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The Effect of Leaf Age on Leaf Resistance and CO_2 Exchange of the CAM Plant *Bryophyllum fedtschenkoi**

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Summary. Leaves of different ages from the CAM (Crassulacean acid metabolism) plant *Bryophyllum fedtschenkoi* Hamet et Per. differ in their ability to accumulate titratable acidity during the night. Measurements of leaf resistance to water vapour diffusion and net CO_2 exchange during the day and night show differing patterns of behaviour dependent upon leaf age. Young leaves do not exhibit CAM; they behave like typical mesophytes with low resistances and a net uptake of CO_2 during the day and a net output of CO_2 at night. Mature leaves exhibit CAM and have high leaf resistances during the day and lower resistances at night but their pattern of CO_2 exchange is complex, with a net output early in their day followed by a net uptake which continues at a reduced rate through the night. Intermediate leaves are intermediate in their behaviour. The presence of CAM in older leaves may simply be the result of increased cell vacuole size.

Leaf resistance measurements are discussed in relation to the possible control of stomatal opening by substomatal CO_2 concentrations.

Introduction

Leaves of many succulent plants have a high acid content at the end of the night and this acid level is considerably depleted during the subsequent day (Ranson and Thomas, 1960). The accumulation of acid at night is associated with the ability to fix carbon dioxide from the atmosphere non-autotrophically and the enzyme involved in this fixation has been shown to be PEP-carboxylase (Kluge and Osmond, 1972; Sutton and Osmond, 1972). It has been suggested that this crassulacean acid metabolism (CAM) is an adaptation to xerophytic conditions which enables water loss through open stomata to be limited to the night when potential evaporation demand is lowest, water use efficiency thus being increased (Meinzer and Rundel, 1973). Measurements of transpiration and stomatal resistances in CAM plants have shown that there is indeed a reversal of the 'normal' behaviour of stomata with maximum opening during the night (Nashida, 1963), however other measurements have indicated that night opening is not as large as the maximum opening in the light (Ting *et al.*, 1967). These anomalies might be explained by differences in environmental conditions during the pretreatment and measurements as Neales (1973) has suggested that low night temperatures are most conducive to the development of night opening of stomata in CAM plants.

The ability of leaves of CAM plants to accumulate acids at night has been shown to develop as they unfold, increase until they are fully expanded, and

* *Abbreviations*: CAM, Crassulacean acid metabolism; PEP, Phosphoenolpyruvate.

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decrease as they become senescent (Ranson and Thomas, 1960). Recently Lerman *et al.* (1974) have indicated, using isotope discrimination methods, that PEP-carboxylase is more active in mature than young leaves.

This paper describes the diurnal changes in leaf resistance and net CO_2 exchange observed in leaves of different ages from a CAM plant grown under controlled conditions.

Methods

The CAM plant used in this study was a member of the Crassulacae, *Bryophyllum fedtschenkoi*. Cuttings were propagated in the greenhouse and after rooting the plants were maintained on short days (8 h light, 16 h dark) in a growth room where day temperature was 23°C, night temperature 19°C and the light intensity at the top of the plants 29.2 J m^{-2} s^{-1}.

For the purposes of the experiment the leaves of the plant were divided into three 'types': young, middle and mature. Young leaves were the first pair of expanding leaves with an area greater than 2 cm^2. Middle leaves were the second and third pair of leaves below the young leaves, and the mature leaves were all those below, but excluding leaves from the original cutting.

Titratable acidity in leaves was determined using a method similar to that of Milburn *et al.* (1968) which extracts 97% of the total acid content of the plant material. Results were expressed as m.eq. acid (monobasic) per 100 g fresh weight of leaf tissue.

Leaf resistance to diffusion of water vapour (R_L) was measured using a clamp-on diffusion porometer (Ennis Associates, Calif.). The instrument was calibrated under the conditions of the experimental treatment using brass plates, perforated with a series of pores, placed between the porometer cup and wet filter paper (Turner and Palange, 1970). The limit of sensitivity of the porometer was reached at about 80 s cm^{-1} when transit times became excessively long (>2 min). Leaf resistances of the abaxial surface were measured during two full light-dark cycles with at least six measurements being made on each type of leaf at each determination and no single leaf being used more than once. To facilitate a full night of measurements the cycle of light and dark was reversed in the growth room after the day measurements had been made. Four days were allowed to elapse before 'night' measurements commenced under a green safe-light.

Net CO_2 exchange of single leaves was determined in an open circuit incorporating an IRGA (Grubb Parsons, SB2). Leaf chamber temperature was maintained at 22°C with a screen of circulating water and a level of illumination equivalent to that in the growth room (29.2 J m^{-2} s^{-1}) was provided by warm white fluorescent tubes.

Results

The titratable acid content of the three leaf types determined at regular intervals throughout the light-dark cycle (Fig. 1) indicates that the mature leaves have the most marked diurnal variation in acid content. The young leaves showed almost no variation while the middle leaves accumulate an intermediate amount of acid at the end of the night.

Leaf resistance measurements showed some marked differences in leaves of different ages (Fig. 2). In mature leaves the resistances were very high during most of the light period (> 80 s cm^{-1}), however, the lowest resistances recorded for mature leaves (about 14 s cm^{-1}) were found during the first half hour in the light. In the dark, R_L for mature leaves is lower than for most of the time in the light (between 30 and 50 s cm^{-1}). By contrast the young leaves have very low resistances in the light and higher resistances during the dark period, highest R_L being found early in the night. The middle leaves are similar to the young leaves

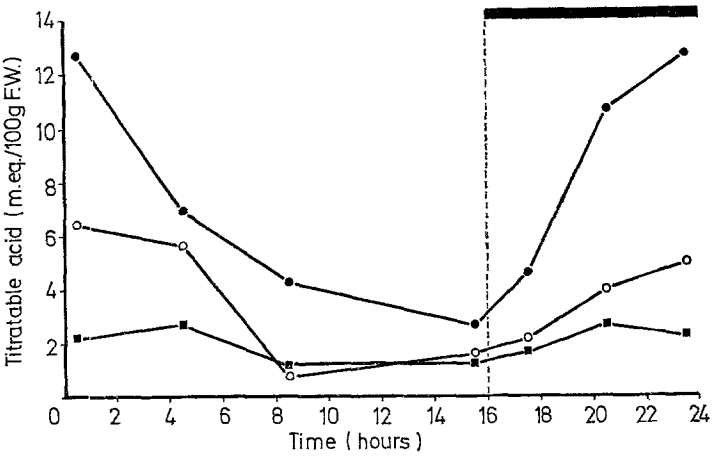

Fig. 1. The diurnal variation in titratable acid content of leaves of different ages from *B. fedtschenkoi*. ■, young leaves; ○, middle leaves; ●, mature leaves. Horizontal black bar indicates time of darkness

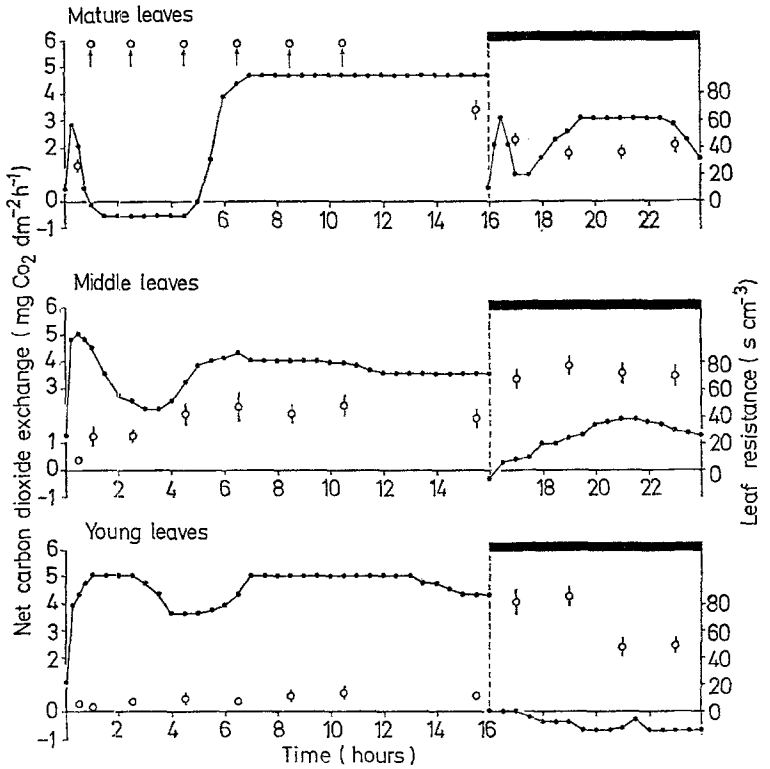

Fig. 2. The diurnal variation in leaf resistance to water vapour diffusion (R_L) (o) and net CO_2 exchange (•—•) of leaves of different ages from *B. fedtschenkoi*. Values of leaf resistance are the means of at least twelve determinations and vertical lines are the standard errors of the means. Conditions during determinations; light intensity 29.2 J m^{-2} s^{-1}, 23°C day, 19°C night. Curves of net CO_2 exchange are typical records for each leaf type. Conditions during determinations; light intensity 29.2 J m^{-2} s^{-1}, constant temperature of 22°C. Horizontal black bar indicates time of darkness

in that resistances are highest in the dark; however, in the light the values of these resistances are considerably higher than those of the young leaves.

The net CO_2 exchange of single leaves from plants similar to those used for resistance measurements was determined throughout a complete light-dark cycle. The plants were maintained at the same light intensity as those in the cabinets but it was not possible to reproduce the different day and night temperatures (23°C day, 19°C night), instead a constant temperature of 22°C was used. Typical records of net CO_2 exchange are presented in Figure 2.

In the light the mature leaves showed an early period when there was a small net output of CO_2, but this later changed to a considerable net uptake of CO_2 which continued for the rest of the day. In darkness there was a continuation of the net uptake of CO_2 but rates were at no time as high as those in the latter part of the light period. In young leaves there was an almost square-wave response typical of most mesophytes, with a net uptake of CO_2 during the day and a net output throughout the night. In the light there was however a characteristic trough in uptake between three and seven hours.

The response of the middle leaves was largely an intermediate one between the young and the mature. In the light there was never a period of net CO_2 output but the trough in uptake in the early part of the day was more pronounced than it was for the young leaves. In darkness, except for the initial few minutes, there was a continued net uptake of CO_2 but the rates were never as high as those for mature leaves in the dark.

Discussion

These results indicate that leaves of the CAM plant *B. fedtschenkoi* show marked differences in both leaf resistance and CO_2 exchange which are closely related to the age of the leaves. Young leaves do not appear to possess the ability to accumulate acids at night and they are similar to 'typical mesophytes' with low leaf resistances and net uptake of CO_2 during the day. As the leaves mature they develop and increase their capacity to accumulate acids at night and associated with this is the net uptake of CO_2 in darkness even though leaf resistances are often high.

There is considerable recent evidence that the development of CAM-type metabolism can be controlled by environmental conditions. Winter (1973a, b) has shown that members of the *Aizoacee* grown in nutrient solutions containing high NaCl concentrations can be induced to develop CAM; and Kluge *et al.* (1973) have shown in *Tillandsia usneoides*, a non-succulent plant, that CAM is enhanced at temperatures between 10 and 20°C and by water stress. However, results presented have demonstrated that in *B. fedtschenkoi* there is a shift with age of tissue from 'typical mesophyte' photosynthesis to CAM which is independent of environmental conditions. Kennedy and Laetsch (1973) have shown that in the C_4 plant *Portulaca oleracea* the stage of leaf development is one of the most important factors determining the operation of particular enzyme systems and they propose that the observed shift toward an increased C_3 photosynthetic activity with increasing leaf age is truly an ontogenetic response and not a function of nutrition, temperature or the flowering status of the plant. It would appear that there is a similar ontogenetic response in the leaves of *B. fedtschenkoi* but

the reason for this response is not clear. Kluge *et al.* (1973) have suggested that the ability of the non-succulent *T. usneoides* to exhibit CAM is associated with the fact that its CO_2-fixing cells possess relatively large vacuoles and are relatively poor in chloroplasts. They therefore suggest that large vacuoles give the tissue a capacity to store acids produced in dark fixation. If this is the case then the ability of older leaves of *B. fedtschenkoi* to exhibit CAM may simply be the result of cell expansion and the development of large vacuoles capable of storing acids.

It can be seen that the mature leaves, which form the majority of the total leaf number on the plant, accumulate large amounts of acid at night through a high rate of CO_2 uptake and their daily cycle of leaf resistance is out of phase with those of the young leaves in that lower resistances are recorded during the night and higher resistances during the day. Now the variable component of leaf resistance is of course the stomatal resistance, and changes in leaf resistance probably reflect changes in stomatal opening. The results presented here show that in a CAM plant different leaves can have differing cycles of stomatal opening. In young leaves there is a behaviour pattern similar to that in non-succulents, but in mature leaves there is a 'night opening' commonly found in other succulent plants.

The question raised is what can cause these different stomatal responses under environmental conditions that are presumably the same for the whole plant. Neales (1973) has proposed that the pattern of nocturnal opening of stomata in CAM plants is caused by the existence of 'dark' CO_2 fixation and it is suggested that the mechanism of this control may be via the internal CO_2 concentration in the substomatal spaces. The lower resistances in mature leaves associated with the 'dark' CO_2 fixation found here would tend to support this theory; however, the ability of some leaves to fix CO_2 in the dark while leaf resistances are in excess of 60 s cm^{-1} suggests that the relationship does not always hold. It should be noted though that the measurement of leaf resistance took place under rather different conditions from those for CO_2 exchange so that the exact relationship between CO_2 exchange and stomatal resistance is clearly not known. Nevertheless, it is interesting to note that CO_2 uptake occurs in both middle and mature leaves while resistances are very high and indeed the highest rates of CO_2 uptake in mature leaves can occur when resistances in excess of 80 s cm^{-1} are recorded.

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