The influence of a pre-infectional heat treatment on the resistance of potato plant to *Phytophthora infestans*.

(Cell Physiological studies on resistance of potato plants to *Phytophthora infestans*. VIII)

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1. Introduction

In the previous paper (2, 5, 6, 7, 8), it was shown that the cell content of potato varieties which are resistant to *Phytophthora infestans*, degenerates according to the following scheme when the cell is infected by the fungus.

In former experiments (9), to ascertain whether such a succession of cell physiological phenomena in the degeneration process was in a causative linkage or not, the influence was examined of a post-infectional treatment with 2, 4-dinitrophenol (DNP) on the hyper-sensitive reaction of a potato cell resistant to infection by *P. infestans*.

DNP is known to be an inhibitor of oxidative phosphorylation and to be poisonous to cytoplasmic streaming. In these experiments, it was found that the death of the invaded cell was remarkably delayed by the post-infectional DNP treatment. These results suggest that the energy liberated from respiratory process (11-14) is closely associated with the death of the infected cell. It is possible, however, that the direct effect of DNP upon the intracellular hyphae gives rise to the delay in the cell death. Therefore in the present experiments, it was planned to study the influence of a pre-infectional heat treatment, which seems to be not influential on the parasite, on the hyper-sensitive reaction of a potato plant cell to the infection of *P. infestans*.

Tochinai and Fukuchi (present author's former name) (1942) (4) showed that in the cells of the seedling of a certain cotton variety, which is resistant to cotton anthracnose when treated with hot water at 55°C for 30 sec. ~ 2 min., hyphal invasion and development were rapid and flourishing. Gradinaroff (1943) (1) showed that living Duke of York and Ackersgen tubers exposed before inoculation to supramaximal temperatures (30°, 35°, 40° and 45°C) acquired a susceptible reaction to fungi incapable of attacking normal material, more particularly *R. stolonifer* and *F. culmorum*. On the other hand, the development of *P. infestans* on pre-treated susceptible tubers was scantier than on the control, indicating that a decline in vitality does not necessarily connote any weakening of resistance to late blight. Yarwood (1956) (16) showed recently that the susceptibility of beans to some viruses and fungi is reduced by a pre-infectional heat-treatment.

2. Experiments on the cortex layer cells of a petiole

a) The influence of a pre-infectional heat treatment on resistance.

Methods and Materials. A potato leaf petiole was cut longitudinally with a razor, and washed with running water in order to eliminate the contents of destroyed cells. It was shown by microscopic examination that almost all the cells of the cut surface were living. Petioles thus prepared were dipped in hot water for a certain time. After the materials had cooled, the cut surfaces were inoculated with zoospore suspension of race O of *P. infestans*, to which the potato variety 41089-8² used in present experiments has been proved to be resistant. The variety 41089-8 has two resistant genes R1 and R4 (Takase (3)). The inoculated materials were kept in a petri dish at 19°C.

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Results. The results obtained are shown in Tables 1 and 2.

On the cut surface of the untreated petiole, remarkable brown necrotic spots came out about a day after inoculation, and no sporulation was observed afterwards; on that of heat treated one, on the contrary, brown necrotic spots did not appear until about 1-2 days after inoculation, and a considerable amount of spores were produced.

b) The influence of a pre-infectional heat-treatment on the growth of intracellular hyphae.

Methods and Materials. On the same materials as described in the preceding section, microscopic examination was made of the growth of intracellular hyphae.

Table 1. Reactions of cortex cells of potato petioles treated with hot water for 5 minutes to the infection by *Phytophthora infestans*.

Temperature °C Hrs. after inoculation	cont.	41.5	44.5	46.5	49.5	51.5	53.5	56.5	61.5
24	+	+	—	—	—	—	—	*	**
42	++	++	+	±	—	—	—		

Remarks: * Discoloration began about one hour after heat treatment.

**..... Discoloration began in the course of heat treatment.

+..... Necrotic brown lesions appeared.

—..... No necrotic brown lesions appeared.

Sporulation A considerable amount of spores was produced on the heat-treated petioles, but not on the control ones.

Table 2. The effects of different durations of pre-infectional heat treatment at 41.5°C on the reactions of petioles of potato plant to infection by *Phytophthora infestans*.

Duration of heat treatment (min.) Hrs. after inoc.	Cont.	5	30	60
22	+	+	—	—
30	+	+	—	—
46	++	++	+	+

Remark: The marks are the same as in Table 1.

Five hours after inoculation, microscopical sections of the inoculated materials were prepared and fixed with formalin-acetic acid-alcohol, and then the length of intracellular hyphae was measured with a micrometer. As will be described in a later section, the penetration of the hyphae of *P. infestans* into the host cell occurs almost simultaneously in tissues of both heated and nonheated petioles. Therefore the values thus obtained may indicate the growth rate of the fungus in living cells.

Results. The results obtained are represented in Table 3.

Table 3. The length of intracellular hyphae of *Phytophthora infestans* in the cortex cells of potato petioles heated with hot water for 5 min. and in cells of untreated ones.

Treatments	Control (non-heated)	Heated at 46.5°C	Heated at 51.5°C
I (Means) *	18.69 (μ)	20.76 (μ)	20.59 (μ)
II "	24.83	21.46	21.94
III "	26.81	26.67	24.76
Means	23.44	22.96	22.43

Remark: * Number of observed cells were about 50~100.

It seems that the pre-infectional heat-treatments at 46.5°C and 51.5°C for five minutes exert no influence upon the growth rate of intracellular hyphae of *P. infestans* in the cortex cells of potato petiole.

c) The influence of a heat-treatment on the occurrence of protoplasmic strands.

Methods and Materials. Observation was made of the occurrence of protoplasmic strands (P. S.) in the cortex cells of the petioles heated with hot water at 46.5°C and 51.5°C according to the same methods as described in the former sections.

A microscopical section made from thus prepared materials was placed in the hanging drop under the cover glass maintained on the hole of section-preserving apparatus (described in a former paper (8)), which was devised for the purpose of keeping sections at constant temperature (at 23°C). Then the number of P. S. which appeared in the cells was counted.

Results. In the cells of a control (non-heated), striking appearances of P. S. s were noted. The P. S., on which an active streaming of cytoplasm was recognized, burst into view, and on some occasions altered their position and then in a short time, disappeared. Such an active appearance of P. S. in the control cells seems to be owing to the act of preparation, because

Table 4. The number of protoplasmic strands in the cortex cells of potato petioles heated with hot water at 46.5°C and 50°C for five minutes.

Treatments		Control (non-heated)		46.5°C		51.5°C	
Hrs. after treatment							
(1 st exp.)	2	range	mean	range	mean	range	mean
(2 nd exp.)	4	1-8	4.9	1-4	2.25	0	0
	23	3-14	7.3	2-13	6.1	0-2	0.8
		3-10	5.8	1-14	4.1	0	0

Remarks: 1) The figure shown is the number of P. S. which was observed at a look.

2) Ten cells were observed in each experiment.

the cells observed were those in the tissue of cut surface of the petiole.

The data shown in Table 4 indicate that the active appearance of P. S. in the control cells was inhibited remarkably by 5 minutes of treatment with hot water especially by the treatment at 51.5°C. It was ascertained by means of both vital staining with neutral red and plasmolysis with 0.5 Mol. sucrose solution that the cells treated for 5 minutes at 51.5°C were still living 23 hours after the treatment.

d) The influence of a pre-infectional heat-treatment on the time which elapsed from penetration of the fungus to hyper-sensitive death of the infected host cell.

Methods and Materials. To the cut surface of petioles prepared by the same methods as described in the former sections, a zoospore suspension of race O of *Phytophthora infestans* was inoculated. At a certain interval of time after inoculation, a microscopical section was made from the inoculated materials; then the state of the penetrating hyphae was observed.

An appressorium was considered as not having penetrated yet into the cell, when the first sign of penetration was not observable under the microscope.

From the graph obtained by plotting the percentages of penetrated cells per total cells attached by appressoria as ordinates and time as abscissas, the time required for the penetration of the fungus into the host cell may be deduced. Both the vital staining method with neutral red and recognition of the existence of P. S. was employed to ascertain the hyper-sensitive death of the infected cells.

These experiments were repeated five times.

Results. From Fig. 1, it seems to be clear that by a pre-infectional heat-treatment, the time required for penetration of the fungus was not affected while the death of the invaded cells was remarkably delayed.

3. Experiments on slices of potato tuber

a) The influence of a pre-infectional heat-treatment on the resistance of the slice to *Phytophthora infestans*.

Methods and Materials. A block of potato tuber was stamped out by a cork borer 22mm in diameter, and sliced 0.9 mm thick by means of microtome, and then washed with running water (12°C). The discs thus washed were inoculated with race 0 and race 1,4 of *P. infestans*: the former race is non-aggressive and the latter is aggressive to the potato variety "41089-8" used in the present experiments. The inoculated discs were kept in a petri dish at 19°C.

Results. In Tables 5 and 6, the results obtained are summarized.

The appearance of necrotic spots in the discs infected by incompatible strain (race 0) was remarkably delayed under the influence of heat-treatment especially at 49°C ~ 50°C, resulting in the production of a considerable amount of spores. Therefore it may be concluded that the pre-infectional heat-treatment reduces the resistance of tuber discs.

The spore production in the discs infected by compatible strain (race 1,4) was also increased by the heat-treatment.

b) The influence of a heat-treatment on the respiration of the slices.

Methods and Materials. About 40-60 minutes after slicing the potato tuber discs, prepared by the same method as in the former section, were dipped in hot water at 45°, 47.5° and 50°C for five minutes.

After heat-treatment, the discs were kept in a petri dish in the thermostat at 19°C. Then the respiration of the discs was measured by means of Warburg respirometer. Evolution of CO₂

Table 6. The reaction of potato tuber slices treated with hot water for 5 minutes to the infection of race 0 and race 1,4 of *P. infestans*.

Time after inoculation		Cont.		49°C		50°C	
		race 0	race 1,4	race 0	race 1,4	race 0	race 1,4
Reaction	18 hrs.	—	—	—	—	—	—
	22 hrs.	±	—	—	—	—	—
	39 hrs.	++	—	±	—	±	—
Sporulation	5 days	non	rich	poor	very rich	poor	very rich

Remark: The marks are the same as in Table 5.

was determined by means of Warburg's "direct method". The solution in the Warburg flasks was 1/15 M. phosphate buffer of pH 5.3. The temperature of the water bath was maintained at 22°C. The potato variety used in the present experiments was "41089-8".

Results. The R.Q.s of potato tuber discs treated with hot water at 47.5°C and those of controls are shown in Table 5. No difference was found between the R.Q.s of the treated and non-treated discs about one day after heat-treatment.

As the R.Q.s of each plot were very close to 1.0, it may be permissible to draw conclusions on the respiratory drifts from the experiments on the O₂-uptake. The O₂-uptake of the discs are shown in Fig. 2. The respiratory rate was depressed remarkably by the heat-treatment, especially in the cases of comparatively higher temperature, and after a more or less continuous fall for some hours, it began to increase. About one day after treatment, the O₂-uptake of the

○...Percentages of penetrated cells per total cells attached by appressoria.
●...Percentages of dead cells per total cells infected by *P. infestans*.

Fig. 1. The influence of a pre-infectional heat-treatment on the penetration of the fungi into the host cell and the time elapsed from their penetration to the death of the infected host cells.

discs treated at 45°C have been restored almost completely, but those of the discs treated at the temperature higher than that were still lower than the O₂-uptake of the control.

Table 7. R. Q. s of the potato tuber discs treated with hot waters for five minutes.

Hrs. after heat-treatment*	Treatments	CO ₂ -output	O ₂ -uptake	R. Q.
21	Cont.	36.59	36.62	1.00
	47.5°C	22.86	22.81	1.00
25	Cont.	35.52	36.07	0.98
	47.5°C	23.04	23.94	0.96
26	Cont.	36.33	37.96	0.96
	47.5°C	31.65	32.50	0.97
Means	Cont.	36.15	36.87	0.98
	47.5°C	25.85	26.42	0.98

Remark: The hot water treatment was carried out about 1 hour after slicing.

4. Consideration

The results obtained from these experiments show that the respiratory rate of a potato tuber discs is depressed remarkably by a treatment with hot water, accompanied with reduction of resistance to infection by *Phytophthora infestans*. The reduction of resistance was learned by the slower and weaker appearance of necrogenous reaction, and by an increased spore-production. Delay of necrogenous reaction by heat treatment seems to mean the delay of the death of infected cells. This presumption has been supported by microscopical observations on the cut surface cells of leaf petioles. The rate of delay of necrogenous reaction under the influence of the heat-treatment seems to go along with the rate of respiratory depression by the same treatment: in the case of the discs treated at temperatures lower than 47.5°C, the respiratory rate of which was restored with relative rapidity, the necrogenous reaction to the infection of the fungus occurred relatively earlier, comparing with that of the discs treated at 50°C, the respiratory rate of which was not easily restored. As both the time required for the establishment of penetration of the fungus into host cell, and the growth rate of the intracellular hyphae are not affected by the pre-infectional heat-treatment, it seems that the heat-treatment has some effect on hyper-sensitive death of infected cells, thus influencing the resistance. This presumption is favoured by the facts, shown in former reports (6,10), that the inhibition of growth of intracellular hyphae in the resistant host cell seems to occur for the first time after the death of the infected host cell.

In the introduction of the present paper, the process of cell physiological changes induced by infection of *Phytophthora infestans* in resistant potato cells was shown schematically. As the present experiments showed that the occurrence of protoplasmic strands in the cells of cut surface of a petiole was inhibited by the heat-treatment, accompanied with the reduction of resistance to infection by *Phytophthora infestans*, it seems to be plausible that these processes are in a causative linkage with one another.

In one of the former reports it was shown that the death of potato plant cells infected by incompatible race of *Phytophthora infestans* was remarkably delayed by post-infectional 2, 4-DNP-treatment. In another paper, it was shown that when the discs of potato tuber, the respiratory rate of which had been accelerated by the act of slicing about one day after slicing of them, were inoculated with incompatible race of *Phytophthora infestans*, the hyper-sensitive death of

Fig. 2. Respiratory drifts of the slices treated with hot water for 5 minutes.

the infected cell was hastened.

From these results, it may be concluded that the hyper-sensitive death of the infected cell has a close connection with the metabolic activity in that cell.

The process of browning after the hyper-sensitive death of the infected cell was described in former papers (6, 15); the growth of intracellular hyphae is inhibited and followed by the death of hyphae, probably owing to the physiological action of metabolism in the neighbouring living cells, especially the deposition of polyphenol compounds and other materials in the infected cell.

5. Summary

1. Tissues of a potato petiole and slices of potato tuber were heated at 45° ~ 53.5°C for five minutes, and then inoculated with *Phytophthora infestans*. The resistance of them to *P. infestans* was reduced remarkably.

2. No influence of the heat-treatment on the time required for penetration of the fungus into the host cell, nor on the growth rate of the intracellular hyphae was found by microscopical observations on the cortex cells of the petioles.

3. The necrogenous reaction of potato-plant tissue to infection by incompatible race of *Phytophthora infestans* was delayed remarkably by pre-infectional heat-treatment. The delay of the hyper-sensitive death of an infected cell was ascertained by microscopical observation on the cortex cells of the petioles.

4. The appearance of protoplasmic strands, in the cut-surface cell of a petiole was reduced remarkably by the heat-treatment.

5. The R.Q. of the potato tuber slice was not affected by the heat-treatment, but the respiratory rate was depressed remarkably by the same treatment. The rate of respiratory depression seems to go along with that of the reduction of resistance.

6. Basis was found for the supposition that the metabolic activity in the cell is closely associated with the hyper-sensitive death of infected cell. (Received on June 25, 1957)

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馬鈴薯疫病抵抗性の細胞生理学的研究 VIII 疫病抵抗性に対する接種前熱処理の影響

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- 1) 馬鈴薯葉柄及び塊茎スライスの組織を5分間45~53.5°Cの温湯に浸漬し、冷却後疫病菌を接種した。抵抗性はこの処理で著しく低下した。
- 2) この処理は菌の侵入時間に影響しなかった。また生細胞内の菌糸伸長速度にも影響しなかった。
- 3) 菌侵入後の寄主植物の過敏感死時間は著しく遅延した。
- 4) 原形質糸の出現頻度は、この処理で著しく低下した。
- 5) 塊茎スライスのR.Q.はこの処理で影響されない(少なくとも20時間後に)。また呼吸はこの処理で著しく低下し、且つ処理終了後もなお低下を続け、ある時間後に再び回復し始める。
- 6) 以上から疫病菌侵入に伴う寄主植物の過敏感死と代謝活性は密接な連関を持つと結論した。