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THE EFFECT OF *GIBBERELLA ZEAE* ON THE PERMEABILITY OF POTATO TUBER TISSUE[1]

Het effect van Gibberella zeae *op de permeabiliteit van het weefsel van aardappelknollen*

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Potato tubers cut into halves were inoculated with conidial spores or mycelium of *Gibberella zeae* in order to study the permeability of the cells adjacent to and at some distance from the tissue invaded by the fungus. Permeability was determined by measuring the exosmosis rate of electrolytes. Within three days of infection the permeability of cells at a mean distance of $3\frac{1}{2}$ mm from the margin of the invaded tissue had doubled. At a mean distance of 6 mm there was no increase of permeability, except, perhaps, of a very slight increase 6 to 8 days after infection. A possible cause of the increase of permeability and its relation to the increased respiration are discussed.

INTRODUCTION

In plants infected with a parasite, not only cells containing the parasite, but also cells in the area adjacent to the invaded tissue show various histological and physiological changes. Surveys of such changes have been given a.o. by ALLEN (1954), SHAW (1963) and TOMIYAMA (1963).

VERLEUR (1960) studied the increased respiration of potato tuber tissue adjacent to tissue invaded by *Gibberella zeae* (Schw. Petch). The increase was larger and the effect extended deeper into the tissue when the infection was heavier and had lasted longer. As possible causes of the increased respiration VERLEUR (1960, 1964) examined the uncoupling of respiration and phosphorylation and the synthetic activity of the tissue.

Another possible cause of the increased respiration is an increase in cell-permeability. This point has been investigated by THATCHER (1939, 1942) and WHEELER & BLACK (1963). THATCHER working with *Uromyces fabae* in pea tissue and using the plasmometric method of HÖFLER, was able to show an increase in permeability of the cells adjacent to the invaded host-tissue. He also measured an increase in permeability in uninfected tissue after application of an extract of invaded pea tissue. WHEELER & BLACK measured the effect of victorin, the toxin produced by *Helminthosporium victoriae*, upon samples of leaf tissue of oats. Permeability, as indicated by the rate of exosmosis of electrolytes, increased as early as five minutes after administration of victorin. Respiration did not begin to increase until 30 minutes after administration of the toxin. The authors concluded that if a causal relationship exists between permeability and respiration, permeability changes must be the cause rather than the result of changes in respiratory activity.

In the present paper experiments are reported on the effect of *Gibberella zeae* (stat. con. *Fusarium graminearum* Schwabe) upon the permeability of cells adjacent to invaded potato tuber tissue.

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MATERIAL AND METHOD

Potato tubers (*Solanum tuberosum* cv. 'Bintje') were externally sterilized and cut into two halves. These halves were inoculated over the whole surface of the cut with a suspension of conidial spores or with young mycelium of *Gibberella zeae*. After inoculation they were transferred to a sterile glass jar and placed in the dark at 24 °C.

Using the technique described by VERLEUR (1960) tissue cylinders were bored out perpendicular to the surface of the cut at intervals following inoculation. These cylinders were then divided into disks at various distances from the inoculated surface, but always beyond the invaded tissue. The disks were 1 mm thick and 6 mm in diameter. Immediately after they were cut, they were put in a 1 l pyrex beaker containing distilled water. They were vigorously stirred with a perspex stirrer to wash the ions from the cut cells at the surface. After one minute the disks were transferred singly, with the aid of a small glass shovel (Fig. 1 d), to quartz vessels, each of which was filled with 16 ml distilled water (e). These vessels were placed in a water bath at 20 °C with their openings (b and c) just above the water level (g). The bath was closed by a lid (h).

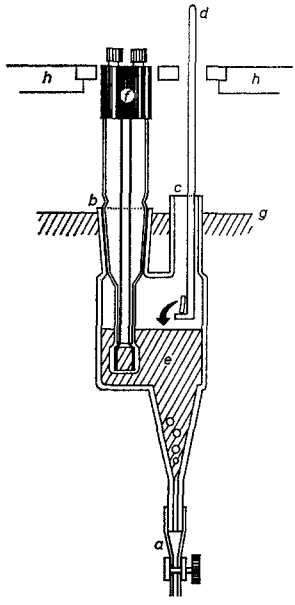

FIG. 1. Apparatus for the measurement of the rate of exosmosis; see text.
Apparaat voor de meting van de exosmose-snelheid; zie tekst.

The distilled water was aerated with carbon dioxide-free air saturated with water vapour, which entered the vessel through the tube-shaped bottom (a). This air not only stirred the water, allowing the exosmosed ions to spread rapidly into the water, but also supplied the revolving disk with the necessary oxygen. The air left the vessel through opening c, through which the disk had been inserted. In order to determine the exosmosis rate the specific resistance of the water was measured with a Philips conductivity measuring cell (type P.R. 9512/01, Fig. 1 f), used in conjunction with a conductivity measuring bridge (Philoscope type G.M. 4249/01). This system was fed by 0.4 V A.C. 50 c.p.s.

The measured specific resistances were expressed as percentages NaCl with the aid of previously determined specific resistances of a series of NaCl-solutions.

Using three vessels it was possible to study three disks at the same time. During the first hour each disk was measured every five minutes, and subsequently every ten minutes. Each experiment lasted at least two hours.

RESULTS

The changes in the specific resistance after the introduction of the disk show that the rate of exosmosis of ions is highest immediately after this introduction and diminishes gradually. This is demonstrated in Fig. 2, which refers to an experiment with three disks cut from a tuber which had been inoculated four days before. The disks were cut at a distance of $3\frac{1}{2}$–$4\frac{1}{2}$, $6\frac{1}{2}$–$7\frac{1}{2}$ and $9\frac{1}{2}$–$10\frac{1}{2}$ mm respectively from the inoculated surface. In this as in all other experiments, none of the disks contained mycelium; the first mentioned distance is always the smallest one at which it was possible to get a mycelium-free disk (VERLEUR, 1960).

Obviously the ion concentration of the water (expressed as percentage NaCl) increases as the distance from the invaded tissue decreases. This demonstrates that the permeability of the cells of the tissue adjacent to the invaded tissue is increased under the influence of the infection.

This experiment was repeated with seven other tubers. The three sampling distances from the inoculated surface (A, B, C) varied according to the thickness of the invaded tissue: A from 2–3 to $3\frac{1}{2}$–$4\frac{1}{2}$ mm, B from 4–5 to $6\frac{1}{2}$–$7\frac{1}{2}$ mm and C from 9–10 to 12–13 mm.

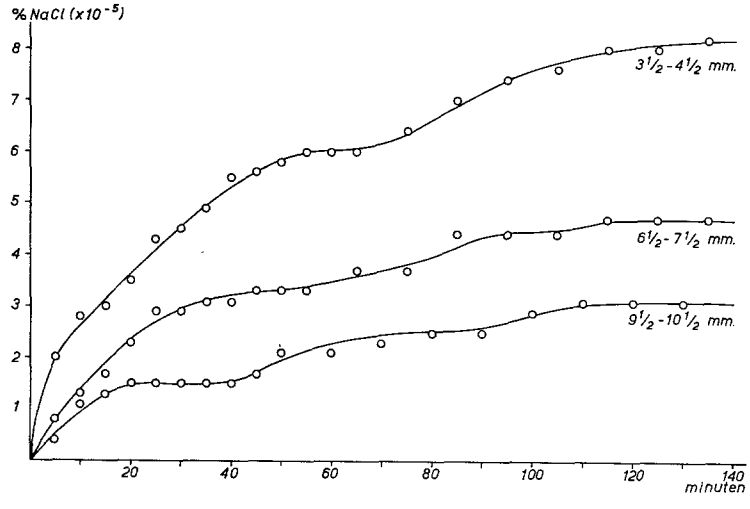

FIG. 2. Exosmosis of electrolytes at various distances from the invaded tissue, expressed as 10^{-5} percentages NaCl; see text.

Exosmose van elektrolyten op verschillende afstanden van het aangetaste weefsel, uitgedrukt in 10^{-5} percentages NaCl; zie tekst.

TABLE 1. Exosmosis of electrolytes expressed as percentages NaCl ($\times 10^{-5}$) at different distances (A, B, C) from the inoculated surface, arranged in order of increasing duration of infection.

Exosmose van elektrolyten, uitgedrukt in percentages NaCl ($\times 10^{-5}$) op verschillende afstanden (A, B, C) van de buitenoppervlakte van het aangetaste weefsel, gerangschikt volgens toenemende infectieduur.

Duration of infection (days)	Distance (mm)			Ion concentration (% NaCl $\times 10^{-5}$)		
	A	B	C	A	B	C
3	2 –3	4 –5	12 –13	7.0	4.6	4.0
4	3½–4½	6½–7½	9½–10½	8.0	4.8	3.0
5	2½–3½	6½–7½	10½–11½	10.5	5.8	6.8
5	3½–4½	6½–7½	9½–10½	7.5	5.1	4.4
5	3½–4½	5½–6½	12 –13	5.7	3.0	4.2
6	3 –4	6 –7	10 –11	10.2	7.2	6.0
7	2½–3½	4½–5½	9 –10	11.0	5.8	4.5
8	2½–3½	4½–5½	9 –10	9.0	5.4	3.2

It is not necessary to discuss the whole course of the exosmosis. To obtain comparisons it will be sufficient to discuss the ion concentrations achieved in the water surrounding the disks after a standard period, such as two hours (Table 1).

Assuming an effect of the fungus on permeability it is clear that this effect decreases with increasing distance. In the experimental conditions this effect has not yet reached the tissue at distance C, the mean exosmosis at this distance (4.5×10^{-5}) being about the same as the mean exosmosis of disks cut from healthy tubers (4.4×10^{-5}; mean of values between 2.0×10^{-5} and 6.2×10^{-5}). Moreover, there is no indication of a relation between the exosmosis and the duration of infection. It may therefore be concluded that the effect of the fungus in the experimental conditions had not yet penetrated as far as distance C. This is in accordance with the experiments of VERLEUR (1960) who found no or hardly any increase in respiration at this distance.

The WILCOXON test proved that the differences in permeability between A and C are significant. This led to the conclusion that the permeability was increased in close proximity to the invaded tissue. The differences between B and C, taken at random, proved to be not significant. Notwithstanding this fact, it is possible that the permeability at B might be increased after a long period of infection but not after a short period, owing to slow penetration of the effect of the fungus into the tissue.

In order to investigate this possibility the results were divided into three groups with durations of infection of 3 to 4, 5, and 6 to 8 days respectively. The mean ion concentration of each of these groups at distances A, B and C is shown in Table 2.

Clearly there is no difference in the rate of exosmosis between B and C after a duration of infection of 3 to 4, and 5 days. After a duration of 6 to 8 days the measured exosmosis at B is greater than at C but the number of measurements is too small to allow calculation of the degree of significance.

TABLE 2. Relation between duration of infection and the depth at which increased exosmosis is found.

Verband tussen infectieduur en binnendringen van de verhoging van de exosmose.

Duration of infection (days)	Ion concentration (% NaCl × 10^{-5})		
	Distance		
	A	B	C
3 to 4	7.5	4.7	3.5
5	7.9	4.6	5.1
6 to 8	10.1	6.1	4.6

The experiments have shown that the exosmosis at distance A is about twice as large as the exosmosis at distance C. Before attributing this result to an increase in permeability in consequence of the infection some other possible explanations have to be eliminated.

The increased exosmosis might be a consequence of a higher concentration of free ions in the cells. However, the concentration in the cells at distance A was found to be only 1.25 times the concentration in the cells of non-infected control tubers. Therefore the increased concentration of electrolytes can only partly explain the measured twofold increase in exosmosis.

The same is true in respect of an insufficient washing out of the cut cells before the experiments, as a cause of the increased exosmosis. On this basis the exosmosis could rise to 1.25 times the normal one at the most, unless one assumes an increased permeability of the membranes of nucleus, plastids and mitochondria. Furthermore, after washing, the cut cells did not contribute significantly to the leakage of ions into the surrounding water. This was clearly shown by a comparison of disks with thicknesses of one and two mm. The latter have a volume twice that of the former, but a surface which is only 1.25 times as large and consequently also the volume of their cut cells. If the ions were released exclusively by these cells then the ion concentration in the water surrounding the 2 mm disks should be 1.25 times the concentration in the water surrounding the 1 mm disks; if the ions were released from the intact cells then this proportion would be two. An experiment showed that the value of this proportion was 1.7 with disks from control tubers and 2.5 with disks, cut at distance A, from infected tubers. The mean value 2.1 indicates that the exosmosis of ions is proportional to the volume of the intact cells and not to the cut ones.

DISCUSSION

The increase in permeability caused by a parasite or by a toxin produced by a parasite, as determined by THATCHER (1939, 1942) and WHEELER & BLACK (1963) has been corroborated. The latter workers also obtained an increase in respiration, beginning somewhat later than the increase in permeability. From this they concluded that if a causal relationship exists between permeability and respiration, permeability changes must be the cause rather than the result of changes in respiratory activity.

A comparison of the measurements of the permeability in the present paper with the determinations of the respiration made by VERLEUR (1960) suggests that the increase of respiration begins earlier and advances more quickly into the tissue than the increase of permeability. After a duration of infection of one day the respiration in cells at a distance of 2–3 mm had already increased clearly. After a duration of infection of seven days this increase had already advanced to a distance of 8–9 mm. After the same duration of infection the tissue at a distance of 9–10 mm (Table 1) had not yet increased its exosmosis. Even if one assumes that after 6 to 8 days of infection the increase of permeability has advanced to a distance of $4\frac{1}{2}$ to 7 mm (which is quite uncertain) the increase of respiration begins earlier and cannot be the result of an increased exosmosis rate.

However, this does not exclude the possibility that the increase of respiration is caused by an increase of permeability. It may be assumed that the measured exosmosis is caused chiefly by ions permeating from the vacuole through tonoplast and plasmalemma into the surrounding water. In addition, the membranes enveloping the mitochondria may also have increased in permeability. This could lead to no or practically no increase in exosmosis and could therefore not be observed. It is possible that these membranes might increase their permeability earlier than tonoplast and plasmalemma, allowing an increase of respiration before the increase of the exosmosis rate.

The cause of the increase of permeability of the semi-permeable membranes is as yet quite hypothetical. Because the cells are going to die as a consequence of the infection (the immediate vicinity of the invaded tissue at times consists of a "soft zone" containing cells which have largely lost their turgor) the increase of permeability might be one of the first symptoms of this dying process, during which the cell content and consequently also the semi-permeable membranes disintegrate. Perhaps the death of the cells may be attributed to a toxin produced by the fungus or to a substance synthesized in cells injured by the fungus.

The discrepancy with the results of WHEELER & BLACK might be due to the difference in host-parasite combination, or to their application of a toxin to the tissue fragments causing a more rapid intoxication than is the case with tissue which had received, in situ, a prolonged exposure to a much smaller dose of toxin.

SAMENVATTING

Halve aardappelknollen werden op het snijvlak geïnfecteerd met conidiosporen of mycelium van *Gibberella zeae* (stat. con. *Fusarium graminearum*) om de permeabiliteit te meten van cellen dichtbij en verder verwijderd van het door de schimmel aangetaste weefsel. De permeabiliteit werd bepaald door de exosmose-snelheid van elektrolyten te meten (Fig. 1).

Drie dagen na de infectie was de permeabiliteit van de aan het aangetaste weefsel grenzende cellen op een gemiddelde afstand van $3\frac{1}{2}$ mm van het buitenoppervlak van dit weefsel reeds verdubbeld. Op een gemiddelde afstand van 6 mm was er geen toename van de permeabiliteit. Op deze afstand is er misschien zes tot acht dagen na de infectie een zwakke toename (Fig. 2).

De mogelijke oorzaak van de permeabiliteitstoename en haar verband met de verhoogde ademhaling worden besproken.

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