

EFFECT OF MINERAL NUTRITION OF POTATO PLANTS ON RESPIRATION OF THE TUBERS

BY

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(received June 25th, 1955)

INTRODUCTION

It is a well-known fact that variation in mineral nutrition of plants may affect the chemical composition of the latter. This holds true not only for mineral constituents but also for organic compounds such as proteins, carbohydrates and presumably many others. Although this effect has been taken into account for a long time by those research workers who are concerned with plant and animal nutrition, many workers studying fundamental physiological problems like respiration, protein synthesis etc. employed plant materials of unknown origin. Such plants may have grown under widely differing soil and nutritional conditions.

In the present investigation the effects of nitrogen, phosphorus, potassium, magnesium and copper supply of potato plants on respiration and related physiological processes have been investigated. For this purpose potato plants were grown on experimental fields under carefully controlled nutritional conditions. Since large differences in plant growth and in yield were obtained as a result of the manurial treatments, it may be assumed that the tubers grown on the control plots were highly deficient in the lacking elements.

Relatively little is known about the effect of mineral nutrition of plants on physiological processes. GREGORY and RICHARDS (1929) have studied the effects of nitrogen, phosphorus and potassium nutrition of barley plants on the respiration rate of the leaves. They found lower values in leaves from nitrogen-deficient plants than in those from fully manured plants. In the case of phosphorus deficiency no marked effect on respiration rate was observed but in leaves from potassium-deficient plants considerably higher values were found than in those from normal plants. These results were confirmed as far as potassium and nitrogen were concerned by RICHARDS (1932) and GREGORY and SEN (1937). In a further paper by RICHARDS (1938) a pronounced effect of phosphorus supply on respiration rate was observed. Leaves from phosphorus-deficient plants were found to respire considerably less intensively than those from plants with an adequate P-supply.

Calculated on a protein basis no differences in CO₂-evolution were found between leaves from P-deficient and normal plants.

PETRIE and WILLIAMS (1938) found lower respiration values per unit of dry matter in leaves of Sudan grass deficient in nitrogen than in those from plants dressed with nitrogen. Calculated on a protein basis, however, the N-deficient tissue gave considerably higher values than the normal, apparently due to the fact that part of the protein was inactive in metabolic processes. Leaves from phosphorus-deficient oat plants were found by these authors to have lower respiration rates than those from normal plants. In contrast to the results of RICHARDS (1938) the differences became more pronounced if they were calculated on a protein basis. Apparently this was due to the fact that the fully manured oat plants were deficient in nitrogen.

AMBERGER (1953) found higher respiration rates, calculated on a dry-matter basis, in leaves from potassium-deficient spinach and sugar beet plants than in those dressed with potassium. Similar results were obtained with perennial rye grass. In the alga *Hydrodictyon reticulatum* a considerable increase in oxygen uptake was found in the case of potassium deficiency (NEEB, 1952). Nitrogen-deficient cultures had decreased respiration rates when calculated on a dry-matter basis. Upon transference to complete nutrient solutions the respiration rate of K-deficient algae decreased considerably within a few hours whereas that of N-deficient cultures rose. PIRSON and SEIDEL (1950) observed an increased respiration rate in potassium-deficient roots of *Lemna minor*.

MATERIAL AND METHODS

Certified seed potatoes of the varieties Noordeling and Voran were planted on soils poor in nitrogen, phosphorus, potassium, magnesium and copper, respectively. In some cases the soils used were deficient in more than one nutrient element. The potato plants were dressed with different amounts of the element in which the soil was deficient; the other nutrient elements were mostly supplied in optimum amounts. The tubers were harvested and transported carefully to the laboratory where they were stored in shallow layers in wooden boxes at a temperature varying from 5° to 10° C.

For the *respiration* experiments with tubers, bottles of 1 l capacity containing about 250 g tubers were mostly used. Air freed from carbon dioxide was passed through these bottles at the rate of approximately 20 l per h. The CO₂ produced was absorbed in baryta. Temperature 25° C. Before the tubers were transferred to the respiration bottles they were incubated for a few days at this temperature. In a number of experiments, particularly in those in which different gas mixtures were employed, oxygen uptake was measured manometrically by the Warburg technique. For this purpose large vessels of 150 ml capacity in which the potato tubers were placed on a little net over KOH were used.

Many experiments were carried out with tissue disks 1 mm thick, and 1 cm in diameter. These disks were cut by a microtome from cylinders obtained by means of a cork borer. Before use the disks were washed approximately 25 times in tap water or in distilled water. They were transferred as soon as possible after cutting to large Warburg vessels of the type used for the tubers. In contrast to the orthodox methods the tissue disks were not suspended in buffer solution but respired in moist air. The advantage of this procedure is that difficulties caused by inadequate diffusion of oxygen into the buffer solutions do not occur. The same is true of bacterial infections which practically do not take place and if they occur can be readily recognized. A slight loss of water may occur under these experimental conditions.

This does not affect the experimental results as was shown in control experiments. Generally the vessels were kept in water of 25° C in which diffuse light could penetrate. In control experiments no effect of this weak light on respiration could be detected. Only in those cases where a light effect was to be expected (experiments with CO-treated disks) light was completely excluded.

Protein and soluble non-protein nitrogen were determined in the ground tubers as described by MULDER (1949), trichloroacetic acid being used for precipitating the protein.

Tyrosine was determined according to LUGG's method (1937), *o*-dihydric phenols by the method of ARNOW (1937).

Tyrosinase was determined in macerates of tuber tissue by means of the usual Warburg technique. 5 g portions of tuber tissue were ground in a mortar and brought up with distilled water to 100 ml. 0.5-ml and 1-ml portions respectively of this suspension were pipetted in Warburg vessels. For the determination of the mono-phenolase activity the side arms contained 4 mg cresol and 0.05 mg dopa (3, 4-dihydroxy-phenylalanine) in 1 ml 0.06 M phosphate buffer of pH 6.8; for the determination of di-phenolase activity: 4 mg catechol and 8 mg ascorbic acid in 1 ml 0.06 M phosphate buffer, pH 6.8. In some experiments the amount of tyrosine disappearing in an aerated suspension of tuber macerate supplied with tyrosine was determined (MULDER, 1949).

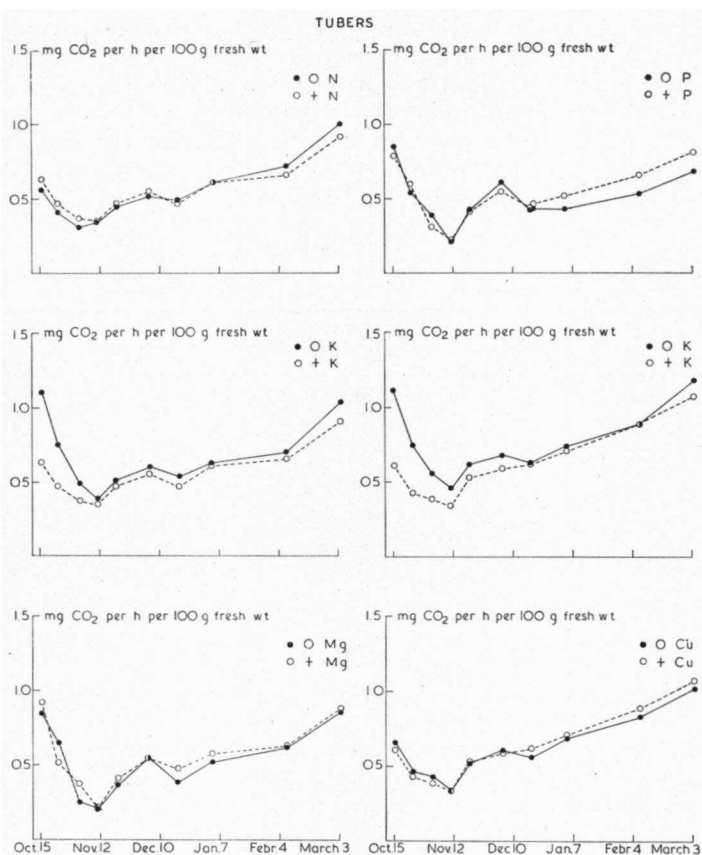


Fig. 1. Effect of mineral nutrition of potato plants on CO₂-output of the tubers.

RESULTS

Fig. 1 gives the respiration rates, calculated as mg CO₂ per hour per 100 g of fresh tissue, of potato tubers deficient in nitrogen, phosphorus, potassium, magnesium and copper, respectively, as compared with tubers grown on the same experimental field in the presence of adequate amounts of these nutrient elements. The experiment started soon after harvesting the potatoes in the beginning of October 1951 and was continued until March 1952. During this period the tubers were left untouched in the vessels.

Although the potatoes used in this experiment came from plants which had shown severe deficiency symptoms (cf. the yield data in Table I), no differences in respiration rate were observed in the case of nitrogen, magnesium and copper. In the case of phosphorus a small difference in respiration rate was found between deficient and normal tubers in the second part of the experiment.

Clear differences were obtained between potatoes with a different potassium supply. K-deficient tubers respired more intensively than tubers with an adequate K-supply. This was particularly clear in the beginning of the experimental period. In the course of this period the differences became smaller. Towards the spring they tended to increase.

Since it is a well-known fact that potatoes with a different manurial treatment may have a different dry-matter content and a different protein content, the respiration rates of this experiment were also calculated on a protein basis.

TABLE I
Yield data of potatoes used in the respiration experiments (cf. Fig. 1).

Experimental field, Variety	Manurial treatment (kg per ha *)	Yield, q per ha (1 q = 100 kg)
1011, Noordeling	no potassium, 50 CuSO ₄	69
	300 K ₂ O, 50 CuSO ₄	179
	300 „ 0 Cu	170
1181, Noordeling	40 N, 300 K ₂ O	216
	160 „ „ „	294
	160 „ 25 „	163
1113, Voran	no phosphorus	53
	140 P ₂ O ₅	294
1220, Voran	5 MgO	161
	160 „	383

*) Basic dressing was applied in optimum amounts.

In this case the nitrogen-deficient tubers gave higher values than those supplied liberally with nitrogen. Apparently the protein formed in excess of the protein of nitrogen-deficient tubers did not take part in metabolic processes.

In the case of phosphorus the differences between deficient and normal tubers became more pronounced if calculated on protein,

whereas with potassium the differences were reduced. This was due to the fact that the protein content of P-deficient as well as that of K-deficient tubers was higher than that of normal tubers.

Nitrogen

The effect of nitrogen nutrition of potato plants on respiration rate of the tubers was studied in a number of further experiments with tubers as well as with tissue disks. In some cases the tubers from plants dressed amply with nitrogen had somewhat higher respiration rates than those from plants with a poor nitrogen supply. In other experiments no differences in respiration values were obtained although large differences in yield occurred. The results of the experiments on oxygen uptake with tissue disks agreed with those on CO₂-emission of intact tubers.

Phosphorus

In addition to the experiment mentioned above, the effect of phosphorus nutrition on respiration rate of the tubers was investigated in a few further experiments. In general, tubers from plants highly deficient in phosphorus had somewhat lower respiration rates than those from normal plants.

Magnesium

Although the tubers used for these experiments came from plants with pronounced symptoms of magnesium deficiency, no differences in respiration rate between deficient and normal tubers were observed. This was true of tubers as well as of tissue disks.

TABLE II

Effect of potassium nutrition of Noordeling*) potato plants on the respiration rate of the tubers.

Experiment and date	Time	Yield, q per ha		mg CO ₂ per hour per 100 g fresh weight	
		0K	+K	0K	+K
589, Jan. 6, 1949	12-17	169	384	1.07	0.69
" " " "	17-24			2.00	1.34
" " " "	24-10			2.16	1.34
" " " "	10-16			2.20	1.32
" " " " 7, 8, 1949	17-9			2.24	1.17
589, March 7, 8, 1949	23.30-9.30	327	340	1.74	1.18
" " 8, "	9.30-16			1.89	1.51
" " " "	18-9.30			1.98	1.76
1011, April 20, 1950	17-19	136	250	3.22	1.82
" " 21, "	10-14			3.60	1.74
1011, Sept. 5, 6, 1950	18-9	74	193	3.27	2.38
" " " "	11-16			3.09	1.86
" " 6, 7, "	17-9			2.82	1.77
1181, " 25, 26, 1950	17-9	127	274	3.14	1.63

*) Tubers of this variety were used in all subsequent experiments.

Potassium

Potassium supply of potato plants has a pronounced effect on respiration rate of the tubers. This can be seen from the results of the two experiments plotted in Fig. 1 and also from a number of further experiments, of which the data are recorded in Table II. It will be seen that the tubers from K-deficient plants always produced considerably more carbon dioxide than those from plants dressed normally with potassium.

The differences in CO_2 -production between K-deficient and normal potatoes were most pronounced at the beginning of the experiment and decreased when the tubers remained for a prolonged period of time in the respiration vessels. This suggested that they depend on a different sensitivity to handling. To verify this hypothesis the following experiment was carried out. Tubers from potassium-deficient and normal plants were dropped once from a height of one metre on a wooden floor before being transferred to the respiration vessels. Fig. 2 shows the results of this experiment. It will be seen that in the

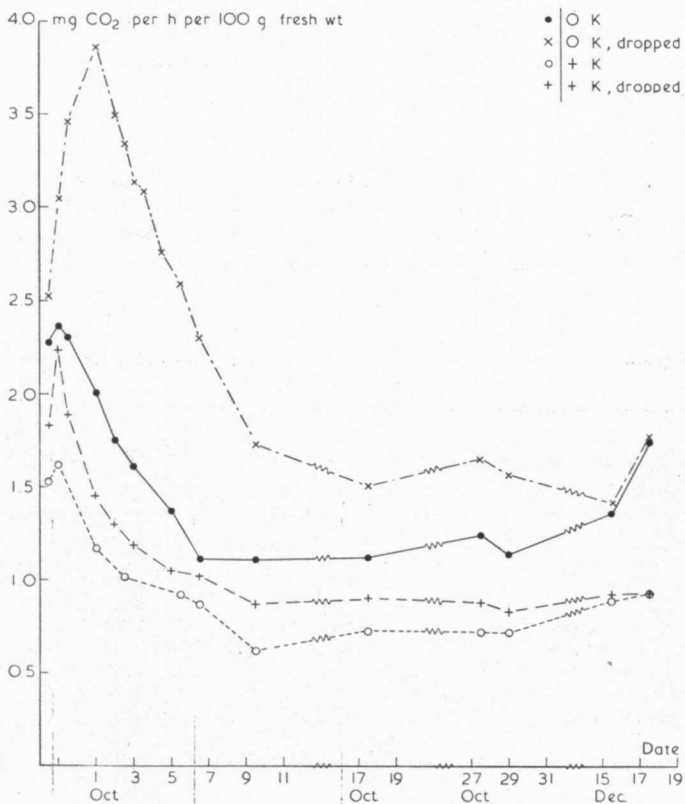


Fig. 2. Effect of bruising of potassium-deficient and normal potato tubers on CO_2 -output. The tubers were dropped once from a height of 1 m on a wooden floor on Sept. 28, 1950.

case of potassium-deficient tubers carbon-dioxide production rose considerably when they were dropped. The increased respiration rate was maintained for a considerable period. Potato tubers from normal plants had also increased respiration rates when being dropped but the rise in CO_2 -production was much less pronounced than in the case of potassium-deficient tubers.

The results of this experiment are in agreement with the results of earlier experiments on the enzymatical blackening of potato tubers (MULDER, 1949).

Tubers from potassium-deficient potato plants were found to be very liable to stem-end blackening. Rough handling of such tubers gives rise to the formation of bluish-black discolorations at the stem end of the tubers. Pl. I, Fig. 1 shows the results of an experiment in which potatoes with a different K-supply were shaken in a bottle for 5 minutes before being halved and exposed to the air. In the case of potassium deficiency reddish discolorations appeared soon after cutting which gradually changed into a bluish-black. This blackening did not appear in tubers with a normal potassium supply.

The phenomenon was found to be due to the enzymatic oxidation of tyrosine and *o*-dihydric phenols to melanin. In normal (undamaged) tissue this reaction does not proceed, presumably due to the fact that substrate and enzyme occur separately in the cell. It is also possible that in actively living cells the dehydrogenase system keeps tyrosine and other phenols in the reduced state. When the cells are damaged these compounds are oxidized by tyrosinase, first to red compounds and then to the black melanin.

Two factors were found to be responsible for the extreme tendency of potassium-

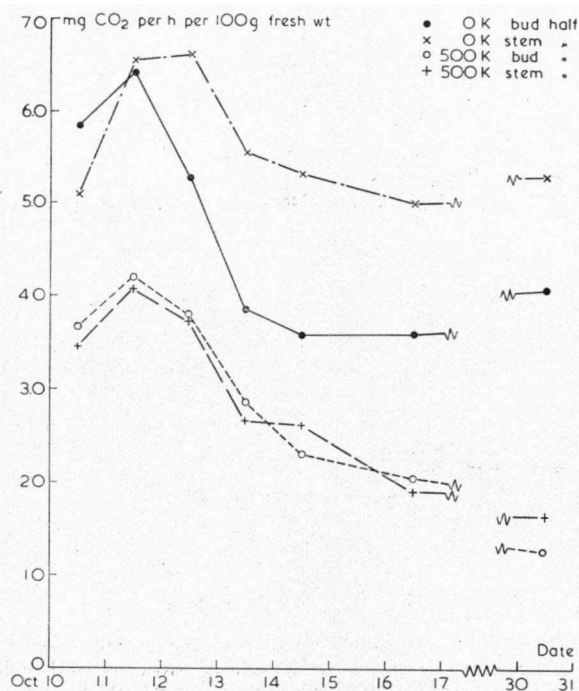


Fig. 3. CO_2 -output of bud and stem halves of K-deficient and normal potatoes. The tubers were halved on October 10, 1950.

deficient tubers to blacken, viz. the high content of free tyrosine and the liability of the tissue to sustain injury. Owing to the operation of the latter factor tyrosine and *o*-dihydric phenols become subjected to tyrosinase activity and in consequence an irreversible oxidation to bluish-black melanin takes place.

That the increased respiration rate of K-deficient potato tubers observed in the above-mentioned experiments was due to the bruising of part of the tissue may be concluded from the following results: (a) Stem halves of potassium-deficient tubers which are much more liable to bruising than top halves respire more intensively than the latter (Fig. 3).

(b) Potassium-deficient potatoes harvested and transported to the laboratory in such a way that any rough handling was prevented respired at a rate similar to that of normal tubers. A slight movement of these tubers (removing of sprouts, dropping in a box covered with cotton-wool) brought about differences in respiration rate (see arrow in Fig. 4). In this case no black lesions could be detected in the potassium-deficient tubers in contrast to the experiments in which large differences in CO_2 -production were observed between tubers with a different potassium supply.

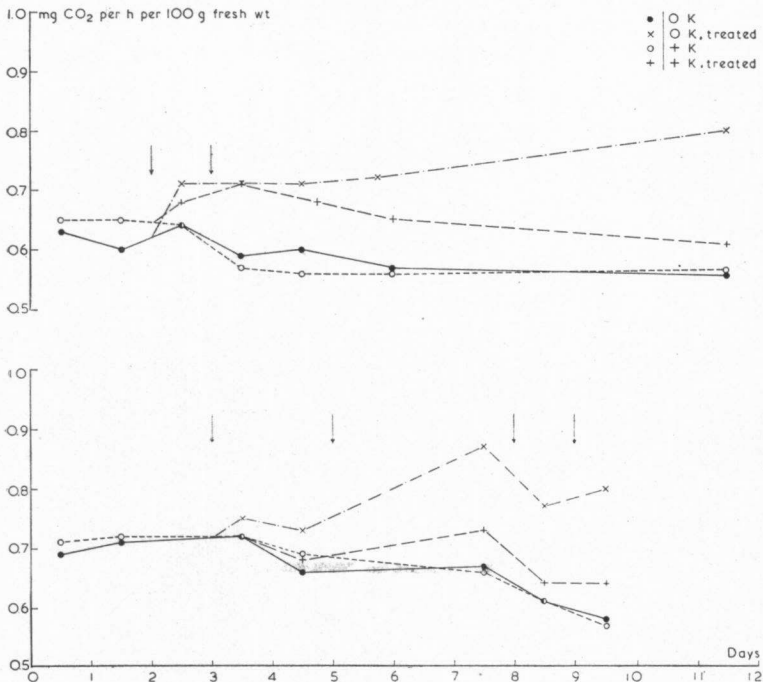


Fig. 4. CO_2 -output of potassium-deficient and normal potato tubers harvested and transferred very carefully. At arrow: a number of tubers were dropped in a box covered with cotton-wool. Tubers transferred to respiration bottles on December 8, 1954 (lower graphs) and on January 5, 1955 (upper graphs). Measurements of respiration rate started 5 days later.

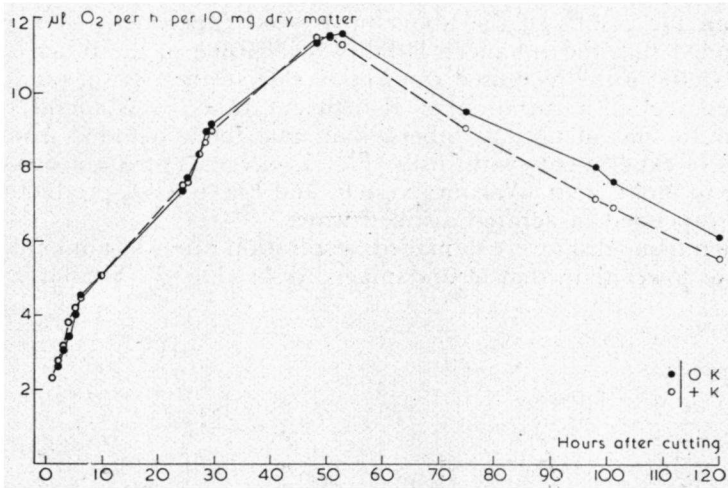


Fig. 5. Effect of potassium nutrition of potato plants on oxygen uptake of 1 mm thick tuber disks respiring in moist air. The drop in respiration rate at the third day was partly due to loss of water (cf. the curves of Fig. 6). Disks cut on January 24, 1955.

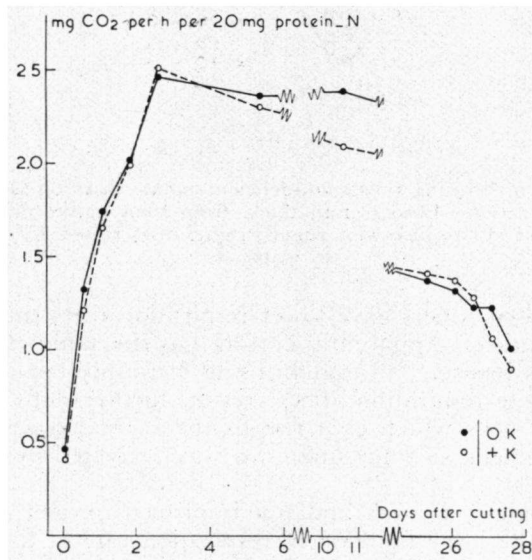


Fig. 6. Effect of potassium nutrition of potato plants on CO₂-output of 1 mm thick tuber disks respiring in aerated distilled water. Disks cut on October 4, 1950.

The cause of the increased respiration rate of K-deficient potatoes

From the results of the above-mentioned experiments it may be concluded that the enhanced liability to bruising of the tubers is the main cause of the increased respiration rate in the case of potassium deficiency. Cell respiration of K-deficient tubers was found to be similar to that of normal tubers. This may be concluded from the results of experiments with disks (Fig. 5, oxygen uptake of disks respiring in moist air in Warburg vessels, and Fig. 6, CO_2 -production of disks suspended in aerated distilled water).

When tissue disks were damaged, respiration rate was not enhanced but was lower than that of undamaged disks (Fig. 7). Similarly disks

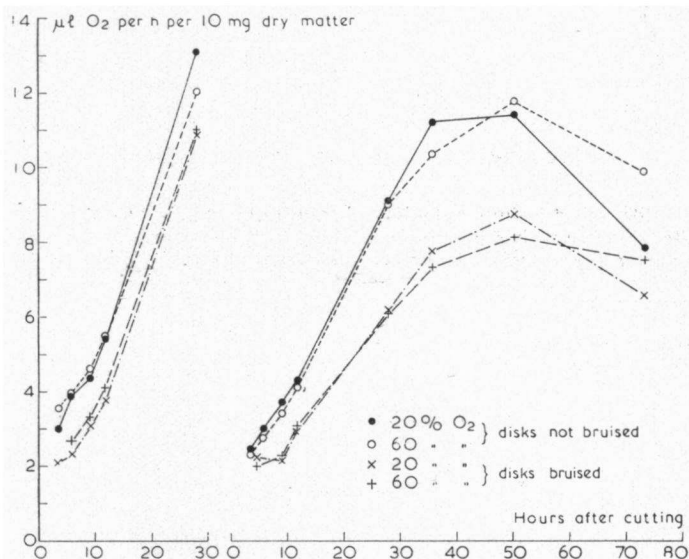


Fig. 7. Effect of bruising potassium-deficient potato disks on O_2 -uptake at two oxygen concentrations. Disks 1 mm thick, from stem halves of the tubers, cut January 28, 1951. Left: disks not rinsed; right: disks rinsed 25 times in distilled water.

cut from bruised tubers gave lower respiration rates than those from undamaged tubers. Apparently cutting has the same effect on respiration rate as bruising, so that bruising of freshly cut disks does not further promote respiration. These results further show that it is not the wounded cells which give rise to the increased respiration rate but the living cells, in some unknown way activated by the wounded cells.

That wounding as such and not improved oxygen supply is responsible for the large increase in respiration rate of tissue disks and wounded potassium-deficient potatoes was shown by the following experiments. Potassium-deficient and normal tubers were cut longitudinally and rinsed quickly. Subsequently the cut surface was covered

with paraffin wax to prevent penetration of oxygen through this surface.

TABLE III
Effect of halving on CO_2 -production of potato tubers.

Treatment	mg CO_2 produced per h per 100 g of fresh tissue	
	0K	+K
Control (untreated)	3.14	1.63
halved	5.80 (2.66) *	4.70 (3.07)
halved + paraffin wax	5.06 (1.92)	4.14 (2.51)

*) in parentheses: increase in respiration.

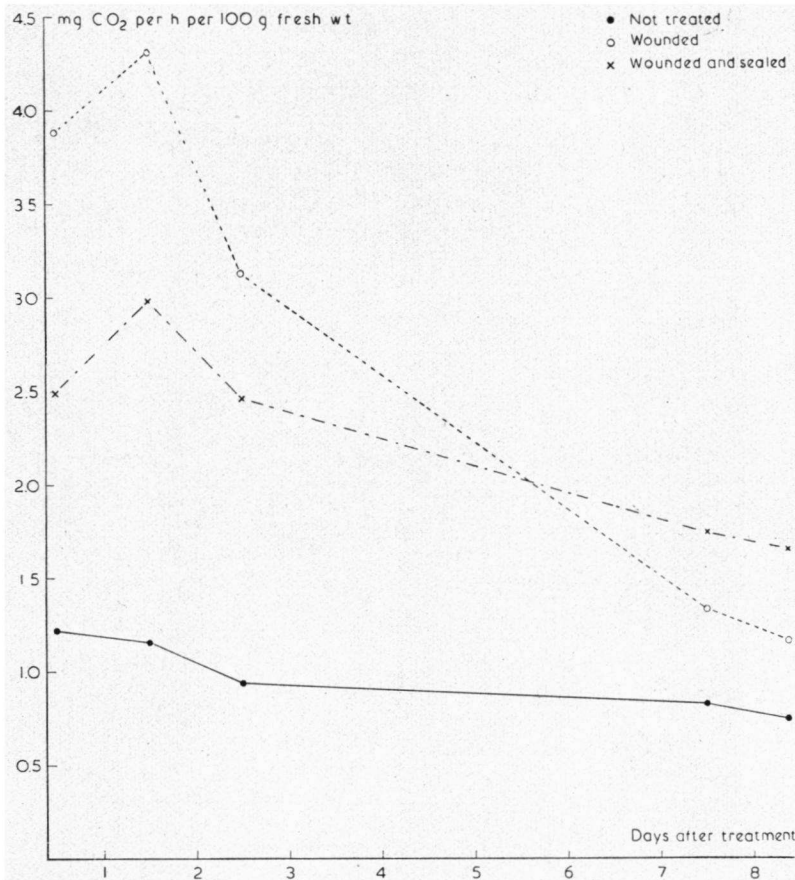


Fig. 8. Effect of wounding on CO_2 -output of potato tubers from normal plants. Wounding was brought about by pushing a cork borer into the stem and bud ends respectively of the tubers (October 10, 1952). In one series the wounds were immediately sealed with paraffin wax so that gas exchange through the wounded surface was prevented.

As will be seen from the data of Table III, increase of CO_2 -production of such tubers was nearly as high as that of uncovered halved tubers. Similar results were obtained in an experiment in which a sterilized cork borer was pushed for 2 cm into the stem and but ends of the tubers. Immediately after its pulling back the wound was covered with paraffin wax. The results of this experiment are plotted in Fig. 8. It will be seen that a pronounced response to wounding was obtained with both covered and uncovered wounds. In the former case respiration

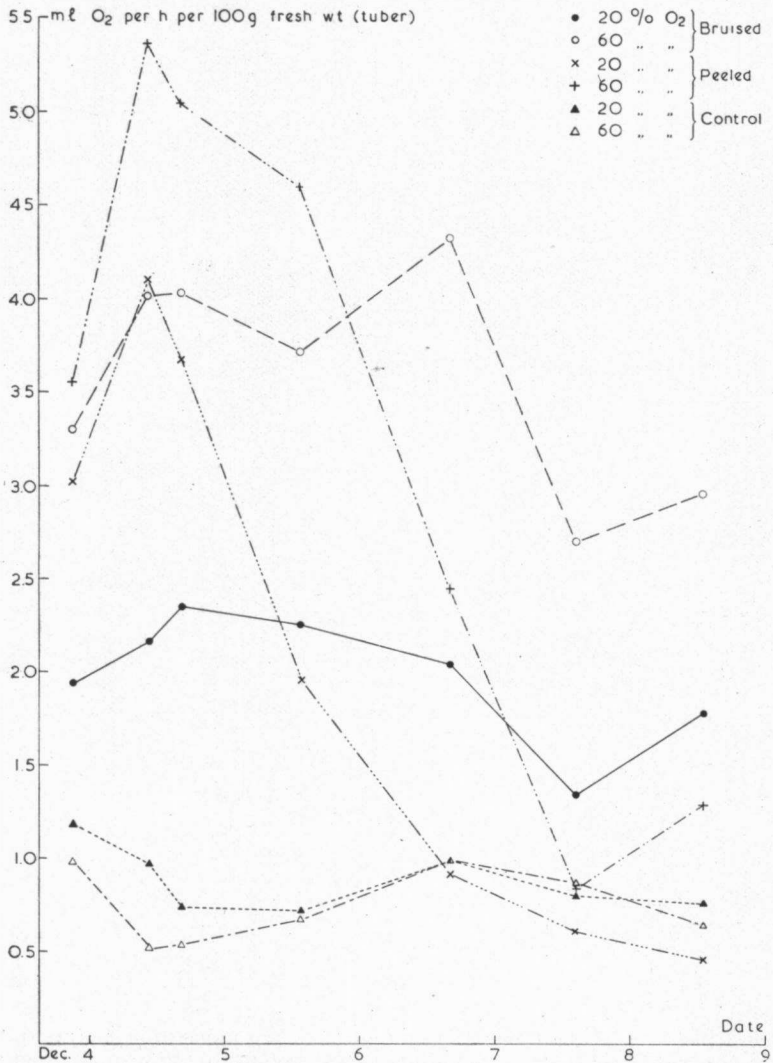


Fig. 9. Effect of oxygen concentration of the atmosphere on O_2 -uptake by variously treated potassium-deficient potato tubers. Treatments carried out on December 3, 1951.

rate rose less sharply and was continued for a longer period of time than in the latter. Apparently oxygen supply through the existing gas spaces is inadequate for proceeding of wound reactions at optimum speed. This was also shown in an experiment in large Warburg vessels in which damaged and peeled potassium-deficient potatoes were kept at two different O_2 -concentrations, viz. 20 and 60 % (Fig. 9). Although in the case of undamaged tubers both O_2 -concentrations in the gas space gave equal results, 60 % O_2 gave considerably higher values than 20 % when the tubers were wounded. When the tubers of this experiment were halved after being kept for 5 days at the respective O_2 -concentrations, those kept at 20 % oxygen were found to be blackened considerably more strongly than those kept at 60 %. Apparently 20 % O_2 was too low for supplying the wounded tissue adequately with O_2 , as a result of which more cells died and gave rise to blackening.

That the effect of wounding on respiration rate is not confined to the cells adjacent to the cut surface can be concluded from the following experiments. Oxygen uptake was measured of disks of 0.5, 1

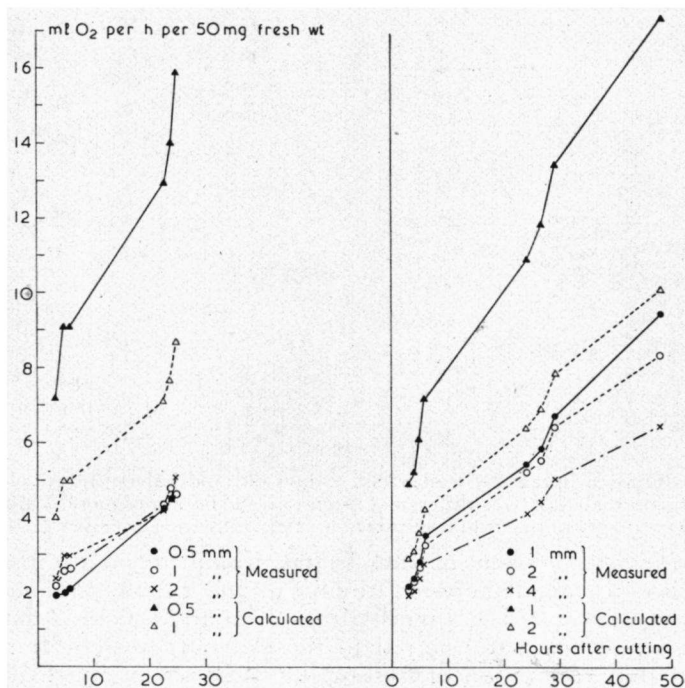


Fig. 10. Oxygen uptake by potato disks of varying thickness. Calculated values were derived from the measured values of 2-mm (left-handed graph) and 4-mm (right-handed graph) disks. The former represents oxygen-uptake rates which would have been obtained for 0.5- and 1-mm disks (left-handed curves) and 1- and 2-mm disks (right-handed curves) if respiration rate were proportional to the area of wounded surface. Tubers from plants with optimum manurial treatment. Disks cut June 6, 1952 (left) and December 16, 1954 (right).

and 2 mm, and 1, 2 and 4 mm thickness respectively. At equal weights of these disks the wounded area decreased with increasing thickness at a ratio of 3,2: 1,7: 1 and 2,7: 1,6: 1. As will be seen from the data of Fig. 10 the respiration rate was proportional to the weight of the disks and not to the area of wounded surface. This means that stimulation of respiration rate owing to wounding was extended throughout the disks. When the slices grew older a surface effect was apparent, presumably due to the fact that the wounds became sealed so that entry of oxygen was hampered.

Comparison of respiration mechanism of wounded and intact potato tissue

As may be seen from Fig. 5 and 8 wounding of potato tubers brings about a considerable rise of respiration rate. In the case of tissue disks a five- to six-fold rise often may be found within 2 days. This phenomenon was also observed by STEWARD and PRESTON (1940) and by THIMANN *et al.* (1954).

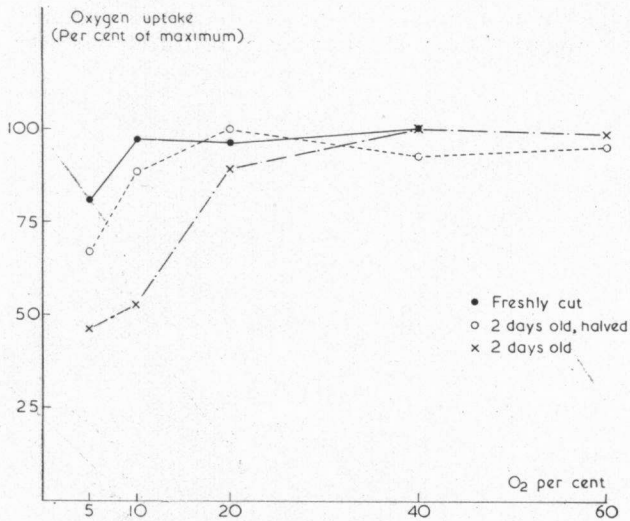


Fig. 11. Oxygen uptake by freshly cut, 2-days old and halved 2-days old potato disks from normal tubers at different O₂-tensions of the atmosphere. Values given as percentages of maximum respiration rate. Disks cut December 2, 1954.

To study respiration of intact and wounded potato tissues, a comparison was made between freshly cut and 2-days old tissue disks. The latter were stored in a moist atmosphere at 20° C for 2 days after cutting before being transferred to the Warburg vessels. It may be assumed that respiration of freshly cut tissue is not much different from that of intact tissue. Pronounced differences in metabolic behaviour between both types of tissue disks were observed. Freshly cut disks have a considerably lower optimum O₂-concentration than 2-days old disks (Fig. 11). They have a much lower tendency to produce carbon dioxide under anaerobic conditions (Fig. 12), and their respiration is more sensitive to inhibiting substances like cyanide, carbon

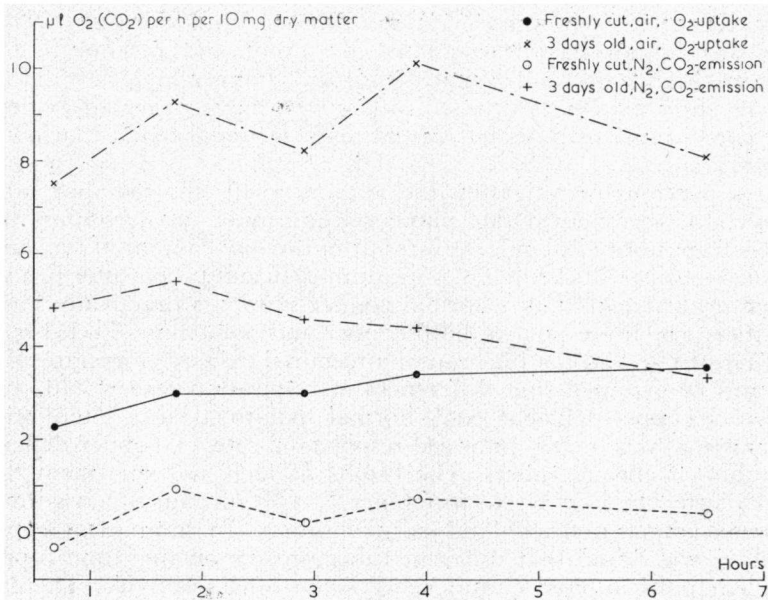


Fig. 12. Oxygen uptake (in air) and CO₂-emission (in N₂) of freshly cut and 3-days old potato disks obtained from normal tubers. Disks cut April 9, 1952.

monoxide, and sodium fluorid. Furthermore, their catalase and peroxidase content is much lower than that of 2-days old disks. Their content of DPN (diphosphopyridine nucleotide) is also much lower.

Some of these differences have also been observed by LEVY and SCHADE (1948) and THIMANN *et al.* (1954), viz. different optimum O₂-concentrations and different behaviour against cyanide and carbon monoxide. These authors suggest that the different behaviour of both types of disks could be ascribed to a different type of terminal oxidase in young and old disks.

The results obtained in the present study provide some evidence that at least part of the different behaviour of young and old disks may be ascribed to a hampered entry of oxygen and of the inhibiting substances into the 2-days old disks as compared with that of freshly cut disks. This is concluded from the fact that 2-days old disks of 2mm thickness divided into two disks of 1 mm each behave more or less like freshly cut disks as to optimum concentration of oxygen (Fig. 11) and sensitivity with respect to cyanide and carbon monoxide. Sensitivity to NaF which in young disks is very high, was only partly restored by halving of 2-days old disks.

Respiration rate of the disks was only slightly affected by halving in these experiments.

Copper

The copper-containing enzyme tyrosinase is considered by some authors to play the role of a terminal oxidase in potato tubers (BARRON,

LINK, KLEIN and MICHEL (1950) and BOSWELL and WHITING (1938)). This assumption has been denied by LEVY and SCHADE (1948), SCHADE, BERGMANN and BYER (1948) and SCHADE and LEVY (1949) and recently by THIMANN *et al.* (1954) who have provided evidence that cytochrome oxidase acts as the main terminal oxidase in potato tubers.

In a previous investigation the author (1949) showed that tubers from copper-deficient potato plants contain much less tyrosinase than tubers from normal plants. As a result of the low content of tyrosinase the enzymatical blackening of potassium-deficient tubers after bruising which occurs readily at a normal copper supply is practically absent in tubers which are poor in both copper and potassium (Pl. I, Fig. 2).

If tyrosinase had the function of a terminal oxidase in potato tubers it could be assumed that differences in respiration rate would occur between copper-deficient and normal potato tissue. Therefore a comparison was made between respiration rate of copper-deficient and that of normal tubers. The results of such an experiment have been plotted in Fig. 1. No difference in CO_2 -production was found between copper-deficient and normal tubers. In subsequent experiments it was found that different tubers grown on the same copper-deficient plots differed considerably in tyrosinase activity. Therefore only those tubers were used which were found to be practically free from tyrosinase. This was tested by cutting a small piece of tissue from the tubers and reacting with a solution of dopa. Since the tyrosinase content of potatoes was found to decrease when the tubers grew older, most respiration experiments have been carried out with relatively old tubers.

Although many experiments with potatoes which were practically free from tyrosinase have been carried out, no differences in CO_2 -output between these tubers and tubers supplied normally with copper have been found. Peeling of such tubers, although considerably increasing respiration rate, did not bring about a difference between both types of tubers.

In experiments with disks cut from copper-deficient and normal potatoes, equal respiration rates in both types of tissue were found in a number of cases, although tyrosinase was completely absent in the copper-deficient tissue. Since complete absence of tyrosinase was found only in old potato tubers these conclusions apply to these tubers only (see Fig. 13). It will be seen that rise in respiration rate after cutting which is found in young tubers did not occur in the disks from these old tubers.

In a number of cases, however, it was found that the copper-deficient disks had lower respiration rates than disks from normal tubers. Since old copper-deficient potatoes were often found to have a very poor turgidity, it was assumed that the observed effect of copper on respiration rate in this case was due to some secondary effect of this element.

In a number of experiments with relatively young potatoes in which respiration rate rose normally after cutting, it was found that rise in

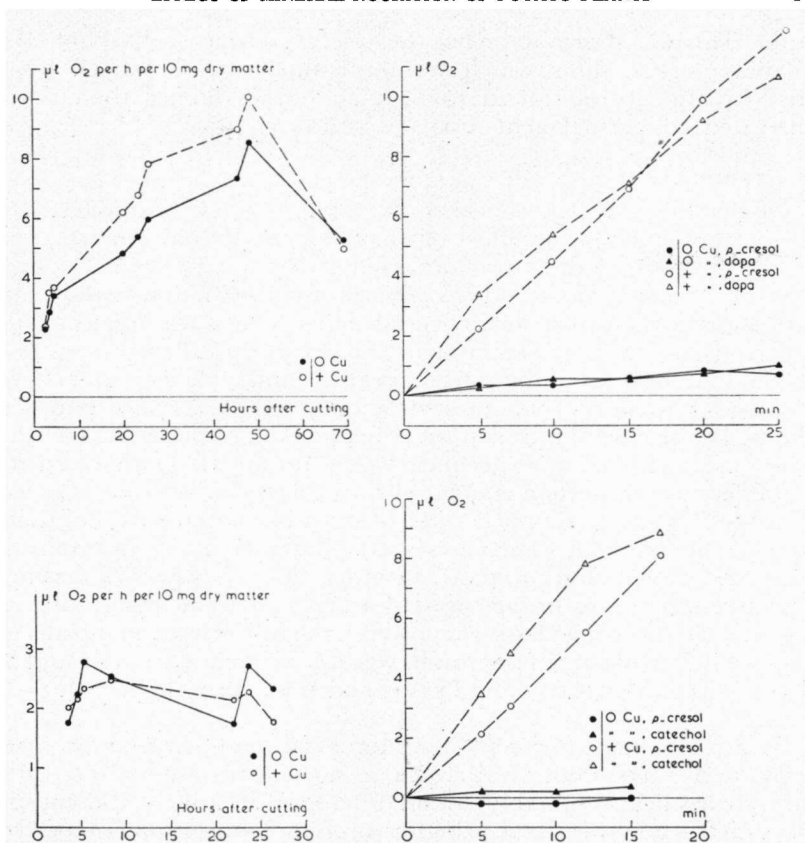


Fig. 13. Oxygen uptake and tyrosinase activity of tissue disks cut from copper-deficient and from normal tubers, respectively. Upper two graphs: disks from relatively young tubers (stored from October — February); lower two: disks from old tubers (stored from October — June).

respiration of normal disks was more pronounced than that of copper-deficient disks (see Fig. 13). This may be an indication that copper has something to do with the stimulation of respiration after wounding.

To show that also in tissue disks in which the respiration rate had increased considerably after cutting, tyrosinase did not function as the terminal oxidase, the following experiment was carried out. Disks from copper-deficient and normal potatoes were exposed to a cyanide atmosphere for half an hour and thereafter respiration was compared with that of untreated tubers. In both cases respiration rate was reduced considerably, presumably due to inhibition of the metal-containing terminal oxidase. If this oxidase were tyrosinase, inhibition of this enzyme by the dilute HCN should be proportional to that of respiration rate. Since respiration rate of HCN-treated plus-Cu disks was lower than that of untreated copper-deficient disks, tyrosinase activity of the former in this case should also be lower than that of the

latter. This was shown to be not the case. Tyrosinase activity of HCN-treated plus-Cu disks which was only moderately reduced by the HCN-treatment was found to be many times higher than that of untreated copper-deficient disks.

DISCUSSION

Of the five nutrient elements (N, P, K, Mg, Cu) studied in the present investigation no effect of *magnesium* supply on respiration rate of tubers or tuber disks was observed.

In the case of *nitrogen* and *phosphorus* a small reduction in respiration rate of tubers and tissue disks from deficient plants was found in some experiments. In other experiments, however, no differences in respiration rate between tubers from healthy and deficient plants were observed, although pronounced deficiency symptoms occurred in the plants. In the case of nitrogen the differences in respiration rate which were observed in some experiments were presumably connected with the differences in protein content of the tubers. The amount of protein of the tubers amply supplied with nitrogen formed in excess of that of N-deficient tubers in some cases was apparently active in respiration processes (cytoplasmic protein), in other cases, however, it has to be considered merely as storage protein. In the latter case respiration rate calculated on a protein basis was lower in the tubers from plants dressed amply with nitrogen. Similar results were obtained by GREGORY and SEN (1937) with barley leaves and PETRIE and WILLIAMS (1938) with Sudan grass.

In experiments in which phosphorus-deficient tubers gave lower respiration rates than those with a normal P-supply, the differences depended presumably not on differences in protein content since the P-deficient tubers had a higher protein content than those from fully manured plants. Calculated on a protein basis differences in respiration rate between P-deficient and normal tubers therefore became more pronounced. Similar results have been obtained by PETRIE and WILLIAMS (1938) with oat leaves. In their experiments the leaves of the fully manured oat plants were deficient in nitrogen, however, so that high respiration rates per unit of protein were found. In the present experiments the fully manured plants were dressed liberally with nitrogen so that it is improbable that nitrogen deficiency occurred in the tubers from these plants. Apparently in the present experiments P-deficiency in some way affected substrate supply to the respiratory enzymes.

Although pronounced differences in respiration rate between *potassium*-deficient, and normal plant tissues have been observed by various authors it is quite clear that the differences in respiration rate observed in the present study do not depend on a different cell metabolism but on a different sensitivity to handling of potassium-deficient and of normal potatoes. It would be interesting to know whether the results obtained with leaves from K-deficient and normal plants as described in the literature depend entirely on differences in cell respiration or whether also in these experiments a different sensitivity to handling

played a part. The fact that both AUDUS (1935) and GODWIN (1935) found that the handling of cherry-laurel leaves increased respiration rate may be an indication that in leaf tissue, too, the above-mentioned potassium effect may occur. Respiration experiments with potassium-deficient and fully manured potato leaves will be carried out in this laboratory in the near future.

About the nature of the observed respiration stimulation in handled potato tubers the following may be said. Apparently two types of stimulation occur, one due to a slight handling in which no damaging of tissue is observed (Fig. 4) and one due to rough handling in which in the case of K-deficiency a considerable bruising and blackening of the tissue occurs (Fig. 2). The former type of response to handling is presumably identical with the response obtained by AUDUS (1935) and GODWIN (1925) in cherry-laurel leaves. Both types of response were found to be much more pronounced in K-deficient potatoes than in fully manured. In the latter, blackening of the tissues never occurs.

The effect of bruising is apparently identical with the wound effect as it occurs after cutting or peeling of potato tubers. This may be concluded from the fact that disks from bruised tubers had a somewhat lower respiration rate than those from undamaged tubers and also from the fact that bruising of disks did not further increase respiration rate.

The nature of the wound effect on respiration rate has been discussed in a number of papers (BARKER (1935), BOEHM (1887), HOPKINS (1927), JOHNSTONE (1925), RICHARDS (1896) and STICH (1891)).

In addition to increased metabolic activity of the injured tissue, facilitated gas exchange has been considered by some authors to be the main cause of increased respiration rate of wounded plant tissues JOHNSTONE (1925). In the case of cutting, the favoured gas exchange may undoubtedly play some part. That this effect is of a secondary character, however, may be concluded from the fact that sealing of the wounded surface (Fig. 8) did not reduce the effect of wounding on respiration a great deal. It modified its effect, however, presumably due to the fact that the processes following wounding require a considerably higher oxygen supply for proceeding at an optimum rate than can be obtained by diffusion of air into the interior parts of the tubers.

The fact that increased oxygen concentration of the atmosphere did not affect respiration rate of intact potatoes*) but increased that of bruised tubers (Fig. 9) provides further evidence that improved oxygen supply is not the cause of the increased respiration rate of cut or bruised tubers.

As to the effect of wounding on liberation of large amounts of CO_2 during the first hours after cutting of potato tubers as described by

*) In some cases a prolonged exposure of potato tubers to 60 or 100 % O_2 was found to cause a sudden increase of respiration rate with several hundred per cent. This effect which was obtained in K-deficient as well as in normal potatoes was found irregularly. So far no explanation of this can be given. It is not due to bacterial or fungal infections.

RICHARDS (1896), it may be assumed that this effect played only a minor part in the present study. In general, CO_2 -determinations were started approximately 4 hours after cutting or wounding. Liberation of accumulated CO_2 after this period of time was presumably completed.

The most likely explanation of the increase in respiration rate of bruised potato tubers is that wounding brings about a regeneration of cell metabolism, presumably due to increased catalytic activity of the protoplasm. The large increase in catalase, peroxidase and DPN-content of 2-days old tissue disks as compared with freshly cut tissue disks may be considered as some evidence of this assumption. This regeneration process is not confined to the cells adjacent to the cut surface but apparently occurs throughout the tissue disks (approximately 50 cell layers in 4 mm disks; see Fig. 10). The older the potato tubers, the poorer their regeneration capacity and the smaller the increase in respiration rate after wounding (Fig. 14). Very old tubers

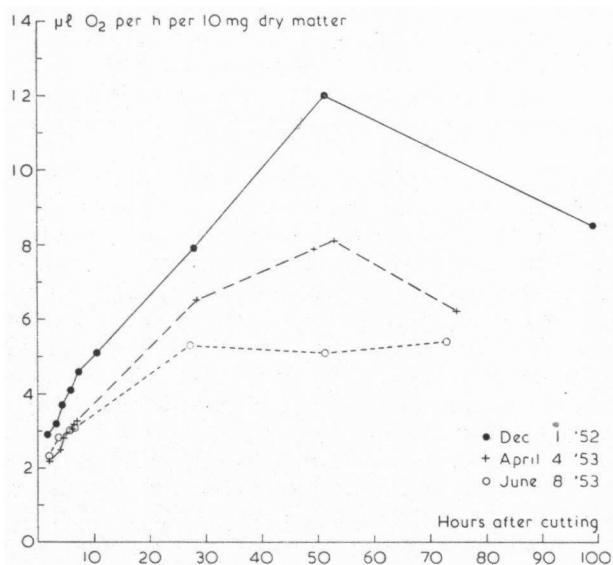


Fig. 14. Effect of time of cutting on respiration rate of tuber disks, 1 mm thick, from fully manured plants.

may have entirely lost their capacity of responding to the wounding presumably because they have lost their regeneration capacity. Such tubers have a poor vitality, as may be concluded from the fact that after cutting they are easily infected by saprophytic micro-organisms.

Regeneration of metabolic activity is presumably related with the formation of active protoplasm. This does not necessarily mean increase of total protein but presumably mainly transference of storage protein into metabolic protein. Table IV gives average respiration rates as mg CO_2 produced per h per gram protein-N of intact potatoes and of tissue disks of 1 mm thickness cut from these tubers.

TABLE IV

Respiration rates of various plant tissues calculated per 1 g of protein-N.

Plant tissue	Author	mg CO ₂ per h per 1 g protein-N
Potato tubers	MULDER (1955)	3—10
Disks of potato tubers, 1 mm thick, 2 d after cutting	" "	100—225
Barley (leaves)	GREGORY and SEN (1937)	184
Barley (leaves)	RICHARDS (1938)	165
Sudan grass (leaves)	PETRIE and WILLIAMS (1938)	43
Oats (leaves)	PETRIE and WILLIAMS (1938)	77

TABLE V

Protein- and soluble non-protein-N in potato disks in relation to respiration rate.

Condition of potato plants	Date of experiment	Treatment of disks	Respiration rate, mg CO ₂ per hour per 100 disks	Protein-N, mg per 100 disks	Soluble non- protein-N mg per 100 disks
K-deficient	Oct. 10, '50	Before treatment ¹⁾	1.63	29.5	26.0
	" " "	3 d in aer- ated H ₂ O	2.98	31.7	19.3
	" " "	3 d in 0.05 M KCl	3.60	31.4	17.9
Ample K	" " "	Before treatment ¹⁾	1.47	26.3	21.0
	" " "	3 d in aer- ated H ₂ O	2.89	30.0	17.0
	" " "	3 d in 0.05 M KCl	3.77	30.0	14.8
K-deficient	Oct. 16, '50	Before treatment ¹⁾	0.36	28.2	21.7
	" " "	3 d in aer- ated H ₂ O	2.80	31.4	14.4
	" " "	3 d in 0.06 M NaCl	3.54	31.1	14.3
Ample K	" " "	Before treatment ¹⁾	0.52	26.7	22.0
	" " "	3 d in aer- ated H ₂ O	2.76	32.8	16.8
	" " "	3 d in 0.06 M NaCl	3.65	—	—
Ample K	April 25, '55	Before treatment ¹⁾	0.81	11.1 ²⁾	8.3 ²⁾
	"	3 d in moist air	2.30	10.4 ²⁾	8.7 ²⁾

¹⁾ In the case of respiration 'before treatment' means first period of respiration in aerated H₂O. Length of this period: 12 h (1st), 4 h (2nd) and 8h (3rd experiment).

²⁾ mg per 1 g dry tissue. A paper-chromatographic analysis of these disks revealed practically no differences in amino-acid composition of the non-protein fraction before and after incubation.

For comparison some values recorded in the literature for leaves of barley and Sudan grass are given.

These data clearly show the great rise of respiration rate of potato tissue after cutting disks of 1 mm thick. The values found in the latter tissue are similar to those of young leaves of cereal plants.

Although in a number of experiments an increase in protein content of tuber disks was observed, three days after cutting, this was not always the case (see Table V).

The data of Table V show that in the young tubers in which the rise in respiration rate was more pronounced than in the old tubers protein formation took place during the incubation period. In the case of K-deficiency this synthesis was less important than in tubers from fully dressed plants. Incubation in KCl which gave increased respiration values (due to 'salt-respiration') did not affect protein formation. The latter is in disagreement with the results of STEWARD and collaborators (1940, 1954), who found a relationship between increase in respiration, protein formation, and K-uptake by potato disks. In the present investigation NaCl was found to affect respiration rate similarly to KCl.

The experiments with *copper*-deficient potato tubers have shown that the copper-containing enzyme tyrosinase does not act as the terminal oxidase in respiration. Ascorbic oxidase, which is present in potato tubers, has not this function either. In agreement with the results of THIMANN *et al.* (1954) optimum activity of the latter enzyme was found at 100 % O₂. This is in contrast with the respiration of potato disks which three days after cutting were found to respire at an optimum rate at 40 % O₂, but after halving at 20 %.

SUMMARY

Respiration rates have been determined of potatoes grown under varying nutritional conditions. Intact tubers as well as tissue disks were used in these experiments.

No effect of magnesium supply on respiration rate was found. Tubers and tuber disks from nitrogen-deficient and those from phosphorus-deficient plants had in some cases somewhat lower respiration rates than those from fully manured plants.

Potassium-deficient tubers were found to have considerably higher respiration rates than tubers grown on the same field at an optimum K-supply. This difference was shown to be due to a different sensitivity to bruising of K-deficient and of normal tubers. The effect of bruising on respiration rate was assumed to be identical with the wound effect as it was found in tuber disks. Some evidence was obtained that the wound effect depends on increased activity of potato protein after wounding. No effect of potassium supply on cell respiration has been observed.

In experiments with copper-deficient potatoes evidence was obtained that tyrosinase does not function as the terminal oxidase in the respiration of the tubers.

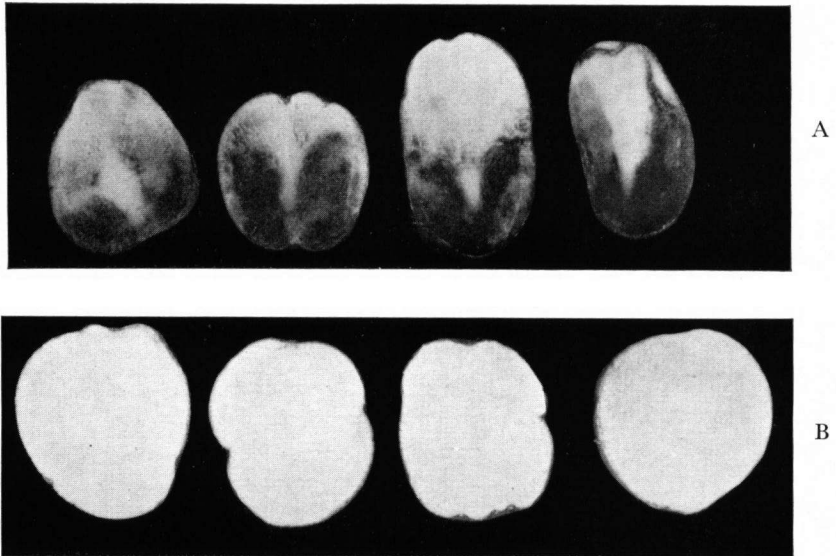


Plate I, Fig. 1. Tubers from potassium-deficient (A) and healthy (B) potato plants. Stored from October until February, cut longitudinally, rinsed with tap water and exposed to air overnight. Tubers shaken for five minutes in a bottle before halving. Black tissue mainly confined to stem ends of the tubers.

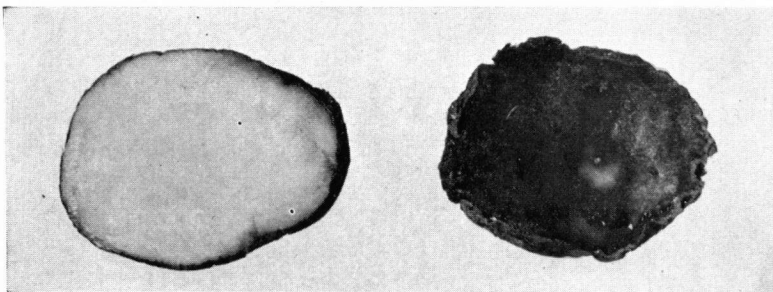


Plate I, Fig. 2. Potassium-deficient potato tubers, bruised, halved and exposed to the air. Left: tuber from a copper-deficient plant, right: tuber from a plant dressed with copper sulphate.

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