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## Fluorine Substitution Affects Decarboxylation of 2,4-Dichlorophenoxyacetic Acid in Apple


*Abstract.* Marked differences exist in the rate at which 2,4-dichlorophenoxyacetic acid (2,4-D) is broken down by different plant species and varieties. This activity has been measured by collecting carbon dioxide from leaves treated with carboxyl-$C^{14}$ labeled compounds. The substitution of fluorine for chlorine in the 4-position of 2,4-D inhibits this decarboxylation in leaves which are able to break down 2,4-D rapidly.


It has been reported by Luckwill and Loyd-Jones (*1*) and Edgerton (*2*) that 2,4-dichlorophenoxyacetic acid (2,4-D) is decarboxylated in the leaves of several varieties of apple. The rate of breakdown or decarboxylation was found to be different in several varieties and would appear to be the basis for the resistance of some varieties to the regulatory effects of this compound on growth. With the McIntosh variety, on which sprays of 2,4-D are ineffective in delaying fruit and leaf petiole abscission, the compound is rapidly decarboxylated in the leaf. In Stayman and Winesap varieties, which show a pronounced response to 2,4-D sprays, decarboxylation occurs at a much slower rate (*2*).

In some studies on petiole abscission and fruit drop in 1959 with several growth regulators, it was found that 2-chloro,4-fluorophenoxyacetic acid (2,4-F) had a marked effect on preventing abscission in McIntosh as well as in several other varieties. The formative effects of 2,4-F on shoot and leaf growth of McIntosh were similar to those produced by 2,4-D on a susceptible variety such as Stayman (Fig. 1). Thus the substitution of fluorine for chlorine in the 4-position of the phenoxy ring appeared to block the decarboxylation of 2,4-F in McIntosh leaves.

To investigate this possibility, tests were conducted with 2,4-F (*3*) similar to those previously carried out on 2,4-D. Carboxyl-labeled 2,4-F was prepared having a specific activity of 1 mc/mmole identical with the carboxyl-labeled 2,4-D which had been used in the earlier decarboxylation studies and with which it was compared. Equipment was assembled to supply apple shoots with dilute solutions of the two carboxyl-labeled compounds and to collect the $C^{14}O_2$ given off by the leaves. The shoots were cut from trees of the desired varieties and transferred immediately to small flasks containing the carboxyl-labeled solutions. These were then placed in large jars through which air could be drawn and the $CO_2$ absorbed in NaOH solutions. Light was excluded during the respiration periods in order to collect the maximum amount of $CO_2$ from the leaves. The carbonate was precipitated from the NaOH solution, and the precipitate was collected on filter paper for counting.

It was found that the decarboxylation of the 2,4-F-1-$C^{14}$ occurs at a much slower rate in McIntosh leaves than does the decarboxylation of the 2,4-D-1-$C^{14}$ (Table 1). In 24 hr only 4 percent of the $C^{14}$ was recovered as $C^{14}O_2$ from the McIntosh shoots supplied with 2,4-F-1-$C^{14}$, while 33 percent of the $C^{14}$ was recovered from the shoots supplied with 2,4-D-1-$C^{14}$. Even in Stayman, where decarboxylation of 2,4-D is extremely slow, the breakdown of the compound is further retarded by the substitution of the fluorine. Bioassays have shown that the decarboxylated form of 2,4-D is comparatively inactive.

Autoradiographs have been made of McIntosh and Stayman leaves treated with these carboxyl-labeled compounds. They show a rapid disappearance of $C^{14}$ from McIntosh leaves supplied with 2,4-D-1-$C^{14}$. Similar leaves supplied with

Table 1. Production of $C^{14}O_2$ by apple leaves on detached shoots treated with 2,4-D-1-$C^{14}$ and 2,4-F-1-$C^{14}$.

| Treatment | Amount absorbed* (μg) | Absorbed $C^{14}$ recovered as $C^{14}O_2$ (%) 4 hr | 24 hr |
|---|---|---|---|
| | *McIntosh* | | |
| 2,4-D-1-$C^{14}$ | 40 | 3.25 | 33.04 |
| 2,4-F-1-$C^{14}$ | 45 | 0.43 | 4.13 |
| | *Stayman* | | |
| 2,4-D-1-$C^{14}$ | 40 | 0.04 | 0.56 |
| 2,4-F-1-$C^{14}$ | 50 | 0.03 | 0.14 |

* After the 4-hr absorption period the shoots (8 leaves per shoot) were transferred to distilled water and the respiration run continued for an additional 24 hr.

an equal amount of 2,4-F-1-$C^{14}$ show much higher $C^{14}$ activity. Thus it is apparent that 2,4-F may be absorbed by McIntosh leaves and move through the leaves to abscission sites without being inactivated.

Further evidence of the stability and effectiveness of 2,4-F in delaying abscission is its effect on the flowers. The application of 2,4-F either as dilute aqueous sprays or in a lanolin paste to flowers of several varieties including McIntosh was found to inhibit their abscission. In fact, it was observed in 1960 that nearly all of the flowers persisted throughout the summer on the McIntosh trees treated the previous fall with 2,4-F for control of preharvest drop, even though the proportion of flowers that formed mature fruits was reduced by the treatment (Fig. 1). This is similar to the stability of 2,4-D in Stayman. It has been reported (*4*) that Stayman trees sprayed with 2,4-D for delay of drop one year may show significant reduction in preharvest drop

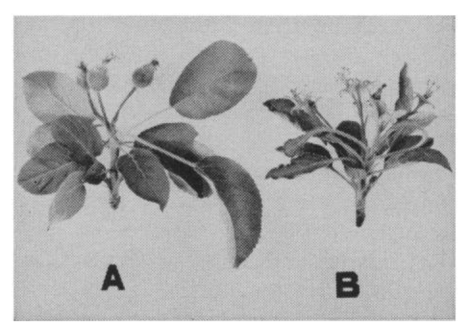


Fig. 1. Representative spur from untreated McIntosh tree (*A*) and from tree sprayed with 20 ppm 2,4-F (*B*). The spray was applied Sept. 1959; spurs were photographed 13 June 1960. Note the leaf distortions and persistent flowers on spur from treated tree. The leaf symptoms include enlargement of leaf veins, shortening and narrowing of the leaves, and development of marginal ruffles similar to the distortions produced by 2,4-D on susceptible varieties and species.

the following year without further application of 2,4-D. The substitution of fluorine in 2,4-D would appear to make it equally stable in varieties that otherwise decarboxylate it.

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## Variation in Clones of Penstemon Growing in Natural Areas of Differing Radioactivity

*Abstract.* A study to determine whether or not the relatively high radioactivity of a natural area has produced detectable morphological effects on plants of the species *Penstemon virens*, a plant of rather plastic morphology, has been conducted in the Central City region of Colorado for over 2 years. While no differences in kinds of anomalies were found to exist between plants growing in the sites of highest and lowest radioactivity, the number of anomalies was greater in plants from sites of greatest radioactivity.

A study was made of 26 clones of *Penstemon virens* growing along the crest of a dike of nonporphyritic quartz bostonite. The ground-surface gamma radioactivity varied from 0.05 to 0.40 mr/hr. According to Phair (*1*), this dike may be the most radioactive igneous rock known on the North American continent. Except in radioactivity, the microenvironments of the clones appeared to be quite similar.

Plant organs that are commonly known to be radiosensitive were examined and measured. The total heights and internodal lengths were measured on 206 flowering spikes; stage of development was determined on more than 4000 floral elements; and the total numbers of nodes, peduncles, and anomalies such as those shown in Fig. 1 were counted for each spike. Simultaneously the gross radiation environment of each clone was determined with a portable Geiger counter, and by securing segments of flowering spikes and selected samples of the adjacent soil for radioassay.

Preliminary analysis of the field data indicate several differences between clones: The range of morphological variability was greater, the percentage of aborted flowers was larger, the numbers of nodes per flowering spike were less, and the number of gross anomalies was higher in clones from the most radioactive sites; however, the average height, numbers of floral elements (buds, flowers, and fruit), and numbers of peduncles of the flowering spikes were very nearly the same in all sites.

Several hypotheses to explain the differences can be suggested: The differences may result from (i) chance, (ii) errors in measurement, (iii) slight variation in microenvironment (other than radioactivity), and (iv) variations in radioactivity. The first two hypotheses can be disregarded because of the large number of comparative measurements made. The several thousand measurements are the aggregates of measurements made by eight to ten different people, and approximately 75 percent of the measurements were independently double checked. Data placed in questionable and arbitrary categories (such as "immature or aborted") were disregarded.

Although the clones all appeared to be growing in similar microenvironments, the possibility exists that very subtle differences in amounts of light, temperature, insect damage, and moisture during critical periods of plant growth and differentiation could account for the differences between clones. It is known that gene action, even in the uniform environment of a single clone, can vary in expression. However, one observation suggests that this is not the reason for the differences in numbers of anomalies between the clones in this case. A study, in 1959, of *Penstemon* growing in a wide range of habitats provided evidence that soil drought, excessively high temperatures, and other environmental factors can account for various "anomalies" but that one of the first general effects of such factors is that the plant is somewhat stunted. In the present study the clones growing in the more radioactive sites were as tall as those in the less radioactive sites. Furthermore, clones from the habitats examined in 1959, including those of the extremely dry and exposed rock outcrop, never showed as many anomalies as the clones from the most radioactive habitats.

There is a final consideration. The radioactivity of the substrate is high compared with that of other natural areas, but alone it would probably be regarded by most radiobiologists as too low to cause detectable morphological

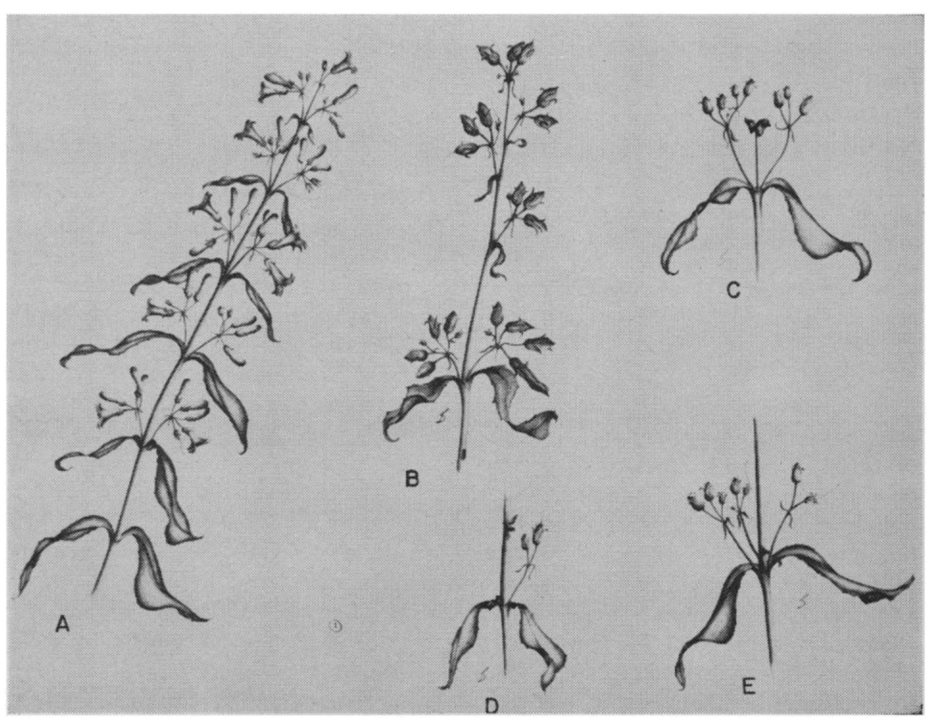


Fig. 1. (*A*) Typical flowering spike of *Penstemon virens*. Note opposite leaves, one axillary peduncle on each side of node, absence of aborted buds, and regularity of internodal lengths. (*B*) Spike has opposite and alternate arrangement of leaves and several aborted buds near the top. (*C*) Terminal development incomplete. (*D*) Blocked development of one peduncle and fragmentary nodal development. (*E*) Extra peduncle.