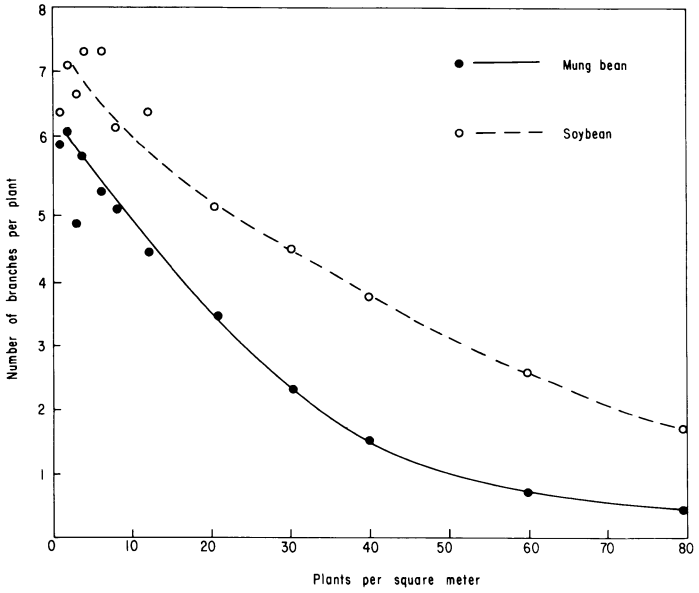

Fig. 11. Effect of plant density on number of branches per plant.

number of pods per plant that the soybean cultivar did when plant densities exceeded 100,000 plants per hectare (10 plants per m^2). Yield per pod did not vary substantially between the 2 species. Hence, yield levels for the mung bean cultivar would be expected, and were found, to be only one fourth those of the soybean.

It appears, based on these results, that there should be little or no detrimental effect of increased plant densities on the other yield components or on most of the other characters studied. In fact, it can be argued that a higher plant densities, plant branching would decrease thereby reducing interplant shading.

An understanding of the response of mung bean and soybean to increased plant density will allow exploration of our mung bean germplasm collection for types which better withstand the stresses of higher plant density. We are hopeful that the genetic resources will be found that will allow mung beans to be bred for more intensive cultivation.

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Diffusible Abscisic Acid and Its Relationship to Leaf Age in Tea Crabapple[1]

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Abstract. Abscisic acid (ABA) and its water soluble glucoside diffused from leaves through petioles and along the axes of seedlings of tea crabapple (*Malus hupehensis*, Rehd.). The apparent mobility of the glucoside was considerably greater than that of free ABA. Concentrations of extractable and diffusible ABA were greatest in growing shoot tips. ABA from mature leaves diffused down the petiole more readily than from younger leaves. The enhanced liberation of inhibitor from older leaves may result in greater quantities of mobile ABA as the season progresses.

Many woody perennials from northern latitudes cease shoot growth and form terminal buds in midsummer. Control mechanisms resulting in development of rest are poorly understood, however, evidence for the involvement of leaves in the cessation of shoot elongation is increasing (9, 12). This influence may be mediated by plant growth inhibitors including ABA (1, 13).

In this report we describe studies involving the contribution of leaves to the level of transportable ABA, and the relative mobility of ABA from leaves of different physiological ages. Analysis of ABA diffusing from leaf tissue may provide insight into source-sink patterns of ABA movement since diffusates will contain only compounds which are transportable and which, therefore, may act at sites remote from regions of production or accumulation.

Materials and Methods

Plant material. Seeds of the tea crabapple, an apomictic crab species, were planted in December, 1973. Seedlings were grown in a glasshouse (24°C day/18°C night) under natural illumination except where noted. Periodically, vigorously growing seedlings, 50 to 100 cm in height, were selected for experimentation.

Effect of defoliation on diffusible ABA. Twelve seedlings were cut off at ground level. Following defoliation of 6 plants, each of the 12 trees was placed vertically in a separate beaker in the dark at 22°C with its cut basal end bathed in 10 ml of 20 mM aqueous EDTA (disodium salt) at pH 7.0 (4). Diffusates, i.e., substances released from the seedlings through the cut were collected over a 6-hr period, the diffusates of each of the 12 seedlings were analyzed separately.

Time course of ABA diffusion. Two greenhouse-grown seedlings were employed to investigate the time course of ABA diffusion over a 24-hr period. Fully expanded leaves were detached from basal, medial, and apical regions of the seedlings. Three leaves per nodal region per seedling were employed. Petioles were recut under water and each 3-leaf sample was placed vertically in a separate vial containing the EDTA solution discribed previously. After the initial 6-hr diffusion period samples were removed to fresh EDTA solution. The process was repeated after the second 6-hr diffusion period. The experiment was terminated 12-hr later.

Diffusible ABA gradients. These were investigated on 3 occasions over a 6-month period. Seedling age and size varied among experi-

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ments; however, trees utilized in any one experiment were visually uniform. Shoot tips (including expanding leaves) were excised and fully expanded leaves detached from basal, medial and apical regions of seedlings. Samples consisted of one shoot tip or 3 leaves per nodal region per tree and were diffused for 6 hr in the dark as described previously.

Diffusible versus extractable ABA. Prediffusion (endogenous) levels of ABA were determined from leaves extracted immediately upon excision from 3 seedlings. Diffusion from leaves detached from 3 comparable seedlings was followed by post-diffusion extraction of these same leaves. This permitted determination of residual ABA after diffusion.

ABA analysis. ABA extraction and analyses were performed essentially as described by Seeley and Powell (11). Tissue was frozen in liquid N powdered, and dropped into cold 80% methanol (MeOH). Extraction was obtained by soaking for three 24-hr periods at 2-3°C in the dark, using freshly redistilled MeOH each time. Extracts were bulked and evaporated to the aqueous phase. Following acidification free ABA was partitioned into methylene chloride ($MeCl_2$). Exudates were acidified directly and partitioned into $MeCl_2$. The aqueous phase of both extracts and exudates was base hydrolyzed (pH 10.5) for 45 min at 60°C to release ABA from its glucoside. Solutions were acidified (pH 2.7) and the released ABA partitioned into $MeCl_2$. Additional cleanup was achieved using silica gel partition columns (6) prior to methylation with diazomethane. Sample aliquots were injected into a Barber-Colman gas chromatograph fitted with an EC detector. A glass U-column 6′ × 3 mm i.d. was packed with 1% XE – 60 supported on Anachrom – ABS.

Results

Effect of defoliation on diffusible ABA. Five times as much ABA diffused from nondefoliated seedlings as defoliated ones (Table 1), emphasizing the importance of the leaves as a source of diffusible ABA.

Time course of ABA diffusion. ABA diffused from detached leaves in greatest amounts the initial 6-hr regardless of leaf age (Fig. 1). In the youngest fully-expanded leaves the diffusion of ABA subsequent to the initial 6-hr period was minimal. Movement of ABA from older leaves was both greater and of longer duration with the exception that only trace amounts of ABA occurred in the diffusate of leaves positioned medially on the seedling axis during the 6–12 hr diffusion period.

Diffusible ABA gradients. The levels of ABA diffusing from detached leaves and shoot tips varied considerably from experiment to experiment and among trees within a given experiment (Table 2). This variation, however, did not appear random. With each seedling, leaves detached from medial or basal nodes released more ABA than younger leaves. In 5 of the 8 trees employed, basal leaves released more ABA than leaves excised from medial or apical regions of the seedlings. In the other 3 trees, the most ABA was found in diffusates of leaves detached from the mid-portion of seedling axes. The higher levels of ABA in foliar diffusates from seedlings grown in a growth chamber under long days (experiment 2) was conspicuous.

Expressed as concentration, i.e., ng ABA in diffusate per g fresh wt of the source leaf, the greater diffusion of ABA from older leaves remained evident. These data are presented in Fig. 2. Growing shoot tips generally diffused more ABA per unit wt than leaves; however, the total contribution of the foliage to the level of transportable ABA far exceeded that of the shoot tip.

Diffusible versus extractable ABA. The levels (Table 3) and concentrations (Fig. 2) of ABA glucoside in diffusates were several fold greater than ABA in the last 2 experiments; however, in the youngest and smallest plants used (experiment 1) this pattern was not evident. Eighty-two percent of the total (ABA + ABA complex) in foliar diffusates was in the bound form (Table 3). The disparity between the levels of free and bound ABA was greatest in the diffusate of basal leaves (Table 3).

Leaves detached from medial and basal nodes not only released more ABA than younger (Table 2; Fig. 2), but released ABA more readily. We have introduced a relative mobility (RM) factor defined as the ratio between ABA present in the diffusate and post-diffusion extractable ABA in these same leaves (Fig. 3).

About twice as much bound ABA diffused from the tissue during the 6-hr period as could be extracted from freshly-harvest (prediffusible) leaves (Table 3; 294 ng vs. 152 ng).

Discussion

Terminal bud formation in deciduous fruit trees corresponds to the period of leaf maturation and precedes considerably cold-induced growth cessation. Previous investigators have noted the build-up of plant growth inhibitor(s), apparently originating in leaves prior to dormancy (9, 12). In at least one instance the inhibitor has been identified as ABA (13). Results of the present investigation indicate that ABA moved readily from leaves and along the seedling axis. The data support the role of the leaves as a source of ABA in apple diffusates and an important contributor to the level of translocatable ABA.

An enhanced mobility of ABA from mature as compared with the youngest fully-expanded leaves was strongly suggested. This could result in increased transport of ABA to shoot tips as the season progresses and leaves mature.

Despite considerably variability in absolute levels of diffusible ABA among seedlings, a trend toward increasing ABA mobility with increasing leaf age was apparent within each tree. Experiments were performed over an 8-month period, during which time environmental

Table 1. ABA in diffusate of defoliated and nondefoliated tea crabapple seedlings following 6-hr diffusion through the basal cut.

Seedling treatment	Number of seedlings	Amount of ABA[z] diffusing per seedling (ng)
Defoliation prior to diffusion	6	184.0 ± 43.5
Non-defoliation	6	880.8 ± 188.3

[z] Mean ± one standard error of the mean.

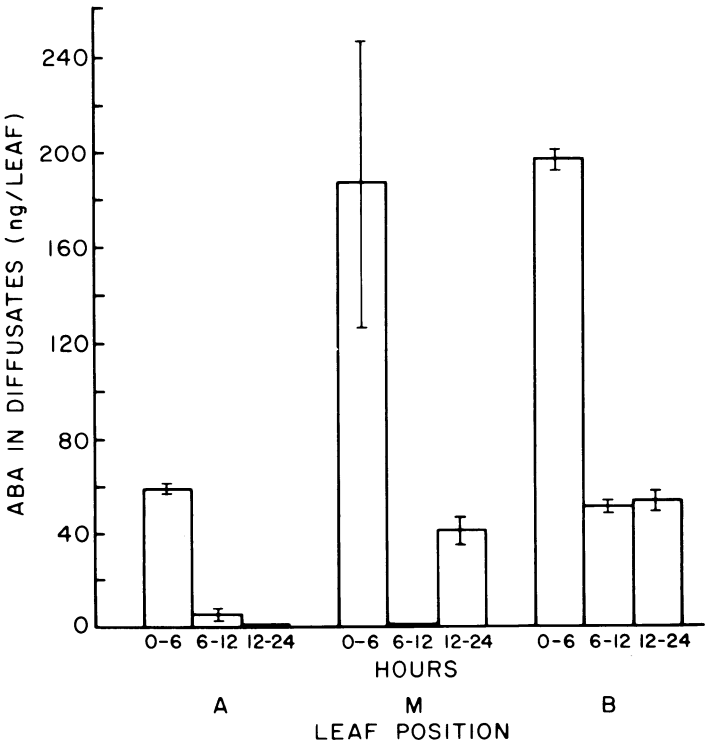

Fig. 1. Time course of ABA diffusion over 24-hr from tea crabapple leaves of different ages. (A = apical, M = medial, B = basal). Vertical bars indicate the standard error of the mean.

conditions varied. Thus, differences in levels of diffusible ABA among experiments may be attributed partially to physiological differences in the seedling populations. Variability among trees within experiments is more difficult to explain. Rapid and extreme fluctuations in extractable ABA have been associated with changes in leaf water potential (15, 16), and subtle differences in the water status of the seedlings employed may have contributed to this variability.

An increased diffusion of ABA from detached older leaves relative to younger leaves occurred in the absence of a 'sink' organ, suggesting a predisposition of mature leaves to release ABA. The enhanced diffusion of ABA from older leaves may be related to membrane integrity. Damage to membranous cell structure is an important deteriorative mechanism of cellular aging (14) and could be related to the release from cellular compartments (8). Alternatively, permeability changes, e.g., in chloroplast membranes, may facilitate access of ABA precursors, cofactors, etc., to sites of ABA biosynthesis (3, 5). Diffusates of the basal leaves did not invariably contain more ABA than medial leaves. However, since our knowledge of the relationship of leaf age to ABA biosynthesis and transport is limited, we may only speculate that among basal leaves releasing little ABA, the biosynthetic capacity has been reduced and/or the levels of transportable ABA were low. Further work is needed to determine the nature and magnitude of ABA pools in leaf tissue.

The physiological significance of the greater mobility of the glucoside versus free ABA is not apparent. The glucoside has not been shown to possess biological activity and, to the authors' knowledge, there is no evidence of *in vivo* hydrolysis of the complex. The data (Table 3) imply synthesis of the complex during diffusion, but, definitive evidence is lacking.

Although the data support previous correlations between leaves and growth inhibitory activity, evidence for ABA as a primary effector of growth cessation is not convincing. The comparatively high concentrations of extractable (10) and diffusible ABA in growing shoot tips is difficult to reconcile with its proposed inhibitory role. Mobilization of ABA by vegetative meristems has been demonstrated (2, 7); however, the active site of growth inhibition may be inaccessible to ABA in vigorously growing systems.

Table 2. ABA in diffusates from tea crabapple excised shoot tips and leaves detached from different regions of seedlings axes.

Experiment	Tree	Organ			
		Shoot[z] tip	Leaves[y]		
			Apical	Medial	Basal
1	1	390.00	11.75	58.50	66.25
	2	83.50	14.60	22.92	24.00
	3	60.00	7.75	8.75	21.60
2	1	99.00	61.00	272.50	205.00
	2	55.00	58.00	103.00	190.00
3	1	58.75	8.33	18.00	8.33
	2	85.32	27.67	59.00	21.33
	3	75.91	13.67	12.00	19.33

[z] Ng ABA diffusing per shoot tip over a 6-hr period.
[y] Ng ABA diffusing per leaf over a 6-hr period.

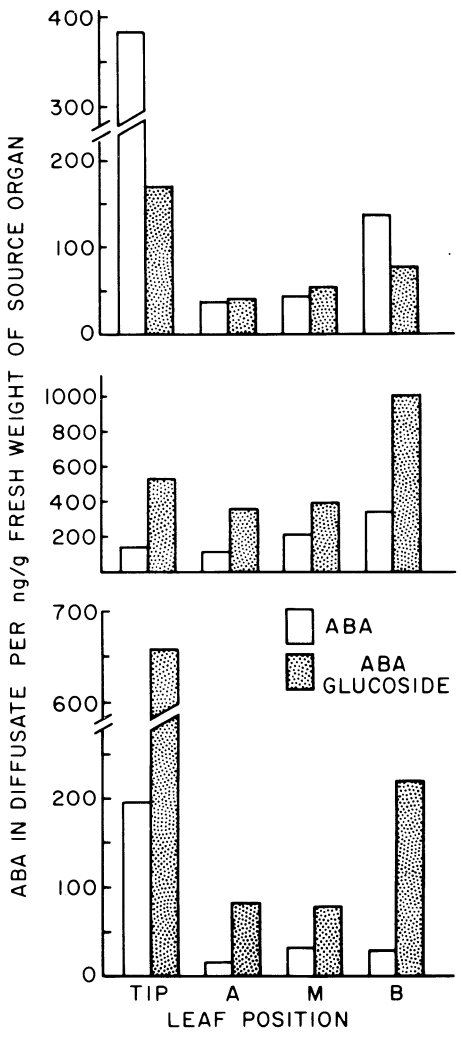

Fig. 2. Concentrations of free ABA and ABA glucoside diffused from tea crabapple growing shoot tips and leaves of different physiological ages. Top, experiment 1; center, experiment 2; bottom, experiment 3. (A = apical, M = medial, B = basal.)

Table 3. ABA and ABA glucoside in diffusates and leaf extracts of tea crabapple before and after diffusion[z].

Leaf position	Source of ABA and ABA glucoside					
	Seedlings 1, 2, 3				Seedlings 4, 5, 6	
	Leaf diffusates		Leaf extracts: post-diffusible		Leaf extracts: pre-diffusible (endogenous)	
	ABA (ng)	Bound ABA (ng)	ABA (ng)	Bound ABA (ng)	ABA (ng)	Bound ABA (ng)
Apical	16.59 ± 9.20[y]	87.67 ± 56.23	96.67 ± 45.80	47.78 ± 56.97	52.00 ± 16.90	51.00 ± 14.17
Medial	29.67 ± 23.63[x]	72.89 ± 54.37	90.22 ± 57.10	37.78 ± 19.47	90.78 ± 44.77	69.89 ± 19.20
Basal	16.44 ± 6.53	133.78 ± 138.33	34.55 ± 14.37	33.34 ± 12.60	55.33 ± 17.63	31.77 ± 26.83
Total	62.70	294.34	221.44	118.90	198.11	152.66

[z] Each value is the mean of 3 samples.
[y] The range about the mean represents 90% confidence limits.
[x] Ng per leaf.

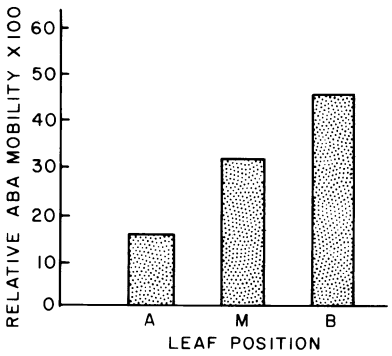

Fig. 3. Relative mobility (RM) of ABA as a function of tea crabapple leaf age. RM defined as the amount of ABA present in the diffusate divided by the amount of residual ABA in leaves following exudation. (A = apical, M = medial, B = basal.)

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Effect of Washing 'Hamlin' Orange on Chlorophyll and Carotenoid Changes During Degreening[1]

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Abstract. 'Hamlin' oranges were washed by hand or with various mechanical-washer procedures. The latter included variations in washing and brushing time and brush-bristle size. Washing procedures had little effect on subsequent chlorophyll loss during degreening. Carotenoid synthesis was significantly reduced by washing before degreening. These reductions in carotenoid levels were not appreciably affected by changes in washing time or brush-bristle size. Hand-washing oranges with a sponge reduced carotenoid synthesis less than mechanical washing. Carotenoid synthesis was reduced by less brushing than was required to clean the fruit, but "over" brushing had little further effect.

Early-maturing cultivars of citrus are normally degreened to improve their color before packing and marketing. Degreened fruit are usually not washed until after they are colored and ready for packing. Processes which follow washing, however, such as grading or color sorting (4), would be more effective if done before degreening. Washing also may contribute substantially to decay control (3, 5, 6) where *Collectotrichum gloeosporioides* Penz., or possibly *Diplodia natalensis* Pole-Evans, is a problem, and particularly when followed by a fungicide application (5, 6). Previous work has indicated that washing retards degreening (2); although in our work, washing had little or no effect on the rate of chlorophyll loss in several cultivars (3, 4). Some reduction in carotenoid synthesis was noted in tests on 'Hamlin' orange, however (3). Since there would be advantages to washing before degreening, work on 'Hamlin' orange was continued to determine if other washing procedures might minimize the color-development problem.

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Materials and Methods

Fruits of 'Hamlin' orange [*Citrus sinensis* (L.) Osb.] grown on rough-lemon [*C. limon* (L.) Burm. f.] rootstock were harvested and prepared for degreening within 6 hr (except where delay was a treatment). Oranges were washed with a commercial washer with 6 transverse brushes rotating at ca 150 rpm. Single samples of 20 fruits each were washed with city tap water and a nonfugicidal cleaner. A brusher with 10 brushes rotating at ca 200 rpm followed the washer. A water rinse was provided between the washer and brusher. Fruits were dried in a warm-air (63° C) roller-drier. The degreening room was held at 29° C with 90 to 95% relative humidity and 5 to 10 ppm ethylene. Oranges were left in ethylene to complete degreening, then transferred to 21°, except in 1973. In the 1973 tests, oranges were left in ethylene at the treatment temp continuously to avoid effects of changing conditions on pigment responses. Changes in chlorophyll were determined by a light-transmittance difference meter (1) with an integrating sphere sample system and filters for measurements which were recorded as ΔOD 695-740 nm. Changes in carotenoids were measured by using a reflectance attachment to the difference meter