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Interrelationships of Nitrate and 6-Azauracil in the Growth, Enzymology, and Sucrose Production of Immature Sugarcane

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INTRODUCTION

Precise control of nitrate[2] supply has been an excellent tool for the study of sugar synthesis and storage in sugarcane. The ripened status is readily simulated by withholding NO_3 in sand culture, while high NO_3 levels induce the succulent growth and profuse tillering characteristic of pre-ripening stages. By gradually increasing or decreasing NO_3, it has been possible to observe the triggering of extremely important physiological and biochemical reactions. Among these are enzymatic changes which make possible a predominance of sugar synthesis over utilization, of utilization over storage in young plants, and of storage over utilization in mature cane. A valuable treatment in its own right, controlled NO_3 is of further use in establishing a several-dimensional context in which physiological treatments may be scrutinized for mode of action and persistence.

Recent growth-control studies in Puerto Rico have shown considerable promise for the pyrimidine analog 6-azauracil (*7*).[3] This chemical appears to stimulate growth, to lessen growth, or to terminate growth completely as a function of concentration. Foliar gibberellic acid (GA) was found to improve the young plants' capacity to survive, but did not eliminate growth suppression by 6-azauracil (*7*). Enzymes were generally retarded while sucrose increased as an apparent consequence of invertase and amylase suppression

Although GA has been employed in attempts to break the growth inhibition (*7*), no studies have been made with plants having an established status of either succulent growth or of advanced ripening. During the present study extreme N regimes were established with variable NO_3 in

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[2] Abbreviations: Nitrate, NO_3; nitrogen, N; adenosine triphosphate, ATP; β-glycerophosphate, β-GP.

[3] Italic numbers in parentheses refer to Literature Cited, pp. 91–2.

sand culture. There were three objectives: 1, To establish a range of N growth stimuli and to measure their perseverance in the presence of foliar 6-azauracil; 2, to evaluate 6-azauracil as a growth-retardant in rapidly growing plants, and as a ripening agent in plants simulating a near-harvest condition; and 3, to explore the effectiveness of 6-azauracil as an enzyme inhibitor under extreme conditions of N stress.

EXPERIMENTAL PROCEDURE

PREPARATION OF PLANT MATERIALS

Plants of the variety P.R. 980 were grown in the greenhouse using a sand-culture technique previously described (*1*). All seedlings were given tapwater for 4 weeks and thereafter received a complete nutrient solution until they were 10 weeks of age.[4]

Three levels of NO_3 were supplied daily in nutrient solution, and three levels of 6-azauracil were applied once as foliar sprays. The treatments were combined factorially in a randomized block design with three replicates for each of the nine treatments. Nitrate treatments of 0.5, 5.0, and 50.0 meq./liter were begun at 10 weeks. Each container of plants was given 1 liter of nutrient solution and 1 liter of water daily. At 12 weeks the 6-azauracil solutions were applied as foliar sprays. Concentrations of 0, 0.01, and 0.05 percent were applied with a hand-sprayer until runoff began. This required about 220 ml./treatment/replicate (about 18 plants). Tween 20 was used as wetting agent at the rate of 1 ml./liter of solution.

A single harvest was made 60 days after initiating NO_3 treatment, and 46 days after foliar treatment with 6-azauracil. From each replicate four uniform canes were cut at the sand surface. Total fresh weight, millable stalk weight, and the internode length of millable stalks was recorded. Leaves +1 to +4 and immature storage tissue were quickly frozen in a mixture of Dry Ice and acetone. These were lyophilized, ground to a fine powder, and stored at about 0° C. Numerical ratings were taken of visible growth-inhibitor symptoms just prior to harvest.

LABORATORY ANALYSES

Clarified water extracts of leaf and immature storage tissues were analyzed for total ketose by the resorcinol method of Roe (*16*), and for sucrose by the modification of Cardini *et al.* (*13*). Fructose was estimated by subtracting sucrose values from those of total ketose. Mature stalks were ground with a small laboratory mill for Brix and polarization analyses.

[4] Nutrient levels, expressed as meq./liter, were supplied as follows: NO_3, 10; phosphorus, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfur, 2. Micronutrients, expressed as p.p.m., were given as follows: Iron, 1.0; boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; and molybdenum, 0.01.

Protein was precipitated from clarified extracts of leaf and immature storage tissue with solid ammonium sulfate. The 0 to 80-percent fraction was retained and used for enzyme assay without dialysis. The method of Sutherland *et al.* (*18*) was used to measure both the water-soluble protein of tissue samples and the protein content of enzyme preparations.

Particular attention was given to hydrolytic and oxidative enzymes which have previously shown correlations with the sugar level in cane. Acid phosphatase and ATP-ase were measured by techniques described earlier (*6*), as was amylase (*3*), invertase (*2*), polyphenol oxidase (*5*), and peroxidase (*4*).

TABLE 1.—*Total fresh weights, millable stalk weights, and internode lengths of immature sugarcane treated with foliar 6-azauracil and supplied with variable nitrate in sand culture*[1]

NO_3 (meq./l.)	Mean values for—											
	Total fresh weight (g./plant)				Millable stalk weight (g./stalk)				Internode length (in.)			
	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean
	0	0.01	0.05		0	0.01	0.05		0	0.01	0.05	
0.5	280	233	117	210	133	109	49	97	4.04	3.44	2.08	3.19
5.0	380	488	292	387	173	236	147	185	3.32	4.62	3.14	3.69
50.0	448	462	359	423	229	189	159	192	3.83	3.82	3.15	3.60
Mean	369	394	256		178	178	118		4.06	3.96	2.79	

[1] Each figure represents the computed mean of 3 replicates; 4 uniform plants were harvested from each replicate.

RESULTS AND DISCUSSION

GROWTH EFFECTS OF NITRATE AND 6-AZAURACIL

6-azauracil succeeded in suppressing growth regardless of NO_3 supply (table 1). Nitrate increased growth even in the presence of "high" 6-azauracil (0.05 percent). 6-azauracil was most effective when NO_3 was withheld, but NO_3 gave relatively greater growth increases when the inhibitor was also present. Ratings of visible 6-azauracil injury indicate that symptoms were most pronounced when NO_3 was low (table 2).

It is concluded that the growth-inhibitor can be operative in the presence of an extremely high N supply. This is of far-reaching importance in that a truly efficient growth-controlling agent should be able to act against a powerful growth stimulus. Research in Puerto Rico has shown that N application to ratoons older than 15 weeks can significantly reduce the

sucrose content of the variety P.R. 980 (*17*). Workers in Hawaii have stressed earliness, with split N applications preferred to a single treatment, for maximum sugar yield and N utilization (*12, 19*). It is now inferred that N fertilization might be prolonged, in the interests of greater tonnage, with

TABLE 2.—*Numerical ratings of visible injury caused by foliar 6-azauracil among sugarcane plants given variable nitrate in sand culture*[1]

NO_3 (meq./l.)	6-azauracil (percent solution)—			Mean
	0	0.01	0.05	
0.5	1.3	3.3	4.7	3.1
5.0	1.0	2.3	3.3	2.2
50.0	1.0	1.7	3.0	1.9
Mean	1.1	2.4	3.7	

[1] Each figure represents the computed mean of 3 replicates. A value of 1.0 indicates no visible injury by 6-azauracil. A value of 5.0 represents severe growth restriction, death of meristem, and dessicated foliage. Ratings were recorded 46 days after foliar treatment.

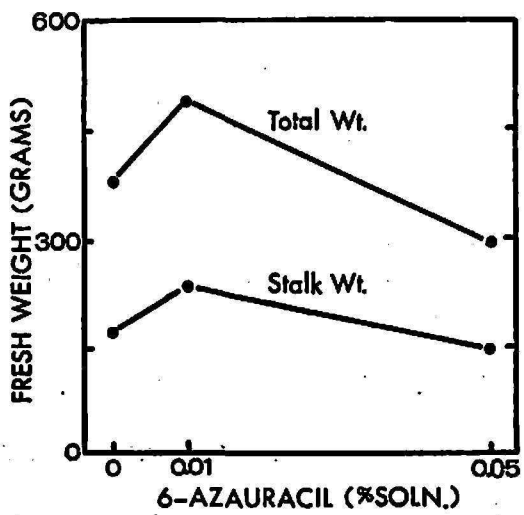

FIG. 1.—Cane growth stimulation by a 0.01-percent solution of 6-azauracil, and growth suppression by the 0.05-percent level.

the certainty that growth can be retarded at will by an appropriate chemical agent. Conversely, more N might be used as a postharvest treatment to offset 6-azauracil growth effects and to regain vigorous ratoon development.

A curious response to 6-azauracil was an apparent growth stimulation by the 0.01-percent solution (fig. 1). This was evident, at the medium NO_3 level, for total fresh weight, weight of millable stalks, and internode length (table 1). When NO_3 was excessively high or in short supply the growth

increase did not appear. A similar effect was observed earlier in plants treated with 0.005-percent 6-azauracil plus 0.10-percent GA (*7*). A proposed explanation for this is the "overcompensation" theory. It has been suggested that plants given a moderate but nonlethal shock can overcompensate in their recovery (*8*). Presumably a small amount of damage to the nucleic acid system is caused by traces of 6-azauracil, and in repairing this damage the plant may provide itself with a more effective system than the original. Increasing chemical concentrations would progressively destroy the capacity to recover and lead to growth inhibition.

TABLE 3.—*Leaf sugar content of immature sugarcane treated with foliar 6-azauracil and supplied with variable nitrate in sand culture*[1]

NO_3 (meq./l.)	Leaf sugars (mg./g. of dry weight)—											
	Total ketose				Sucrose				Fructose			
	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean
	0	0.01	0.05		0	0.01	0.05		0	0.01	0.05	
0.5	93.3	89.2	85.6	89.4	91.8	86.9	82.5	87.1	1.5	2.3	3.1	2.3
5.0	97.2	96.2	78.3	90.6	86.4	77.9	65.2	76.5	10.8	18.3	13.1	14.1
50.0	101.0	98.3	92.6	97.3	76.4	78.2	64.0	72.9	24.6	20.1	28.6	24.4
Mean	97.2	94.6	85.5		84.9	81.0	70.6		12.3	13.6	14.9	

[1] Each figure represents the computed mean of 3 replicates.

SUGAR RESPONSES

Lowering NO_3 supply generally increased sucrose values for leaf and immature storage tissues (tables 3 and 4), and raised the Brix and polarization values for mature storage tissues (table 5). These effects were anticipated.

6-azauracil increased sucrose of immature storage tissue. This increase was greatest when NO_3 was low, and least when NO_3 was high. Combining high 6-azauracil with high NO_3 brought sucrose values back up to the level recorded with low NO_3 and zero 6-azauracil (table 4). Thus, while the growth inhibitor countered sucrose losses due to increasing NO_3, the additional growth gained with high NO_3 increased the net sucrose yield.

Brix and polarization data recorded in table 5 reflect sugar increases with 6-azauracil, but only when NO_3 was low (fig. 2). The growth inhibitor caused moderate decline of both Brix and polarization values when 5.0 and 50 meq./liter of NO_3 were given. 6-azauracil therefore seems to have failed a most important test with regard to its ripening capacity. Yet the desirable

growth, enzyme, and sucrose effects of 6-azauracil have otherwise been so consistent that it would be unwise to discount a potential for ripening.

Three approaches should logically be explored in evaluating the ripening potential of 6-azauracil, or related agents, under conditions of excessive N

TABLE 4.—*Sugar content of immature storage tissue from sugarcane treated with foliar 6-azauracil and supplied with variable nitrate in sand culture*[1]

NO_3 (meq./l.)	Sugars (mg./g. of dry weight)—											
	Total ketose				Sucrose				Fructose			
	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean
	0	0.01	0.05		0	0.01	0.05		0	0.01	0.05	
0.5	230	247	—[2]	239	77.0	104.0	—[2]	90.5	153	143	—[2]	148
5.0	248	285	261	265	47.0	87.7	93.7	76.1	201	197	167	188
50.0	279	295	262	279	50.6	65.6	75.5	63.9	228	229	186	214
Mean	252	276	261		58.2	85.8	84.6		194	189	118	

[1] Each figure represents the computed mean of 3 replicates.
[2] Tissues were killed by the 6-azauracil treatment.

TABLE 5.—*Polarization and Brix values for sugarcane treated with foliar 6-azauracil and supplied with variable nitrate in sand culture*[1]

NO_3 (meq./l.)	Brix				Polarization			
	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean
	0	0.01	0.05		0	0.01	0.05	
0.5	12.3	13.3	14.2	13.3	36.0	38.6	50.2	41.6
5.0	11.9	10.0	9.2	10.4	28.1	21.2	21.4	23.6
50.0	10.9	9.0	8.3	9.4	23.3	12.9	11.9	16.0
Mean	11.7	10.8	10.6		29.1	24.2	27.8	

[1] Each figure represents an aliquot sample from 3 combined replicates.

supply. 1, Higher levels of inhibitor should be tested. 2, The growth inhibitor should be combined with a compatible and specific inhibitor of cane amylase and invertases. Silicon is a logical agent for this role (*9,10*).

Both of the above approaches assume that the ripening effect of the growth inhibitor is simply overwhelmed by a still more powerful, NO_3-induced growth stimulus, and that N metabolism remains an indirect and

nonlimiting factor with regard to sucrose level. Thus a third approach must take into account the biochemical imposition by abnormally high N, and the reactions employed by the plant to achieve its utilization. Nitrate reductase is a key enzyme the relationship of which to sucrose has long demanded investigation. Transaminase action and amino acid synthesis also require study under conditions of controlled NO_3 supply. Of particular interest is the amino acid arginine. Recent work in Hawaii has implied that arginine may be a limiting factor for growth of sugarcane (*15*) and for growth of excised cane parenchyma tissues (*14*).

ENZYME RESPONSES

Leaf enzyme activity was not significantly altered by 6-azauracil (table 6). Increasing NO_3 usually raised enzyme activity levels. The general

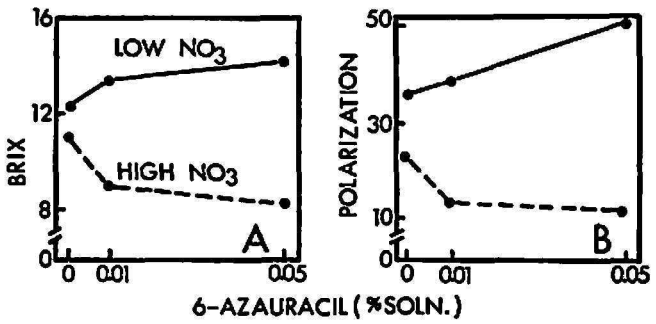

FIG. 2.—Effects of foliar 6-azauracil on Brix and polarization values for immature sugarcane supplied with variable NO_3 in sand culture. A, Increase of Brix when NO_3 was low (0.5 meq./liter), and decline when NO_3 was high (50 meq./liter). B, Increase of polarization with low NO_3 and decline with high NO_3.

failure of 6-azauracil to damage leaf tissues and leaf enzymes apparently reflects a rapid translocation of the chemical to storage tissues. This is highly desirable in that inhibitor concentrations may be focused in growth-critical areas of tissue initiation and elongation, while the leaf tissues remain relatively undamaged and photosynthetically active.

Enzymes of immature storage tissue were clearly suppressed by 6-azauracil (table 7). Increasing NO_3 yielded greater enzyme activity, yet some of the 6-azauracil effect remained evident regardless of NO_3 supply. Figure 3 illustrates a continuing 6-azauracil capacity to retard invertase at the high NO_3 level, but it is also evident that the slopes for sucrose increase and invertase decline have become greatly flattened. The 50-percent suppression of invertase was apparently not sufficient to prevent excessive sucrose inversion in the presence of high NO_3. Amylase was even less affected by 6-azauracil. Although clearly retarded, amylase action among the high NO_3 × high 6-azauracil samples was nearly twice that of the low NO_3 × low inhibitor samples. The failure of 6-azauracil to retard amylase

and invertase severely probably accounts for the decline of Brix and polarization values in plants given high NO_3. Both amylase and invertase suppression are regarded as common denominators in the biochemistry of ripened cane (*8*).

At the moment it is concluded that 0.05-percent 6-azauracil cannot achieve severe growth and enzyme inhibition in the presence of excessive NO_3. Nevertheless encouraging trends are evident. Since earlier work has shown that leaf damage begins above the 0.10-percent concentration (*7*), it is possible that more effective NO_3-inhibitor responses can be obtained by

TABLE 6.—*Specific activity values for leaf enzymes of immature sugarcane treated with foliar 6-azauracil and supplied with variable nitrate in sand culture*[1]

NO_3 (meq./l.)	Phosphatase				ATP-ase			
	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean
	0	0.01	0.05		0	0.01	0.05	
0.5	2.7	5.6	5.0	4.4	9.1	9.7	8.9	9.2
5.0	8.2	8.6	10.8	9.2	13.6	16.4	19.1	16.4
50.0	14.1	15.5	12.8	14.1	21.7	31.7	30.1	27.8
Mean	8.3	9.9	9.5		14.8	19.3	19.4	
	Amylase				Peroxidase			
0.5	20.7	14.5	18.5	17.9	28.3	30.8	43.0	34.0
5.0	42.4	44.7	56.2	47.8	52.3	58.9	64.5	58.6
50.0	91.8	87.6	89.0	89.5	80.0	86.6	76.0	80.9
Mean	51.6	48.9	54.6		53.5	58.8	61.2	

[1] Each figure represents the computed mean of 3 replicates.

raising 6-azauracil to this level. The possibility of combining the growth analog with an invertase inhibitor such as silicon should also be explored.

SUMMARY

Sugarcane grown in sand culture was subjected to extreme levels of nitrate (NO_3) to simulate conditions of restricted growth and ripening, moderate growth, and succulent growth. The pyrimidine analog 6-azauracil was supplied concurrently as a foliar spray. There were three objectives: 1, To measure the perseverance of the growth regimes in the presence of a powerful growth inhibitor; 2, to evaluate 6-azauracil as a growth-retardant in high-nitrogen (N) stimulated plants, and as a ripening agent for slow-growing cane in a simulated ripening condition; and 3, to investigate the

TABLE 7.—*Specific activity values for enzymes from immature storage tissue of sugarcane treated with foliar 6-azauracil and supplied with variable nitrate in sand culture*[1]

NO_3 (meq./l.)	Phosphatase				ATP-ase			
	6-azauracil (percent solution)			Mean	6-azauracil (percent solution)			Mean
	0	0.01	0.05		0	0.01	0.05	
0.5	15.7	5.9	—[2]	10.8	19.3	8.4	—[2]	13.9
5.0	21.1	14.6	11.9	15.9	23.8	18.2	17.2	19.7
50.0	20.3	19.1	13.1	17.5	17.8	18.9	16.2	17.6
Mean	19.0	13.2	12.5		20.3	15.2	16.7	
	Invertase				Amylase			
0.5	11.6	5.1	—[2]	8.4	28.3	19.1	—[2]	23.7
5.0	13.0	10.6	5.9	9.8	51.1	50.4	37.7	46.4
50.0	14.0	13.4	7.0	11.5	69.7	70.8	54.7	65.1
Mean	12.9	9.7	6.5		49.7	46.8	46.2	
	Polyphenol oxidase							
0.5	20.6	10.6	—[2]	15.6				
5.0	24.9	22.1	18.4	21.8				
50.0	35.0	22.8	16.3	24.7				
Mean	26.8	18.5	17.4					

[1] Each figure represents the computed mean of 3 replicates.
[2] Tissues were killed by the 6-azauracil treatment.

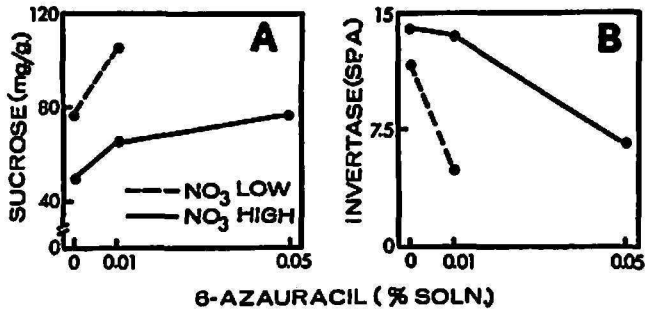

FIG. 3.—Moderating effects of high NO_3 (50 meq./liter) on 6-azauracil-induced sucrose and invertase changes in immature storage tissue. A, Lessened sucrose increase; B, less pronounced invertase decline.

effectiveness of 6-azauracil as an enzyme inhibitor under extreme conditions of N stress.

6-azauracil (0.05 percent) succeeded in suppressing growth regardless of NO_3 supply. High NO_3 (50 meq./liter) alleviated but did not overcome the

inhibitor effect. It was suggested that N fertilization might be prolonged to increase cane tonnage, with subsequent growth termination being based upon a chemical agent rather than the withholding of N.

Low NO_3 (0.5 meq./liter) reduced growth, increased sucrose content of leaf and immature storage tissues, and raised Brix and polarization values for mature storage tissue. 6-azauracil also produced a ripening effect, as evidenced by increasing Brix and polarization values in the low NO_3 plants, but failed to do so when NO_3 supply was raised.

Nitrate generally increased the activity of invertase, amylase, phosphatase, ATP-ase, and oxidases. 6-azauracil retarded enzyme action, but was less effective when the NO_3 supply was high. Failure to severely retard invertase and amylase is believed to account for Brix and polarization decline.

Although 6-azauracil was applied as a spray to the cane leaves, the leaf tissues and leaf enzymes did not appear to be damaged by the inhibitor. The chemical is believed to be translocated to meristem and immature storage tissues while leaves remain relatively undamaged, and presumably photosynthetically active.

RESUMEN

Caña de azúcar, sembrada en arena, fue sometida a niveles extremos de nitrato (NO_3) para simular condiciones de crecimiento restringido y madurez, crecimiento moderado y crecimiento suculento. El análogo de pirimidina 6-azauracilo fue suministrado concurrentemente en aspersión foliar. Tres fueron los objetivos: 1, Medir la persistencia de los regímenes de crecimiento en presencia de un inhibidor poderoso del crecimiento; 2, evaluar el 6-azauracilo como un retardador del crecimiento en aquellas plantas estimuladas por cantidades elevadas de (N) y como agente de madurez en las cañas cuyo crecimiento era lento bajo condiciones similares de madurez; y 3, investigar la eficacia del 6-azauracilo como inhibidor de enzimas al aumentarse en extremo el N.

El 6-azauracilo (0.05 por ciento) logró suprimir el crecimiento independientemente del suministro de NO_3. Una aplicación elevada de NO_3 (50 meq./litro) atenuó, pero no superó el efecto del inhibidor. Esto sugiere que el abonamiento con N puede prolongarse para aumentar el tonelaje de la caña, con un subsiguiente cese del crecimiento estando esto basado en un agente químico, en vez de la supresión del N.

Una aplicación baja de NO_3 (0.5 meq./litro) redujo el crecimiento, aumentó el contenido de sacarosa de la hoja y de los tejidos reservantes tiernos y elevó los valores de Brix y polarización en el tejido reservante maduro. El 6-azauracilo también produjo un efecto de madurez al aumentar los valores de Brix y polarización en las plantas con NO_3 bajo, pero no cuando se aumentó el suministro de NO_3.

El nitrato generalmente aumentó la actividad de la invertasa, amilasa, fosfatasa, ATP-asa y las oxidasas. El 6-azauracilo retardó la acción enzimática, pero fue menos efectivo cuando el suministro de NO_3 fue alto. La inhabilidad en cuanto a retardar marcadamente la amilasa y la invertasa se cree responda a la baja de los valores de Brix y polarización.

A pesar de aplicarse originalmente como una aspersión en las hojas de la caña, tanto los tejidos como las enzimas de la hoja no demostraron haber sido afectados por el inhibidor. Se cree que el agente químico se trasloca al meristemo y a los tejidos reservantes tiernos, mientras que las hojas permanecen relativamente sin daño alguno y con toda posibilidad fotosintéticamente activas.

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