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Development of Sinks as an Autocatalytic Feed-back Process: a Test using the Asymmetric Growth of Leaves in Mestha (*Hibiscus cannabinus* L.)

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Differential development of sinks that depend on a common resource pool has been viewed as a consequence of an autocatalytic feedback process of flow of resource units into them. The feed-back process implies that the stronger a sink is relative to its competitors, the greater is its probability of getting further resources as a non-linear function of its resource drawing ability and sink size. We show that this model contrasts with that of the sink-strength dependent model in its prediction of the subsequent development of the initial asymmetry of growing leaves when their resource drawing ability is enhanced. By artificially enhancing the resource drawing ability of the leaves of Mestha (*Hibiscus cannabinus* L.) by external application of growth regulators, we test these predictions and show that the results are in conformity with the autocatalytic model proposed by Ganeshaiah and Uma Shaanker.

Key words: Autocatalytic growth, resource flow, leaf asymmetry, sink-strength, self-organization, *Hibiscus cannabinus* (L.).

INTRODUCTION

Ganeshaiah and Uma Shaanker (1992, 1994) and Uma Shaanker, Ganeshaiah and Krishnamurthy (1995) have shown that the differential development of seeds within a fruit can be viewed as a consequence of an autocatalytic feed-back process of accumulation of resource units into growing sinks. Their argument is based on the assumption that movement of resource units into any developing seed is a non-linear function of its relative dominance among the developing seeds of a fruit for garnering resources. Accordingly, the relatively dominant among them draw more resources; these additional resource units gained enhance their dominance further, which in turn facilitates them drawing more resources. In other words, this autocatalytic feed-back flow of resources results in an aggravated asymmetry or hierarchy among developing seeds leading to abortion of the subordinates. Ganeshaiah and Uma Shaanker (1994) and Uma Shaanker *et al.* (1995) also suggested that such aggravation of asymmetry mediated through the process of self-organized flow of resource units could be the underlying process of the differential development of several plant organs, such as branches and leaves, and flowers and fruits in an inflorescence.

Ganeshaiah and Uma Shaanker (1992) simulated the development of seeds in a fruit as a consequence of such self-organized flow of resource units and developed certain predictions that, though not in conformity with the general belief in plant physiology, are strongly supported by the experimental data and existing literature (Ganeshaiah and Uma Shaanker, 1994; Uma Shaanker *et al.*, 1995).

In this paper, we show that certain predictions of this model for the process of movement of resource units into sinks are upheld even in the development of leaves. We alter the resource drawing ability of leaves that depend on a common resource pool during their early growth and examine the fate of the initial size asymmetry among them. We show that the results conform to the predictions of the autocatalytic growth model proposed by Ganeshaiah and Uma Shaanker (1992, 1994).

THE MODEL AND THE PREDICTIONS

The autocatalytic feed-back process simulated by Ganeshaiah and Uma Shaanker (1992, 1994) is a direct translation of the Polya-Urn equation used to explain the pheromone mediated path selection by ants (Deneubourg *et al.*, 1989; Ganeshaiah and Uma Shaanker, 1994). Consider any two simultaneously developing sinks (say A and B) that are similar in all respects including the initial resource levels (k) in them. Ganeshaiah and Uma Shaanker (1994) proposed that if the two sinks are dependent on a common resource pool, then the probability that a given sink (say A) gets the resource unit can be given by:

$$P(\mathrm{A}) = \frac{(k+r_{\mathrm{A}})^{x}}{(k+r_{\mathrm{A}})^{x}+(k+r_{\mathrm{B}})^{x}} \quad (1)$$

where r_{A} and r_{B} are the additional resource units moved in

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to the sinks A and B, respectively and x is the sink drawing ability or the metabolic activity of every unit of tissue (and hence considered the same for both A and B).

Ganeshaiah and Uma Shaanker (1994) suggested that growth of a set of developing sinks can be simulated as a series of steps of accumulation of discrete units of resources in the sink in accordance with the above equation and with certain additional conditions (Ganeshaiah and Uma Shaanker, 1992) that might be specific to the nature of the growing sinks.

Note that to begin with, since $r_A = r_B = 0$, $P(A) = P(B) = 0{\cdot}5$, and hence both sinks should develop equally. However, if the resource units are assumed to move in discrete units then, purely by random drift, one of the sinks (say A) is likely to accumulate more resources. This reinforces its resource base (r_A) and, as a feed-back process, increases the probability of it getting subsequent resource units; such an autocatalytic feed-back process eventually leads to the dominance of sink A over the others. Thus Ganeshaiah and Uma Shaanker (1994) showed that without any intrinsic biological factors, asymmetry among the developing sinks might arise purely by a random drift. Though this is one of the important predictions of the process of the self-organized movement of resource molecules, we do not set forth to test this here as it has been shown and discussed elsewhere (Deneubourg *et al.*, 1989; Ganeshaiah and Uma Shaanker, 1992, 1994).

But in biological systems, such initial asymmetry need not build up purely by random drift or stochastic process. Rather, factors such as temporal differences in the differentiation of leaves and sequential fertilization of ovules may also lead to initial asymmetry among those respective sinks. Once asymmetry is generated, the feed-back process of resource flow, based on the Polya-Urn equation, defines the pattern in which such asymmetry gets accentuated. Note that the x in eqn (1) defines the extent to which $P(A)$ increases (decreases) with a unit increase in r_A over r_B (or *vice versa*). For $x = 0$, even if $r_A > r_B$, the $P(A)$ would still be equal to $P(B) = 0{\cdot}5$. However for higher values of x, the $P(A)$ increases with increase in r_A. More importantly, $P(A)$ also increases with x; for a given value of r_A (such that $r_A > r_B$), $P(A)$ increases with x (Ganeshaiah and Uma Shaanker, 1992). The power of the equation, x, is hence suggested to represent the metabolic activity or resource drawing ability of every unit of sink, and Ganeshaiah and Uma Shaanker (1992, 1994) have considered this to reflect the metabolic or specific activity of the tissue.

Grossly, it might appear that the argument followed in the usage of the equation is similar to that of the sink strength dependent flow of resources into the developing organs (Peel and Ho, 1970; Evans, 1976; Cook and Evans, 1978). But these two arguments differ with respect to their predictions about the fate of the initial asymmetry among the developing sinks that are dependent on a common resource pool. The sink strength dependent model implies that the available resources move into the competing sinks in proportion to their sink strength (Ho, 1988), which generally is defined as the product of the sink size (r_A or r_B) and its metabolic activity (x). Therefore, if the metabolic activity of the sinks is enhanced equally and simultaneously (say from x to $x + \delta x$), the proportional or the relative sink strength of all the sinks would still remain the same and hence the initial asymmetry among them in their growth (and hence in size) would also remain unaltered. For instance the initial asymmetry of the two sinks, A and B [$(r_A x/(r_B x) = r_A/r_B$; or, $r_A x/(r_A x + r_B x) = r_A/(r_A + r_B)$; depending on how the proportion is defined], would remain unaltered on enhancing the sink drawing ability [$(r_A(x+\delta x))/(r_B(x+\delta x)) = r_A/r_B$; or, $(r_A(x+\delta x))/(r_A(x+\delta x) + r_B(x+\delta x)) = r_A/(r_A + r_B)$].

On the other hand, the Polya-Urn model as envisaged by Ganeshaiah and Uma Shaanker (1994) predicts that the initial asymmetry between the sinks becomes aggravated by simultaneously enhancing their sink drawing ability. Since sink strength in this model is defined as a power function of the sink size (r_A), any increase in x, the power of the equation, would increase the probability that a given sink would get the resource units in a subsequent step [see eqn (1)], and hence such a sink would dominate further in drawing the resource units. Thus when the resource drawing ability of the developing sinks is simultaneously and equally enhanced, the autocatalytic model, as envisaged by Ganeshaiah and Uma Shaanker (1994), predicts an enhancement of the initial asymmetry among such sinks while the existing model of sink strength dependent flow of resources does not.

In this study we have attempted to provide a test of this prediction by monitoring the fate of the initial asymmetry between simultaneously developing leaves of Mestha when their resource drawing ability, x, was simultaneously and equally enhanced by the external application of growth regulators. We propose that aggravation of this asymmetry due to application of growth regulators supports the model proposed by Ganeshaiah and Uma Shaanker (1994), while persistence of the original asymmetry supports sink strength dependent flow of resources.

MATERIAL AND METHODS

Plant material

Seedlings of Mestha (*Hibiscus cannabinus* L., Malvaceae) var. AMC 16 (seed source: ICAR adhoc Project on Genetic Improvement of Mestha, Department of Plant Genetics and Breeding, University of Agricultural Sciences, Bangalore, India) were used. In these seedlings, the first pair of primary leaves (P1) emerged 4 or 5 d after the cotyledons turned green and expanded as cotyledonary leaves. Following this, subsequent leaves emerged in pairs at intervals of 4–6 d. The leaves of each pair grew apparently simultaneously from the meristematic tip, but as they expanded they differed in their length and breadth. These leaves occupied the same position on the stem until they grew to their maximum size and hence would be directly competing for the resources; however later, the portion of the stem subtending them started elongating such that they eventually occupied two separate nodes. In such a pair, the leaf that is larger to begin with (older) occupies the lower node. We assume that leaves behave as sinks at least until they grow and expand to their complete (maximum) size, and until then the two leaves of each pair depend on a common resource pool. We used the first three pairs of leaves (P1, P2 and P3) for our experiments.

Growth regulator treatment

Seedlings were raised in pots (30 cm diameter, 30 cm height) filled with sand and soil and were assigned randomly to 12 treatments with four plants as replications for each treatment. We used all 12 factorial combinations of indole 3-acetic acid (IAA; three concentrations *viz*., 0, 10, 100 μM) and 6-benzylaminopurine riboside (BA; four concentrations *viz*., 0, 0·5, 5·5, 14·0 μM) for treating the plants. Our treatments started within about 3–4 d after the seedlings emerged or from the time the cotyledonary leaves started to turn green and to expand. A small cotton swab was placed on the meristematic tip from where the first pair of leaves (embryonic; P1) expand and the later pairs (P2 and P3) differentiate, and 100 μl of growth regulator combinations were applied twice a day in the morning and evening. The growth regulator treatment was repeated every day with fresh cotton swabs until the completion of the observations. Hormones thus applied would diffuse into both leaves of each pair, such that their sink strength is simultaneously and equally enhanced. As argued in the previous section, the consequences of such simultaneous and equal enhancement of sink strength of both leaves would help discriminate the two models.

Observations

Leaves of each pair took about 3–5 d to attain a length of about 1 cm after the leaves of the preceding pair had expanded to this size. These leaves were in direct contact with the externally applied growth regulators only up to this stage and hence observations were recorded from the day the leaves of a pair reached at least 1 cm in length. The length and breadth of the first (L_1 and B_1, respectively) and second leaf (L_2 and B_2, respectively) of each pair was measured every morning to an accuracy of 0·1 mm using a vernier calipers. Observations were stopped for each pair either when the leaves had grown to their maximum size or when the portion of the stem between leaves of each pair started to elongate and so separate the leaves. Until this stage the leaves are actively growing, and hence we consider them to be sinks, drawing resources from external sources.

The asymmetry ratio in leaf size was computed in two ways: (*a*) as a ratio of length (breadth) of first leaf to the second (L_1/L_2 or B_1/B_2), and (*b*) as a proportion of the length (breadth) of first over the total length (breadth) of both the leaves [$L_1/(L_1+L_2)$ or $B_1/(B_1+B_2)$]. Since analysis of both of them yielded similar results we have presented here only the first parameter (ratio of the first to second leaf).

Area of leaves was computed as a product of the length and breadth. On a sample survey we found that this product was highly correlated with the actual area such that the latter was a simple linear function of the product of L and B. However, we have used the product of length and breadth as a measure of area because our analysis was mostly concerned with the ratios of the leaf sizes, and in finding this ratio the conversion factor would in any case vanish; hence the results and conclusions are not affected by using this measure for area. Further, by adopting this method, destructive sampling of the leaves was avoided. Area asymmetry was also represented as the ratio of the area of the first to that of the second leaf [$A_1/A_2 = (L_1 B_1)/(L_2 B_2)$].

RESULTS

General pattern of leaf growth

The pattern of the relative growth of the two leaves in all the three pairs was similar. To begin with, the first leaf of each pair was larger than the second; but its growth stopped much earlier than the second leaf and eventually the second leaf grew larger than the first. Hence the asymmetry ratio of the size of the first to the second leaf (in length or breadth or area) decreased monotonically with days (Fig. 1 for length and breadth of P2; Fig. 2 for area of P1, P2 and P3). Such reduction in the asymmetry ratio between the first and second leaf was true for all the pairs of leaves (Fig. 2); but the three pairs differed in the rates at, and levels to, which their asymmetry ratios decreased. The number of days for which each pair was observed differed because of the criteria adopted to terminate the observations (see Material and Methods).

Effect of growth regulators

Asymmetry. Application of growth regulators enhanced the initial asymmetry between the first and second leaf (A_1/A_2) in each pair (Figs 3–5), and such enhanced

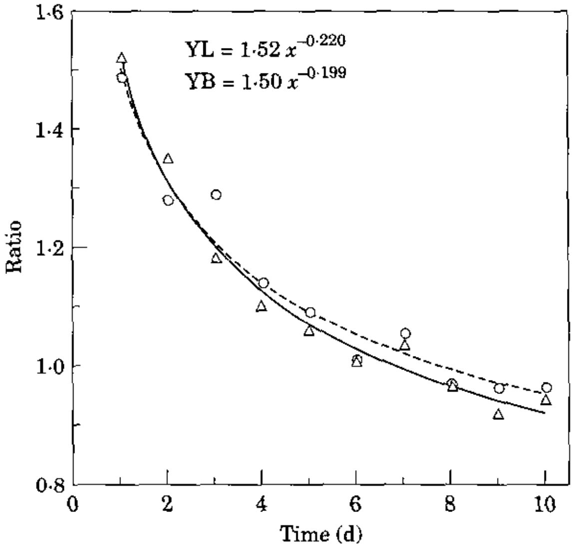

FIG. 1. Decrease in asymmetry ratios (ratio of first to second leaf) of length (△) and breadth (○) of leaves of pair 2 during the observation period in control plants. The solid (length ratio; YL) and dashed (breadth ratio; YB) lines refer to the best fit equations.

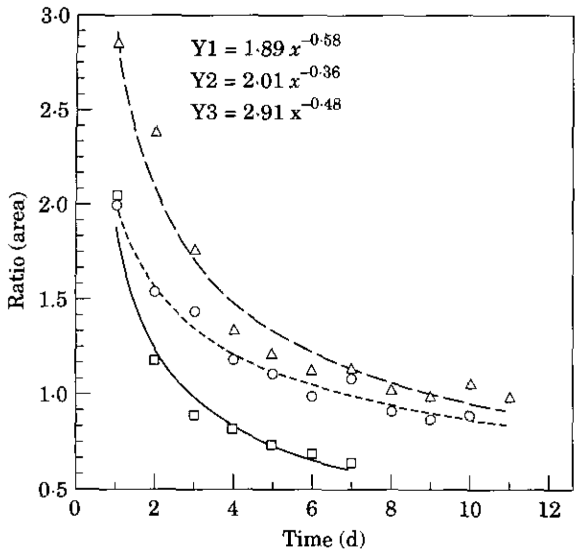

FIG. 2. Decrease in asymmetry ratios of areas (ratio of area of first to that of second leaf) of pair 1 (□, ——; Y1), pair 2 (○, ······; Y2) and of pair 3 (△, ---; Y3) during the observation period in control plants. The lines refer to the best fit equations.

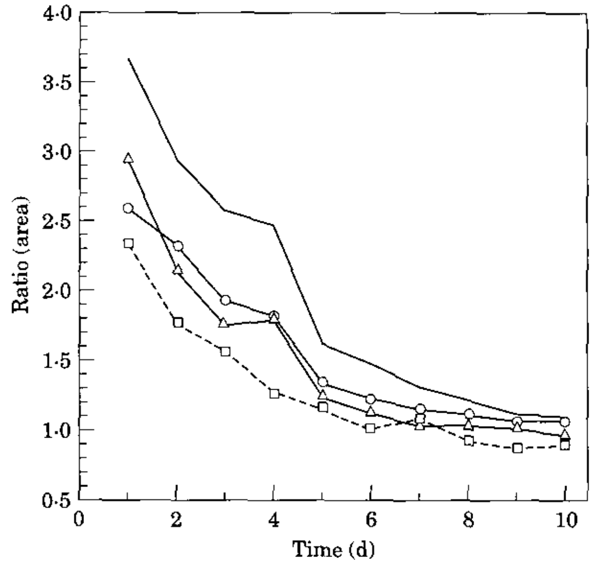

FIG. 4. Decrease in asymmetry ratios of areas of leaves of the second pair of control (0 μM BA; □, ---), 0·5 μM BA (△, ——), 5·5 μM BA (○, ——) and 14 μM BA (——) treated plants.

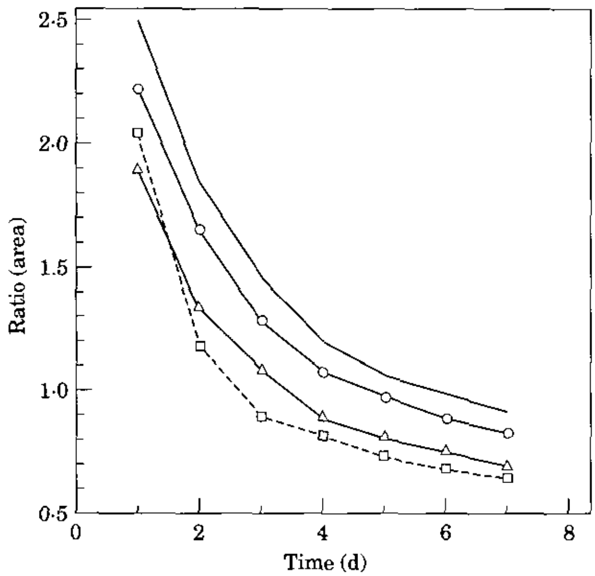

FIG. 3. Decrease in asymmetry ratios of areas of leaves of the first pair of control (0 μM BA; □, ---), 0·5 μM BA (△, ——) 5·5 μM BA (○, ——) and 14 μM BA (——) treated plants.

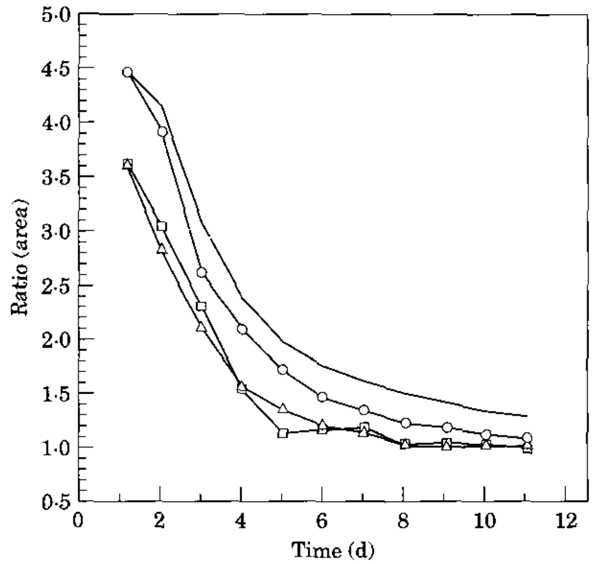

FIG. 5. Decrease in asymmetry ratios of areas of leaves of the third pair of control (0 μM BA; □, ---), 0·5 μM BA (△, ——), 5·5 μM BA (○, ——) and 14 μM BA (——) treated plants.

asymmetry persisted throughout. The effect of BA on enhancing the asymmetry was more prominent than that of IAA. In fact, the effect of IAA on asymmetry was significant only on the second, third and fourth day of observations for P1, and only on the third day for P2 (Table 1). IAA did not affect the asymmetry between the leaves of P3 (Table 1). On the other hand, BA significantly enhanced the asymmetry of all three pairs of leaves, and this effect persisted almost until full growth of the leaves (Table 1, Figs 3–5). IAA and BA did not, however, interact in their effect on asymmetry ratios (Table 1). For this reason, and because the effect of IAA was not very prominent, we discuss below only the effect of BA on leaf size.

Leaf size. The enhanced asymmetry could be due to the enhanced size of the first leaf, and/or reduction in the size of the second leaf. However, the results suggested that the effect of BA on enhancing the asymmetry was found to be primarily a consequence of the enhanced size of the first leaf, the larger of the two to begin with. In all the pairs, the first leaf was larger in the plants treated with 14 μM BA than the corresponding leaf in the control, and these differences were also significant on several days of observation (Figs

TABLE 1. *Analysis of variance (mean squares) for the asymmetry ratio for area of all the three pairs of leaves*

		Day										
Source	d.f.	1	2	3	4	5	6	7	8	9	10	11
Pair 1												
IAA	2	1·298	0·557*	0·393*	0·149*	0·022	0·008	0·006				
BA	3	0·879	0·770**	0·489**	0·317**	0·236**	0·173**	0·136**				
Interaction	6	0·171	0·029	0·005	0·006	0·012	0·023	0·030				
Error	34	0·570	0·126	0·090	0·037	0·018	0·018	0·020				
Pair 2												
IAA	2	1·79	1·73	1·44*	0·646	0·194	0·138	0·141	0·024	0·043	0·023	
BA	3	4·49*	2·63*	2·25*	2·075*	0·502**	0·424**	0·188*	0·172**	0·127**	0·124**	
Interaction	6	0·22	0·140	0·310	0·253	0·064	0·036	0·024	0·012	0·004	0·009	
Error	34	1·39	0·73	0·342	0·652	0·083	0·073	0·045	0·016	0·017	0·016	
Pair 3												
IAA	2	0·018	1·16	0·48	0·034	0·199	0·136	0·071	0·028	0·027	0·051	0·064
BA	3	0·217	6·07	1·78	2·086*	1·18**	0·778**	0·537**	0·549**	0·405**	0·259**	0·246**
Interaction	6	0·209	0·98	0·27	0·26	0·003	0·005	0·031	0·014	0·013	0·085	0·034
Error	34	0·228	2·36	1·65	0·53	0·181	0·105	0·079	0·053	0·058	0·040	0·050

* Significant at $P < 0{\cdot}05$; ** significant at $P < 0{\cdot}01$.

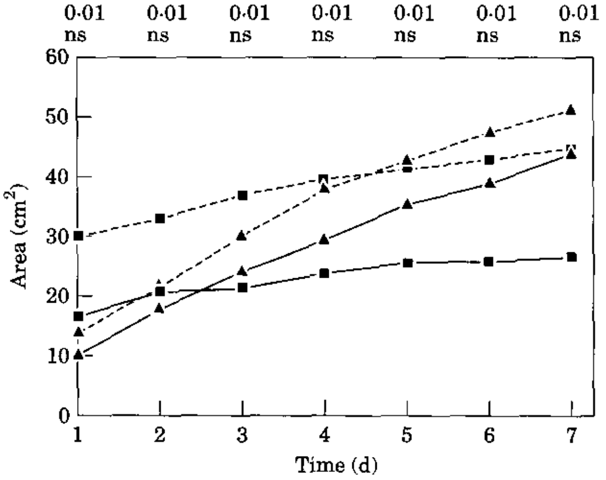

FIG. 6. Area of first (■) and second (▲) leaf of first pair in control (——) and 14 μM BA treated (–––) plants. The significance for differences between control and treated plants for different days is indicated on the top of the graph. The first row of values refers to the first leaf and second row for second leaf.

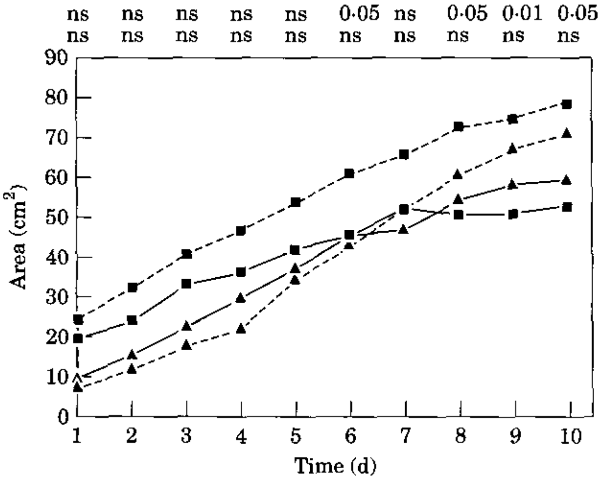

FIG. 7. Area of first (■) and second (▲) leaf of second pair in control (——) and 14 μM BA treated (–––) plants. The significance for differences between control and treated plants for different days is indicated on the top of the graph. The first row of values refers to the first leaf and second row for second leaf.

6–8). However, interestingly, the size of the second leaf, always the smaller of the two to begin with, was either equal or occasionally smaller in treated compared with control plants (statistical test of significance for the differences between the control and treated plants are provided on the top of Figs 6–8; the first row corresponds to first leaf). Though the second leaf of P1 in the plants treated with 14 μM appeared to be large compared to that in the control, these differences were not significant on any of the 7 d of observations (Fig. 6). Rather, the size of this second leaf in P2 and P3 was smaller in plants treated with 14 μM BA compared to that in the control, and these differences were also significant at least on a few days of observation (Figs 7 and 8). This may be because, unlike P2 and P3, leaves of P1 are differentiated at the embryonic stage itself and hence the application of the hormones on them may not have as significant an influence as it would on leaves of the later pairs.

DISCUSSION

The results show that when the metabolic activity of the leaves developing at the meristem is enhanced, the asymmetry in their size becomes aggravated. This is in conformity with the predictions of the model proposed by Ganeshaiah and Uma Shaanker (1994) for the movement of resources into the developing sinks. Their model suggests that the fate of any developing sink depends upon its history; among the competing sinks, those that have already received more resources dominate over the others and hence derive further resources and so aggravating the dominance hierarchy.

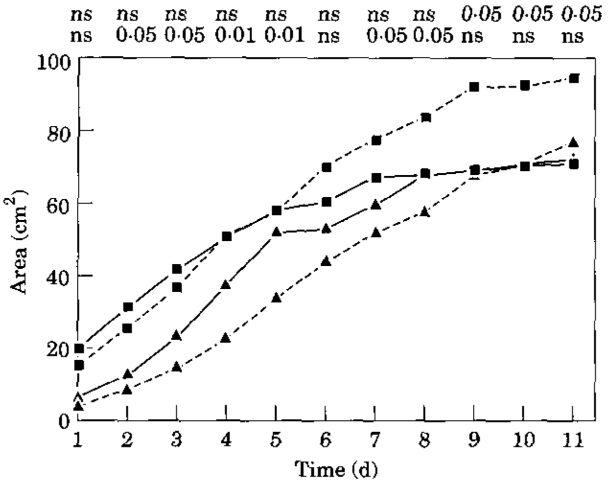

FIG. 8. Area of first (■) and second (▲) leaf of third pair in control (——) and 14 μM BA treated (---) plants. The significance for differences between control and treated plants for different days is indicated on the top of the graph. The first row of values refers to the first leaf and second row for second leaf.

Such an autocatalytic feedback process of resource flow is in fact supported by the observations made by Peel and Ho (1970) who used aphid colonies drawing plant sap as sinks. Using two colonies of aphids that differed in numbers as competing sinks for common resources from the host plant, they estimated the radioactive sugar moved in to the honeydew secreted by the aphids as a measure of resource flow to sinks. They found that the specific activity of the honeydew from aphids of the large colony was disproportionately greater than that obtained from the small colony, and concluded that 'the relationship between mass transport and colony size is not generally a linear one'. Though these results were inferred to reflect interaction among the demands made by the individual aphids of a colony, the mechanistic basis of such non-linear resource flow into developing sinks of plants was not clear; the model by Ganeshaiah and Uma Shaanker (1994) seems to offer a physical basis for such a process.

An important consequence of such a pattern of flow of resources is that the larger among the sinks draws relatively more assimilates compared to the smaller. In other words, within each pair, the leaf that is relatively large to begin with can be expected to grow larger in the treated plants while the smaller of the two can be expected to be the same as in the control (if additional resources do not flow to it) or smaller (if its resources are utilized for maintenance or if drawn away by the dominant sink). This pattern was in fact evident from the differential growth of leaves within each pair.

The first (large) leaf was generally larger in BA treated compared to that in control plants. The second leaf of each pair was either equal to or smaller than in the BA treated plants compared with corresponding leaves in control plants. In P1, though, the leaf that was initially large was larger in the treated compared with that in the control plants; the smaller was not different from the corresponding leaf in the control. This may be because the primary pair of leaves are differentiated in the embryo itself and hence are not completely amenable to the influence of the external growth regulator application as are the subsequent pairs. Nevertheless, the asymmetry ratio of the first pair of leaves was also higher in the BA treated plants than in the control. The subsequent pairs are exposed to growth regulator application almost from the early stages of their differentiation and probably for this reason they also exhibited a greater reduction in the size of the second (small) leaf.

The asymmetry ratio eventually decreased below one suggesting that the second leaf (small to begin with) has become larger than the first. This happens despite the initial dominance of the first leaf because, intrinsically, the two leaves grow to different maximal sizes on their full development. The first leaf (the older among the two) grows to a smaller maximum size than (as in P1), or equal to that of (as in P2 and P3), the second (younger) and hence attains its plateau much earlier. In other words, the growth rate of this first leaf is intrinsically reduced much before that of the second leaf. Despite this the initially aggravated asymmetry among the leaves of the treated plants persists until the end such that the first leaf of the pair in the treated plants is still larger than the corresponding leaf in the control.

As argued earlier, the sink-strength dependent model of resource flow predicts a constancy in the asymmetry among the developing sinks on enhancing their resource drawing ability. Though application of BA significantly enhanced the asymmetry among leaves, that of IAA did not alter it as prominently. Such persistence of asymmetry could suggest that the flow of resources occurs in a sink-strength dependent manner. However, the effect of IAA in enhancing the sink activity is not found to be as prominent as that of BA (Thomas, 1985). In other words, IAA is not likely to enhance the sink activity of the tissues and hence the persistence of asymmetry on IAA application may not constitute evidence for the sink-strength dependent model. Also, on certain days of observations, IAA application enhanced the asymmetry probably due to its positive but mild influence on the sink activity of the growing leaves.

In other words, the results do not support the existing model of sink-strength dependent resource flow. In fact the sink-strength dependent model was epitomized by Evans (1976) by citing from the Bible: 'For unto every one that hath shall be given and he shall have in abundance...'. Interestingly the Polya-Urn model while conforming to this, additionally implies what is said further 'but from him that hath not shall be taken away even that which he hath' (Matthew 25:29). Precisely, this is illustrated by the substantial increase in the size of the first (large) and occasional decrease in the size of the second (small) leaves of the pairs on enhancing their sink drawing ability.

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