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THE ALCOHOL-SOLUBLE AND ALCOHOL-INSOLUBLE NITROGEN FRACTIONS OF POTATO-TUBER TISSUE AFTER INFECTION WITH GIBBERELLA SAUBINETII (MONT.) SACC.

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ABSTRACT

The respiratory increase observed in potato-tuber tissue after infection with *Gibberella saubinetii* might be related to the synthesis of proteins in the host. Therefore the alcohol-soluble and -insoluble N-fractions have been determined in non-infected and infected tuber halves in samples cut at various distances from the surface. Samples were cut starting from the surface. Consequently the samples at 0–0.5 mm from the surface contained mycelium of the invading parasite; the other samples were mycelium-free.

In the samples at 0–0.5 mm from the surface of the infected halves, and to a lesser extent in the corresponding samples of the non-infected halves the alcohol-insoluble N increased while the alcohol-soluble N decreased. The total N-content was unchanged. Apart from a wound-reaction which may have occurred during the incubation period due to the cutting of the tubers into two halves before inoculation of one half, it is most likely that the shift in the ratio insoluble N/soluble N of the samples at 0–0.5 mm from the infected surface is the consequence of the presence of mycelium in the tissue disks.

As no change in soluble N and insoluble N could be demonstrated in the samples cut at 0.5 mm or more from the surface, no evidence has been obtained for a parallel behaviour of the respiration rate and the synthesis of insoluble nitrogenous compounds in the host tissue after infection. The possibility that synthesis and breakdown of proteins occurred simultaneously without any change in the total amount of insoluble N is discussed.

INTRODUCTION

Metabolic changes that occur in higher plants infected by micro-organisms have been a subject of investigation for many years. As a general feature an increase in respiratory activity was demonstrated in several host-parasite combinations, although only a few investigators succeeded in separating the pathogen and the parasitically affected host tissue (AKAZAWA and URITANI, 1956; AKAZAWA, 1956a, b).

As already reported in a previous paper (VERLEUR, 1960), *Gibberella saubinetii* growing on potato-tuber tissue penetrates only the outer cell layers and slowly spreads inward with simultaneous collapse of the superficial cells. Hence, separation of the pathogen and the affected tissue proved highly feasible. In samples of mycelium-free tissue from the area adjacent to the parts invaded by the fungus, the respiratory activity appeared to be greatly enhanced. Experiments with 2,4-dinitrophenol (DNP) indicated that the respiration rate of healthy tuber tissue was limited by the rate of oxidative phosphorylation. Uncoupling of oxidation and phosphorylation as a factor in the

increase of respiratory activity after infection proved to be unlikely, as parallel to the acceleration of the respiration rate an increase of organic, not-easily hydrolyzable phosphate compounds and an enhanced incorporation of P^{32} from $Na_2HP^{32}O_4$ into the "nucleotide" fraction could be observed. The latter findings suggested especially that the respiratory increase in infected potato-tuber tissue is caused by the activation of metabolic processes that utilize high-energy phosphate (ATP) leading to an increased phosphate-acceptor regeneration.

After investigations on the mechanism of disease-resistance and on the mechanism of respiratory increase following fungus-infection several investigators came to suggest a relation between these phenomena and the nitrogen metabolism and protein content of the host. A breakdown of proteins would make the leaves of the potato plant more susceptible to *Phytophthora infestans* (GRÜMMER, 1955). On the other hand, TOMIYAMA et al. (1955) suggest that resistance of the potato plant to this fungus is accompanied by a stimulation of protein synthesis in this plant. However, the results as shown in their tables, do not lend support to their conclusion, as there appears to be hardly any difference between the infected samples and the non-infected controls.

Infection of both sweet potato and white potato with *Ceratocystis fimbriata* appeared to give rise to an increase of the acid-insoluble nitrogen and a concomitant decrease of the acid-soluble nitrogen in the tissue adjacent to the infected parts of the tubers (AKAZAWA and URITANI, 1956; AKAZAWA, 1956a). KASAI, YAMAZAKI and SUZUKI (1957), too, report an increase of the insoluble nitrogen and a decrease of the soluble nitrogen in sweet potatoes after infection with *Helicobasidium mompa.*

The experiments described in this paper aim at answering the question whether infection of potato-tuber tissue with *Gibberella saubinetii* induces a shift in the alcohol-soluble and the alcohol-insoluble nitrogen fractions of the host.

MATERIAL AND METHODS

Sound potato tubers (*Solanum tuberosum L.* var. Bintje) were externally sterilized and then cut into two halves, one of which was inoculated with mycelium of *Gibberella saubinetii.* Determination of the two nitrogen fractions was carried out after 1–2 or 6–7 days incubation in sterile glass jars kept at 25° C.

Using the method previously described (VERLEUR, 1960), disks measuring 6 mm in diameter and 0.5 mm in height were cut from the tuber halves. From each tuber half the disks cut at equal distance from the surface [1]) were put together as tissue samples (10 disks per sample), and weighed (fresh weight).

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[1]) The surface of the cut by which the tuber was divided into two halves si called 'the surface'.

The alcohol-soluble nitrogen fraction was obtained by extracting the separate samples of 10 disks with 10 ml ethanol 80 % during the night at room temperature. The extracted disks were washed with ethanol several times, the ethanol added to the extract, and the extract made up to 25 ml.

The extracted disks contained the alcohol-insoluble nitrogen fraction. After the disks had been dried at 25°C overnight, the samples were weighed. This weight is assumed to be the dry weight of the samples.

The nitrogen content of both the extracts and the dried disks was determined using the micro-Kjeldahl method: destruction in H_2SO_4 98 % using Selenium as a catalist (100 mg Se-dioxide, 75 mg $CuSO_4 \cdot 5H_2O$ and 4750 mg anhydric Na_2SO_4 dissolved in H_2SO_4 98 % up to 40 ml). In the case of the extracts two drops of conc. H_2SO_4 were added to 5 ml extract in a Kjeldahl-flask after which the mixture was almost completely evaporated by boiling. Then the conc. H_2SO_4 and the Selenium-mixture were added for the destruction. The dried extracted disks could be destructed without further treatment.

After complete destruction the amount of nitrogen was determined colorimetrically using Nessler's reagent (containing 15 % NaOH) and measuring the intensity of the developed colour at 480 mμ in a Unicam spectrophotometer.

The amount of nitrogen was calculated in μg nitrogen per 100 mg fresh weight and per 10 mg dry weight. The total amount of nitrogen was obtained by adding the amounts of soluble and insoluble nitrogen of each sample. Preliminary experiments showed the maximum error of the N-determinations to be about 5 percent.

Experiments

Although the present author intended to study the behaviour of the host tissue itself after infection, it seemed of interest to include in the experiments the analysis of the tissue that had been invaded by the fungus. Therefore, the mycelium-containing discoloured parts from the tissue cylinders bored out of the infected tuber halves were not discarded, but they, too, were cut into disks and put together in tissue samples. Consequently, the series of samples from each tuber half start from the surface. The samples at 0–0.5 mm from the surface of the infected tuber halves covered nearly all the mycelium-containing tissue; in a few experiments very small parts of the disks at 0.5–1 mm from the surface still consisted of discoloured tissue.

In 7 out of the 13 experiments the dry weights were determined in addition to the fresh weights of the samples. The tubers of all experiments gave the same picture after infection. However, the individual tubers appeared to have rather different levels of nitrogen content: the total N-content ranged between about 200 μg N per 100 mg fresh weight in the tuber with the lowest content and about 375 μg N per 100 mg fresh weight in the tuber with the highest N-content. On a dry weight basis the levels ranged between about

TABLE 1

The alcohol-soluble and -insoluble nitrogen fractions of the samples cut at various distances from the surface of the infected halves and the non-infected halves of potato tubers, calculated in μg N per 100 mg fresh weight. The values are averages of 13 experiments.

Distance from surface	Infected tuber halves				Non-infected tuber halves			
	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N
mm	μg	μg		μg	μg	μg		μg
0 – ½	105	342	3.3	447	129	190	1.5	319
½–1	175	135	0.8	310	174	135	0.8	309
1 –1½	151	142	0.9	293	172	134	0.8	306
1½–2	173	128	0.7	301	168	134	0.8	302
2 –2½	154	132	0.9	286	181	139	0.8	320
2½–3	158	123	0.8	281	173	139	0.8	312
3 –3½	157	127	0.8	284	175	144	0.8	319
3½–4	165	125	0.8	290	—	—	—	—
4 –4½	161	127	0.8	288	—	—	—	—
4½–5	160	124	0.8	284	—	—	—	—
6 –6½	—	—	—	—	176	132	0.8	308
8 –8½	173	124	0.7	297	—	—	—	—
Average ½–8½	163	129	0.8	292	174	137	0.8	311

TABLE 2

The alcohol-soluble and -insoluble nitrogen fractions of the samples cut at various distances from the surface of the infected halves and the non-infected halves of potato tubers, calculated in μg N per 10 mg dry weight. The values are averages of 7 experiments.

Distance from surface mm	Infected tuber halves				Non-infected tuber halves			
	Alcohol-soluble N μg	Alcohol-insoluble N μg	Insoluble N / Soluble N	Total N μg	Alcohol-soluble N μg	Alcohol-insoluble N μg	Insoluble N / Soluble N	Total N μg
0 – ½	39	125	3.2	164	72	109	1.5	181
½–1	104	79	0.8	183	98	85	0.9	183
1 –1½	104	77	0.8	181	101	78	0.8	179
1½–2	118	79	0.7	197	97	73	0.8	170
2 –2½	96	77	0.8	173	100	71	0.7	171
2½–3	106	81	0.8	187	95	73	0.8	168
3 –3½	104	76	0.7	180	—	—	—	—
3½–4	99	73	0.7	172	—	—	—	—
4 –4½	96	72	0.8	168	—	—	—	—
4½–5	96	70	0.7	166	—	—	—	—
6 –6½	—	—	—	—	101	70	0.7	171
8 –8½	97	66	0.7	163	—	—	—	—
Average ½–8½	102	75	0.7	177	99	75	0.8	174

100–275 μg N per 10 mg dry weight. Therefore, average values are given in the tables.

Within each tuber the nitrogen proved to be evenly distributed, the N-determinations in parallel samples from the same healthy tuber yielding N-values with a 10–15 percent variation.

For each zone of the infected tuber halves and the corresponding non-infected halves the Tables 1 and 2 show the average values found for the two nitrogen fractions as calculated in μg N both per 100 mg fresh weight (average of 13 experiments) and per 10 mg dry weight (average of 7 experiments), as well as the ratios alcohol-insoluble N/alcohol-soluble N for each zone.

Obviously neither in the infected halves nor in the non-infected halves a shift in the ratio insoluble N/soluble N can be demonstrated, except in the samples at 0–0.5 mm from the surface. In the non-infected halves the shift in the superficial tissue is apparently the result of the cutting of the tubers into two halves.

On an average the amounts of nitrogen in the tissue at more than 0.5 mm from the surface of the infected and the non-infected halves prove to be the same. This illustrates the equal distribution of the nitrogen compounds in the central parts of the tubers, but at the same time indicates that infection did not affect the amounts of nitrogen in the fractions at more than 0.5 mm from the surface.

Whereas the amounts of soluble and of insoluble N did not change at a greater distance from the surface (Fig. 1), a decrease of the soluble N and a concomitant increase of the insoluble N was found in the samples at 0–0.5 mm from the surface. When calculated per 10 mg dry weight (Fig. 1 C, D) this decrease of soluble N was compensated by the increase of insoluble N resulting in the same level of total N as in the other samples of the tuber halves. The same holds for the non-infected halves when calculating the N-values per 100 mg fresh weight (Fig. 1 B). However, the amount of total N per 100 mg fresh weight in the samples at 0–0.5 mm from the surface of the infected tuber halves (Fig. 1 A) proved to be much higher than the other samples owing to a much sharper increase of the insoluble N-fraction as compared with the decrease of the soluble N-fraction.

It will be clear that the two ways of calculation do not lead to the same picture as far as the tissue near the surface of the infected tuber halves is concerned. Hence, in order to obtain reliable conclusions, it is necessary to reason out which is the picture of what really happened.

A higher total N content in the superficial cell layers might be the result of an accumulation of nitrogenous substances in this zone. This, however, implies a corresponding decrease of the total N content in the adjacent tissue, but it will be easily seen from the data in the Tables 1 and 2 that the facts do not lend support to such an assumption.

It is already stated that the penetration of *Gibberella saubinetii* into the tissue causes a collapse of the superficial cells. Microscopic observations show a number of shrunken cells just below the surface.

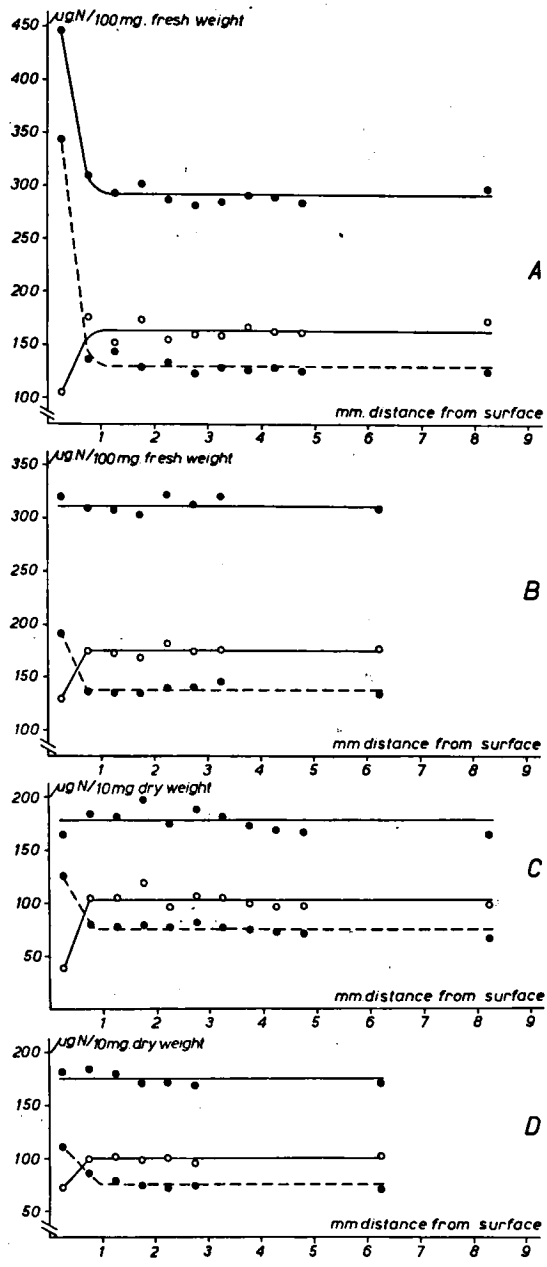

Fig. 1. The alcohol-soluble and -insoluble nitrogen fractions of potato-tuber tissue as influenced by infection. The N-values of the samples cut at various distances from the surface of the infected halves (A, C) and the non-infected halves (B, D) of the tubers, calculated in µg N/100 mg fresh weight are averages of 13 experiments, those calculated in µg N/10 mg dry weight are averages of 7 experiments.

○——○ alcohol-soluble nitrogen

●- - - -● alcohol-insoluble nitrogen

●——● total nitrogen (soluble + insoluble N)

Therefore, it may be expected that disks cut from this area contain more cell layers than do disks of equal height cut at greater distances from the surface.

The higher total N content per 100 mg fresh weight of the samples at 0–0.5 mm from the surface of infected tuber halves seems to be the result of the greater number of cells in these samples, the more so as the total N content per 10 mg dry weight does not show any sign of an increase.

By calculating the dry weights as percentages of the fresh weights it might be possible to obtain a measure for the number of cells in the samples. The 7 experiments in which dry weights have been determined yielded an average dry weight of 27 percent (ranged between 17 % and 36 %) for the zones at 0–0.5 mm from the surface of the infected halves against an average of 17 percent (ranged between 10 % and 24 %) for the other zones. These figures suggest that the superficial disks on an average contain 1.6 times the number of cells of the deeper ones.

As the conclusion may be drawn that the calculation per unit of dry weight produced the most real picture of the behaviour of the N-fractions after infection, only the values per 10 mg dry weight will be considered in the following pages.

1. *The effect of the length of the period of incubation*

The 7 experiments in which dry weights of the samples have been determined can be divided into two groups, *viz.* a group of 4 experiments in which the tuber halves have been incubated at 25° C during 1–2 days, and a group of 3 experiments with an incubation period of 6–7 days. The average N-values for each zone of tissue are given in the Tables 3 and 4.

The general picture here is similar to that described above from the data of Table 2. In the infected and in the non-infected tuber halves no change in the soluble N and the insoluble N could be demonstrated except in the samples cut at 0–0.5 mm from the surface. The increase of the insoluble N content and the decrease of the soluble N content are of the same order and do not lead to a change in the total N content.

The shift in the ratio insoluble N/soluble N in the samples at 0–0.5 mm from the surface of the infected tuber halves as well as of the non-infected controls appears to be greater after 6–7 days of incubation than after an incubation period of only 1–2 days.

2. *The effect of the season*

The 7 experiments of the previous section can also be divided into two groups according to the time of the year in which they have been undertaken. Four experiments with "old" tubers took place in spring (April-May) and 3 experiments were carried out during the next autumn (September-October) with tubers of the new crop. The water content of the tubers in spring amounted to about 80 percent

TABLE 3

The alcohol-soluble and -insoluble nitrogen fractions of the samples cut at various distances from the surface of the infected halves and the non-infected halves of potato tubers, calculated in μg N per 10 mg dry weight. The values are averages of 4 experiments with tubers incubated after infection during 1–2 days at 25° C.

Distance from surface	Infected tuber halves				Non-infected tuber haives			
	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N
mm	μg	μg		μg	μg	μg		μg
0 – ½	52	126	2.4	178	86	109	1.3	195
½–1	107	94	0.9	201	104	84	0.8	188
1 –1½	122	91	0.7	213	109	79	0.7	188
1½–2	118	91	0.8	209	109	77	0.7	186
2 –2½	110	90	0.8	200	109	74	0.7	183
2½–3	119	93	0.8	212	108	79	0.7	187
3 –3½	116	84	0.7	200	—	—	—	—
3½–4	111	81	0.7	192	—	—	—	—
4 –4½	105	81	0.8	186	—	—	—	—
4½–5	100	80	0.8	180	—	—	—	—
6 –6½	—	—	—	—	110	70	0.6	180
8 –8½	101	70	0.7	171	—	—	—	—
Average ½–8½	111	85	0.8	196	108	77	0.7	185

TABLE 4

The alcohol-soluble and -insoluble nitrogen fractions of the samples cut at various distances from the surface of the infected halves and the non-infected halves of potato tubers, calculated in μg N per 10 mg dry weight. The values are averages of 3 experiments with tubers incubated after infection during 6–7 days at 25° C.

Distance from surface	Infected tuber halves				Non-infected tuber halves			
	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N
mm	μg	μg		μg	μg	μg		μg
0 – ½	22	125	5.7	147	53	110	2.1	163
½–1	84	58	0.7	142	91	85	0.9	176
1 –1½	79	59	0.7	138	91	76	0.8	167
1½–2	85	64	0.8	149	81	69	0.9	150
2 –2½	78	60	0.8	138	88	68	0.8	156
2½–3	90	65	0.7	155	79	65	0.8	144
3 –3½	87	65	0.7	152	—	—	—	—
3½–4	82	64	0.8	146	—	—	—	—
4 –4½	83	59	0.7	142	—	—	—	—
4½–5	90	58	0.6	148	—	—	—	—
6 –6½	—	—	—	—	90	69	0.8	159
8 –8½	81	57	0.7	138	—	—	—	—
Average ½–8½	84	61	0.7	145	87	72	0.8	159

TABLE 5

The alcohol-soluble and -insoluble nitrogen fractions of the samples cut at various distances from the surface of the infected halves and the non-infected halves of potato tubers, calculated in μg N per 10 mg dry weight. The values are averages of 4 experiments carried out in spring (April–May).

Distance from surface	Infected tuber halves				Non-infected tuber halves			
	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N
mm	μg	μg		μg	μg	μg		μg
0 – ½	31	100	3.2	131	44	92	2.1	136
½–1	53	69	1.3	122	79	76	1.0	155
1 –1½	77	67	0.9	144	77	69	0.9	146
1½–2	76	67	0.9	143	73	65	0.9	138
2 –2½	73	67	0.9	140	74	60	0.8	134
2½–3	81	69	0.9	150	71	61	0.9	132
3 –3½	70	63	0.9	133	—	—	—	—
3½–4	70	66	0.9	136	—	—	—	—
4 –4½	73	63	0.9	136	—	—	—	—
4½–5	75	61	0.8	136	—	—	—	—
6 –6½	—	—	—	—	71	61	0.9	132
8 –8½	77	61	0.8	138	—	—	—	—
Average ½–8½	73	65	0.9	138	74	65	0.9	139

TABLE 6

The alcohol-soluble and -insoluble nitrogen fractions of the samples cut at various distances from the surface of the infected halves and the non-infected halves of potato tubers, calculated in μg N per 10 mg dry weight. The values are averages of 3 experiments carried out in September–October.

Distance from surface	Infected tuber halves				Non-infected tuber halves			
	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N	Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N
mm	μg	μg		μg	μg	μg		μg
0 – ½	50	159	3.2	209	109	132	1.2	241
½–1	142	91	0.6	233	124	95	0.8	219
1 –1½	139	90	0.6	229	133	90	0.7	223
1½–2	141	96	0.7	237	130	85	0.7	215
2 –2½	128	91	0.7	219	136	87	0.6	223
2½–3	139	98	0.7	237	128	90	0.7	218
3 –3½	149	92	0.6	241	—	—	—	—
3½–4	136	83	0.6	219	—	—	—	—
4 –4½	129	83	0.6	212	—	—	—	—
4½–5	123	83	0.7	206	—	—	—	—
6 –6½	—	—	—	—	142	81	0.6	223
8 –8½	113	69	0.6	182	—	—	—	—
Average ½–8½	134	88	0.7	222	132	88	0.7	220

whereas the tubers of the new crop contained about 90 percent of water.

It appears from the Tables 5 and 6 that both groups of experiments yielded a picture similar to that of the previous sections, irrespective of the season. The only difference is that in the experiments during spring the ratio insoluble N/soluble N seems to change already in the samples at 0.5–1 mm from the surface. However, as this shift is chiefly caused by a slight decrease of the soluble N-fraction while the insoluble N-fraction was unchanged, it is difficult to draw any conclusion.

3. *The nitrogen fractions of the mycelium*

From the previous sections it will be clear that a shift in the ratio insoluble N/soluble N is almost limited to the samples cut at 0–0.5 mm from the surface. Although this could also be observed in the non-infected tuber halves, the shift always appeared to be greater in the infected halves.

As the growth of the mycelium in the tuber tissue is limited to an area just below the surface of the infected tuber halves, it seems reasonable to suppose that the chemical composition of the mycelium in the tissue of these samples will affect the analytical results of the samples as a whole.

For an exact interpretation of the results, therefore, it was necessary to determine the N-fractions and the ratio insoluble N/soluble N of the mycelium. Now, it is impossible to separate the mycelium from the cells in which it is growing, but, fortunately besides invading the cells the fungus also forms aerial mycelium, which could be easily harvested from the tuber halves. However, although the mycelium grown on potato tuber halves may be expected to resemble best the mycelium in the tissue samples, an influence of the culture-medium on the chemical composition of the mycelium may not be excluded without any further experiment. Therefore, mycelium grown in

TABLE 7

The alcohol-soluble and -insoluble nitrogen fractions of the mycelium of *Gibberella saubinetii* grown on agar media and on potato-tuber tissue, calculated in μg N per 10 mg dry weight.

Mycelium inoculated		Alcohol-soluble N	Alcohol-insoluble N	Insoluble N / Soluble N	Total N	Number of experiments
from	to	μg	μg		μg	
potato agar	potato agar	106	469	4.4	575	3
potato agar	malt agar	115	539	4.7	654	3
malt agar	potato agar	108	454	4.2	562	3
malt agar	malt agar	89	498	5.6	587	3
potato agar	potato tuber	131	598	4.6	729	2
malt agar	potato tuber	128	616	4.8	744	2

culture tubes both on potato agar and on malt agar was inoculated on (1) potato-tuber halves, and in petri dishes on plates of (2) potato agar or (3) malt agar, incubated at 25°C during one week, harvested and analysed using the method described above. The results are summarized in Table 7.

The nature of the medium on which the mycelium had been grown before and during the experiments appears to have hardly any effect on the analytical results. The ratios insoluble N/soluble N of nearly all mycelial samples from the agar media agree very well with those of the samples from potato-tuber tissue. Only two points are worth mentioning: (1) there is some indication that mycelium grown on tuber tissue has a higher total N content than mycelium grown on agar media; (2) whereas the ratios insoluble N/soluble N in unaffected potato-tuber tissue are less than unity, the ratios for the mycelium have an average value of about 4.7.

Discussion

Apart from some quantitative differences due to the time of the year in which the experiments were undertaken and as a consequence of differences in the period of incubation after infection, all experiments show the same general picture, *viz.* a decrease of the soluble N and a concomitant increase of the insoluble N in the samples at 0–0.5 mm from the surface, but no changes at greater distances from the surface.

Now, one should bear in mind that it is exactly the zone of tissue including the mycelium of the parasite which appeared to have a high ratio insoluble N/soluble N. Therefore, the increase of this ratio in the superficial tissue only, might be merely caused by the presence of the mycelium with its high ratio.

Moreover, the shift found in the superficial tissue of the non-infected halves, apparently as a result of the cutting of the tubers into two halves, should also be taken into consideration, although it is uncertain whether the reaction to cutting will be the same in infected and in non-infected tuber halves.

In trying to explain the shift in the ratio insoluble N/soluble N on the basis of the chemical composition of the invading mycelium, it may be stated that the only sources of nitrogen for the mycelium were the nitrogenous constituents of the potato-tuber tissue, the quantity of total N in the samples at 0–0.5 mm from the surface being equal to that of the other samples of the series. When it is further assumed that the growing mycelium took up its nitrogen from the soluble fraction of the potato tissue, one can calculate the amount of mycelium that should have been present in the samples in order to produce the observed shift of the ratio insoluble N/soluble N.

As shown in Table 2 the samples at more than 0–0.5 mm from the surface give an average value of 0.7 for the ratio insoluble N/soluble N. Supposing that the superficial tissue had the same composition before infection, this tissue would have contained 67.5 μg

insoluble N, 96.5 μg soluble N (ratio 0.7) and 164 μg total N per 10 mg dry weight. After infection, however, the composition proved to be 125 μg insoluble N and 39 μg soluble N (ratio 3.2) per 10 mg dry weight, *i.e.* an increase of 57.5 μg insoluble N.

On the assumption that this increase of insoluble N is merely due to the presence of mycelium, the mycelium in this tissue would have contained 57.5 μg insoluble N. The average content of insoluble N in mycelium grown on potato-tuber tissue appeared to be 607 μg N per 10 mg dry weight (Table 7). So, about 1 mg mycelium (dry weight) would have been present in the samples per 10 mg dry weight, *i.e.* on this basis about 10 percent of the samples consisted of mycelium.

Considering the fact that mycelium appeared to have a dry weight of about 20 percent of the fresh weight, whereas the samples at 0–0.5 mm from the surface had an average dry weight of 27 percent of the fresh weight, the calculated amount of about 1 mg dry mycelium/10 mg dry sample equals about 5 mg fresh mycelium/37 mg fresh sample or about 13.5 mg fresh mycelium/100 mg fresh sample, *i.e.* about 13.5 percent of the fresh samples would have been mycelium.

When the effect of cutting the tubers into two halves, as found in the non-infected halves, is taken into account, the amount of mycelium necessary to explain the situation in the samples at 0–0.5 mm from the surface of the infected halves will be smaller.

From Table 2 it appears that in the uppermost cell layers of the non-infected halves the ratio insoluble N/soluble N increased to a value of 1.5. Supposing that in the superficial tissue of the infected halves the same reaction to cutting has occurred, then this tissue would have contained 164 μg total N per 10 mg dry weight divided into fractions of 98.4 μg insoluble N and 65.6 μg soluble N (ratio 1.5). Analysis after infection, however, showed that in fact 125 μg of the nitrogen was insoluble while 39 μg of the nitrogen was soluble, *i.e.* a further increase of 26.6 μg insoluble N.

On the assumption that, apart from the reaction to cutting, the increase of the insoluble N is due to the presence of mycelium, 26.6 μg insoluble N should be located in the invading mycelium.

As the average insoluble N content of mycelium amounted to 607 μg N per 10 mg dry weight (Table 7), the mycelium-content of the samples would have been about 0.5 mg dry mycelium/10 mg dry sample or about 6.5 mg fresh mycelium/100 mg fresh sample, *i.e.* about 5 percent, resp. 6.5 percent.

Percentages of the same order were obtained for the groups of experiments summarized in the Tables 3–6. As could be expected, the percentage of mycelium necessary to explain the changes in the N-fractions in the experiments with tuber halves incubated 6–7 days (10.5 %) was a little higher as compared with the percentage after 1–2 days incubation (7.5 %). The percentage calculated from the experiments during autumn (12 %) also proved to be higher than that from the experiments carried out in spring (6.5 %).

The calculated percentages for the amount of mycelium present in the samples at 0–0.5 mm from the infected surface make it reason-

able to conclude that apart from the wound-reaction which may have occurred, the composition of the fungal hyphae present in these samples account for the changes of the N-fractions described.

As previously reported (VERLEUR, 1960), dependent on the period of incubation and the severity of the fungal attack, a respiratory increase could be demonstrated in tissue samples up to about 5–10 mm from the infected surface. In the non-infected tuber halves an enhanced respiration rate was found up to 2–3 mm from the surface. Now a shift in the ratio insoluble N/soluble N of the samples from the non-infected halves could only be demonstrated in the samples at 0–0.5 mm from the surface and the same proved to be the case in the infected halves. So, apart from the wound-reaction there is no evidence at all for a parallel behaviour of the respiratory activity and the synthesis of insoluble nitrogenous compounds after infection.

No changes could be found in the soluble and insoluble N of the mycelium-free samples at more than 0.5 mm from the surface of the infected halves. This is in contradiction with the results obtained by AKAZAWA (1956a) in experiments with potato tubers infected by a different parasite, *Ceratocystis fimbriata.*

However, the possibility cannot be excluded that synthesis and breakdown of proteins occurred simultaneously without any change in the amount of N in the insoluble fraction as a whole. In sweet potato certain proteins increase, others decrease after infection with *Ceratocystis fimbriata* (URITANI and STAHMANN, 1961). The soluble protein fraction of potato tubers showed some changes in the elution pattern in column-chromatography after infection with *Phytophthora infestans* (STAHMANN, URITANI and TOMIYAMA, 1960), while OKANENKO and BERSHTEIN (1960) found a different amino acid composition of the protein fraction of potato tubers infected with *Synchytrium endobioticum*, and even found amino acids in the proteins which did not occur in the proteins of non-infected tubers. Differences in the proteins after infection have been demonstrated in experiments with other hosts and parasites by the use of immunochemical and serological methods and electrophoresis (AKAZAWA, UMEMURA and URITANI, 1957; STAHMANN, URITANI and TOMIYAMA, 1960; HEITEFUSS, BUCHANAN-DAVIDSON, STAHMANN and WALKER, 1960).

Although one should be careful in comparing investigations with different host-parasite combinations carried out under different circumstances, it is clear that a rearrangement of proteins and a synthesis of new proteins may take place in infected plants. If these proteins have a key-position in the pattern of metabolic processes and the regulation of metabolic activity, one can imagine that an extra utilization of high-energy phosphate (ATP) and consequently an increase of the respiration rate after infection might be the result. Whether a qualitative change in the composition of the insoluble nitrogenous compounds occurs in potato-tuber tissue after infection with *Gibberella saubinetii* has to be decided by further experiments.

REFERENCES

AKAZAWA, T. 1956a. J. Biochem. Japan **43**: 589.
———. 1956b. J. Biochem. Japan **43**: 597.
———, Y. UMEMURA and I. URITANI. 1957. Bull. Agric. Chem. Soc. Japan **21**: 192.
——— and I. URITANI. 1956. J. Biochem. Japan **43**: 579.
GRÜMMER, G. 1955. Phytopathol. Z. **24**: 1.
HEITEFUSS, R., D. J. BUCHANAN-DAVIDSON, M. A. STAHMANN and J. C. WALKER. 1960. Phytopathology **50**: 198.
KASAI, K., Y. YAMAZAKI and N. SUZUKI. 1957. Bull. Natl. Inst. Agric. Sci. Japan ser. C. **8**: 61.
OKANENKO, A. S. and B. I. BERSHTEIN. 1960. Dokl. Akad. Nauk. SSSR **134**: 727 (Ber. Wiss. Biol. **162**: 24).
STAHMANN, M. A., I. URITANI and K. TOMIYAMA. 1960. Phytopathology **50**: 655.
TOMIYAMA, K., N. TAKASE, R. SAKAI and M. TAKAKUWA. 1955. Ann. Phytopath. Soc. Japan **20**: 59.
URITANI, I. and M. A. STAHMANN. 1961. Plant Physiol. **36**: 770.
VERLEUR, J. D. 1960. Acta Bot. Neerl. **9**: 119.