growth regulating activity using the dicotyledonous plants, Pinto bean, large seeded sunflower, Arlington White Spine cucumber, and the monocotyledonous plants Wong barley, and U. S.-13 hybrid corn. The lanolin assay method was used on dicots and the coated sand assay method on monocots (*9*).

At intervals of 2, 4, 7, and 14 days following treatment, the degree of growth modification induced by the various compounds was estimated and scored according to the intensity of growth responses. Responses studied were: stem curvature, growth inhibition, epinasty, formative effects, and induced cell proliferation (gall formation). Table IV shows responses observed 14 days after treatment, to conserve space the other values have been omitted. These data are representative, although they do not show the rate of response or relative progressive effectiveness of the compounds tested. The experiments were designed to indicate growth regulating properties rather than the herbicidal potentialities of the compounds.

Results with Plant Tests. In general, coupling of halogenated phenoxy acids with *p*-toluenesulfonamide, *p*-aminobenzoic acid, and *p*-aminosalicylic acid and terpenoids resulted in compounds that induced plant-growth responses, but the magnitude of the different responses was less than that induced by the parent regulating compounds (Table IV). From the standpoint of utilization of these new regulating compounds, the parent phenoxy acids sometimes induce unwanted responses when used for a specific purpose—for example, to improve fruit set. The present data indicate that some derivatives used here may possess limited growth regulating properties of interest. For example, it is desirable to induce fruit set chemically without marked side effects such as modification and suppression of leaf growth. Regulators that induce a localized response are of interest. In the present tests, 2,4,5-trichlorophenoxyacetic acid induced marked responses (formative effects) at a distance from the site of application when applied to bean plants. The hydronopol derivative of this acid, on the other hand, induced a moderate response which was apparently localized within the treated region of the stem.

In a few cases, coupling of the phenoxy acids resulted in compounds with increased growth-regulating properties. When applied to cucumbers, for example, the lactic acid derivative, DL-2-(2 - methyl - 4 - chlorophenoxyacetoxy) propionic acid, methyl ester was more effective than its parent acid, 2-methyl-4-chlorophenoxyacetic acid.

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Received for review April 2, 1958. Accepted September 24, 1958. Division of Agricultural and Food Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958. Mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of similar nature not mentioned. Commercially supplied compounds were obtained through courtesy of the Amchem Products, Inc., Dow Chemical Co., Glidden Co., Hercules Powder Co., and Monsanto Chemical Co.: DL-2-(2,4-Dichlorophenoxyacetoxy) propionic acid N-ethylamide was prepared by C. Neufeld, Western Regional Research Laboratory, Albany, Calif. Analyses were by L. Nutter (Extension Center, Pennsylvania State University, Abington, Pa.), R. B. Kelly, and K. Zbinden. Paper 7 in a series on growth regulators.

FRUIT DROP CONTROL

Evaluation of Chlorine-Substituted Phenoxyacetic Acids and Amides for Retarding Abscission of Apple Fruits

PAUL C. MARTH, WILLIAM V. AUDIA, and JOHN W. MITCHELL

Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

The amide forms of chlorine-substituted phenoxyacetic plant regulators were more effective in retarding mature fruit abscission than the corresponding acids. When chlorine was substituted in all possible combinations on mono-, di-, tri-, tetra-, and penta- positions on the benzene nucleus, in all instances where the 6 position contained chlorine the compound was rated as relatively inactive. To a lesser degree chlorine substitution in the 3 position also reduced the effectiveness of a compound. The 17 acids and 17 amides were compared directly on three varieties of mature apple fruits; the complete series of 17 amides was applied to six varieties.

PLANT-REGULATING ACTIVITIES of chemicals can be compared or evaluated in various ways. Retardation of the abscission of mature fruits of apple was used as the response in the present study, because that type of plant reaction appears to be very different from the cell elongation or multiplication, formative growth effects, parthenocarpy, modification of organs, and repression or stimulation of root or vegetative bud meristems or others (*11*, *15*) commonly used as measures of response.

According to McCown (*4*) the harvest drop of mature apple fruits involves dissolution changes in the walls and lamellae of old, lignified cells. The possible role of plant-regulating chemicals in controlling the metabolism of pectic substances through enzymatic processes has been suggested by Neeley *et al.* (*10*). Other workers have shown that chemical changes associated with the ripening of fruits can be stimulated or

retarded by application of plant regulators to the attached (*5, 6*) or detached (*8*) fruit. Treatment of snap bean fruits with certain plant-regulating chemicals caused them to take up and retain metabolic water against strong forces of drying (*9*).

Plant-regulating chemicals have been used to help retard the harvest drop of mature apple fruits since 1939 (*1*) and have been of considerable economic value to commercial growers throughout this country and abroad. Naphthaleneacetic acid and naphthaleneacetamide used in the first successful experiments are still used by many apple growers, particularly on early-maturing varieties. These chemicals are effective over a rather short period, especially on McIntosh, Winesap, Stayman Winesap, and York Imperial varieties. At present, many growers are using 2,4,5-trichlorophenoxy-α-propionic acid (2,4,5-TP), which is rather effective in retarding abscission of most leading varieties. This chemical may be applied 3 weeks before harvest and is effective over a long period. Perhaps a disadvantage of this phenoxy compound is that it tends to advance maturity of certain summer- and early-fall-maturing varieties. In the search for new plant-regulating compounds to retard apple fruit abscission in the present study, 2,4,5-TP was used as the standard for comparison.

The comparative effectiveness of a complete series of mono-, di-, tetra-, and pentachlorophenoxyacetic acids in retarding the abscission of mature fruits of six varieties of apples has been reported (*7*). This work was extended in the study reported here to include the comparative effects of the acid and the amide form of each chlorinated phenoxyacetic acid on abscission of fruits of each of three fall-maturing varieties of apples, and the effectiveness of the amide series on three additional late-summer varieties. The chemicals were prepared and furnished by J. M. F. Leaper and J. R. Bishop, American Chemical Products Co., who reported the physiological activity of the various members of the acid series in inducing formative effects, epinasty, and inhibition of roots of young seedlings (*3*).

Material and Methods

The chemicals were applied in a lanolin paste, because only a few milligrams of some difficult to synthesize chemicals were available, and it was desirable to compare directly the entire group of compounds.

The lanolin mixtures were prepared by placing a weighed amount of each phenoxy compound in a small vial and dissolving the chemical in a weighed quantity of Tween 20 by gentle heat. Weighed melted lanolin was then added and the entire mixture was stirred until it cooled to a creamy paste. Each chemical was applied at 1% concentration in the carrier, which consisted of 1 part of Tween 20 to 4 parts of lanolin. By the use of an applicator stick a narrow band of each mixture was placed around the stem directly over the abscission zone of each of 10 tagged fruits selected for uniformity of size and quality (Figure 1). The individual treatments were applied at random on lower branches around the entire tree about 3 to 4 weeks before the estimated harvest date for the particular variety.

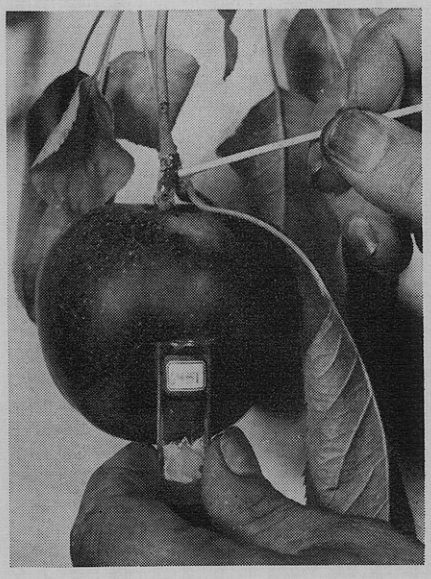

Figure 1. Application of plant regulator to abscission zone of apple

A single large tree of each of six varieties was used. The amide series of compound was applied in most instances to all six varieties (McIntosh, Grimes Golden, Red Delicious, Golden Delicious, York Imperial, and Winesap), but the acid and the amide form of each phenoxy compound were compared on only the last three, the fall-maturing, varieties. The acid and amide forms of 2,3,5-trichloro- were applied only to the Winesap variety, whereas the amide of 2,3,4,5-tetrachloro was applied only to the McIntosh variety. Because only a few milligrams of these chemicals were available for the study, their use was restricted. All untreated fruits except a representative set (control fruits) on each tree were removed, so that in taking records the fruits given the individual treatments could be readily located. In addition to the untreated controls, 10 fruits on each tree were treated at random with a 1% mixture of 2,4,5-trichlorophenoxy-α-propionic acid for comparison. The data presented here were obtained after 100% of the untreated control fruits had fallen. All treatments that induced 60% or more of the fruits to remain on the tree for 3 to 4 weeks past the usual harvest date were designated as highly effective in retarding abscission.

Results

Under the conditions of this experiment, the amide form of several chlorinated phenoxy plant regulators proved to be markedly more effective in controlling fruit abscission than the acid form (Figure 2). All three varieties responded to the amide form of 2-chloro-, whereas the acid was ineffective in retarding fruit abscission. Similarly, the amide form of 3-chloro- and 2,3,5-trichloro- effectively retarded fruit abscission of Winesap, but the acids of both of these chemicals were not effective. Abscission of York Imperial appears to be more difficult to retard than that of the other varieties studied. In four instances the amide forms proved superior to the (2-chloro-, 4-chloro-, 2,3-dichloro-, and 2,5-dichloro-). On York Imperial eight of the amides and four of the acids (3,4-dichloro-, 2,3,4-trichloro-, 2,4,5-trichloro-, and 3,4,5-trichloro-) proved superior to 2,4,5-TP, the standard chemical applied for comparison. There was no marked difference in the various acids or amides except the 2-chloro-amide in causing response of the Golden Delicious variety.

The complete series of chlorinated phenoxyacetamides was applied to fruits of the McIntosh, Grimes Golden, and Red Delicious varieties (Figure 3). It is rather surprising that so many of the amides were outstandingly effective in retarding abscission of McIntosh. This had often proved difficult under commercial conditions, although 2,4,5-TP has been used rather successfully (*2*). In this experiment it was also highly effective. Eleven phenoxy amides—2-chloro-, 3-chloro- and 4-chloro-; 2,4-dichloro-, 2,5-dichloro- and 3,4-dichloro-; 2,3,4-trichloro-, 2,3,5-trichloro-, 2,4,5-trichloro-, and 3,4,5-trichloro, as well as 2,3,4,5-tetrachloro—were highly effective in retarding abscission of McIntosh. The responsiveness of McIntosh to these phenoxy amides is reported here for the first time, so far as the authors know.

Only five amides were rated highly effective in retarding abscission of Grimes Golden variety, the fruits of which tend to abscise rather early in some seasons: 3-chloro-, 4-chloro-, 2,5-dichloro-, 2,4,5-trichloro-, and 3,4,5-trichloro-. 2,3,5-Trichloro- and 2,3,4,5-tetrachloro- were not tested on Grimes Golden.

Harvest drop of Red Delicious is not generally considered difficult to control by the use of plant regulators under most conditions. It is not surprising, therefore, that nine of the amides were rated as highly effective; these include 3,5-dichloro amide, that was ineffective on all the other varieties of the experiment.

Discussion

In evaluating the results of the experiments discussed here, one should remem-

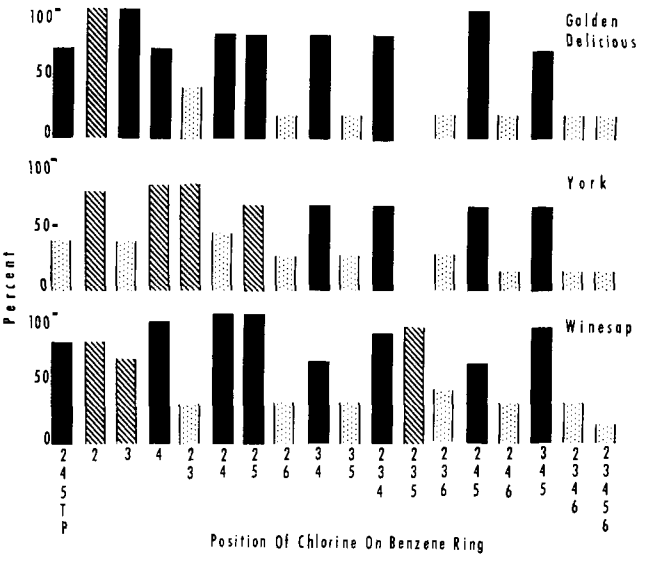

Figure 2. Relative effectiveness of phenoxyacetic acids and corresponding nuclear halogenated amides

Solid bars. Amide highly effective (60% or more of fruit remain attached) and acid not significantly different
Crosshatched bars. Amide highly effective, but acid significantly less effective. Dotted bars. Amide as well as acid ineffective

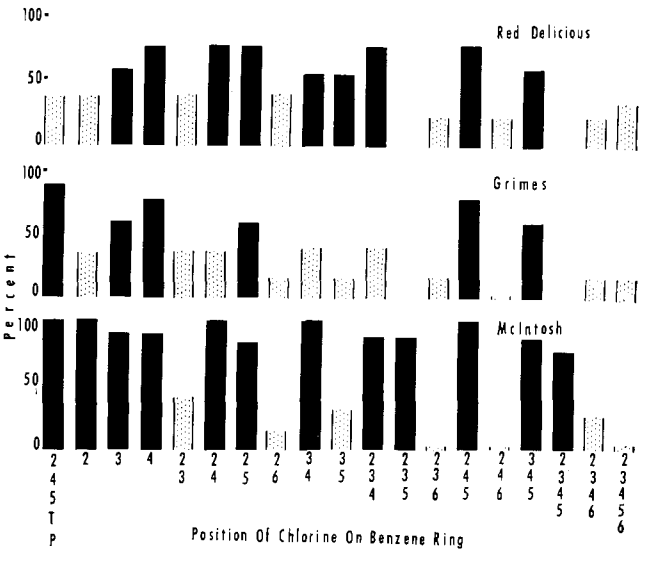

Figure 3. Mature apple fruits remaining on tree 1 month after usual harvest date

Solid bars, treatment induced 60% or more of the fruit to remain on trees 1 month past usual harvest date; dotted bars, less than 60%

ber that the plant response used may be a unique one, which may or may not involve chemical or physical responses that bring about other kinds of plant responses such as cell elongation, cell division, or even others. The chemicals were applied in relatively strong concentrations in a carrier (lanolin), which no doubt kept the compound in close contact with the plant and near the zone used as a measure of activity over a long period (2 months or longer). The lanolin method has been found particularly useful in studying the comparative activity of compounds of which only a few milligrams were at hand. Data obtained by the method, however, cannot always be directly useful for practical purposes.

Presence of an amide group on the side chain of chlorinated phenoxy plant regulators is perhaps almost as important as the position and degree of chlorination on the benzene nucleus. In certain instances, the results may have a direct bearing on other kinds of more commonly studied plant-regulator responses, such as cell elongation or cell multiplication. The responses of test plants such as bean, cucumber, cotton, and tomato to both the amide and the acid series of phenoxyacetic plant regulators have been reported (*14*).

Compounds that had little or no influence on fruit abscission when applied as either the acid or the amide, were: 2,6-dichloro-, 3,5-dichloro-, 2,3,6-trichloro-, 2,4,6-trichloro, 2,3,4,6-tetrachloro-, and 2,3,4,5,6-pentachloro-; five have a chlorine substitution in the 6 position on the ring and all were inactive. The exception, 3,5-dichloro-, suggests that substitution in the 3 position may also result in decreased activity. This suggestion is substantiated by the response to the compound 2,3-dichloro-, the acid form of which was inactive on all six varieties studied and the amide was inactive on five of them. Both the acid and the amide of 2,6-dichloro- were rated low in activity in the fruit-abscission test, whereas Osborne and coworkers (*12, 13*) reported the acid to be inactive and the amide to induce marked plant-regulator responses in the Avena test.

Unfortunately, 2,3,4,5-tetrachloro- compound was available only in limited quantity and only as the amide form and was applied to but one variety. It was rated highly active on McIntosh, a variety considered one of the most troublesome in abscising prior to or at harvest (*2*). Comparing this evidence with the inactivity of both the acid and the amide form of 2,3,4,6-tetrachloro- compounds further substantiates the importance of chlorination at position 6.

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Received for review October 13, 1958. Accepted November 17, 1958. Division of Agriculture and Food Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958.