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A Comparison of the Mode of Floral Abscission in Two Varieties of *Nicotiana tabacum*

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Abstract. The time required for floral abscission in Lizard's Tail variety is less and more uniform than in Little Turkish. Factors which affect abscission in general and which cause a varying effect in the two varieties include auxin concentration, leaves (sources of carbohydrates), methyl donors, and pectic enzymes. Concentrations of 100 ppm, 1,000 ppm, and 10,000 ppm IAA are more effective in retarding abscission in Little Turkish variety; low concentrations (5 ppm) tend to accelerate abscission slightly in Little Turkish and retard the process in Lizard's Tail. It is suggested that carbohydrate levels are higher in Little Turkish and have a varying effect upon abscission in the two varieties. A lack of a methyl donor is more limiting in Little Turkish. Addition of 0.01 percent methionine resulted in a 32 percent decrease in time of abscission in Little Turkish and a 19 percent decrease in Lizard's Tail. Response to higher concentrations is much the same in both varieties. Pectin-methylesterase activity is higher in Little Turkish, and since this enzyme prevents abscission, its greater abundance aids in explaining differences between the two varieties. The results indicate that abscission is controlled by the interaction of many factors and that a variation in the concentration, activation, or transport of any one or any combination affects the mode of abscission generally.

There are numerous differences between Lizard's Tail and Little Turkish varieties of *Nicotiana tabacum.* Differences in size and shape of leaves, the pattern of growth, the formation of floral primordia, the manner of pollination, and the rate of growth were reported by Paleg (1955). Yager (1959*a*) reported differences in floral abscission between the two varieties. These differences include a more regular time of abscission and a shorter interval between anthesis and abscission in Lizard's Tail. In this same study two anatomical factors which may affect the differences in abscission were identified. The vascular strand and the whole pedicel were found to be larger in Little Turkish. Since the separation through the vascular strand must be accomplished mechanically, it follows that this is more difficult to accomplish in Little Turkish and would require more time. Secondly, several tiers of cells are involved in the dissolution reactions in Lizard's Tail. This indicates that an enzyme responsible for dissolving the middle lamella is more abundant in Lizard's Tail. Also, this would suggest that the two reactions would be more sensitive to abscission accelerants and retardants in Little Turkish.

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In addition to such findings based on anatomical studies, there have been several reports of the effect of various physiological factors upon abscission in Little Turkish and Lizard's Tail varieties. Addicott and Lynch (1955), Muir and Yager (1958), and Yager (1957) have reviewed the physiology of abscission and have described the results of specific experiments concerned with reactions involved in abscission. The purpose of this paper is to consider data from several recent reports which identify the effects of specific factors affecting floral abscission in tobacco and to compare these effects in Lizard's Tail and Little Turkish varieties. For example, Yager (1959*b*, 1960*b*) reported various effects of leaves and indoleacetic acid (IAA) upon abscission. Yager and Muir (1958*a*, 1958*b*) reported the effect of methionine upon abscission in the two varieties. Yager (1960*a*) has also reported various effects of enzymes in the regulation of abscission in the two varieties.

Methods

Plants of Little Turkish and Lizard's Tail varieties of *Nicotiana tabacum* were grown under standard greenhouse conditions. Plants of the same age, size, and level of development were selected for use in each experiment. Various leaves were removed to discover possible effects upon abscission. IAA in various concentrations was dissolved in lanolin. It was then added to the leaf nodes following removal of the leaves. It was also added to the pedicel stubs following removal of the ovaries. In addition, IAA in aqueous solution was added to the ovaries of unfertilized flowers by means of a glass tube which had been drawn to a fine point. Methionine was also added to the unfertilized ovaries in a similar manner. In other experiments mixtures of IAA and methionine were added. Variations in the effect upon floral abscission in Lizard's Tail and Little Turkish varieties were tabulated.

To determine pectin-methylesterase (PME) activity, a section of tissue one millimeter thick was removed from the abscission zone of sixty pedicels which abscised at touch. This tissue was treated in a manner described by Yager (1960*a*). The activity was determined by the method proposed by Kertesz (1937). Variation in activity between varieties was observed as well as variation which occurred when IAA and methionine were added to the subtrate.

Results and Discussion

One of the most marked differences between the two varieties is the effect of additions of IAA. Results of treatment with IAA in lanolin and in water are recorded in Tables 1 and 2. In both varieties, addition of 10,000 ppm IAA in lanolin to the nodes of

excised leaves overcame the accelerating effect of the removal of upper leaves. However, additions of 1,000 ppm and 100 ppm IAA resulted in a greater retardation of abscission in Little Turkish than in Lizard's Tail. This effect was particularly apparent when IAA in lanolin was added to the pedicel stubs and to the ovaries in aqueous solution. In these instances concentrations of 1,000 ppm and 100 ppm completely retarded abscission in Little Turkish, whereas it was only partially retarded in Lizard's Tail.

This difference in effect could mean that the same application retards the enzymatic dissolution of the middle lamella to a greater extent in Little Turkish. It could also mean that IAA is more rapidly transported in Little Turkish or that there is a greater supply of endogenous auxin in Little Turkish. Yager (1960*a*) reported that IAA added to tissue cultures containing various pectic enzymes resulted in similar effects upon dissolution of the middle lamella, whether Little Turkish or Lizard's Tail tissue. Yager (1957) reported a retention phenomenon concerning some of the first flowers that reach anthesis in Little Turkish. These observations are evidences that there is a difference in endogenous auxin in the two varieties.

Table 1

Comparison of Effects of Leaves and Additions of IAA Upon Floral Abscission in Little Turkish and Lizard's Tail Tobacco[a]

Leaves Removed	Experimental Variation	Average Number Days from Anthesis to Abscission (± one standard error) Lizard's Tail	Little Turkish
None	None	8.4 ± 1.15	10.3 ± 0.87
None	None	8.6 ± 0.94	10.2 ± 0.56
Upper 2/3	None	4.7 ± 0.36	4.9 ± 0.41
Upper 2/3	None	4.8 ± 0.46	5.3 ± 0.72
Upper 2/3	100 ppm IAA added[b]	6.1 ± 0.66	9.0 ± 0.74
Upper 2/3	1,000 ppm IAA added[b]	6.3 ± 0.31	9.9 ± 1.26
Upper 2/3	10,000 ppm IAA added[b]	8.5 ± 0.62	10.4 ± 0.73
Upper 2/3	Ovaries Removed	1.3 ± 0.21	3.6 ± 0.16
Upper 2/3	100 ppm IAA added[c]	8.4 ± 1.24	14.0[d]
Upper 2/3	1,000 ppm IAA added[c]	16.0[d]	30.0[d]
Upper 2/3	10,000 ppm IAA added[c]	30.0	30.0[d]

[a]Data from fall plants.
[b]To nodes where leaves had been excised (mixed with lanolin).
[c]To pedicel stubs following removal of ovaries (mixed with lanolin).
[d]When 30.0, none abscised during 30 days plants observed; if less than 30.0, ⅓ abscised in time indicated—remainder requiring 30.0 days.

Table 2

Comparison of the Effect of Additions of Aqueous Solutions of IAA and Methionine to Unfertilized Ovaries Upon Floral Abscission in Little Turkish and Lizard's Tail Tobacco[a]

Addition of: IAA (ppm)	Methionine (%)	Average Number Days from Anthesis to Abscission (± one standard error) Lizard's Tail	Little Turkish
0 (no addition)	0	5.8 ± 0.42	8.1 ± 0.28
0 (addition of water)	0	3.7 ± 0.36	7.4 ± 0.44
5	0	4.4 ± 0.48	7.0 ± 0.73
100	0	12.0[b]	30.0[b]
1,000	0	30.0[b]	30.0[b]
0	0.01	3.0 ± 0.67	5.0 ± 0.91
0	0.1	2.2 ± 0.58	3.6 ± 0.82
0	0.5	1.3 ± 0.32	1.7 ± 0.21
0	1.0	0.8 ± 0.22	1.1 ± 0.52
5	0.5	1.4 ± 0.36	1.1 ± 0.52
100	0.5	5.0 ± 0.44	7.0 ± 0.61
1,000	0.5	9.8[b]	30.0[b]

[a]Data from spring plants.

[b]When 30.0, none abscised in 30 days plants observed; if less than 30.0, ⅓ abscised in time indicated—remainder requiring 30.0 days.

Another difference between the two varieties is the effect of adding very small amounts of IAA to the tissue. In most cases 5 ppm IAA accelerated abscission in Little Turkish, whereas it retarded abscission in Lizard's Tail. One such example is found in Table 2. Although some of the findings concerning additions of 5 ppm IAA were not statistically significant, the fact that such a difference occurred repeatedly indicated a difference between varieties. This probably reflects differences in endogenous levels again. Yager (1960*a*) has reported that additions of 5 ppm IAA increased the activity of various enzymes which catalyze the dissolution of the middle lamella. In this instance no difference between the effect on Little Turkish and Lizard's Tail was observed. However, tissue sections were taken from the pedicel and placed in Petri cultures where effects of materials from surrounding cells were negligible. Gaur and Leopold (1955) reported that such low levels of IAA accelerated abscission in other tissues.

Removal of leaves produced some variation in effect between varieties. Removal of two-thirds of the upper leaves reduced the time of abscission by approximately one-half the original time in both varieties. Removal of lower leaves produced more effect upon abscission in Little Turkish than in Lizard's Tail although a much lesser effect than removal of upper leaves. In addition, removal of lower leaves decreased the number of flower buds which reached maturity to a high degree in Little Turkish when compared to Lizard's Tail. Since the leaves are the primary sources of carbo-

hydrates, removal of large areas of leaves disturbed the internal levels of carbohydrates as well as other chemical factors affecting abscission. Carbohydrate levels and transport have been reported to affect abscission by Chandler (1925).

Results of other experiments with leaf removals produced the following results. When all leaves were removed when the first flower reached anthesis, three times as many ovaries matured into ripened fruit in Little Turkish. This suggests greater carbohydrate reserves in the stems of Little Turkish which enable more ovaries to reach maturity following fertilization. When no leaves were removed, Lizard's Tail averaged twice as many mature fruits as Little Turkish. A greater production of auxin or a greater effect upon limiting the development of additional ovaries in Little Turkish is suggested. Muir (1942) showed that fertilized ovaries contained high levels of auxin and Paleg (1955) showed that fertilized ovaries and auxin production affected the development of additional ovaries to a greater degree in Little Turkish variety. Although a relationship to auxin synthesis, activation, and transport is suggested, the effect of carbohydrate levels is an additional difference between the varieties.

Differences between the two varieties were also observed in the relative availability of methyl groups necessary for the completion of abscission. As seen in Table 2 addition of only 0.01 percent methionine resulted in a 32 percent decrease in time of abscission, whereas similar application in Lizard's Tail produced a decrease of only 19 percent. Apparently a lack of methyl donor is more limiting in Little Turkish than it is in Lizard's Tail. This is an additional explanation of the difference in the two varieties in the time required for abscission to occur. When higher concentrations (1 percent) were applied to the unfertilized ovaries, the response was much the same in both varieties. At this level there is seemingly some other limiting factor in further acceleration of the process.

Table 3 illustrates another difference between the two varieties. Without exception Little Turkish tissue displayed a greater PME activity. It was expected that separation would naturally occur more slowly in Little Turkish since there is more enzyme which favors the deesterification reaction (the reverse of the abscission reaction). The effects of IAA and methionine additions also paralleled expected effects when factors which accelerate or retard abscission were considered. Additions of high concentrations of IAA were more effective in increasing PME activity in Little Turkish than in Lizard's Tail.

Table 3

Comparison of Pectin-Methylesterase Activity in Little Turkish and Lizard's Tail Tobacco

Addition of: IAA (ppm)	Methionine (%)	Mean Activity /g. Fresh Tissue (± one standard error) Lizard's Tail	Little Turkish
0	0	12.17 ± 0.31	13.95 ± 0.15
5	0	11.62 ± 0.62	12.81 ± 0.23
100	0	13.33 ± 0.41	14.46 ± 0.53
1,000	0	16.68 ± 0.21	16.90 ± 0.38
0	0.5	9.52 ± 0.66	10.55 ± 0.81
100	0.5	12.56 ± 0.30	14.81 ± 0.31
1,000	0.5	15.11 ± 0.27	15.30 ± 0.62

The difference in PME levels provides evidence that different enzyme levels between varieties is probable. Since two varieties contain varying amounts of PME, it is likely that they also contain varying amounts of an esterification enzyme or some other enzyme involved in the dissolution of the middle lamella. The limitation of the separation of cells to only a few tiers supports the view that there is a lesser amount of active enzyme in Little Turkish. Also, when identical amounts of an enzyme capable of dissolving the middle lamella are added to sections of tissue from Little Turkish and Lizard's Tail, the rates and mode of separation are nearly identical. Hence, if there were similar amounts of such an enzyme in the plants, times of abscission would be similar.

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