A Field Survey of Respiration Rates in Leaves of Arctic Plants

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ABSTRACT. In a survey of several plant species found at Churchill, Manitoba, in the transition zone between the low and subarctic regions, we measured leaf respiration in terms of total respiration and alternative pathway respiration rates. Leaves of arctic plants exhibit higher rates of total respiration and alternative (cyanide insensitive) respiration than temperate species. There is a negative correlation between plant height and alternative pathway activity. Shorter plants have higher rates of alternative pathway respiration. More alternative pathway activity may mean that there is less energy in the form of ATP available for growth. A shorter growth habit keeps these plants in the still air close to the ground. This prevents cooling, water loss and physical damage due to wind abrasion. Thus plants with high rates of alternative pathway respiration may be better adapted to the arctic environment. The alternative pathway respiration of *Orchis rotundifolia* was shown to be under the influence of the biological clock.

Key words: Subarctic, plant respiration, alternative pathway, cyanide insensitive

RÉSUMÉ. Lors d'une étude sur plusieurs espèces de plantes qui poussent à Churchill, au Manitoba, dans la bande de transition entre l'Arctique inférieur et la zone subarctique, on a mesuré la respiration des feuilles en termes de taux de respiration totale et par voie alterne. Il en ressort que les feuilles des plantes de l'Arctique ont des taux plus élevés de respiration totale et alterne (insensible au cyanure) que les espèces des régions tempérées. Il existe une corrélation négative entre la hauteur des plantes et la respiration par voie alterne. Les plantes plus petites ont des taux de respiration par voie alterne plus élevés. Une plus grande activité par voie alterne pourrait signifier qu'il y a moins d'énergie sous forme d'ATP (adénosine triphosphate) disponible pour la croissance. En raison de leur petite taille, ces plantes se maintiennent dans l'air calme près du sol, ce qui évite le refroidissement, la perte d'eau et les dommages physiques causés par l'érosion éolienne. Les plantes qui ont des taux élevés de respiration par voie alterne sont donc peut-être mieux adaptées à l'environnement arctique. On a trouvé que la respiration par voie alterne de l'*Orchis rotundifolia* était influencée par l'horloge biologique.

Mots clés: subarctique, respiration des plantes, voie alterne, insensible au cyanure

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INTRODUCTION

Churchill (58°45'N, 90°4'W) is situated on Hudson Bay at the mouth of the Churchill River in northeastern Manitoba, Canada. This places it in the transition zone between the low arctic and subarctic regions. A wide variety of plant families, including those with a more temperate distribution (i.e., *Spiranthes Romanzoffiana*, commonly known as ladies' tresses) and those with a more arctic/alpine distribution (i.e., *Gentia propinqua*, commonly known as gentian) occur in the same area. Therefore Churchill provides a good location to survey respiration rates in different species found in the low Arctic.

Respiration is easily measured in terms of oxygen consumed or carbon dioxide released. Arctic plants typically demonstrate very high rates of respiration (Billings and Mooney, 1968; Chabot, 1979; Mawson *et al.*, 1986) both in the field and when grown in controlled conditions of constant light and temperature. When plants grown at low temperature are transferred to a higher temperature, their respiration rates experience a transient increase (Smakman and Hofstra, 1982). The subsequent decline has been interpreted as a manifestation of thermal acclimation. During acclimation the respiration rates are brought under a new control system. Clearly respiration rates are influenced by temperature pretreatment. Recent studies (McNulty and Cummins, 1987) have shown that in *Saxifraga cernua* (an arctic herb commonly known as nodding saxifrage) the magnitude of respiration rate is directly proportional to the length of the photoperiod. This suggests that the accumulation of the products of photosynthesis leads to increased respiration.

The present study was designed to determine the magnitude and range of respiration rates in arctic and subarctic plants in the field. In many species, some respiration can continue in the presence of inhibitors such as cyanide (CN) and antimycin A, which inhibit normal cytochrome respiration. Such CN-insensitive or alternative pathway respiration is itself sensitive to hydroxamic acids (Schonbaum *et al.*, 1971) and tetraethylthiuram disulfide (disulfiram; Grover and Laties, 1981).

Alternative pathway respiration takes electrons from the ubiquinone pool in the mitochondrial electron transport chain (Palmer, 1976; Moore and Rich, 1980; Hemrika-Wagner *et al.*, 1986). No ATP is generated along this alternative pathway and no membrane potential is generated along the inner mitochondrial membrane by it (Moore and Bonner, 1982). Energy usually conserved as ATP through the "normal" cytochrome pathway is instead lost as heat when the alternative pathway is engaged (McCaig and Hill, 1977; Moore and Rich, 1980).

We have recently discovered that *Saxifraga cernua* and other arctic plant species demonstrate relatively large rates of alternative pathway respiration (APR) when grown in growth chambers. In some cases the APR amounted to as much as 70-80% of the total measurable respiration in the leaves of such species. The APR reputed for temperate crop plants such as tomato, wheat and peas amounts to 17, 45 and 2% of total respiration respectively (Lambers *et al.*, 1983). Clearly arctic plants are capable of extraordinarily great APR.

This study was designed to determine the rates of APR in arctic plants at Churchill and to check the results of the controlled environment studies. For the study species were chosen that represent both temperate species at the northern extreme of their range and arctic species near the south of their range of distribution. If one assumes that APR rate represents a loss of potentially useful energy then one would predict that plants with a large APR/TDR (total dark respiration) ratio would grow less rapidly and hence display smaller stature. This prediction was tested.

Rates of leaf respiration in species such as wheat (Azcon-Bieto et al., 1983) vary with leaf sugar levels. Further, certain plant species show higher APR rates when carbohydrate levels are high (Azcon-Bieto et al., 1983; Azcon-Bieto and Osmond, 1983). After a period of photosynthesis one might therefore expect the rates of TDR and APR to be maximal. Arctic plants typically experience periods of long and even constant light during the growing season. Using one species, we conducted a series of experiments to determine the rates of TDR and APR periodically over a 24-hour period. To assess the effect of light exposure on the rates of TDR and APR, half of the plants were covered with blackout curtain in the field during this diurnal period. In this way the role of light and photoperiod in the field could be assessed.

METHODS AND MATERIALS

The site chosen for study was the Churchill Northern Studies Centre near Churchill, in northeastern Manitoba, Canada, situated just off of Hudson Bay. This site was chosen as it lies on the tree line and there is a wide variety of plant species available for testing. Twelve species were chosen for study from around the centre: the biennial herb *Gentia propinqua* (gentian); the perennial herbs *Parnassia palustris* (grass-of-Parnassus), *Spiranthes Romanzoffiana* (ladies'-tresses), *Potentilla multifida* and *Potentilla palustris* (cinquefoils), *Taraxacum lacerum* (dandelion), *Hedysarum Mackenzii* (liquorice-root), *Solidago multiradiata* (goldenrod), *Senecio pauperculus* (groundsel) and *Epilobium angustifolium* (fireweed); the shrubs *Rhododendron lapponicum* (Lapland rose-bay) and *Ribes oxyacanthoides* (wild gooseberry). Plants were identified as in Porsild and Cody (1980). This survey was performed 5-18 August 1985. Respiration measurements were made at midday for each species used. The experiments showing the relationship of respiration to time of day were performed using *Orchis rotundifolia* (orchis) during August 1986.

Within each species, plants used for experimentation were morphologically similar. Average plant heights for each species were measured from the base of the stem to the apex of the plant and ranged from 5 cm for *Rhododendron lapponicum* to 100 cm for *E. angustifolium*.

Rates of TDR, APR and normal cytochrome (cyanide-sensitive) respiration were determined for each species. Leaf material was collected from each plant and tested within 20 minutes of harvest. The lower epidermis was peeled off the 23.8 mm^2 leaf discs, using Millipore MF filter forceps, to facilitate diffusion of gases and inhibitors. Six discs were placed into a darkened 4 ml glass reaction chamber, which was completely filled with 40 mm HEPES (N-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid) buffer (pH 7.2). The medium was agitated at 100 revolutions per minute using a 1 cm teflon-coated magnetic spin bar. Oxygen consumption was measured polarographically using a model 5739 oxygen electrode (YSI Yellow Springs, Ohio). Respiratory measurements were taken at 10 and 15°C for each species. Temperature was maintained by placing the reaction chamber into a small plexiglas water bath. The oxygen electrode was calibrated at both 10 and 15°C, as described by McNulty and Cummins (1987). Briefly, air-saturated tap water was placed in the reaction chamber and the oxygen concentration from air dissolved in water at each temperature was determined from: $O_2 = (pO_2 \alpha) / (760 RT)$, where pO_2 is the partial pressure of oxygen in mmHg; α is the Bunsen coefficient and represents the saturating solubility of oxygen in water (Umbreit et al., 1964).

Initially, the electrode current corresponded to the saturating oxygen concentration at 10 or 15°C. A saturated sodium dithionite solution ($Na_2S_2O_3$) was then injected into the reaction chamber to reduce the dissolved oxygen to water. The resulting electrode current represented an oxygen concentration of zero. In this way the current at each temperature representative of a saturating oxygen concentration was calibrated.

Rates of APR were determined by addition of a small volume of 0.1M SHAM (salicylhydroxamic acid) through a small cannula, with a Hamilton model 801 syringe. The concentration of inhibitor necessary to inhibit APR was determined from an initial dosage curve comparing O_2 consumption rates with SHAM concentration prepared for each species. Rates of APR were defined as that respiration inhibited by SHAM addition. Respiration not inhibited by SHAM was assumed to be attributable to the normal cytochrome pathway. Previous titration experiments with SHAM in the presence and absence of CN as well as experiments with disulfiram (a more specific inhibitor of the alternative pathway) have shown that the contribution to respiration by other cyanide-resistant oxidases is a maximum of 2% (McNulty and Cummins, 1987). Therefore, interpreting inhibition of respiration by SHAM in terms of APR seems justified. Oxygen consumption was expressed as pmoles·O_2 $mm^{-2}·min^{-1}$. TDR and APR were determined at certain times of the day for *Orchis rotundifolia*: dawn (6:00 h), midday (13:00 h), late afternoon (18:00 h) and post dusk (22:00 h). Measurements of *Orchis rotundifolia* were made at 25°C.

All chemicals were obtained from Sigma (St. Louis, Missouri). A water-soluble sodium salt of SHAM was synthesized by titration with NaOH.

RESULTS

Respiration rates increased with increasing temperatures (Table 1). Rates of TDR were highest in leaves of *R. lapponicum* (358 pmol·O_2 $mm^{-2}·min^{-1}$) when measured at 15°C. These are very high when compared with published values of 72 pmol·O_2 $mm^{-2}·min^{-1}$ for spinach leaves (Lambers et al., 1983). The lowest TDR rate of 134 pmol·O_2 $mm^{-2}·min^{-1}$ was measured in leaves of *S. pauperculus* when tested at 10°C. Rates of normal cytochrome (CN-sensitive) pathway respiration were the highest in leaves from *T. lacerum* when measured at 15°C (191 pmol·O_2 $mm^{-2}·min^{-1}$). The lowest rate of normal cytochrome pathway respiration (52 pmol·O_2 $mm^{-2}·min^{-1}$) was observed from leaves of *G. propinqua* when tested at 10°C. The normal cytochrome pathway is more sensitive to measuring temperature than is APR as seen when rates measured at 15°C are compared to those measured at 10°C for the same tissue.

The highest APR rate (228 pmol·O_2 $mm^{-2}·min^{-1}$) was measured from leaves of *R. lapponicum* at 15°C. This represented 68% of the TDR rate. The second highest rate of APR (150 pmol·O_2 $mm^{-2}·min^{-1}$) was measured in leaves from *P. multifida* at 15°C. This represented 61% of the TDR rate. The lowest rate of APR (53 pmol·O_2 $mm^{-2}·min^{-1}$) was measured in leaves of *S. pauperculus* at 10°C.

Table 1 illustrates the fact that some small plants (e.g., *R. lapponicum*) had very high rates of TDR. Respiratory rates can be roughly correlated to plant stature. Figure 1A shows that normal cytochrome respiration shows no clear correlation to plant height. However, when APR rates were graphed vs.

TABLE 1. Rates of TDR, APR and normal cytochrome respiration (pmol·O_2 mm^{-2}·min^{-1}) for different plant species from Churchill, tested at 10 and 15°C[1]

Species	Temperature (°C)	TDR	APR	Normal cytochrome
Rhododendron	15	358(26)	228(28)	130(31)
lapponicum	10	257(15)	183(17)	74(2)
Taraxacum	15	273(19)	81(15)	191(24)
lacerum	10	218(36)	91(52)	127(16)
Solidago	15	257(41)	105(13)	152(40)
multiradiata	10	171(19)	91(27)	80(8)
Potentilla	15	245(15)	150(17)	95(3)
multifida	10	156(21)	98(26)	58(5)
Gentia	15	242(32)	142(38)	100(6)
propinqua	10	147(16)	96(23)	52(7)
Senecio	15	230(28)	87(8)	144(32)
pauperculus	10	134(12)	53(14)	81(2)
Ribes	15	215(20)	100(20)	115(4)
oxyacanthoides	10	179(13)	93(25)	86(12)
Spiranthes	15	215(20)	102(15)	113(8)
Romanzoffiana	10	199(25)	107(39)	92(14)
Epilobium	15	215(20)	78(6)	137(15)
angustifolium	10	170(14)	65(27)	105(14)
Hedysarum	15	190(9)	97(8)	93(3)
Mackenzii	10	152(15)	69(24)	83(9)
Parnassia	15	171(15)	108(12)	63(11)
palustris	10	153(12)	100(15)	53(2)
Potentilla	15	166(13)	58(7)	108(8)
palustris	10	145(9)	56(10)	90(1)

[1]Standard deviation values, where n was at least 3, are indicated in brackets.

height of the plant (Fig. 1B) there was a negative correlation. It is apparent that the APR/TDR ratio decreases with increasing height (Fig. 2). This trend was observed when rates were measured at both 15°C (Fig. 2A) and 10°C (Fig. 2B).

Figure 3 shows the rates of respiration measured at various times of the day in discs derived from either plants covered with blackout curtain or uncovered plants. Most of the large midday increase in respiration can be accounted for by the increase in APR. Surprisingly, the APR, and consequently TDR, rates also increased in tissues that had not been able to photosynthesize since the previous day.

DISCUSSION

Rates of TDR in the species tested in the field are similar to those rates we have measured for the arctic herb *Saxifraga cernua* grown in controlled environment chambers. These rates are much higher than those found in temperate species (Lambers *et al.*, 1983). As well, high rates of APR were observed in all species tested. Plant stature is apparently correlated with APR. Shorter plants display more APR. In APR 67% of the energy normally conserved as ATP through normal cytochrome pathway respiration is lost as heat and is therefore not available for growth. This may account, at least partially, for the low-growth habit observed in many arctic species. This is consistent with Day *et al.* (1985), who found that increased biomass production in *Lolium perenne* was associated with lower rates of mature leaf respiration.

The heat produced by APR can be computed from the APR rates in leaves of *Saxifraga cernua*. When the steady state leaf temperature increase caused by this heat production was calculated using a simple leaf energy budget, it was discovered that APR could account for an increase in leaf temperature of no greater than 0.02°C (McNulty and Cummins, 1987). In this case, at least, it seems doubtful that APR functions to significantly alter the temperature of the leaf tissue. A reasonable physiological role for this process must be sought elsewhere.

Stunting may be beneficial to arctic plants, protecting them from exposure to wind. High winds often occur during the growing season. In August 1986 peak NW winds of 76 km·h^{-1} were recorded twice at Churchill (Environment Canada, 1986). Shorter plants would be better protected from cooling, water loss and physical damage due to abrasion associated with strong winds.

The amount of daylight, and hence photosynthesis, has been shown to affect the rate of APR (Azcon-Bieto *et al.*, 1983). Rates of TDR have been shown to decline during the dark period and to be related to the rate of net photosynthesis that occurred during the previous light period (Ludwig *et al.*, 1975; Pearson, 1979; Azcon-Bieto and Osmond, 1983). Therefore the long photoperiods to which arctic plants are typically exposed may influence the amount of TDR and APR. It is as if there is a point when enough photosynthate is conserved for growth or storage and any more has to be discarded via the process of APR (Lambers, 1982).

Our experiments with *Orchis rotundifolia* grown at Churchill suggest that APR may indeed be under the control of the biological clock. This has subsequently been confirmed through experiments with *S. cernua* grown in an environmental chamber where temperature and photoperiod were rigorously controlled. In *Orchis rotundifolia*, the large midday increase in TDR may be mostly accounted for by an increase in APR that occurs at midday, even if the plants are kept in the dark. Clearly more experiments of this type are called for in order to explore the relationship of APR to circadian rhythms.
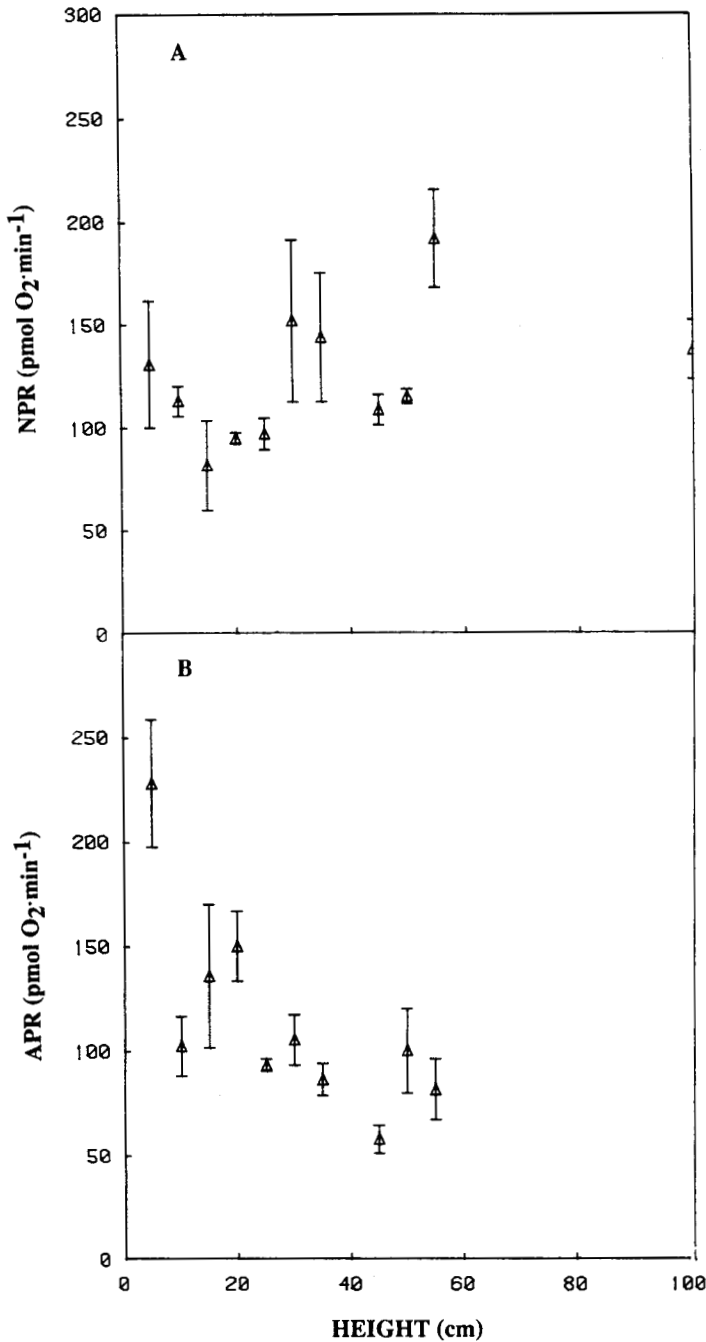
FIG. 1. The rates of A, normal cytochrome pathway respiration (NPR), and B, alternative pathway (CN-insensitive) respiration (APR), measured at 15°C vs. plant height for the 12 species tested in Table 1. Vertical bars represent standard deviation where n was at least 3.
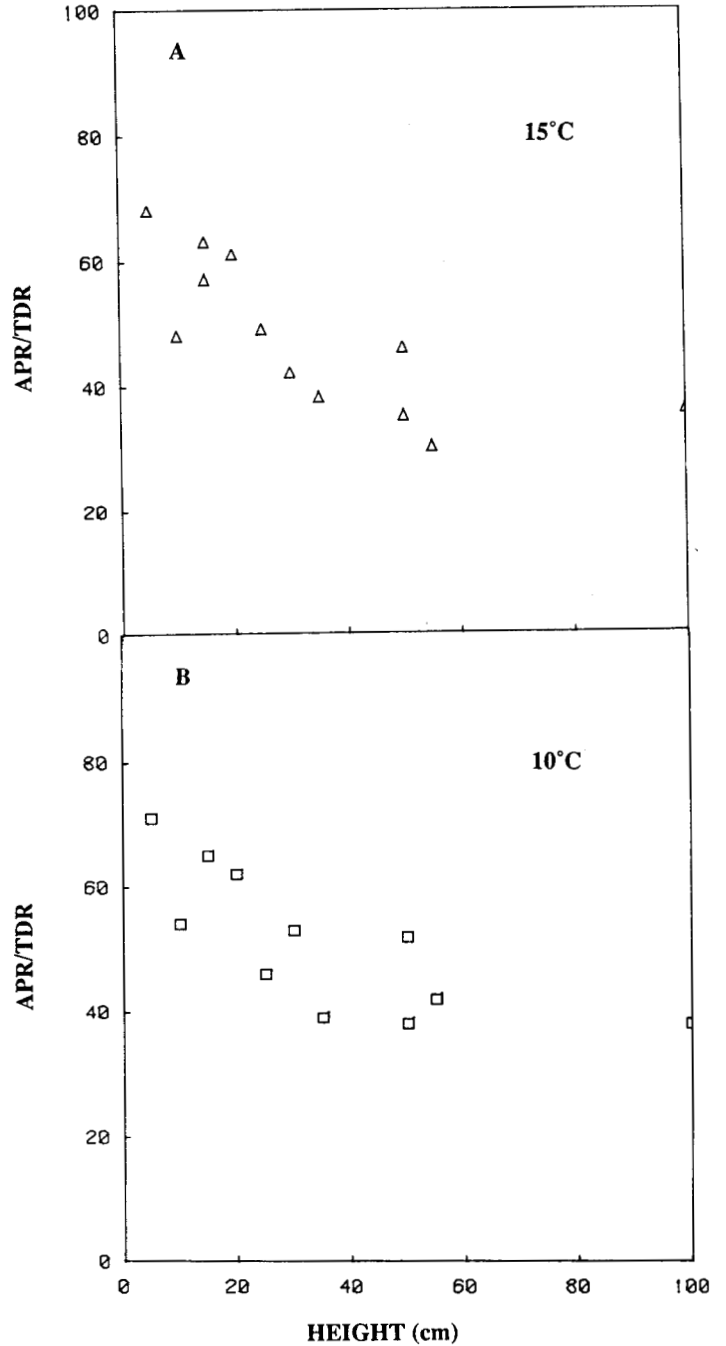

FIG. 2. The ratio of APR/TDR (expressed as percentage) measured at A, 15°C, and B, 10°C, vs. plant height for the 12 species tested in Table 1.

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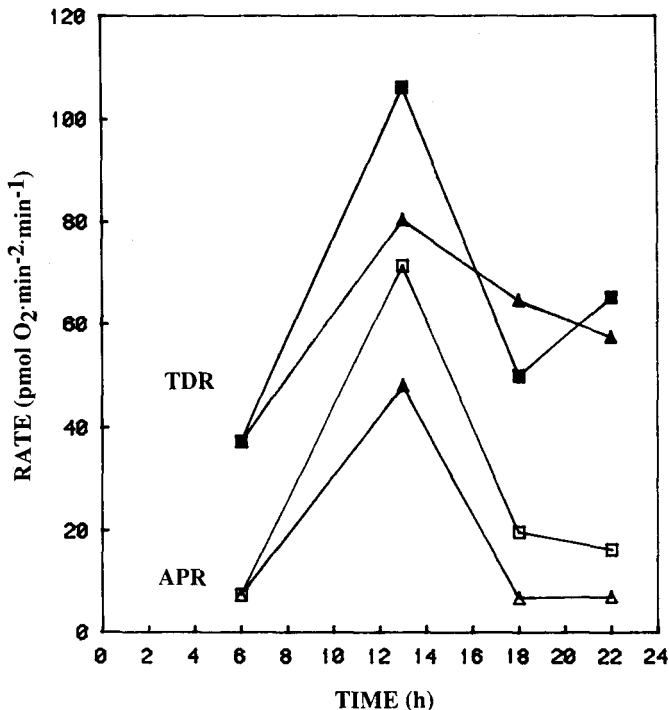
FIG. 3. Respiration rates measured in leaf discs from *Orchis rotundifolia* over a day on 11 August 1986. Closed symbols represent rates of total dark respiration (TDR), while open symbols represent the rates of alternative pathway respiration (APR). Squares represent the rates measured in discs taken directly from plants in the open field. Triangles represent discs taken from neighbouring plants that had been kept from all sunlight since dusk the night before. Each point represents the mean of three trials.

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