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 NO₃⁻—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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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 10⁻²M 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 10⁻²M 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 NO₃⁻ — 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