

Effect of Nitrate on Respiration of *Mentha arvensis*

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Abstract. The supply of nitrate nitrogen caused a marked increase in the rate of respiration of Japanese mint. Sodium azide strongly inhibited the rate of respiration at all the nitrate levels, but the inhibition was more marked at higher levels. Besides this, inhibition caused by azide treatment was less marked in older and nitrogen deficient tissues than in younger ones at higher nitrogen levels. The addition of sodium diethyl dithio-carbamate (DIECA), an inhibitor of copper containing enzymes resulted in an increase in the respiration of mint leaves which increased further with an increase in the nitrate supply. The same concentration of DIECA which stimulate the respiration of leaves caused an inhibition in the respiration of roots. The inhibition was greater at lower levels and decreased consistently as the supply of nitrate increased. The sensitivity of root respiration to DIECA observed with varying levels of nitrate indicated that unlike the leaf, the roots contain copper-containing enzymes which get decreased as the nitrate supply is increased. An increased supply of nitrogen up to 16 me $\text{NO}_3^- - \text{N}$ was associated with an increase in respiratory quotient.

Many studies have shown an appreciable effect on respiration when nitrogen is supplied to the whole plant or to detached roots or leaves (HATTORI 1958, AUSTIN 1960, BERNER 1971). These effects have usually not been related to the oxidase system which plays an important role in the electron transport chain of oxidative phosphorylation. However, OHIRA and MABUCHI (1958) stated that nitrogen deficiency leads to variation in cytochrome-oxidase activity of the plant and reported that nitrogen deficient roots and shoots of rice seedlings have a significantly lower cytochrome-oxidase activity. Information on the metal-containing oxidases with varying supply of nitrate and with different position of the tissues are still meagre. Therefore, the respiration and extent of metal containing oxidases in Japanese mint with varying supply of nitrate in culture medium of a different position of the tissues were studied in the present experiment.

Material and Methods

The Japanese mint (*Mentha arvensis* L. var. *piperascens* HOLMES) was grown in sand nutrient cultures with varying supply of nitrate nitrogen, during the summer of 1971. The methods of growing plants and the procedure

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for sampling were the same as described previously (SINGH and SINGH 1977). Respiratory measurements were confined to the leaves and roots only. The leaves were divided into three categories. The fully opened youngest leaf was designated as recently matured. Two additional leaves, one younger and the other older than the first selected leaf were designated as immature and mature respectively. The roots and leaves were cut into one cm long segments in water. Twenty such segments in each case were used for respiratory measurements.

Respiration was measured in Warburg reaction vessels with a side arm using conventional manometric techniques outlined by UMBREIT *et al.* (1949). The main compartment of the vessel, besides the sample contained 2.5 ml phosphate buffer (pH 5.3), 0.2 ml of 10% KOH solution (W/V) and a small roll of Whatman No. 40 filter paper were placed in the central well. The shaking speed was adjusted to 110 to 120 oscillations per minute. All measurements were made in a bath of 34 °C.

Two inhibitors acting at two different sites in the respiratory pathway were employed. Sodium azide was employed to determine the extent to which metal containing oxidases were active. DIECA (Sodium diethyldithiocarbamate) was used to determine the extent to which copper containing oxidases were active.

In the experiment with DIECA excised roots and leaves were pretreated with 50 ml of 10^{-2} M DIECA in water at room temperature for one hour. The inhibitor solution was frequently changed as suggested by JAMES and GARTON (1952). In the experiments with sodium azide 0.5 ml solution of this at 6 times the final concentration required was taken in the side arm. Thus the final concentration of 10^{-2} M was obtained by dissolving in phosphate buffer of pH 5.3. After measuring the normal respiration for two hours, the manometer was closed again after an equilibration period of 15 min. Readings were taken at one hour intervals after tipping. The inhibition obtained has been presented as a percentage of the average normal respiration during the initial two hour period.

Respiratory Quotient

The respiratory quotient was determined by the method suggested by HSIANG, TSUNG-HSUM T. (1951). Readings were first taken without KOH in the central well, after which KOH was added and readings were taken again after re-equilibration in the same flask. All the measurements were made at 34 °C at hourly intervals.

Results and Discussion

Rate of Respiration as Affected by Levels of Nitrate

Rate of respiration in recently matured and mature leaves and in both young and old roots increased with increasing concentration of nitrate nitrogen. However, in immature leaves a marked augmentation was recorded only up to the concentration of 0.5 me NO_3^- — N per litre beyond which a decreasing tendency was noted (Fig. 1). This is in agreement with the findings reported with nitrogen starved algae and with higher plants, where a marked stimulation in the rate of respiration has been reported through the nitrogen (AUSTIN 1960, HATTORI 1958, BERNER 1971). This seemed to

be due to the following reasons. Firstly, nitrate nitrogen augmented respiration due to diversion of respiratory DPNH for nitrate reduction and its utilization along with respiratory intermediates for amino acid synthesis. Secondly, nitrogen nutrition accelerated respiration through the stimulation of synthetic reactions requiring ATP (BEEVER 1960). Thirdly, salt effect was probably responsible to some extent for the respiratory increase. The augmented effect of nitrate in the case of barley root has also been explained on this basis (WILLIS and YEMM 1955).

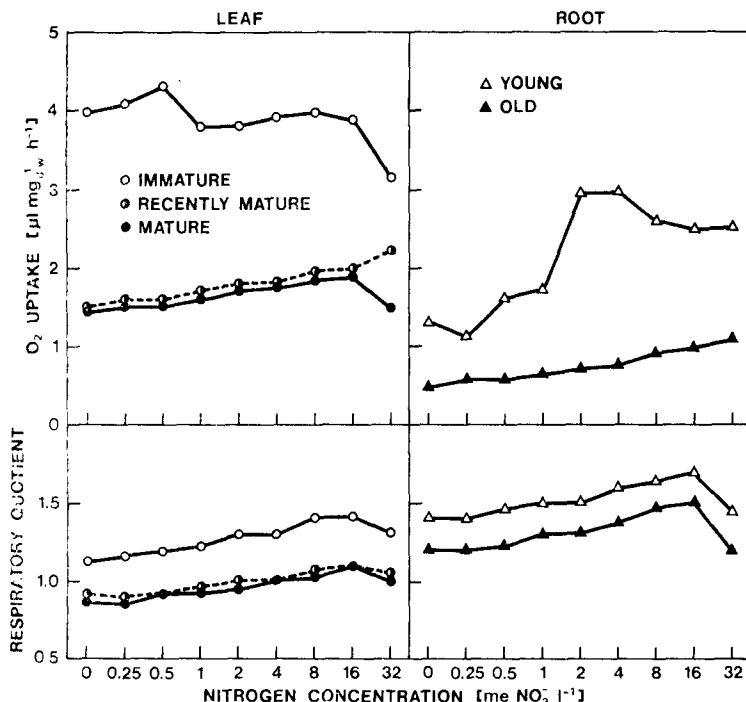


Fig. 1. Effect of varying levels of nitrate nitrogen on the rate of respiration and respiratory quotient of leaves and roots of Japanese mint at full bloom stage.

The rate of respiration was maximum in young tissues which decreased with advancement of age, irrespective of nitrate concentration and part of the plant. This is in agreement with the findings of DICKMAN (1971).

Respiratory Quotient as Affected by Levels of Nitrate

Mature and recently matured leaves had a respiratory quotient below unity but in immature leaves and, young and old roots, it was always higher than unity (Fig. 1). Similar results have been reported in studies with syringa leaves (ULRICH and RUHLAND 1928). The falling value in mature and recently matured leaves was probably due to their diminished photo-synthetic activity, leading to shortage of carbohydrate. An increase in the respiratory quotient, on the other hand, above unity might be caused by the reduction processes connected with the biosynthesis of new cell substances as suggested by KARLSSON and ELLIASSON (1955).

An increased supply of nitrogen was associated with an increase in respiratory quotient up to a 16 me NO_3^- -N per litre beyond which a decrease was noted. Enhanced respiratory quotient with the supply of nitrogen was also reported by FOLKES *et al.* (1952), KESSLER (1953), and SYRETT (1953). Further YAMAGATA (1934) observed that in nitrate fed plants the R.Q. value amounted to 1.5 when sucrose or mannose were used as the substrate and believed that these high values were connected with high growth rate.

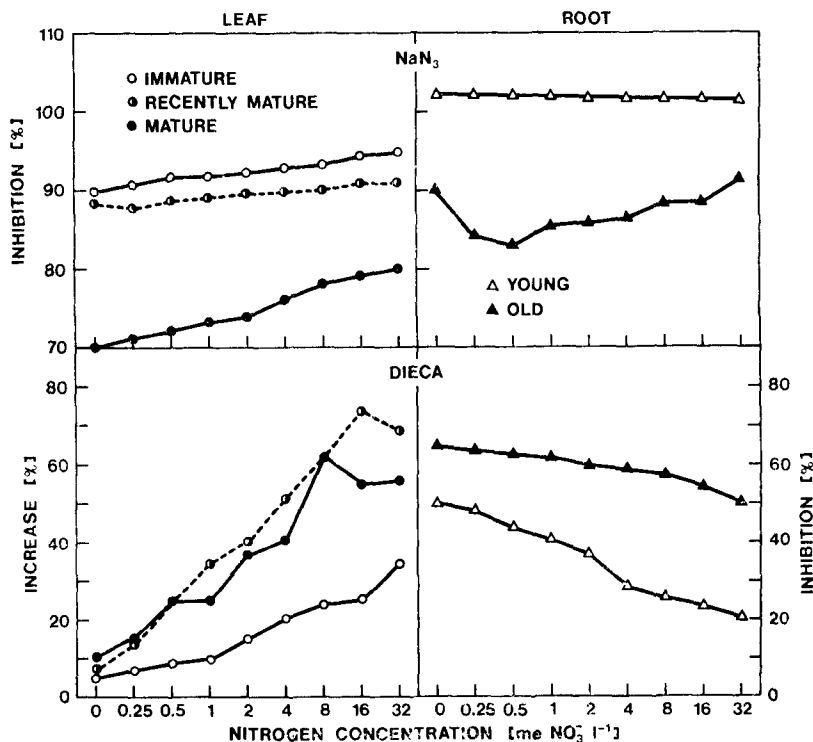


Fig. 2. Effect of sodium azide and sodium diethyl dithiocarbamate on the respiration of leaves and roots of Japanese mint as affected by levels of nitrate nitrogen at full bloom stage.

Effect of Respiratory Inhibitors

a) Sodium azide: Sodium azide strongly inhibited the rate of respiration of different plant parts at all the nitrogen levels (Fig. 2) suggesting participation of heavy metal containing oxidases in respiration. Inhibition due to this inhibitor was more pronounced with an increase in the levels of nitrogen. This suggested that the activity of metal containing oxidases in respiration increased with an increase in the levels of nitrate. Previous experiments with shoots and roots of rice seedlings have also revealed a lower oxidase activity under nitrogen deficient conditions, which resulted in reduced oxygen uptake (OHIRA and MABUCHI 1958).

The sensitivity of respiration to azide treatment was less marked in older and nitrogen deficient tissues. This was perhaps due to the mediation of other mechanisms (such as b_3 and b_7 cytochromes) in the oxidative process of these tissues as suggested by MARTIN and MORTON (1955) and BENDAL

and HILL (1956) in silver beet petioles and *Arum maculatum*. The presence of these cytochromes in older and nitrogen deficient tissues of Japanese mint also seems to be a possibility. However, this needs further investigation.

b) Sodium diethyldithiocarbamate (DIECA): The addition of sodium diethyldithiocarbamate, an inhibitor of copper containing enzymes, resulted in an increase in the respiration of mint leaves which increased further with an increase in the nitrate levels (Fig. 2). This is in disagreement with the finding of SYCHEVA and VASYUKOVA (1972) in the leaves of pea. Despite its well known inhibitory action on respiration, stimulation of respiration due to DIECA has been reported by HARLEY *et al.* (1956) and HIREMATH *et al.* (1974) in beech mycorrhizas and infected *Melanthesea* leaves. These workers believed that copper containing enzymes were apparently not active in their experimental materials. Results of the present experiment indicate that the leaves of Japanese mint plant may also be lacking in copper containing enzymes. This is the first report so far in the literature, however, it needs further investigation.

Another possibility for stimulated respiration of Japanese mint leaves with DIECA might be the uncoupling of oxidative phosphorylation which possibly might have an effect similar to that of 2,4 dinitrophenol. The stimulatory effect of sodium diethyldithiocarbamate in the case of beech mycorrhizas has also been explained on this basis (HARLEY *et al.* 1956).

It was interesting to note that the same concentration of DIECA which accelerated the rate of respiration of the leaves, caused an inhibition in the respiration of the roots. The inhibition was greater at lower nitrogen levels and it reduced consistently as the supply of nitrate increased. The sensitivity of DIECA observed with a varying supply of nitrate nitrogen might be taken as an indication of the fact that unlike the leaf, the roots of the mint plant contain copper containing enzymes which decrease as the nitrate supply is increased.

Besides these effects, older roots were more sensitive to DIECA than younger roots (Fig. 2). This suggests that ascorbic oxidase enzyme plays an important role in the respiration of older tissues of roots. Similar results have been also reported by BARAT *et al.* (1968).

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