METABOLISM OF 2,4-DICHLOROPHENOXYACETIC ACID. III. METABOLISM AND PERSISTENCE IN DORMANT PLANT TISSUE [1]

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In contrast to actively growing, susceptible plants which usually respond very promptly to applied 2,4-dichlorophenoxyacetic acid (2,4-D), certain perennials that have been treated toward the end of a growing season may not exhibit morphological responses until the following season (9, 10, 12, 13). It has been a moot question whether (a) such delayed responses are initiated in sensitive cells of winter buds at time of application of the growth-regulator but become visible only much later when the buds renew development or (b) 2,4-D, or some physiologically active product thereof, persists in the dormant plant and interacts the following season with tissues which become sensitive as they renew development (2, 3, 11, 13, 14).

Previous experiments with C^{14}-labeled 2,4-D have furnished direct evidence of relatively long persistence of the compound in bean plants. Although a portion of the applied growth-regulator is rapidly transformed into a variety of products (5, 15, 16), a significant amount may remain unaltered for at least 42 days (6). Less direct evidence of persistence of an active growth-regulator has been provided also by observations of protracted morphological responses in cotton (4, 7, 8) and of long delayed fruit abscission in apple (10). In these instances it has not been established whether the delayed or protracted responses are due to 2,4-D itself or to a biologically active transformation product.

The present experiment was undertaken with the objective of obtaining direct information concerning the metabolism and persistence of 2,4-D in dormant buds.

EXPERIMENTAL PROCEDURE

2,4-D labeled with C^{14} was applied to dormant buds of potted two-year-old cherry trees (*Prunus avium*) at the end of the second growing season. Half of the plants were treated on September 16, when leaves were still present, and half were treated on November 25, after abscission of all leaves. Separate lots of plants were treated with carboxyl-labeled, methylene-labeled, or ring-labeled 2,4-D, each form of the growth-regulator being applied at each of two doses of approximately 125 μg and 250 μg per bud. Each compound was applied to the five largest buds on each of two plants.

The compounds were applied by placing two or four 0.0025 ml drops of a 25,000 ppm acetone solution on each bud, allowing the surface of the bud to become dry after each addition. Very little of the solution ran off onto the adjacent stem. A considerable portion of the applied 2,4-D was not immediately absorbed through the bud scales and remained as a visible deposit on the surface. In order to allow time for penetration and to avoid possible loss from rain, the plants were taken into the greenhouse at time of treatment and held there during the succeeding two weeks after which they were returned out of doors where they remained exposed to rain and snow throughout the winter. It was shown in a separate experiment that 10.6 % of the 125 μg dose and 20.6 % of the 250 μg dose could be recovered by thoroughly washing the exterior of the buds one week after application. The remainder is presumed to have penetrated into the bud.

On March 6, before any visible growth had begun, the treated buds, together with 2 cm of adjacent stem, were excised. The ten fragments constituting each sample were bulked, comminuted with sand in a mortar, transferred to extraction thimbles, and extracted for five three-hour periods with successive 100 ml portions of boiling 80 % ethanol. The extracts were made to volume and aliquots removed for determination of C^{14} as previously described (15).

After removal of the alcohol by distillation, the aqueous solutions were acidified to pH 1 with sulfuric acid and extracted repeatedly with ether. Aliquots of the ether extracts were taken for C^{14} analysis and the remainder was chromatographed on filter paper with a number of solvents.

RESULTS

Since the results were essentially the same for the three forms and two doses of 2,4-D, only the average values are presented. As shown in Table I, 3.0 to 7.7 % of the applied radioactivity was recoverable from the buds and adjacent tissue several months after application of 2,4-D-C^{14}. The buds treated in November contained more than twice as much C^{14} as those treated in September. More time was, of course, available for metabolism to volatile products or for export from the buds treated at the earlier date; furthermore these processes would be expected to be more rapid at the higher temperatures prevailing between the first and second applications than during the subsequent colder weather.

Even the very incomplete fractionation that was made of the extracts served to demonstrate that part of the 2,4-D was transformed into at least two other products. An appreciable fraction of the recovered C^{14} was in the form of ether-insoluble substances which it was not attempted further to separate. The ether-soluble fraction also comprised two substances which were separable by paper chromatography. The major component, which contained, on the average, 85 % of the total ether-soluble radioactivity, was identified as unaltered 2,4-D by its chromatographic behavior and by bioassay (1). Cochromatograms

[1] Received December 2, 1953.

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN DORMANT CHERRY BUDS AT THREE AND FIVE MONTHS AFTER APPLICATION OF 2,4-D-C^{14} (EXP. 1481)

C^{14} FRACTION	PERCENT OF APPLIED C^{14}	
	TREATED 16 SEPT. 1952	TREATED 25 NOV. 1952
Ethanol-insoluble	0.6	1.3
Ethanol-soluble, ether-insoluble	0.6	1.8
Non-2,4-D acid	0.3	0.5
Unaltered 2,4-D	1.5	4.1
Total C^{14} present in bud	3.0	7.7

with pure 2,4-D gave a single spot at Rf 0.80 with 90 % aqueous *n*-butanol and at Rf 0.73 with 75 % aqueous phenol. The physiological activity was that expected for 2,4-D. The 2,4-D comprised approximately half of the total radioactivity present in the tissue at harvest.

DISCUSSION

These results demonstrate that 2,4-D may undergo certain transformations in "resting" plant organs. At the same time a sufficient quantity of the compound may persist throughout the dormant period to induce morphological abnormalities when growth resumes the following season. It appears, therefore, that delayed responses such as have been observed to follow application of 2,4-D could be due to a reaction of the growth-regulator with cells that are formed long after the treatment as well as to delayed expression of injury induced at time of treatment.

The chromatographic evidence indicates that the same ether-soluble transformation product was obtained from the variously labeled 2,4-D molecules, showing that the carboxyl, methylene, and number 1 ring carbon atoms are all present, and suggesting that the molecule had not been extensively metabolized in the formation of this product.

SUMMARY

2,4-Dichlorophenoxyacetic acid applied in autumn to winter buds of cherry is in part metabolized to other products during the dormant season. A portion, however, remains unaltered until the following spring.

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