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Leaf Thickness and Carbon Isotope Composition in the Crassulaceae

J.A. Teeri, S.J. Tonsor and M. Turner
Barnes Laboratory, 5630 South Ingleside Avenue, The University of Chicago, Chicago, Illinois 60637 USA

Summary. Measurements of leaf thickness and $\delta^{13}C$ value were obtained for twenty species and three intergeneric hybrids of the Crassulaceae. The data include plants growing in their native habitats and also in greenhouse cultivation. There is a strong relationship between leaf thickness and leaf $\delta^{13}C$ values. The plants with the thickest leaves of ca. 7 to 11 mm had $\delta^{13}C$ values ranging from $-11.5‰$ to $-13.8‰$. Plants with leaves that were thinner than 2.0 mm all had $\delta^{13}C$ values that were more negative than $-23‰$. Plants having intermediate leaf thickness possessed intermediate $\delta^{13}C$ values. The leaf tissue of four genotypes spanning the range of leaf thicknesses all exhibited a two-fold or greater nocturnal increase in titratable acidity. It appears that the differences in leaf thickness and $\delta^{13}C$ values among the tested species are genetically determined.

Introduction

Among the species capable of Crassulacean acid metabolism (CAM) there is a wide range of degrees of succulence associated with the photosynthetic organs. At the cellular level, most CAM plants have the capability to store quantities of malic acid in large vacuoles, resulting in a large volume of water being present in the photosynthetic tissue. Such tissue has a large volume to surface area ratio resulting in the morphology termed succulent and many definitions of succulence have been proposed (Kluge and Ting 1978) at the organ, tissue and cellular levels.

The relationship between succulence of CAM photosynthetic tissue and the proportion of day versus night CO_2 fixation is not well understood. There are many species that have been classified as succulent that have been demonstrated to fix CO_2 in the dark via CAM (Kluge and King 1978). Some succulent CAM species which exhibit dark-fixation of CO_2, such as in the genera *Hoya, Euphorbia* and *Sedum* (Kluge and Ting 1978; Teeri in prep.) are related to species that are classified as non-succulent and which do not exhibit CO_2 fixation in the dark. Finally, some CAM species have been classified as having dimorphic photosynthetic organs and dark fixation has been reported in the succulent organs and only daytime (C_3) fixation reported for the non-succulent organs (Kluge and Ting 1978). As indicated above, there is in fact a wide range of degrees of succulence among CAM plants, and it is likely that the widely-used two-parted classification of succulent versus non-succulent is an oversimplification.

The family Crassulaceae is made up of a number of genera and species that exhibit a wide range of degrees of leaf succulence. In addition there is evidence, based on variability in leaf $\delta^{13}C$ values, that suggests there may be considerable variability among these taxa in the relative amount of light and dark fixation of CO_2 that occurs during growth (Knopf and Kluge 1978; Teeri in prep.). The goal of this study was to determine if a relationship exists between the degree of leaf succulence and the leaf $\delta^{13}C$ value of sixteen species of the Crassulaceae possessing a range of leaf morphologies in their native habitats. In addition, the leaf succulence and leaf $\delta^{13}C$ values of eight species and three hybrids were compared for plants grown together in greenhouse cultivation.

Methods

We have used leaf thickness as an index of succulence because of the availability of field information for this character. Leaf thickness and $\delta^{13}C$ values were obtained for plants both growing in their natural habitats and in greenhouse cultivation. Because drying results in large changes in the morphology of the succulent leaves of many of these species (Clausen 1959), it was necessary to obtain a measure of succulence of the living plant growing in its native habitat. Clausen (1959, 1975) has published such data on the thickness of leaves of many species of the Crassulaceae in their natural environments. Leaf thickness refers to the thickness between the adaxial and abaxial surfaces of a fully developed fresh leaf and was measured as described by Clausen (1959), except that for plants grown in greenhouse cultivation leaf thickness was measured to the nearest 0.5 mm. For sixteen species growing under natural conditions, mean leaf thickness data were calculated from the published data of Clausen for live plants in the field (1959, 1975). For each species the mean and standard deviation of leaf thickness are included in Table 1, for additional information on sampling methods see Clausen (1959, 1975). A single $\delta^{13}C$ value was determined for fully developed leaves obtained from a herbarium specimen of each species at the herbarium of the Field Museum of Natural History, Chicago, Illinois. The data are presented in Table 1. The second method of data collection involved measurements made on plants growing in the greenhouses at Cornell Universtiy, Ithaca, New York. Dr. C. Uhl has grown plants of the eight sample species and three hybrids in greenhouse cultivation for a number of years. On each of these plants a leaf thickness measurement and a $\delta^{13}C$ determination were made on the same fully developed leaf. The data are presented in Table 2. In spite of the different sources of data used in this study, the agreement among the data is very strong, as is evident when both the field and greenhouse values of all twenty species and the three hybrids are plotted together in Fig. 1. In some species large differences in leaf thickness have been reported between populations growing in the field (Clausen 1959, 1975). All such species were excluded from the analysis of herbarium data, as it was not possible to know which thickness morph was represented by the herbarium specimen.

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Table 1. Mean leaf thickness and $\delta^{13}C$ values of sixteen species of the Crassulaceae growing in their native habitats

Species	Mean leaf thickness) (mm)	Leaf $\delta^{13}C$ (‰)
Sedum botterii	2.5 (0.4)[a]	−24.1
S. tortuosum	1.1 (0.2)	−26.3
S. dendroideum	4.0 −	−15.7
S. oxypetalum	0.9 (0.1)	−24.3
S. obcordatum	2.0 (0.4)	−23.1
S. stahlii	4.9 (0.7)	−13.1
S. longipes	1.4 (0.5)	−24.9
S. ebracteatum	2.7 (0)	−16.1
S. greggii	1.4 (0.2)	−25.0
S. moranense	0.6 −	−24.3
S. telephioides	1.4 (0.4)	−25.2
S. nevii	1.1 (0.2)	−27.8
S. wrightii	2.3 (0.7)	−22.6
Cremnophila nutans	11.0 (1.9)	−13.8
Lenophyllum texanum	3.0 (1.0)	−13.3
Parvisedum pumilum	1.3 (0.1)	−25.4

[a] One standard deviation

Table 2. Individual leaf thickness and $\delta^{13}C$ values of plants growing in greenhouse cultivation

Species	Leaf thickness (mm)	$\delta^{13}C$ (‰)
Sedum dendroideum	4.5	−13.7
S. praealtum	3.5	−16.2
S. stahlii	5.0	−14.7
S. greggii	1.5	−25.1
S. pachyphyllum	7.5	−11.5
S. rubrotinctum	7.0	−11.6
Cremnophila nutans	9.5	−12.9
C. nutans	9.0	−12.4
C. linguifolia	9.0	−13.2
S. greggii × *C. linguifolia*	4.0	−18.5
C. nutans × *S. praealtum*	6.0	−15.6
C. nutans × *S. stahlii*	7.5	−13.2

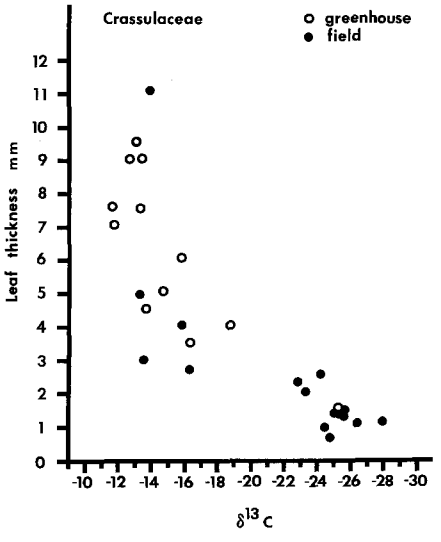

Fig. 1. The relationship between leaf thickness and $\delta^{13}C$ value of the twenty species and three intergeneric hybrids of the Crassulaceae reported in Tables 1 and 2

Table 3. Titratable acidity of three species and one hybrid of the Crassulaceae growing in greenhouse cultivation

Species	Titratable acidity μ equiv. g^{-1} fwt.	
	evening	morning
Sedum greggii	14±1*W*	28±3
Cremnophila linguifolia	12±1	52±2
S. greggii × *C. linguifolia*	13±2	54±5
Sedum rubrotinctum	31±4	120±2

One standard deviation

For $\delta^{13}C$ determination the sample consisted of mature leaves of the current year from each plant. All plant samples were oven dried at 70° C to constant weight and $\delta^{13}C$ values (relative to the PDB standard) measured on ca. 4 mg of photosynthetic tissue as described previously (Teeri and Schoeller 1979). The isotopic data have been corrected for abundance sensitivity, mass spectrometric background, and ^{17}O abundance.

Titratable acidity was measured with the method of Nishida (1977) on plants grown in greenhouse cultivation. The measured plants had grown together for ten months, one plant per four-inch pot, in "promix" with daily watering. At the time of assay the plants were fully-developed and exposed to a 12-h daylength. The samples consisted of recently fully-expanded leaves from each plant. Samples were collected in the evening at sunset and in the morning at sunrise. The mean and standard deviations of the acidity measurements are based on 5 clonal replicates per measurement period, except for *S. rubrotinctum* where there were two replicates per measurement.

Results

The sixteen species used in the field study exhibited a wide range of leaf thicknesses and $\delta^{13}C$ values (Table 1). The five species having the thinnest leaves (ca. 1 mm) also were found to have the most negative $\delta^{13}C$ values (ca. −26‰). In contrast the species that had the thickest leaves (*Cremnophila nutans*, 11.0 mm) had one of the most positive $\delta^{13}C$ values (−13.8‰). Between these two extremes there was observed to be a cline of intermediate phenotypes with the leaf thickness of a species being strongly related to the $\delta^{13}C$ value (Fig. 1).

Similar results were obtained for the greenhouse plants (Table 2). The species having the thinnest leaves (*Sedum greggii*, 1.5 mm) had the most negative $\delta^{13}C$ value (−25.1‰), and the species having the thickest leaves (*C. nutans*, 9.0 and 9.5 mm for two plants) had $\delta^{13}C$ values of −12.4 and −12.9‰, respectively, for the two plants. As in the field grown plants, the greenhouse plants exhibited a cline of intermediate phenotypes with the leaf thickness of a particular plant being strongly related to the $\delta^{13}C$ value (Fig. 1). The different proportions of thin and thick-leaved species in the field and greenhouse data sets reflects only the availability of sample material. All available species were included in this analysis.

Three of the plants included in the greenhouse study were F_1 hybrids between several of the studied species. In all three hybrids the leaf thickness and $\delta^{13}C$ values of the hybrid was intermediate between the corresponding values of the parents (Table 2). Two of the hybrids (*C. nutans* × *S. praealtum* and *C. nutans* × *S. stahlii*) involve crosses between parents having $\delta^{13}C$ values separated by less than 4‰. However, the cross between *S. greggii* and *C. linguifolia* is between parents having $\delta^{13}C$ values that are different by 11.9‰. The hybrid progeny of these parents has a ^{13}C value of −18.5‰, which is very close to being at midrange between the parental values. The

leaf thickness of *S. greggii* × *C. linguifolia* is 4.0 mm which is also near the midrange between the parental values.

All four studied genotypes exhibited a two-fold or greater nocturnal increase in titratable acidity (Table 3) which is similar to many other CAM plants. *Sedum greggii*, which has thin leaves, exhibited the smallest (two-fold) nocturnal increase in acidity. In contrast, *Sedum rubrotinctum* and *C. linguifolia* both have thick leaves and both exhibited a four-fold nocturnal increase in acidity. The four-fold increase in nocturnal acidity of the hybrid *S. greggii* × *C. linguifolia* was indistinguishable from the pollen parent, *C. linguifolia*.

Discussion

Among these tested species and hybrids of the Crassulaceae there is a strong relationship between leaf thickness and leaf $\delta^{13}C$ value. The functional significance of this relationship remains to be fully understood. If the absolute size of the vacuoles of the photosynthetic cells of different species is correlated with the cell's storage capacity for malic acid, then it may be that increased thickness represents an increased capacity of the photosynthetic tissue to accumulate malic acid during dark fixation of atmospheric CO_2. Such an interpretation would be compatible with the observation of increasingly positive $\delta^{13}C$ values accompanying increasing leaf thickness. The published data (Crews et al. 1976; Troughton, 1979; Winter et al. 1978) strongly suggest that increasingly positive $\delta^{13}C$ values indicate an increasing proportion of dark fixation of atmospheric CO_2 into biomass. The preceding argument implies that leaf thickness is related to vacuolar capacity and this possible relationship clearly is in need of further investigation.

It is possible that small amounts of light or dark fixation of CO_2 may occur that do not substantially contribute to the structural carbon and hence the $\delta^{13}C$ value of a fully expanded leaf. In *Sedum acre* for example, Kluge (1977) has shown that the plants appear to utilize only daytime (C_3) CO_2 fixation in their natural habitats. Yet this species has the capability to accumulate malic acid in photosynthetic tissue under certain environmental circumstances. The interpretation is that in *S. acre* CAM is used only during periods of stress as a mechanism to either fix atmospheric CO_2 in the dark or to recycle respiratory-derived CO_2. The comparatively smaller amounts of CO_2 converted to malic acid in the latter case presumably would require much less vacuolar capacity than in the case of a plant that utilized dark CO_2 fixation for all of its photosynthesis. In North America *S. acre* has a reported leaf thickness of 1.6 mm (Clausen 1975) and a $\delta^{13}C$ value of $-24^0/_{00}$ (Teeri in prep.) which is compatible with the above interpretation. The nocturnal increase in titratable acidity of both thick and thin leaved plants in this study (Table 3) strongly suggests that the four tested genotypes all exhibit some degree of CAM. The thin-leaved species *S. greggii* exhibited the smallest nocturnal increase in acidity. Additional experiments on stomatal rhythm and diel patterns in CO_2 exchange will be required to demonstrate if in this species the nocturnal increase in acidity is due to dark fixation of atmospheric CO_2 or the internal recycling of CO_2 from respiration.

The present data are based on observations of species in four genera and three intergeneric hybrids. Taken together, they strongly suggest that the relationship between leaf thickness and $\delta^{13}C$ value is a general relationship in at least these taxa of the Crassulaceae. It is apparent that there is continuous variation among the tested genotypes in both leaf thickness and $\delta^{13}C$ values (Fig. 1). The species grown together under greenhouse cultivation (Table 2) exhibited similar differences in leaf thickness and $\delta^{13}C$ values as they did in their natural habitats. Thus a genetic basis is suggested for the quantitative differences in leaf thickness and $\delta^{13}C$ values observed among these species. The three hybrids all of which exhibited phenotypes intermediate between their parents provide additional support for the genetic determination of the among-species differences. These very limited data also suggest that differences in leaf thickness and $\delta^{13}C$ values may be highly heritable. Additional information will be required from larger breeding experiments to further elucidate the genetic control of these characters.

Finally it appears that in these species leaf thickness and $\delta^{13}C$ values are not susceptible to environmental modification to a very large extent. Two lines of evidence support this contention. First the range of phenotypes exhibited in the field and in the greenhouse is equally broad. Secondly, four of the species for which field data are available were also grown in the greenhouse study. Each of these four species (*S. dendroideum, S. stahlii, S. greggii* and *C. nutans*) exhibited a greenhouse leaf thickness and $\delta^{13}C$ value that was very similar to the corresponding field phenotype (Tables 1, 2). A similar observation was previously reported for a comparison of field and greenhouse $\delta^{13}C$ values of several other species of the Crassulaceae (Rundel et al. 1979). Further research will be required to determine what relationships between leaf thickness and $\delta^{13}C$ value occur in other taxonomic groups.

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