High Productivity and Photosynthetic Flexibility in a CAM Plant

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Summary. In the annual succulent *Mesembryanthemum crystallinum* growing *in situ*, the balance between C_3 and CAM carbon fixation shifted rapidly in response to changes in water availability. When water was plentiful, *M. crystallinum* fixed carbon dioxide by the C_3 pathway and grew at rates comparable to other C_3 species. Under drought conditions, *M. crystallinum* fixed carbon by the CAM pathway at an average rate which exceeded 1 nanomole of carbon dioxide per square centimeter of leaf surface per second, a very high rate for a CAM plant.

Introduction

Crassulacean Acid Metabolism (CAM) — the process by which many succulent plants fix external carbon dioxide to form organic acids in the dark and refix internally generated carbon dioxide to carbohydrates in the light — is generally considered to be an adaptation to arid environments which achieves water conservation at the expense of slow growth. The transpiration ratio (g H_2O transpired/g CO_2 assimilated) of plants using CAM ranges from 25 to 125 whereas that of C_3 and C_4 plants ranges from 450 to 600 and 250 to 350 respectively (Szarek and Ting, 1975). By this measure, CAM plants are at least two times more efficient in their water use than are C_3 or C_4 plants. The maximum carbon fixation rates of C_3 and C_4 plants, however, are typically several times faster than those of CAM plants: C_3 rates vary between 1 and 6 nMoles $CO_2 \, cm^{-2} \, s^{-1}$, C_4 between 2 and 5, and CAM between 0.1 and 0.8 (Black, 1973; Mooney et al., 1976).

Mesembryanthemum crystallinum is an annual CAM plant which has spread rapidly along the coast of California (Moran, 1950; Philbrick, 1972). In the laboratory studies of Winter (Winter, 1974a, b) this species rapidly shifted the balance between C_3 and CAM carbon fixation as water stress varied: C_3

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was the predominant method of carbon assimilation when water stress was low, but CAM assimilation increased as water stress increased. The maximum carbon fixation rates measured in these studies were 0.47 nMole CO_2 cm^{-2} s^{-1} for C_3 and 0.13 nMole CO_2 cm^{-2} s^{-1} for CAM (Winter, 1974b). Such low rates seem inconsistent with *M. crystallinum*'s success in Mediterranean climates. The present study examined whether or not *M. crystallinum* growing under natural conditions exhibited rapid growth rates as well as a flexible carbon fixation response to water stress.

Materials and Methods

A small population of *M. crystallinum* grows along a raised path which borders the salt marsh formed by San Francisco Bay at Palo Alto, California. *Atripex semibaccata, Mesembryanthemum nodiflorum*, and the Eurasian grasses, *Hordeum vulgara, Lolium multiflorum, Bromus rigidus*, and *Bromus mollis*, are the other major species at this site. The present study was conducted entirely on one patch of *M. crystallinum* 3 m × 1.5 m. Such a small sample site was chosen to reduce microclimate variation.

During the 1975 growing season (February through July), measurements were made on one age class of *M. crystallinum* which had germinated in mid-January (Age Class 1) and on another which had germinated in mid-February (Age Class 2). From February to May, both age classes grew as low-lying rosettes which had two major leaf pairs set perpendicularly. The plants assumed at the end of May a reproductive growth form with many branches bearing comparatively small leaves. Flowering occurred from the middle of June through July. By August, most of the plants had set seed and died.

On each sampling date, three soil cores from the soil surface to 7 cm deep were taken at random points in the plot and sealed in cans. Three plants from one age class and three from the other, all of which had been marked the previous sampling date, were photographed against graph paper to determine plant surface area. Six neighboring plants, similar in size and stage of development to the first six, were photographed for plant area and marked for the next sampling date. At dawn, the first six plants were sampled. Discs of known area were punched from a leaf of both major leaf pairs for the Age Class 1 plants and from a leaf of the older leaf pair for the Age Class 2 plants. These discs were either kept on dry ice or sealed in cans. Samples from the opposite leaves of the same plants were taken again at dusk and kept on dry ice.

The soil samples were weighed, dried for 72 h at 105° C, and reweighed to calculate soil moisture in percent dry weight. The leaf samples which were kept on dry ice were weighed, ground with a mortar and pestle, boiled in 15 ml of water for 3 minutes, and titrated to an end point of pH 6.4 with 0.01 normal sodium hydroxide to determine both fresh weight per unit area and titratable acidity per unit fresh weight. In *M. crystallinum*, the change in titratable acidity to a pH of 6.4 results from the accumulation of malate at night and the breakdown of malate during the day by the CAM process (Winter and Lüttge, 1976); therefore, the change in titratable acidity is an accurate measure of the amount of CO_2 fixed by CAM. The leaf samples which were sealed in cans at dawn were dried for 72 hours at 70° C and weighed to calculate plant dry weight per leaf area. The dry plant samples were then analyzed for carbon isotope ratio ($^{13}C/^{12}C$) with a mass spectrometer (Troughton et al., 1974).

The relative growth rates for plants with the rosette growth form were determined using the change in plant area from one sampling date to the next and the conversion factor between leaf area and dry weight. Precipitation was measured at the Palo Alto Weather Station located 3 km from the field site.

Plants for CO_2 and H_2O exchange analysis were excavated with the surrounding soil on March 29 and June 6. The plants were enclosed in a cuvette identical to that pictured in Bartholomew (1973) and were monitored with a CO_2 and H_2O exchange system similar to that described in Björkman et al. (1973). For the measurements of the dark carbon fixation rates, the temperature and humidity of the chamber were changed every hour to match the temperature and humidity
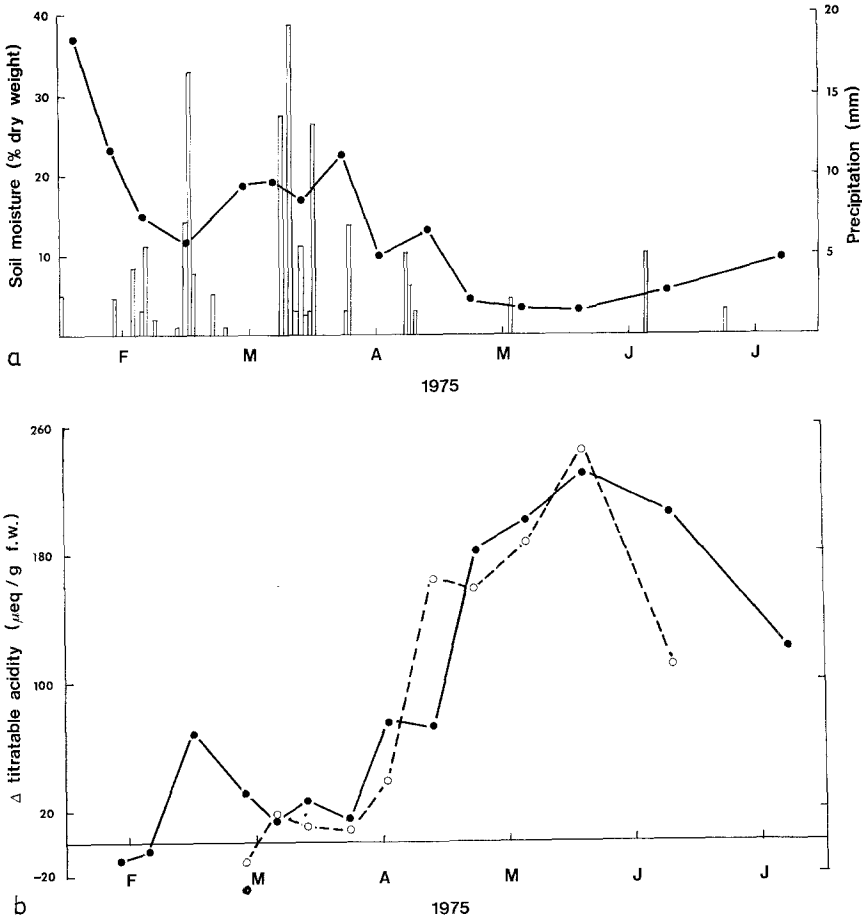
Fig. 1a. Seasonal course of precipitation (*bars*) measured 3 km away from the field site and of soil moisture (*dots* and *lines*) at the site. **b** Seasonal course of the change in titratable acidity for an age class germinating in mid-January (*closed dots*) and another germinating in mid-February (*open dots*). Each value is the mean of three plants except the July value for which there was only one plant

at the field site measured the previous night. For the measurements of the C_3 fixation rates, the cuvette temperature and dew point were maintained at 25° and 20° C, respectively, and photosynthetically active radiation was set at $190 \, nE \, cm^{-2} \, s^{-1}$, and the CO_2 concentration was varied to determine the CO_2 compensation point and the fixation rate at ambient CO_2 concentrations.

Results

Over the course of the growing season, soil moisture fluctuated with sporadic rainfall (Fig. 1a). The increase in soil moisture at the end of the season resulted from the summer's heavy early morning fog. The change in titratable acidity for both age classes reflected the fluctuations in soil moisture during this period
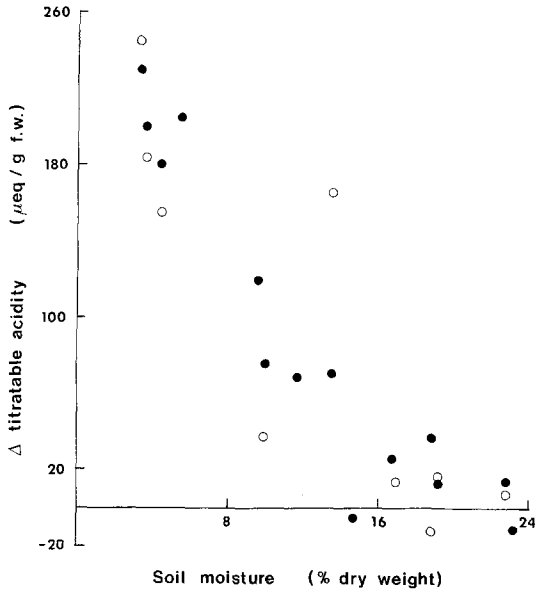
Fig. 2. The change in titratable acidity as a function of soil moisture for an age class which germinated in mid-January (*closed dots*) and another which germinated in mid-February (*open dots*)

Table 1. Seasonal course of relative growth rate (mean of 3 plants ± standard deviation) and carbon isotope ratio for *M. crystallinum* which germinated in January (Age Class 1) and in February (Age Class 2)

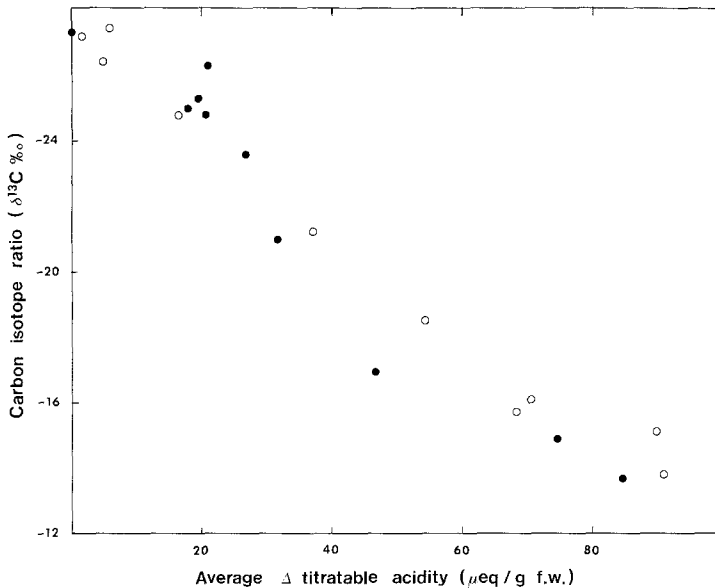| Sample date	Age class	R.G.R. (g/g/day)	$\delta^{13}C$ ($^0/_{00}$)
2/19/75	1	0.074 ± 0.067	−27.3
3/1/75	1	0.016 ± 0.027	−25.0
3/11/75	1	0.122 ± 0.045	−26.3
	2	−	−24.2
3/19/75	1	0.102 ± 0.003	−25.3
	2	0.053 ± 0.064	−27.2
3/27/75	1	0.112 ± 0.056	−24.8
	2	0.073 ± 0.072	−26.4
4/6/75	1	0.036 ± 0.024	−24.6
	2	0.012 ± 0.021	−27.4
4/16/75	1	0.111 ± 0.016	−23.6
	2	0.094 ± 0.033	−24.8
4/26/75	1	0.044 ± 0.019	−21.0
	2	0.039 ± 0.038	−21.2
5/7/75	1	0.065 ± 0.015	−16.4
	2	0.006 ± 0.011	−18.5
5/19/75	1	0.031 ± 0.028	−
	2	0.053 ± 0.031	−16.1
6/3/75	1	−	−14.9
	2	−	−15.1
6/23/75	1	−	−13.7
	2	−	−13.8

Fig. 3. The carbon isotope ratio at different times in the season as a function of change in titratable acidity for an age class averaged from the first sampling until that particular sampling. *Closed dots* are values for samples from the older age class; *open dots* are values for samples from the younger

(Fig. 1 b). Figure 2, a plot of the change in titratable acidity versus soil moisture, points out the strong relationship between these variables ($r = -0.95$, $P < 0.001$ for Age Class 1, $r = -0.83$, $P < 0.01$ for Age Class 2).

The relative growth rates (R.G.R.'s) of *M. crystallinum* were remarkably uniform while the plants remained in their vegetative growth form (Table 1). This accounts for the non-significant ($P > 0.05$) correlation between R.G.R. and soil moisture or change in titratable acidity for both age classes. The seasonal average (R.G.R. ± standard deviation) was 0.071 ± 0.039 g/g/day for Age Class 1 and 0.047 ± 0.031 g/g/day for Age Class 2. The maximum R.G.R. found during this study was 0.17 g/g/dday for an Age Class 1 plant between March 19 and 27. The change in titratable acidity of this plant at that time was only 10 microequivalents per gram fresh weight.

The carbon isotope ratios ranged from $-27.4^0/_{00}$ in the beginning of the season to $-13.7^0/_{00}$ at the end (Table 1). Since a CAM plant's carbon isotope ratio indicates its relative dependence on CAM fixation, the carbon isotope ratio of an *M. crystallinum* sample collected on a given date is plotted in Fig. 3 against the change in titratable acidity of its age class averaged from the value measured on the first sampling date to the value measured on that particular date. Figure 3 reveals the strong correlation ($r = -0.98$, $P < 0.001$) between the carbon isotope ratio and the average change in titratable acidity.

The CO_2 and H_2O exchange analysis of the plants collected from the field showed a shift from C_3 carbon assimilation to CAM. The March 29 plant had no positive net CO_2 uptake in the dark and no detectable change in titratable

acidity. In the light, it used only C_3 fixation as indicated by a CO_2 compensation point of 50 μbars and had a rate of 2.0 nMoles cm^{-2} s^{-1} at the normal atmospheric CO_2 concentration of 320 μbars. The June 6 plant had a maximum dark fixation rate of 1.3 nMoles cm^{-2} s^{-1}, a nightly average of 0.8 nMoles cm^{-2} s^{-1}, and an acid accumulation of 358 μeq/g f.w. in the leaves, but had no positive net CO_2 uptake in the light.

Discussion

M. crystallinum growing in situ by San Francisco Bay demonstrated a flexible carbon fixation response which seemed independent of plant age. In two age classes which germinated a month apart, the amount of CAM fixation—as indicated by diurnal acid fluctuations—varied inversely with water availability throughout the growing season and could double or halve within a ten day period (Figs. 1 b and 2). These results suggest that *M. crystallinum* used CAM in proportion to the extent that it was water-stressed.

The carbon isotope ratios ($^{13}C/^{12}C$) of *M. crystallinum* sampled during the season (Table 1) provide an assessment of the balance between C_3 and CAM fixation. RuBP carboxylase discriminates against ^{13}C to a greater extent than PEP carboxylase; consequently, C_3 plants which use only RuBP carboxylase have a lower isotope ratio with an average of $-27^o/_{oo}$ than plants fixing CO_2 by CAM alone or by C_4 with an average of $-13^o/_{oo}$ (Lerman, 1975). In *M. crystallinum*, the low carbon isotope ratios (ca. $-27^o/_{oo}$) at the beginning of the season and the high ratios (ca. $-14^o/_{oo}$) at the end, show that this species went from fixing all its carbon by the C_3 pathway to fixing it exclusively by the CAM pathway. Since the average change in titratable acidity is directly related to the average amount of CO_2 that a plant has fixed by CAM, the linear relationship between the carbon isotope ratio and the average change in titratable acidity (Fig. 3) implies that the amount of CAM carbon fixation in *M. crystallinum* was inversely proportional to the amount of C_3 carbon fixation.

The levels of titratable acidity reached by *M. crystallinum* are exceptionally high. On a sample date early in June, the average change in titratable acidity for all six plants was 237 microequivalents of acid/g fresh weight (Fig. 1b). On separate occasions, three different plants had values exceeding 350 microequivalents/g fresh weight. The value for the plant collected on June 6 was 358 μeq/g fresh weight. Such an accumulation of acid is roughly equivalent to a gross carbon fixation rate of 1.1 nMole CO_2 $cm^{-2} s^{-1}$:

[(358 μeq/g f.w.) (500 nMoles malate/μeq/g) (1 nMoles CO_2 fixed/nMole malate) (0.2 g f.w./cm^2)(1/3.4 × 10^4 s)] = 1.1 nMole CO_2 cm^{-2} s^{-1}.

The assumption that dark respiration was 30% of gross dark fixation as in *Kalanchoë daigremontiana* (Kaplan et al., 1976) leads to a net fixation rate of 0.8 nMole CO_2 cm^{-2} s^{-1}. The CO_2 exchange measurements on this same plant yielded a maximum net CAM fixation rate of 1.3 nMole CO_2 cm^{-2} s^{-1}

and a nightly average of 0.8 nMole CO_2 cm^{-2} s^{-1} and, thus, fully support the estimate from diurnal acid fluctuations. To compare with other CAM species, the highest previously reported maximum and nightly average rates of CAM fixation are 0.74 and 0.56 nMole CO_2 cm^{-2} s^{-1}, respectively, for *Agave* (Neales et al., 1968).

The relative growth rate (R.G.R.) of *M. crystallinum* was also very high with a maximum of 0.17 g/g/day and seasonal averages of 0.071 and 0.047 for Age Classes 1 and 2, respectively. Accounting for the changes in dry weight attributable to salt (Bloom, 1979) and using the conversion factor between CO_2 fixed and saccharides formed which is given in Sestak et al. (1971), the maximum R.G.R. implies a carbon fixation rate of 1.3 nMoles CO_2 cm^{-2} s^{-1}:

$$[(0.17 \text{ g/g/day}) (1 \text{ g d.w. plant material} - 0.1 \text{ g d.w. salt})$$
$$(9.9 \times 10^{-3} \text{ g d.w./cm}^2) (1.54 \text{ g } CO_2 \text{ fixed/g saccharide formed})$$
$$(2.27 \times 10^7 \text{ nMole } CO_2/\text{g}) (1/4 \times 10^4 \text{ s})] = 1.3 \text{ nMole } CO_2 \text{ cm}^{-2} \text{ s}^{-1}.$$

The maximum R.G.R. was achieved in a plant which was using very little CAM fixation and, thus, presumably using C_3 fixation. Consistent with this estimate, the C_3 fixation rate of *M. crystallinum* measured by gas exchange on March 29 was 2.0 nMoles cm^{-2} s^{-1}. For contrast, *Avena fatua, Bromus mollis,* and *Lolium multiflorum*, three Eurasian grasses with which *M. crystallinum* competes, were found at a site 7 km away to have maximum R.G.R.'s of 0.077 g/g/day and seasonal averages of up to 0.025 g/g/day during the two previous growing seasons (Gulmon, 1974). The maximum carbon fixation rates for greenhouse grown *Bromus mollis* was 1.6 nMoles cm^{-2} s^{-1} (Turitzin, 1976).

These data suggest that *M. crystallinum* has rapidly replaced the Eurasian grasses in many areas of coastal California (Moran, 1950; Philbrick, 1972) because it has a higher productivity. Yet *M. crystallinum* cannot invade undisturbed grasslands (Vivrette and Muller, 1977). Differences in the photosynthetic light response of *M. crystallinum* and the grasses explain this apparent contradiction. My experiences and those of N. Vivrette (personal communication, 1977) indicate that *M. crystallinum* grows poorly under low light conditions. Turitzin (1976) found that greenhouse grown *Bromus mollis* had a light compensation point of 1 nE cm^{-2} s^{-1} and a light saturation point of 60 nE cm^{-2} s^{-1}; preliminary measurements on greenhouse grown *M. crystallinum* showed the much higher light compensation and saturation points of 35 and 120 nE cm^{-2} s^{-1}, respectively (Bloom, 1979). Thus, *M. crystallinum* barely has a positive CO_2 uptake rate at the light intensity which supports *Bromus mollis'* maximum rate. Although *M. crystallinum* has a higher maximum growth rate than the competing grasses, the grasses would have a higher growth rate in a light limited environment. Dissimilar growth forms accentuate the differences in light response: the grasses with their erect form can easily shade *M. crystallinum* with its prostrate growth form. In dense grasslands, *M. crystallinum* never receives enough light to support rapid growth. Only in disturbed areas of the grassland where light is abundant can *M. crystallinum* become established.

M. crystallinum is a strong competitor in Mediterranean climates because of its high capacity for both C_3 and CAM fixation and its flexibility to adjust

quickly the balance between those two pathways to changes in water availability. Winters with sporadic, unpredictable precipitation and summers with predictable drought are characteristic of Mediterranean climates. *M. crystallinum*, after it germinates with heavy winter rains, fixes CO_2 solely by the C_3 pathway and achieves growth rates at least equal to the competing grasses. As the soil dries out with the onset of summer or with mid-winter drought, such as that which occurred during the 1975 season, *M. crystallinum* progressively employs more CAM fixation and less C_3 fixation. Under dry conditions which the competing grasses cannot survive, it exclusively uses CAM fixation at rates which are lower than the rates attained when it used C_3 fixation but which are still comparable to the rates of many C_3 plants. Thus, the lifetime productivity of *M. crystallinum* can be very high.

Recently, Winter (Winter et al., 1978; Winter and Troughton, 1978) reported on *M. crystallinum* growing along the coast of Isreal. He found that the plants also shifted from predominately C_3 fixation at the beginning of the growing season to CAM fixation at the end. He could not exclude the possibility, however, that the shift might have resulted from the plants reaching a particular stage of development. This possibility seems unlikely in view of the present study. At the California site, the diurnal acid fluctuations of the older age class increased from -4.5 to $69.1 \, \mu eq/g$ fresh weight during the drought in February, 1975, and decreased to $11.6 \, \mu eq/g$ fresh weight with the rains in March. Even the younger age class which germinated in mid-February showed a similar increase and decrease in acid fluctuations over this period (Fig. 1b). The shift back and forth between the two carbon fixation methods which occurred in both age classes indicates that developmental stage was unimportant.

The diurnal acid fluctuations in *M. crystallinum* at the California site were much larger than in the plants at the site in Israel (Winter et al., 1978). The largest values at the California site occurred in June, 1975. Measurements of acid fluctuations at the Israel site terminated in May, 1977. Perhaps, if the study in Israel had been continued into summer when the plants experienced more extreme water stress, higher levels of acidification might have been observed.

In other aspects, the two studies agree closely. The average R.G.R.'s ranged from 0.12 to 0.01 g/g/day in California and from 0.11 to 0.03 g/g/day in Israel. The carbon isotope ratios increased from -27 to $-14^0/_{00}$ in California and from -26 to $-16^0/_{00}$ in Israel. That two independent studies conducted on widely separated populations obtained similar results provides convincing evidence for the natural occurrence of a shift from C_3 to CAM in *M. crystallinum*.

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