WOUND HEALING OF THE POTATO TUBER IN RELATION TO INFECTION BY *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY

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Summary

The protective action of healing potato tuber tissue against *Phytophthora infestans* (Mont.) De Bary is made manifest in two ways:

(i) In the tissues adjacent to the injured surface the speed of reaction to the pathogen, as evidenced in production of necrotic changes, is altered; it increases with increasing time interval between injury and infection. In close association with this, development of aerial mycelium on the inoculated face decreases, sporangiophore production ceasing completely after an interval of 4 days.

(ii) A marked decrease in susceptibility to infection occurs in the healing face; this becomes obvious before any cell divisions take place in the tissues which will form wound periderm. A statistical analysis showed that the probability of an individual spore being able to penetrate the protective barrier of wound periderm is decreased by at least 92 per cent. when an interval of 6 days has occurred between injury and exposure to the parasite, the tubers being kept at temperatures around 20°C.

The results are discussed in the light of the following hypotheses: (1) as a result of mechanical injury there is an increase in sensitivity towards the parasite produced in the tissues adjacent to the injured face (change of reactivity towards suprasensitivity), and (2) the development of a mechanical factor (possibly deposition of suberin in the cell layer adjacent to the injured surface) makes it more difficult for the parasite to gain a footing in the host tissue (increase of "passive resistance" to infection).

I. Introduction

Peridermal tissues are commonly regarded as effective barriers against infections by pathogenic microorganisms. Infections of potato tubers by *Phytophthora infestans* (Mont.) De Bary, for example, are considered to occur only through bud initials, lenticels, stolons, or wounds, the unbroken skin being impenetrable to fungal or bacterial parasites. This was confirmed by previous inoculation experiments carried out with tuber initials. As soon as the epidermal cells of the young tuber had undergone two divisions in peridermal formation, infection through the skin no longer took place, (Müller, unpublished data).

A similar effect is produced by peridermal tissues which are developed in response to mechanical wounding, e.g. in potatoes. Where a continuous layer of wound cork has been established, adjacent tissues have been found to be largely protected against subsequent infections whether bacterial or fungal. The majority of writers on this subject attribute this protection to the deposition of suberin in the wound periderm cells. It follows then that the more quickly the wound cork is developed, the greater is the protective effect; but the influence of environmental

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factors on the speed of wound periderm formation causes a considerable variation in the time necessary for the development of this protective mechanism (Appel 1906; McKee 1954, 1955; Smith and Smart 1955).

The protective effect of wound periderm against *Phytophthora* infection has not previously been investigated systematically, though Meyer (1939) mentions that 4 days after wounding, the parasite is still able to penetrate into the parenchyma of the tuber, but the reaction of the tuber to the parasite had changed. Necrotic changes in response to infection appeared much sooner in a tuber which had been cut 3 days before inoculation, than in one inoculated immediately after cutting; also fructification was much less in the former than in the latter. In the course of wound periderm formation an accumulation of phenolic substances occurs in the cells adjacent to the cut face, and Meyer concludes that the inhibition of the parasite is due to these phenolic or tannin-like substances.

McKee (1955) attributes the inhibition of *Fusarium caeruleum* (Lib.) Sacc. and *F. avenaceum* (Fr.) Sacc. in sap from wounded tissue to an increased concentration of solanine occurring in wounded tissue after 6 days.

Numerous infections of potatoes with *Phytophthora* occur around harvest time, when soil is wet and contains a high proportion of infective spores and where wounds have been inflicted during lifting. It therefore seemed worthwhile to investigate quantitatively the relationship between wound periderm formation, the probability of infection, and the clinical reaction produced by the tuber in response to those hyphae which do succeed in penetrating into the tissue.

II. Materials and Methods

Sebago variety of tubers was used throughout these experiments; this variety is susceptible to all known *Phytophthora* strains. The *Phytophthora* strain used was of B group and was very virulent on Sebago tubers (belated necrosis and abundant fructification seen on inoculated faces).

Dishes lined with moist blotting-paper and covered with a glass sheet were used as containers. The tubers were cut longitudinally on different days in order to have a variety of time intervals between cutting and inoculation. These were placed in the containers, with the cut surface upwards, kept at 20°C, and, finally, all were inoculated at the same time with the one inoculum preparation.

The following tests and observations were made:

(i) The suberization of the cut surface, and the appearance of cell divisions in the adjacent layers.

(ii) The probability of individual spores being able to penetrate through the changing surface layer.

(iii) The reaction of tuber parenchyma to the invading hyphae of the parasite.

Methods used were as follows:

In (i) the tissues adjacent to the healing face were hand-sectioned, and the sections examined microscopically for suberization and cell division in surface layers. The conventional stains were used to assist observation.

In (ii) the tubers were left for varying times after cutting, then slices 1 mm thick were taken from the cut surface of each. From these slices disks 10 mm in diameter were cut with a cork borer, and placed at random on wet filter paper in dishes, with the healing surface upward. Each dish contained 56 disks, random samples taken from the slices which had been cut from healing surfaces after intervals of 1, 3, 6, and 10 days. Zoospore suspensions of different densities were sprayed on these disks, the dishes being rotated during spraying to ensure equal distribution of the spores. After 24 hr the disks were inverted to allow for easier exit of fructifications. After a further 4 days the diseased and non-diseased slices were counted and the results obtained analysed statistically.

In (iii) filter-paper disks, 5 mm in diameter, were soaked in a dense zoospore suspension and placed on the cut surfaces, five to each half tuber. The effect was recorded in terms of necrotic changes on the inoculated face and the aerial mycelium produced thereon by the parasite.

III. Results

Microscopical examination of the cut surfaces showed that suberin formation had started earlier than 24 hr after cutting the tubers. The first cell divisions, however, were observed much later, the earliest appearing after 2 days. During the following 2–3 days, the number of cell divisions increased sharply so that a nearly continuous layer of divided cells had been formed by the end of this time. These observations are in good conformity with those of other workers (Appel 1906; Artschwager 1927; Smith and Smart 1955).

The response to infection of the tissues adjacent to the cut surface change markedly with increasing time after cutting. This can be seen in Table 1. The tubers inoculated immediately after cutting showed no definite necrotic changes at points of inoculation; the majority of cells, in this inoculated area, remained turgid for 6 days, and abundant fructification had occurred. The tubers, which were inoculated after 1 day's interval between cutting and inoculating, showed a marked browning on the inoculated face and the fructification was much less than in the case of the freshly cut tuber examined 6 days after inoculation.

It can also be seen from Table 1 that the greater the interval between cutting and inoculating, the more striking was the difference in the response of the tissues to the parasite. Accordingly, in the tubers which had had an interval of 5 days between cutting and inoculating, only some necrotic spots were seen at the point of inoculation and no fructification was produced. These results are consistent with those obtained by Meyer (1939).

Within the tubers also, certain differences were observed between those inoculated immediately after cutting and those which had been subjected to an interval between cutting and inoculation. In the former no necrotic changes were seen in the tissue; where an interval had occurred, however, the tissues showed browning until in the case where 5 days had elapsed before inoculation, brown traces with sharply bordered edges were seen in tissue near the inoculated face. Thus there was reason to assume that even the tissue which lies at a distance from these healing

layers is also to some extent, and in some way, affected by those changes taking place at the cut surface.

Accordingly a further test was made by quartering some of the non-inoculated half tubers already used, and uniformly inoculating those freshly cut surfaces which lie at right angles to the original healing surfaces. No marked effect was seen on the freshly cut surface, except for a slight reduction of fructification in the layers

TABLE 1

INTERACTION BETWEEN HOST TISSUE AND PATHOGEN AFTER INCREASING INTERVALS BETWEEN CUTTING AND INOCULATION OF THE TUBERS

Time after Cutting (days)	Necrotic Changes of Host Tissue after 6 Days		Fructification of the Fungus after 6 Days
	At Cut Surface	Below Cut Surface	
0	None	None	Abundant
1	Diffuse brown discoloration in and around inoculated areas	Diffuse brown discoloration	Scattered; aerial mycelium rather poor
2	Necrotic patches more or less sharply defined	Not examined	Similar, but markedly less than before
3	Necrotic patches to necrotic spots	Not examined	Very little to none
4	Necrotic spots only	Diffuse necrotic traces near the inoculated face	None
5	Fewer necrotic spots	Sharply defined necrotic traces	None

closely adjacent to the wound periderm tissue. This negative result suggests two possibilities, either (1) that the pathogen, in passing through the region of developing wound periderm, undergoes some change which influences the interaction between host and pathogen, so that necrosis occurs much earlier in the adjacent tissue; or (2) that after a fresh surface is cut, the conditions within the tissues concerned are altered and the original equilibrium between host and pathogen is restored. At present no decision between these alternatives is possible.

Tubers which had been inoculated 5 days after cutting showed only a few necrotic changes in the inoculated area. This indicated that only a very small proportion of the spores placed on the cut surface had been able to penetrate into the tuber parenchyma, and the majority of these had not established an infection. We may conclude, therefore, that with increasing time after cutting the probability

(P_i) of an individual spore being able to penetrate the healing surface decreases considerably. This was proved by experiments carried out as follows:

Three separate experiments were set up, using five or six dishes in each case, with disks from the differently "treated" tubers distributed at random among the dishes. These were divided into three lots and inoculated with "light", "medium", and "heavy" doses of the pathogen. As Table 2 shows, the number of successful spores decreases markedly with the time which elapses after cutting and before inoculation. After 10 days, although a heavy spore load was applied, only a small proportion were successful in penetrating.

TABLE 2

PERCENTAGE OF INFECTION ON TUBER DISKS EXCISED FROM THE CUT SURFACES AT DIFFERENT INTERVALS AFTER CUTTING

Spore Load	Days after Cutting				
	0	1	3	6	10
Light	90·5	84·0	38·9		
Medium	100·0	100·0	50·8	16·1	
Heavy			60·4	32·4	13·5

In order to have a quantitative measure of the probability of a single spore becoming infective, a statistical analysis of the data was made. It showed variation between the replicates in excess of the errors of chance. Nevertheless, the trend to progressively lower infection with increase in period after cutting is quite significant with all the intensities of spraying. An estimate of the relative likelihood of infection is given by $\log_e p$, where p is the proportion of tuber slices which did not show infection. Through the three statistical estimates of the experimental values, the three intensities of spraying at 3 days and the two estimates at 6 days, combined estimates over all dosages were determined for the relative probabilities of a spore causing infection. The value at 3 days was arbitrarily taken as 1·00. Relative values are tabulated as follows:

Days after cutting and before inoculation	0	1	3	6	10
Relative probability of spore causing infection	4·76	3·71	1·00	0·36	0·14

From these results it is seen that inoculation at zero interval after cutting is *c.* five times as effective as inoculation 3 days after cutting, and more than 30 times as effective as inoculation 10 days after cutting.

The actual differences must necessarily be greater than the above for the following reasons: in preparing and spraying the disks, freshly cut surfaces are exposed to the pathogen and these also would become infected, obviously affecting the final values given above. The 0·14 value at 10 days could be only the result of

these freshly cut surfaces and if this is subtracted from each value, then the relative differences of probability become much greater than shown in the above tabulation.

In the graph shown in Figure 1 these values and observations on wound periderm formation and the interaction between host and parasite are plotted against each other. From this it can be seen that a marked reduction of P_i occurs before the first cell divisions begin; the same is true for the intensity of fructification of the pathogen on the cut surface. On the other hand, there is a marked increase in the rapidity of appearance of the first necrotic changes in response to an infection. This indicates that the cell division itself in wound tissue is not the only, and perhaps not even the essential, factor in the protective mechanisms produced after mechanical injury. The suberization of these outer cells, however, coincides with the first changes inhibitory to the pathogen.

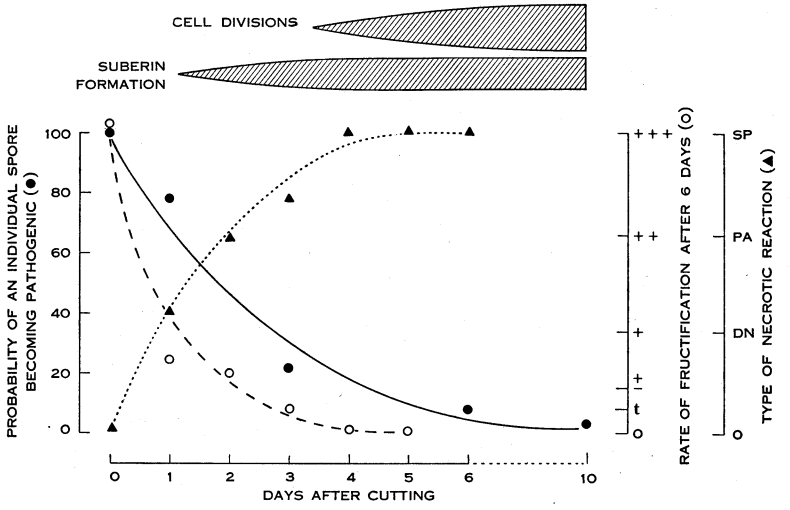

Fig. 1.—Diagram illustrating the time relationship of wound periderm formation, reaction of the wounded tissue to infection, fructification of the pathogen, and the probability of an individual spore becoming pathogenic. Onset and intensity of cell division and suberin formation are indicated by the hatched diagrams above the graph. 0, no necrotic change; *DN*, diffusing necrosis; *PA*, necrotic patches; *SP*, necrotic spots (clearly defined); +++, abundant fructification; *t*, traces of fructification; −, no fructification—other symbols on this axis are intermediates between the extremes.

IV. Discussion

It has been found that there is a gradual increase in protection against pathogenic infection in the tuber tissues which are changing to form wound periderm. This protective effect increases with time, the first marked changes being seen after 24 hr. This result is in good accord with those obtained by previous authors working with bacterial diseases.

The most interesting outcome of this work is that the protective action of healing tissue is made manifest in two ways: firstly, in a change in the reaction of the host tissues, adjacent to the wounded surface, towards infection by the invading

pathogen, and secondly in that the proportion of spores able to establish an infection decreases as the time interval between injury and inoculation increases.

Meyer (1939) suggested that the decrease of the activity in the growth of the pathogen was due to an accumulation of phenolic substances in those cells which are changing into wound periderm. McKee (1955), so far as *Fusarium* infections are concerned, suggests that the presence of solanine, which he found to be present in greatly increased amounts in tissues below the cut surface, is the cause of the inhibition of the pathogen. But there is a striking correlation between the rate of necrotic change of the infected tissue and the decrease in growth activity of the pathogen. This means that the greater the necrotic disintegration of the host cell, in response to the infection, the greater is the inhibiting effect on the pathogen. This would indicate that within the tissue affected by the mechanical injury, an increase of sensitivity and with this an increase of reactive resistance towards the pathogen takes place. The simplest explanation of this sensitizing effect may be the assumption that in the actual healing process a toxic principle is produced which is the precursor of, or even identical with, that factor which retards the pathogen, and which at the same time causes the disintegration of the host cell. Whether this hypothesis is in conformity with the truth must be left for further research to decide.

As far as the reduction of the number of spores able to establish an infection is concerned, there is good reason to assume that here the protective effect is a completely passive one (mechanical or chemical or both). In the early stages of healing there will be gaps between the groups of dividing cells forming wound periderm; with the passage of time these gaps become fewer and smaller until finally the periderm tissue is continuous. Therefore, the chance of penetration by the pathogen automatically becomes less as the time between injury and contamination increases. Here then the whole question would be reduced to a statistical problem, namely the combined effect of both the number of infective units, each of which is a possible initiator of disease, and the total area of sites available for infection, on the clinical result of contamination of a susceptible host with a pathogen. The question, however, whether the actual protective mechanism is solely a mechanical one (suberization of the outer walls of wound periderm?) or a chemical one which locally prevents, by virtue of its toxicity, penetration of the parasite into the host tissue, is again a problem which must be left for further research to decide.

The same principle must be taken into account when we consider the probability of an infection being established under field conditions during harvest time. It is self-evident that both the amount of viable individuals of the pathogen in the soil and the speed with which wound periderm formation proceeds, co-determine the incidence of tuber blight due to wound infections which take place after the tubers have been lifted. The greater the latter the greater is the quantity of spores necessary to ensure that an infection becomes established. But no direct proportionality between these two factors can be expected. According to our results, the relative importance of the number of spores per unit volume of soils depends largely on the speed with which wound periderm is formed. In the late autumn, wound periderm formation proceeds rather slowly and therefore at that time relatively great differences of spore load are somewhat insignificant unless the density of the pathogen in the soil is

comparatively low. On the other hand, in the early autumn when temperatures are higher and wound periderm develops relatively quickly differences of spore load even at high levels may have a great influence on the incidence of tuber blight due to wound infection. Under these circumstances reduction of the pathogen's population can have a marked effect on the incidence of blight infections established after the tubers have been lifted. This may be one of the reasons why the preventative effect of premature killing of foliage and stalks by spraying the crop with toxic chemicals, for instance sulphuric acid, in order to stop spore production, varies so much with date of spraying.

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