that the White McCormick potato or some hybrid of this variety would be resistant to the fungus when grown in the San Joaquin Valley and would thus solve

the problem of the control of this disease, but no field tests have been made for varietal susceptibility.

If the fungus enters the potato cell by breaking through the cell walls mechanically, it is of course necessary that there be some support from which this pressure may be developed. This support would be readily furnished where the fungus filaments were against the opposite cell wall—for instance, when the fungus is within the tissue. It seems probable also, as has been brought out earlier in this paper, that the fungus may attach itself to the cell walls of its host. In the penetration of the host tissue from the outside, as it took place in this study, it is of course necessary to have an attachment of the fungus hyphae to the host tissue. This may be accomplished by the newly formed wall of the fungus hyphae adhering to the cell wall of the potato. Blackman and Welsford (5) considered that the mucilaginous membrane of the germ tube of Botrytis formed an attachment sufficiently strong to withstand the pressure necessary for the puncturing of the cuticle of broad bean leaves. In natural infections the fungus hyphae are frequently thrust deep into the tissues of the potato, and a support from which the pressure could be developed would readily be found by the closing of the wound in the tuber.

If in its growth in the potato this fungus breaks its way through the tissue mainly by mechanical means, as seems quite possible, it is in keeping with the manner in which roots grow through potato tissue. Peirce (24) has shown that roots of *Pisum* sp. and *Vicia faba* can force their way through potato tissue mechanically, and one of the present writers has frequently observed potatoes in the San Joaquin Valley with roots growing through them. A somewhat analagous condition is found in the penetration of the stigma and style of certain Rubiaceae by the pollen tube, as described by Lloyd (19).

While it has not been proved in this investigation that *Pythium debaryanum* penetrates the cell walls of the potato by mechanical pressure, there is considerable evidence that the main factor in this penetration is the growth pressure of the fungus filament and that the resistance of the White McCormick potatoes to this disease is due to cell walls that are more resistant to mechanical puncture than are the cell walls of extremely susceptible varieties.

SUMMARY

(1) It has been shown in this paper that *Pythium debaryanum* destroys the pentosans, starch, and sugar of the potato tuber in rotting it.

(2) The fungus secretes a toxin which kills the cells of the potato. It also secretes an enzym which breaks down the middle lamellae of the cells but apparently has little or no effect on the secondary thickening.

(3) More pressure was required to puncture the tissues of White McCormick potatoes, which are comparatively resistant to the disease,

than to puncture the tissues of the two susceptible varieties, Bliss Triumph and Green Mountain. Correlated with this resistance to puncture is a resistance to infection by *Pythium debaryanum*.

(4) The resistance to puncture in McCormick tubers is also correlated with a higher crude-fiber content, which was considered to be due to more secondary thickening in the cell walls.

(5) The cut surface of the cortex of Bliss Triumph and Green Mountain when dried for three hours was much more resistant to puncture than the freshly cut surface. Here also there is a correlation between resistance to infection by this fungus and resistance to mechanical puncture.

(6) The osmotic pressure within the fungus filament, as determined by plasmolysis in this work, was sufficient to develop the pressure necessary to puncture the cell walls in the potato tubers in all cases in which infection occurred, with one exception. It was not sufficient to develop the pressure necessary to puncture the tissue of the potatoes in the cases where no infection occurred, with three exceptions.

(7) Mechanical pressure exerted by the fungus hyphae seems to be the most important factor in cell-wall penetration by this fungus, and resistance to infection is apparently due to resistance of the cell walls to mechanical puncture. Microscopical observations of cell wall penetration by the fungus hyphae seem to corroborate this theory.

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PLATE 35

A.—Apparatus used in determining the pressure required to puncture the tissue of potato tubers.

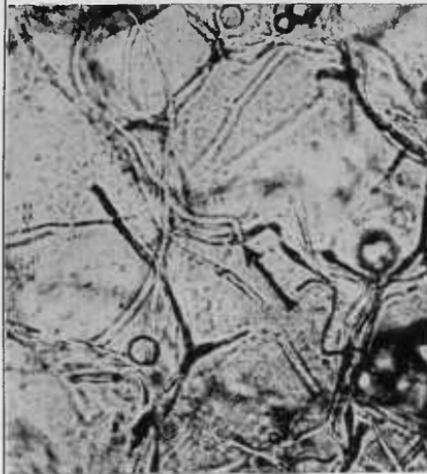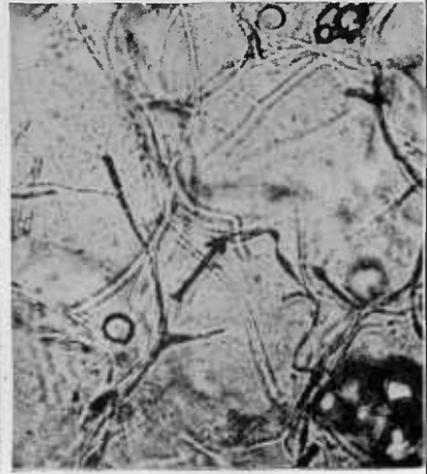B.—Glass rod with attached needle. About actual size. Photographs by J. F. Brewer.

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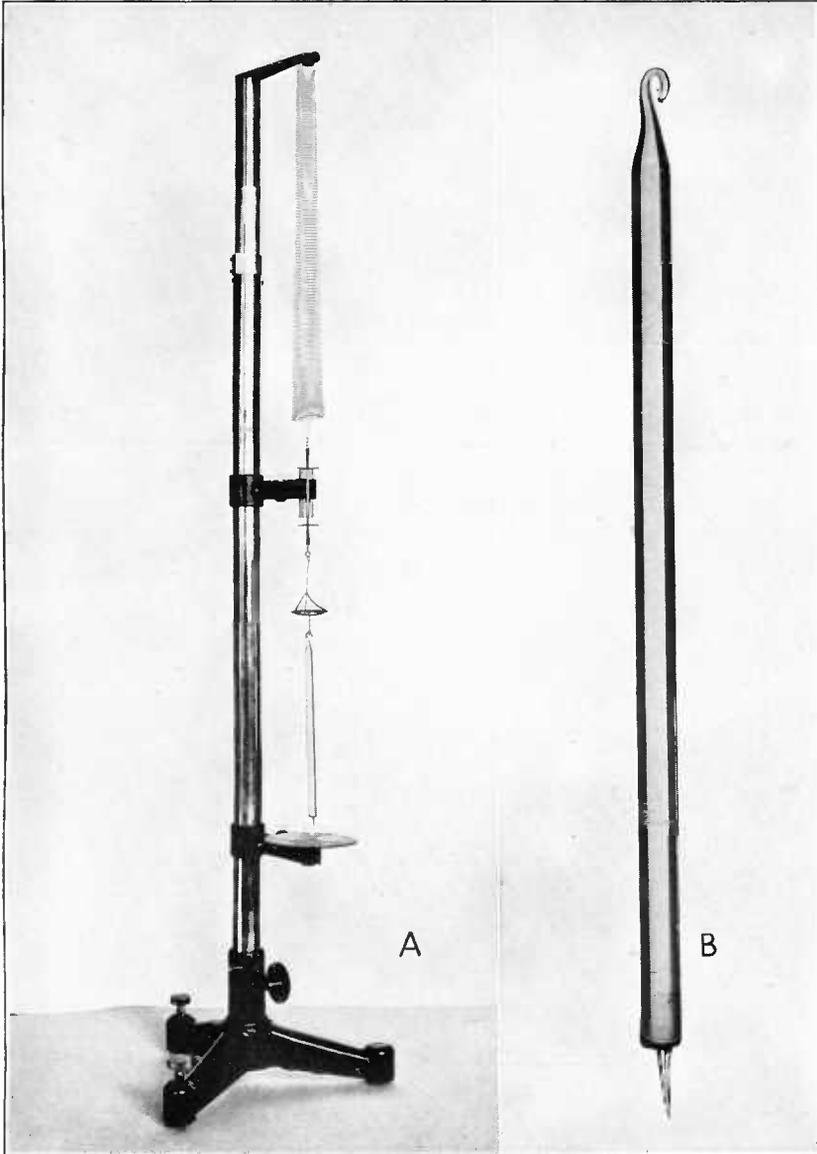
A
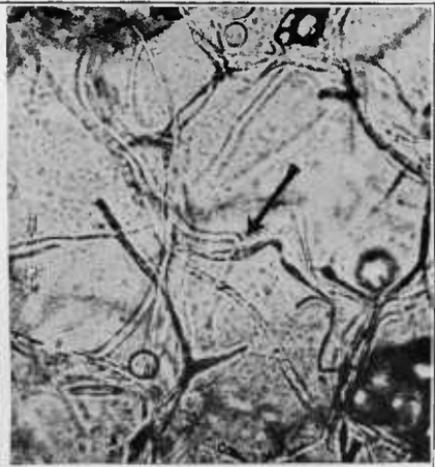
B

C

D

PLATE 36

Photographs enlarged from portions of a motion photomicrograph, showing the method of cell wall penetration by Pythium hyphae.

A.—Shows hypha growing toward the potato cell wall.

B.—Shows hypha attached to wall and about to penetrate.

C.—The tip has just broken through the wall.

D.—The penetration is complete. Note the black line at the point where the hypha penetrates the wall. This may be due to a rolling up of the potato cell wall about the hypha or to a difference in refraction caused by compression of the wall.
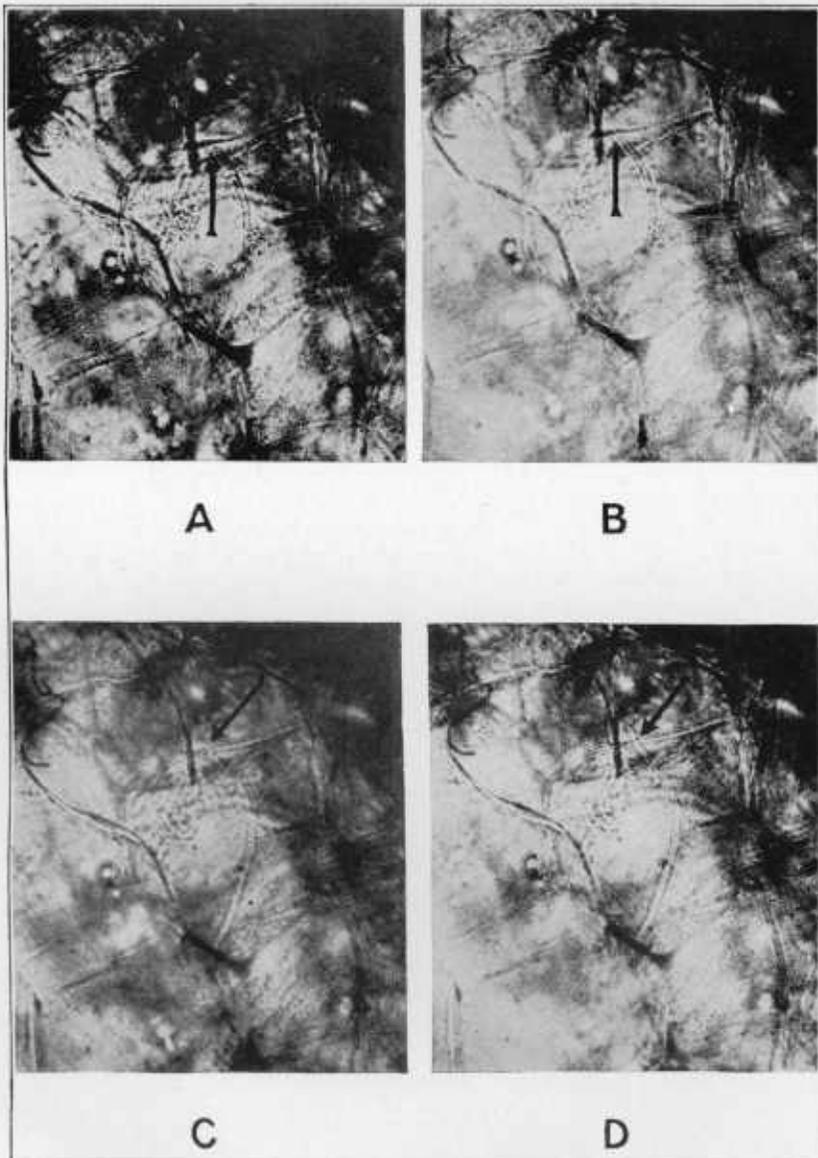
PLATE 37

Photographs enlarged from portions of a motion photomicrograph, showing the method of cell wall penetration by Pythium hyphae.

A.—Shows the hypha growing against the potato cell wall. Sufficient pressure has already been applied to cause the hypha to bend. Notice that this bending increases in later photographs.

B.—A little later stage than A.

C.—The tip has broken through as a small tube.

D.—Penetration is complete. Notice the constriction of the hypha at the point where it penetrates the potato cell wall.

A

B

C

D