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CARBOHYDRATES AND FLOWERING IN *HESPERALOË FUNIFERA* (KOCH) TREL. (SAMANDOQUE)

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Abstract. Several researchers have noted that flowering in Agavaceae requires substantial resources, but few studies have attempted to directly measure such resources. This study addresses the hypothesis that fruit set in *Hesperaloë funifera* is limited by available carbohydrates. The accumulation of total non-structural carbohydrates (TNC) prior to flowering was measured, and total requirements for carbohydrate were estimated. *Hesperaloë funifera* was found to accumulate fructans, and roots were an important organ for storage of accumulated carbohydrates. Carbohydrates stored in the plant prior to flowering are sufficient to meet only about one-third of the carbohydrate needed to produce an average inflorescence with 1% to 2% fruit set. All of the carbohydrate produced by photosynthesis from May through August is needed to support flowering and fruit production. Low percentage fruit set in *Hesperaloë funifera* is probably due to a deficiency of carbohydrate resources.

Key words: Agavaceae, carbohydrates, flowering, fructan, fruit set, *Hesperaloë*.

Resumen. Varios investigadores han notado que el desarrollo de la inflorescencia en las agaváceas necesita recursos importantes, pero pocos han tratado de medirlos directamente. La acumulación de todos los carbohidratos no-estructurales (TNC) antes de florecer fue medida, y todos los requisitos de carbohidratos fueron calculados. Se encontró que *Hesperaloë funifera* acumula fructanos, y sus raíces son órganos importantes para el almacenamiento de carbohidratos. Estos son almacenados antes de florecer y son suficientes sólo para satisfacer una tercera parte de las necesidades para producir una inflorescencia media, con 1% a 2% de fructificación. Todos los carbohidratos producidos por fotosíntesis desde mayo hasta agosto fueron necesarios para la floración y la fructificación. El porcentaje bajo de fructificación en *Hesperaloë funifera* probablemente es debido a la falta de carbohidratos.

Palabras clave: Agavaceae, floración, fructanos, fructificación, *Hesperaloë,* carbohidratos.

Most species of Agavaceae consist of monocarpic rosettes that produce an often massive inflorescence after several years of vegetative growth (Nobel, 1994). The plants must allocate significant amounts of resources, including carbohydrates, water, and nutrients, to produce their large inflorescences (Nobel, 1977; James *et al.*, 1994). These resources must come from accumulated reserves, current assimilation, or both. Certain plants in the Agavaceae are known to accumulate high levels of carbohydrates prior to flowering (Nobel, 1994); these stored carbohydrates are tapped and utilized in the manufacture of the distilled alcoholic beverages tequila, mescal, and bacanora.

Hesperaloë is a small genus in the Agavaceae consisting of 5 species, all from northern Mexico: *H. campanulata* Starr from Nuevo León; *H. funifera* (Koch) Trelease, the most widespread species, known from Coahuila, Nuevo León, and San Luis Potosí; *H. nocturna* H. S. Gentry known only from northeastern Sonora; *H. parviflora* (Torrey) J. M. Coulter found in Texas and Coahuila; and *H. tenuifolia* Starr known only from southern Sonora (Starr, 1997). All *Hesperaloë* species produce acaulescent rosettes which reproduce sexually by seed and vegetatively by the production of secondary or lateral rosettes, usually on short rhizomes. *Hesperaloë funifera* is the largest species producing leaves up to 2 m in length and branched flower stalks up to nearly 6 m in height.

The individual rosettes of *H. funifera* are monocarpic; however, the leaves have a long "post-flower-

ing half-life" (Schaffer and Schaffer, 1977). The leaves of a flowering rosette remain physiologically active during and after flowering (apparently for up to a year or more), continuing to contribute to the carbon and water economy of the plant. After flowering a rosette produces no new leaves but does produce new lateral rosettes.

Researchers at the University of Arizona have been investigating *H. funifera* as a potential source of high-value specialty fibers for the pulp and paper industry (McLaughlin, 1995, 1996). In order to plant enough acreage of *H. funifera* to support a small pulp mill, significant quantities of seed, not currently available, would be required (McLaughlin, 1996). We have observed very low fruit set (1% - 2%) in *H. funifera* for plants grown in cultivation with irrigation and fertilization. *H. funifera* is an obligate outcrosser and appears to require a pollen vector. The rotate-campanulate flowers open at night. It is not known what organism(s) pollinates this species in nature; in cultivation in Arizona flowers appear to be pollinated by honeybees, hummingbirds, and other diurnal visitors; potential nocturnal pollinators have not been observed in plots at Tucson, Arizona.

Possible explanations for the low percentage of fruit set in *H. funifera* include: *1*] insufficient energy (carbohydrates); *2*] insufficient water; *3*] insufficient nutrients, and *4*] lack of pollinators. We have pollinated flowers by hand at Bioresources Research Facility (BR on several occasions and have observed little or subsequent fruit set. However, when we severe pruned the inflorescences on which flowers had be hand pollinated —effectively reducing the number competing sinks for available resources— a high pr portion of fruit set was observed. We have thus h pothesized that it is the availability of carbohydrat to support production of flower stalks, flowers, a fruits which limits the number and percentage of fr set. The objective of this paper is to further exa ine this hypothesis by estimating the amount of c bohydrates required for flowering and fruit productio and comparing these values with the amount of c bohydrates stored in the plant prior to flowering a produced by the plant during flowering.

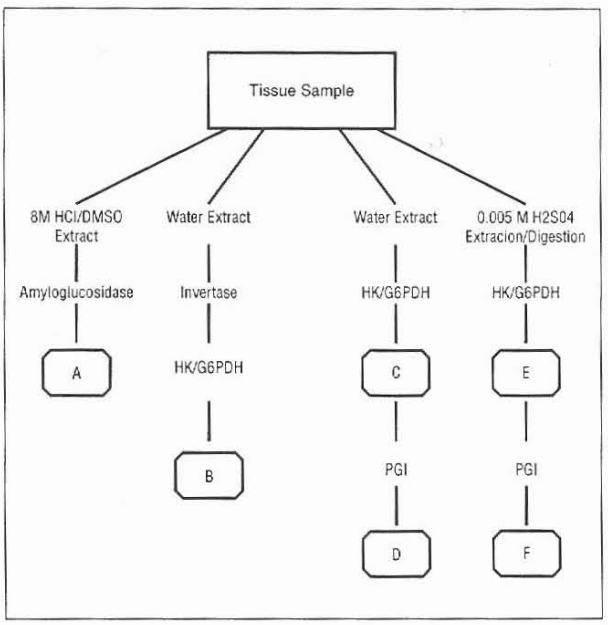

Figure 1. Diagram of analytical procedures used to determine non-structural carbohydrates in *Hesperaloë funifera* plants.

Methods

Plants used for analysis of carbohydrates were grow at the University of Arizona's Maricopa Agricultur Center (MAC), located in central Arizona (33° 4' 111° 58' W) at an elevation of 365 m. Additional da reported here come from field plots maintained the Bioresources Research Facility (BRF) south Tucson in southern Arizona (32° 8' N, 110° 58' W at an elevation of 760 m. Both MAC and BRF are l cated in the Sonoran Desert and have arid climat with hot summers and mild winters.

All plants at MAC were grown from seed harveste from plants growing at BRF. The plants at BRF ori nated from seed harvested from a small group of plan growing in a private landscape in Tucson. The ul mate geographic and genetic origin of our experime tal populations is not known.

Thirty 3-year-old plants from MAC were selected f analyses of non-structural carbohydrates. On Mar 27, 1997, 10 plants that were just beginning to i tiate flower stalks were harvested. On June 5, 199 20 additional plants were harvested-10 with fu emerged flower stalks, and 10 plants not flowering 1997. All 30 plants from MAC were of similar siz averaging about 600-800 g dry weight. Leaves we harvested from the plants at a height of 2-3 cm abo ground level. Plants were then carefully excavated remove the crowns and recover as much root ma as possible. As used in this paper, *crown* actually i cludes the short, subterranean stem plus the attach leaf bases.

Roots were separated from the crowns and wash thoroughly. Fresh weights were obtained for leav crowns, roots, and flower stalks. Roots and leaves we split and crowns were cut into 4 segments to fac tate drying. Plant parts were oven-dried at 37 °C

constant weight and then ground to a powder in a Wiley mill with a 0.5-mm mesh screen.

We measured samples of leaf, crown, root, and inflorescence tissues for glucose, fructose, sucrose, starch, and fructan (figure 1) using a modification of the methods of Chatterton *et al.* (1987). Fraction **A** (see figure 1) contains glucose from starch plus free glucose and was produced by treating a tissue sample with 8 M HCl and dimethysulfoxide (DMSO) to solubilize the starch, followed by digestion with amyloglucosidase. Fraction **B** contains glucose from sucrose plus free glucose and was obtained by treating a water extract with invertase, hexokinase (HK), and glucose-6-phosphate dehydrogenase (G6PDH). Fraction **C** contains free glucose only and was obtained by treating a water extract with HK and G6PDH. Fraction **D** contains glucose from free fructose and free glucose and was produced by treating fraction **C** with phosphoglucoseisomerase (PGI). Fraction **E** contains glucose from fructans plus free glucose and was produced by extracting a tissue sample with 0.005 M H2SO4 to digest fructans followed by treatment with HK and G6PDH. Finally, Fraction **F** contains glucose from free fructose, glucose from fructans, and free glucose and was obtained by treating fraction **E** with PGI.

Weights of each fraction were expressed as a percentage of the starting material and percentages of the five non-structural carbohydrates were calculated as follows: free glucose = **C**; free fructose = **D** - **C**; sucrose = **B** - **C**; starch = **A** - **C**, and fructan = **F** - **D**. Total non-structural carbohydrates (TNC) was calculated as TNC = free glucose + free fructose + sucrose + starch + fructan.

Results

Results of the analyses of carbohydrates are shown in Table 1 and figure 2. Table 1 gives the percentages of starch, fructan, glucose, fructose, sucrose, and total non-structural carbohydrates (TNC) in *Hesperaloë funifera* plants not producing inflorescences (Group A), plants initiating inflorescences (Group B), and in plants with fully-emerged inflorescences (Group C). Group A plants had a high percentage of TNC in their roots but had not accumulated high levels of TNC in crowns or leaves. Fructan, a polymer of fructose with

Table 1. Percentage of non-structural carbohydrates in leaves, crowns, roots and inflorescences from non-flowering *Hesperaloë funifera* plants, plants initiating inflorescences, and plants with fully-developed flower stalks. TNC = total nonstructural carbohydrate. Table entries are means of 10 individuals ± the standard error.

Carbohydrate	Roots	Crowns	Leaves	Flowerstalks
		Group A. Plants not flowering		
Starch	2.29 ± 0.35	0.47 ± 0.17	0.13 ± 0.08	
Fructan	17.67 ± 1.93	1.15 ± 0.37	-0.13 ± 0.21	
Glucose	4.79 ± 0.61	1.93 ± 0.15	1.26 ± 0.11	
Fructose	3.58 ± 0.40	1.92 ± 0.15	1.06 ± 0.17	
Sucrose	4.19 ± 0.40	.71 ± 0.28	0.17 ± 0.06	
TNC	32.53 ± 1.82	6.18 ± 0.66	2.49 ± 0.62	
		Group B. Plants initiating inflorescences		
Starch	3.86 ± 0.34	2.78 ± 0.22	1.95 ± 0.39	1.34 ± 0.55
Fructan	24.04 ± 1.87	18.16 ± 1.41	0.42 ± 0.44	8.34 ± 1.14
Glucose	5.62 ± 0.47	2.22 ± 0.21	2.78 ± 0.21	4.80 ± 0.40
Fructose	3.21 ± 0.22	3.31 ± 0.27	2.67 ± 0.24	6.60 ± 0.53
Sucrose	6.62 ± 0.40	4.50 ± 0.24	4.60 ± 0.70	5.82 ± 0.53
TNC	43.36 ± 1.86	30.97 ± 1.31	12.41 ± 0.86	26.89 ± 2.05
		Group C. Plants with fully-developed flower stalks		
Starch	1.01 ± 0.30	0.01 ± 0.08	-0.03 ± 0.06	0.06 ± 0.01
Fructan	9.22 ± 1.35	0.19 ± 0.39	-0.11 ± 0.04	0.04 ± 0.02
Glucose	4.75 ± 0.33	1.51 ± 0.12	1.11 ± 0.12	0.12 ± 0.02
Fructose	4.51 ± 0.45	1.33 ± 0.18	0.85 ± 0.10	0.08 ± 0.02
Sucrose	3.81 ± 0.36	0.66 ± 0.07	0.18 ± 0.05	0.06 ± 0.02
TNC	23.30 ± 2.10	3.80 ± 0.61	1.99 ± 0.20	0.36 ± 0.04

a terminal glucose molecule, was the primary storage carbohydrate found in roots. Leaves and crowns contained mostly glucose and fructose.

Plants initiating inflorescences (Group B) had accumulated high levels of TNC in roots and crowns; leaves and crowns had 5 times higher percentage of TNC compared to plants not flowering (Group A). Again, the principal storage carbohydrate in the roots and crowns was fructan; levels of sucrose, glucose, and fructose were relatively high in the leaves. The emerging flower stalks had comparatively high levels of fructan, fructose, and sucrose.

Compared to plants initiating inflorescences, plants with fully-emerged inflorescences (Group C) were depleted in carbohydrates, particularly in their crowns and leaves. Fructan levels showed the greatest declines, with essentially all of the fructan having been mobilized from the crowns. Fructan levels in the roots had decreased (relative to plants initiating inflorescences) by over 60%. The tissues in the fully-developed flower stalks had very low levels of non-structural carbohydrates.

The plants in Group A had a mean dry weight of 598 g consisting of 59.6% leaves, 20.3% crowns, and 20.1% roots. Mean dry weight of the vegetative parts of plants in Group B was 611 g consisting of 53.2% leaves, 26.8% crowns, and 20.0% roots; the emerging flower stalks for plants in Group B averaged just 9.2 g. For the plants in Group C with fully-emerged flower stalks, mean dry weight of the vegetative parts was 641 g consisting of 55.9% leaves, 24.2% crowns, and 19.9% roots. Average dry weight of the emerged flower stalks in this latter group was 215.7 g.

The *pools* of available non-structural carbohydrates in these three groups can be calculated by multiplying the percentage carbohydrate for each plant part times the biomass of that plant part. These carbohydrate pools are shown in figure 2. In non-flowering plants (Group A) the largest pool of TNC is found in the roots. For plants initiating inflorescences (Group B) the sizes of the non-structural carbohydrate pools in the leaves, crowns, and roots were generally similar. Based on a comparison of plants with fully-emerged flower stalks (Group C) and those initiating flower stalks (Group B), production of the flower stalk consumed nearly all of the stored carbohydrates from the leaves and crowns but less than half of the carbohydrate stored in the roots.

Discussion

Various authors (e.g., Nobel 1994) refer to the accumulation of *starch* in the rosettes of *Agave* spp. prior to flowering. This study found that *Hesperaloë funifera* accumulates comparatively little starch, and that fructans are the primary storage carbohydrates. The accumulation of fructans in species of Agavaceae has been reported previously (Pollock and Chatterton, 1988). Aspinall and Das Gupta (1959) and later Dorland *et al.* (1977) found fructans in the stem of *Aga-*

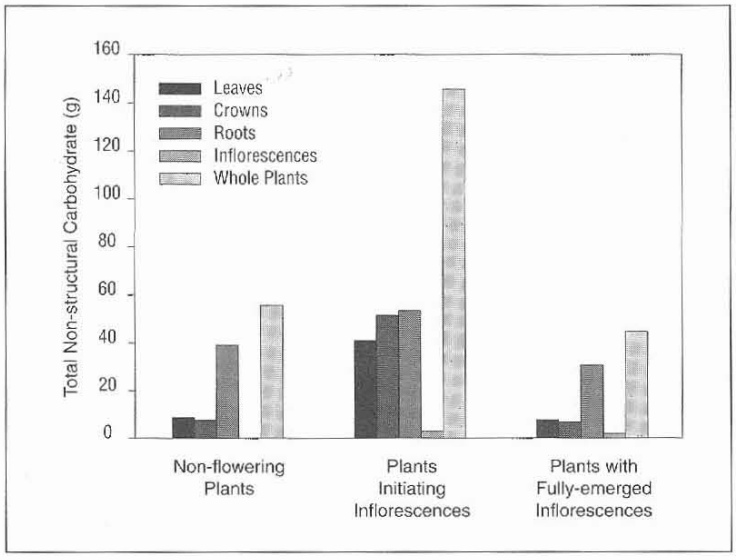

Figure 2. Non-structural carbohydrates pools in leaves, crowns, roots, and inflorescences in *Hesperaloë funifera* plants not flowering (left), plants initiating flower inflorescences (center), and plants with fully-emerged inflorescences (right). N = 10 for each bar.

Table 2. Carbohydrate requirements for flowering in *Hesperaloë funifera*. Substrate requirements, the number of grams of glucose required to produce a gram of flower stalks, flowers, capsules, or seeds, are based on a study of *Yucca* spp. (Reynolds and Cunningham, 1980).

		Substrate	Carbohydrate
Inflorescence Part	Dry Weight (g)	Requirement (g g^{-1})	Requirement (g)
Flower stalk	357	1.50	536
Flowers	511	1.39	710
Capsules	112	1.21	135
Seeds	131	1.67	219
Total	1111		1600

ve vera-cruz (= *A. lurida* Aiton according to Gentry, 1982), Bhatia and Nandra (1979) discussed synthesis of fructans in *A. americana* L., and Wang and Nobel (1998) reported on phloem transport of fructans in *A. deserti* Engelm. Valenzuela-Zapata (1994), discussing the processing of *Agave tequilana* Weber, stated that cooking the *cabezas* (crowns) hydrolyzes complex carbohydrates such as inulin (a type of fructan) to fructose and sucrose. Olivares and Medina (1990) discussed the physiological role of fructans in the leaves *Furcraea humboldtiana* Trel. *Hesperaloë* apparently differs from other Agavaceae in using roots as the primary organ for storage of carbohydrates.

The results presented here on accumulation of nonstructural carbohydrates can be combined with data on inflorescence mass and fruit set, directly related to the demand for carbohydrate, and plant size, directly related to the supply of available carbohydrate, to evaluate the hypothesis that carbohydrate supplies limit fruit set. Studies of flowering in *Hesperaloë funifera* conducted at the BRF found that these plants began flowering in May and continued producing flowers for several months. Plants flowering for the first time had flower stalks 352 ± 10 cm (mean ± standard error) (McLaughlin and R. A. Ravetta, unpublished results). In this same sample mean inflorescence dry weight was 1111 ± 108 g, consisting of support structures (i.e., the flower stalk itself) with a mass of 357 ± 33 g, flowers with a total mass of 511 ± 62 g, and capsules (including the seeds) with a total mass of 243 ± 31 g. The large biomass of flowers was a consequence of the large number of flowers (4523 ± 548) produced over the long flowering season.

Reynolds and Cunningham (1980) studied growth and reproduction in 2 species of *Yucca*, *Y. elata* Engelmann and *Y. baccata* Torrey. According to the phylogenetic studies of Bogler and Simpson (1996), *Yucca* is the genus most-closely related to *Hesperaloë*. Flowers, capsules, and seeds of *Yucca* and *Hesperaloë* are morphologically similar. Reynolds and Cunningham (1980) estimated the glucose requirements for biosynthesis of various organs of *Yucca*, including flower stalks (1.50 g g^{-1}), flowers (1.39 g g^{-1}), capsules (1.21 g g^{-1}), and seeds (1.67 g g^{-1}). Applying their values to *Hesperaloë*, we can estimate that the total carbohydrate requirement for production of an average inflorescence is about 1600 g glucose equivalent (table 2).

Hesperaloë funifera usually does not flower in cultivation until its fourth year. An average plant in the BRF field plots (at a density of 13,500 plants ha^{-1}) has a mass of approximately 1800 g after three years of growth. A typical individual flowering during its fourth year probably has a mass of about 2 400 g consisting of about 55% leaves, 25% crowns, and 20% roots. Assuming that TNC prior to flowering for plants of this size averages 15% of the leaves, 30% of the crowns, and 40% of the roots (approximate values from table 1), the pool of TNC in the plant would be 198 g in the leaves, 180 g in the crowns, and 192 g in the roots for a total of 570 g in the plant. This would only amount to about 35% of the estimated 1600 g required for an average inflorescence. The remaining carbohydrate would have to come from current assimilation.

Photosynthetic rates of *Hesperaloë funifera* are rather low during the summer months (May-August), averaging about 200 mmol CO_2 m^{-2} d^{-1} on a 1-sided leaf basis (Ravetta and McLaughlin, 1996). Photosynthesis in this species increases during the autumn with decreasing night temperatures to a rate closer to 400 mmol CO_2 m^{-2} d^{-1}. A plant with a mass of 2 400 g would have a leaf surface of approximately 1.15 m^2 on a 1-sided basis. Current production of carbohydrates through photosynthesis over a 120-day period from May through August would be approximately 830 g (as glucose). Current photosynthesis during the summer plus estimated storage reserves totals 1 400 g or about 87% of the estimated TNC needed for flowering.

Photosynthesis during a 60-day period during September and October would produce an additional 830g carbohydrate. Not all of this would be available for allocation to flowering and fruit production, however, since secondary rosettes are produced after the inflorescence emerges and are rapidly expanding at the same time (McLaughlin, 1995). Thus fruit production and growth of the secondary rosettes would compete for available carbohydrate during the autumn months.

Production of the flower stalk and flowers appears to require about 1 250 g of non-structural carbohy-

drate, approximately equal to all of the TNC stored in leaves, crowns, and roots prior to flowering plus carbohydrate produced by photosynthesis from May through August when most of the flowering and fruit set occurs. Very little carbohydrate remains to support production of capsules and seeds. Therefore it does seem very likely that fruit set in *Hesperaloë funifera* would be limited by the energy available as stored carbohydrate and current assimilation, even when other resources (water, nutrients, pollinators) are abundant.

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