Diurnal Behavior of Sugarcane Acid Invertase in Early-Juvenile and Early-Adult Plants Treated with Polaris[1]

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ABSTRACT

The diurnal behavior of sugarcane acid invertase was examined in 5- and 14-week old plants treated with Polaris (N,N-bis [phosphonomethyl] glycine). A commercial plant growth regulator and in vivo inhibitor of cane invertase, Polaris was administered as an aqueous foliar spray at 0645 h. Immature stem samples were harvested at 2-h intervals from 0630 to 1830 h on day of treatment and throughout the following two days. In plantlets, Polaris produced a major shift in the time of maximum invertase action within 2 h after application and without inhibiting the enzyme. Polaris persistently inhibited invertase during the third day without distorting the time or magnitude of enzyme fluctuation. Similar effects were produced in young-adult plants having greatly lengthened and elaborated transport pathways. Neither the natural fluctuations nor chemical-induced changes were accompanied by changes in tissue sucrose, reducing sugars, or soluble protein. Results are discussed in the context of two control systems for acid invertase: one governing its synthesis in immature stem tissue and inhibited by Polaris, the other governing its diurnal behavior, possibly located in leaf tissues, and only temporarily distorted by Polaris.

INTRODUCTION

Activity levels for sugarcane acid invertase fluctuate several fold between early morning and late afternoon (6, 5, 2). This capacity for rapid change is consonant with the enzyme's roles in storing and utilizing the large sucrose influxes received daily by sugarcane sink tissues (6, pp. 332–56, 11, 10). On the other hand, the regulatory system governing these changes, and the extent to which they are isolated from the general production of catalytic protein, are largely unknown.

The plant growth regulator Polaris[3,4] is a very powerful and persistent in vivo inhibitor of sugarcane acid invertase (2, 3). As a chemical ripener, its maximum effect is usually recorded together with stem quality improvement several weeks or more after its application. As a physiological tool, however, it offers an insight into the invertase regulatory system by forcing the latter to respond to an imposed stress whose

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[3] N,N-bis (phosphonomethyl) glycine. Monsanto Agricultural Products, Co., St. Louis, Mo.

[4] Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

timing and ultimate effects are known. For the present studies, Polaris was used as a stress-inducing agent in order to evaluate diurnal invertase behavior during a 3-day time course spanning the onset of chemical inhibition.

MATERIALS AND METHODS

Plants of the interspecific hybrid PR 980 were propagated in quartz sand with controlled water and nutrient regimes as previously described (4). Two experiments were performed, the first with 5-week old plantlets, and a second with young-adult plants 14 weeks old. In each experiment, expanding stem tissues were harvested for enzyme, sugar, and soluble protein analyses at 0630 h on day 0, and at 2-h intervals thereafter through 1830 h. Samples were similarly harvested on the following two days (days 1 and 2).

Polaris was administered at 0645 h on day 0, as an aqueous foliar spray containing 3000 p/m active material plus 0.10% Tween 20 as wetting agent. Control plants received distilled water plus wetting agent. There were three replications of each treatment arranged in an incomplete randomized block design.

Tissue samples were initially frozen in a mixture of dry ice and acetone. They were lyophilized, ground to a fine powder, and extracted with distilled water containing 2-mercapto-ethanol (0.005M) as a phenol oxidase inhibitor. Protein was precipitated with solid ammonium sulfate, redissolved in water, and dialyzed against a 0.001M solution of 2-mercapto-ethanol. The protein obtained between 30 and 55 percent saturation was used for assay of acid invertase. Total soluble protein was determined with the 0 to 95 percent fraction. Sucrose and reducing-sugar analyses were performed directly with clarified aqueous extracts. Procedures for the sugar, protein, and invertase assays are detailed in a previous report (4). Statistical comparisons of control and Polaris values at each harvest were performed in accordance with the Student *t* test.

RESULTS

Maximum invertase activity in plantlets was shifted to an earlier hour by Polaris (fig. 1), and the effect was produced within two hours after chemical application. Repetition of the experiment with young-adult plants, i. e. in a vastly lengthened and elaborated source-to-sink system, produced a similar effect within four hours after application. Invertase repression became general during the third day of treatment for both experiments. Characteristic diurnal action patterns were retained in spite of Polaris repression of absolute activity. Neither the chemical-induced changes nor natural fluctuations for invertase were

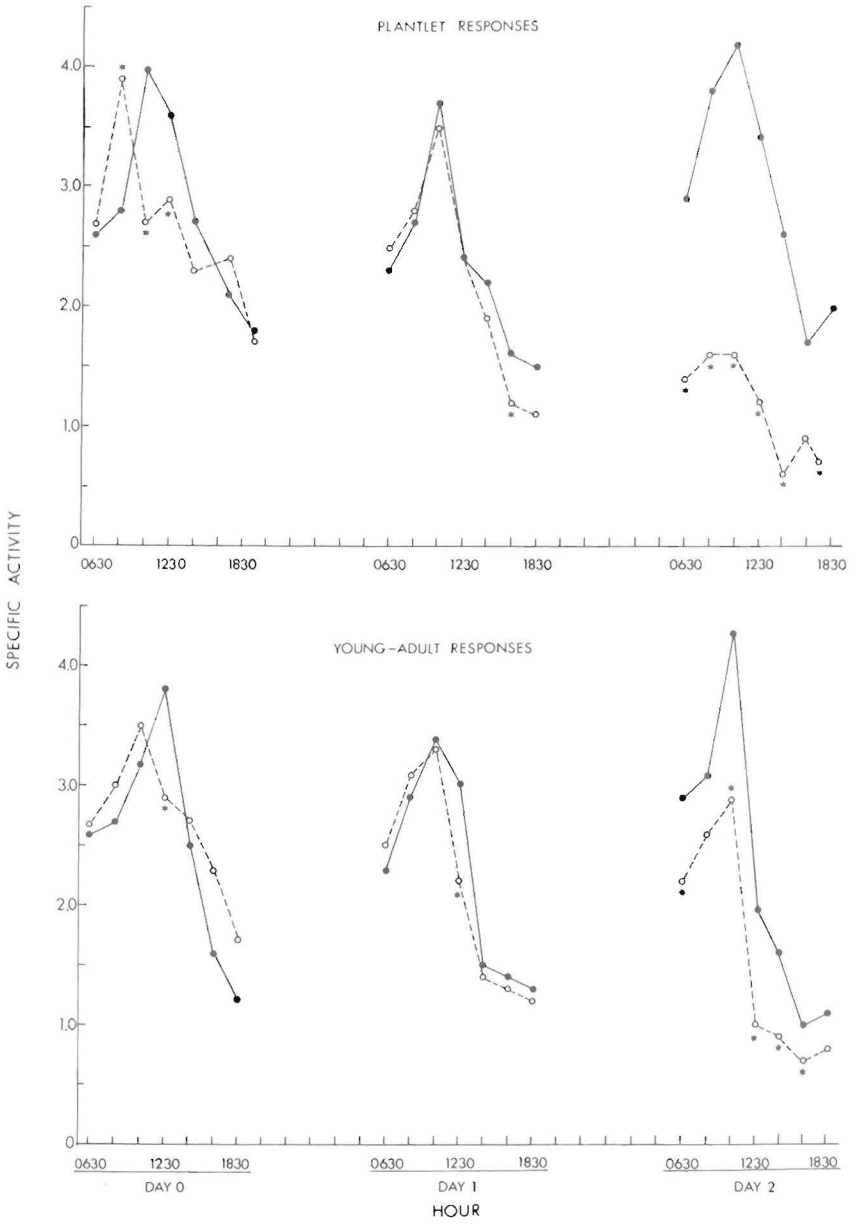

FIG. 1.—Diurnal behavior of sugarcane acid invertase in early-juvenile and early-adult plants following treatment with Polaris. Symbols: (—●—) Control; (--○--) Polaris. Asterisks indicate a significant deviation of Polaris values from the corresponding control values ($P < .05$).

accompanied by similar changes in soluble protein, sucrose, or reducing sugars (table 1).

DISCUSSION

While in general terms the endogenous levels of both substrate and product can affect the performance of a given enzyme, the present diurnal changes for acid invertase were not directly related to either factor. The rapidity and magnitude of change, and the concurrent lack of change in soluble protein, are consistent with the operation of a hormonal regulatory system specifically directed toward acid invertase. This interpretation for cane invertase regulation was developed by workers in Australia and has been reviewed in detail (6, pp. 359–82). On the other hand, the early distortion of peak activity by Polaris, plus the persistence of a diurnal fluctuation pattern after chemical repression was underway, offer several qualifications to prior understanding of invertase behavior.

The diurnal shift in maximum invertase activity, occurring within a few hours after Polaris application, suggests that the enzyme's change is governed by a timing system sensitive to the intrusion of a powerful growth regulator. The quickness of change in plantlets was at first considered to be an artifact of their abbreviated transport pathway, consisting only of 2 to 3 leaves closely connected with a miniature sink. If it is assumed that Polaris is translocated in cane via the phloem conducting pathway[5] developed with ^{14}C-labeled sucrose (6, pp. 274–88), at rate and velocity levels proportional to sucrose, traces of the chemical would begin to arrive at the plantlet sink about 40 minutes after leaf penetration. For young-adult plants the arrival of inhibitor at the apical meristem should require 2 to 3 hours[6]. The bulk of transient Polaris would disperse along an extended sink, comprising 8 to 10 stem internodes of varying maturity, over a time course of about 24 hours. Repetition of the experiment with young-adult plants again produced an early distortion of maximum enzyme activity (fig. 1), indicating that the effect was not reliant upon an undeveloped transport pathway.

It can be argued that Polaris or a Polaris-induced inhibitor might be translocated more efficiently than sucrose. This is unlikely in view of

[5] There is evidence that the bipyridilium desiccant Diquat produces a reversal of metabolite transport from phloem to xylem conducting pathways in *Lycopersicon* (17); however, this appears to be an artifact of tissues approaching death and has not been reported with respect to chemical ripening.

[6] These estimates are based on a minimum transport pathway, that is from basal lamina tissue of the leaf blade having the shortest conducting distance to the apical meristem.

TABLE 1.—*Diurnal levels of expanding-stem tissue components of early-juvenile and early-adult sugarcane treated with Polaris*

Day	Hour	Early-juvenile components, mg/g –						Early-adult components, mg/g –					
		Sucrose		Total red. sugars		Soluble protein		Sucrose		Total red. sugars		Soluble protein	
		Control	Polaris[1]	Control	Polaris	Control	Polaris	Control	Polaris	Control	Polaris	Control	Polaris
0	0630	201	193	127	128	51	56	180	202	194	229	41	46
	0830	215	162	146	142	59	59	207	193	226	227	48	44
	1030	196	264	150	144	61	54	173	164	213	226	48	46
	1230	234	259	149	136	42	41	197	207	229	220	38	49*
	1430	220	233	134	125	55	48	233	241	209	241*	45	35
	1630	205	230	169	170	59	42*	237	221	210	223	44	45
	1830	240	236	125	139	43	42	235	208*	200	216	48	39
1	0630	193	175	145	116*	66	88	196	172	218	230	49	52
	0830	183	152	142	137	95	90	175	154	239	245	45	42
	1030	227	169	136	118	64	65	212	185	236	239	45	41
	1230	217	209	131	122	72	72	240	260	229	235	37	45
	1430	204	191	108	146	64	73	293	306	226	219	38	43
	1630	198	221	124	107	80	67	278	268	220	227	41	46
	1830	196	197	135	118	90	80	286	263	209	217	45	43
2	0630	134	159	109	91	39	46	199	200	198	198	45	45
	0830	123	183*[2]	101	88	44	38	202	197	210	206	51	47
	1030	179	204	95	97	44	40	206	212	225	213	47	44
	1230	187	275*	114	89*	44	36	249	257	217	200	38	54
	1430	226	282*	107	88	33	30	277	275	212	192	36	42
	1630	244	272	107	101	28	36	289	261	200	190	36	42
	1830	193	286*	93	93	30	30	263	277	196	202	36	36

[1] Polaris was administered at 0645 h on day 0, as an aqueous foliar spray containing 3000 p/m active ingredient.

[2] Asterisks indicate a significant deviation of the Polaris value from the corresponding control value ($P < .05$).

TABLE 2.—*Acid invertase activity over a three-day time course in early-juvenile and early-adult sugarcane treated with Polaris*

Plant age (weeks)	Average specific activity[1] values, for day—					
	0		1		2	
	Control	Polaris	Control	Polaris	Control	Polaris
5	2.8	2.7	2.3	2.2	2.9	1.1
14	2.5	2.7	2.3	2.1	2.3	1.5
	Daily max. activity/Daily min. activity					
5	2.2	2.3	2.5	3.1	2.5	2.7
14	3.2	2.1	2.6	2.8	3.9	4.1

[1] Specific activity = activity units/mg of protein. One activity unit equalled the amount of enzyme required to produce 0.10 mg of reducing sugar under the prescribed conditions of the assay (4).

the complex anatomy of sugarcane conducting tissues, and of transport logistics in a conduit system already burdened with sucrose (6, pp. 321–26). A more plausible interpretation is that small amounts of Polaris had confounded an endogenous timing or "clock" mechanism governing the diurnal pattern for invertase activity. Moreover, this mechanism might be located in leaf tissues where translocation time is not a decisive factor. The main support for this hypothesis derives from the rapidity of the early changes effected by Polaris, and the fact that these were not changes in absolute activity (table 2), but rather in the timing of maximum activity. The traditional Polaris repression was not clearly underway until the third day (fig. 1, table 2).

The hypothetical timing mechanism for invertase is apparently not damaged or permanently distorted by Polaris. Invertase changes recorded shortly after the chemical's application were not repeated during the second day, nor during the third day when activity repression in sink tissue was clearly underway[7] (fig. 1). At this time the magnitude of diurnal flucuation in Polaris-treated plants remained proportionately equal to the fluctuation in control plants (table 2). This is interpreted in terms of a net loss of invertase-producing capability—presumably a Polaris restriction of invertase m-RNA (9, 15)—while a second system governing the diurnal conformation of the enzyme's remaining work potential continues to operate unimpeded. The continued operation of a diurnal control system is also consistent with the view that some critical level of invertase activity must continue in support of sucrose accumulation processes, even while sucrose inversion for support of growth and respiratory processes is being curtailed.

[7] Presumably, most of the Polaris that penetrated the cane canopy would have exited leaf tissues by the second day.

Hormone-like substances having powerful regulatory effects on plant source and sink processes that are spatially removed from the site of substance production are generally recognized and have been discussed by numerous authors (13, 8, 12, 14, 7). The concept of foliar contributions toward the invertase changes presently recorded in sink tissues is empirically consonant with earlier findings from variable-illumination studies (5, 1). However, the operation of a foliar "clock" for invertase remains a matter of speculation. Polaris, thoughtfully utilized, will serve as a useful tool in examining source regulation of sink reactions.

RESUMEN

Se examinó el comportamiento diurno de la invertasa ácida en plantas de caña de azúcar de 5 y 14 semanas de edad tratadas con Polaris. Este se administró en aspersión foliar acuosa a las 0645 h. Se cosecharon muestras de tallos verdes a intervalos de 2 horas de 0630 a 1830 h el día del tratamiento y durante los dos días siguientes. En las plántulas, Polaris adelantó el tiempo de acción máxima de la invertasa dentro de las 2 horas posteriores a la aplicación, sin retardar la acción de la enzima. Durante el tercer día Polaris retardó persistentemente la invertasa sin alterar el tiempo o la magnitud de fluctuación de la enzima. Efectos similares se produjeron en plantas adultas jóvenes con conductos de transporte alargados y elaborados. Ni las fluctuaciones naturales ni los cambios producidos por el agente químico fueron acompañados por cambios en el contenido de sacarosa del tejido, reducción de los azúcares o cantidad de proteína soluble. En este contexto se discuten dos sistemas de control para la invertasa ácida, uno que gobierna su síntesis y el otro que gobierna su comportamiento diurno, posiblemente localizado en los tejidos de la hoja, el que el Polaris altera temporalmente.

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