DIFFERENCE IN ACTIVITY BETWEEN 2,4-DICHLOROPHENOXYACETIC ACID AND OTHER AUXINS, AND ITS SIGNIFICANCE IN HERBICIDAL ACTION

J. VAN OVERBEEK, RENÉ BLONDEAU AND VESTA HORNE

(WITH THREE FIGURES)

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Introduction

Many reports are available in the literature on the effects of 2,4-D on numerous physiological and biochemical processes. Among these are increased cell proliferation (**5**), increased respiration (**20**), increased carbohydrate depletion (**17**), decreased respiration of roots (**27**), decreased uptake of potassium by roots (**18**), decreased accumulation by the root system of KNO_3 and KCl (**13**), inhibition of lipase activity (**9**), inhibition of ascorbic acid oxidase (**27**), stimulation of phosphatase activity (**15**), stimulation of beta-amylase activity (**22, 28**), inhibition of alpha- and beta-amylase activity (**14**), etc. Useful though these observations are for the understanding of auxin action, they do not tell us if these physiological changes are the specific reasons 2,4-D has such a high herbicidal activity. It would rather seem that these phenomena are not specific for 2,4-D but are caused as well by other auxins such as indoleacetic acid and naphthaleneacetic acid (for terminology see **22**). Thus, an increased respiration, a stimulated proliferation, and a considerable loss of carbohydrate are caused in the Jerusalem artichoke tuber by indoleacetic acid (**8**). Increased phosphatase activity is found in Avena roots after treatment with indoleacetic acid and other auxins (**4**). Inhibition of amylase activity by indoleacetic acid and by a variety of other auxins has been demonstrated (**26**). Indoleacetic acid, though capable of affecting many reactions in a fashion similar to 2,4-D is definitely not an efficient herbicide.

The clue to the solution of why 2,4-D displays such a high herbicidal activity appears to lie in a different direction. Rather than only asking: "What physiological changes does 2,4-D bring about?" it would appear to be more profitable to ask first: "In what fundamental aspect does 2,4-D differ from the other auxins?" The present paper will largely deal with this question.

Materials and methods

Etiolated pea stems of the Alaska variety constituted the plant material. The growing regions of these stems were used either in the section test or in the pea test. Details of these tests may be found elsewhere (**21, 22, 24**). During the testing period, which lasted 16 hours, the cut pea stems remained in 0.05 or 0.01 M phosphate buffer solutions. The higher concentration was

used in earlier experiments, the lower concentration in most later tests. The latter concentration was shown by AUDUS (**1**) to give the most satisfactory results also. To the buffer solutions were added 1% sucrose and auxin of the desired type and quantity. Two pH values were used: pH 5.5 and pH 6.2. These were chosen because the pH of the cytoplasm of plants falls within this range (**19**). It is generally agreed at present that the cytoplasm is the site of auxin activity.

In the section tests seven sections, initially 5.4 mm. in length, were bathed in 2 cc. of solution per 50-cc. beaker on a shaker. In the pea test five split stems were in 30 cc. of solution per 4½-inch stacking dish. The beakers and dishes were sterilized. It was found that this simple expedient was sufficient to keep bacterial contamination of the test solutions to a minimum. Without any aseptic precautions, solutions were found to become cloudy during the test; in such contaminated dishes a reduced auxin reaction was observed. During the testing period, the pea stems remained in darkness at 81 ± 1° F. Each test was run in triplicate and in addition repeated at least once on a different day. The standard error of the mean was calculated, the range of which is indicated in the graphs by the small vertical lines.

The dissociation constant of each auxin was determined by dissolving the substance in 100 cc. of CO_2-free water with the aid of 1 cc. of isopropyl alcohol to give a 0.001 M solution. The pH of this solution was measured rapidly with the glass electrode and the K and pK calculated according to standard procedure (see for instance **16**). The results are summarized in table I, in which the concentrations of undissociated auxin molecules are

TABLE I

DISSOCIATION DATA OF SIX AUXINS ARRANGED IN ORDER OF INCREASING DISSOCIATION

	K	pK	[HA] in 10^{-3} M and pH 6.2	[HA] in 10^{-3}M and pH 5.5
Indoleacetic acid	2.8×10^{-5}	4.55	2.2×10^{-5}	1.03×10^{-4}
Alpha-naphthalene-acetic acid	6.17×10^{-5}	4.21	1.0×10^{-5}	4.9×10^{-5}
Cis-cinnamic acid	1.1×10^{-4}	3.96	5.7×10^{-6}	2.85×10^{-5}
Beta-naphthoxyacetic acid	1.25×10^{-3}	2.89	5.5×10^{-7}	2.75×10^{-6}
2,4-D	1.55×10^{-3}	2.81	4.06×10^{-7}	2.03×10^{-6}
2,4,5-T	2.66×10^{-3}	2.57	2.38×10^{-7}	1.18×10^{-6}

given at the two pH values employed in these tests. The pK values found in the literature have been reported elsewhere (**24**).

The auxins investigated were: indoleacetic acid (IA), now widely recognized as a native auxin, alpha-naphthaleneacetic acid (NA), beta-naphthoxyacetic acid (NO), cis-cinnamic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). 2,4-D and 2,4,5-T are highly effective herbicides. The 2,4,5-T and NO were tested only in the

split pea stem test, while the remaining auxins were tested in both the split pea stem test and the pea stem section test.

Results

When auxin activity in the pea test is plotted against auxin concentration the broken lines of figure 1 are obtained. Evidence has accumulated (**1, 3, 13**) to the effect that auxin activity does not so much depend upon the total auxin concentration, as upon the concentration of undissociated

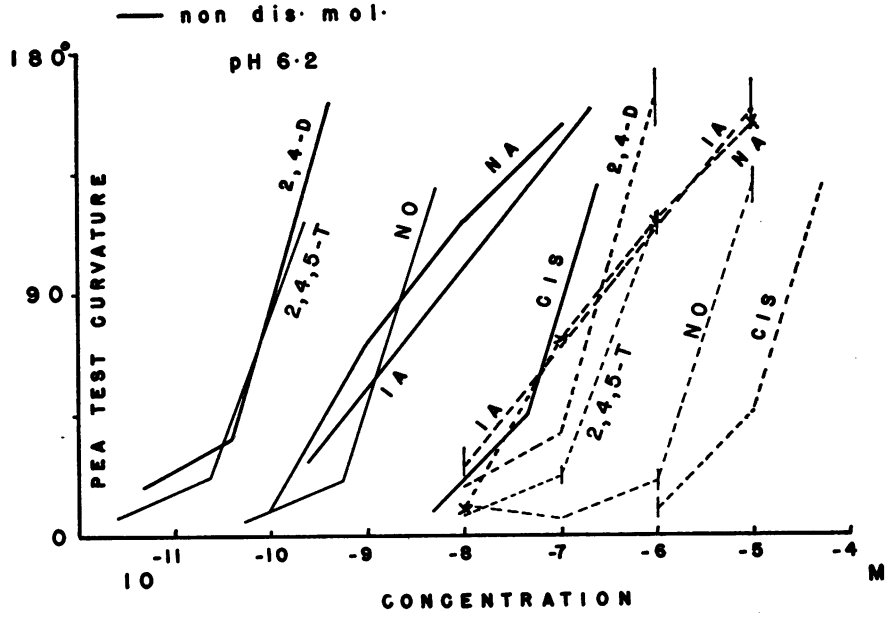

FIG. 1. Curvatures of six different auxins in the pea test as a function of the auxin concentration. Curvature in the buffer solution alone is rated 0. Broken lines plotted on the basis of molar concentration not corrected for dissociation. Solid lines plotted on the basis of undissociated auxin molecules. The short vertical lines indicate the range of the standard error of the mean. 2,4-D = 2,4-dichlorophenoxyacetic acid; 2,4,5-T = trichlorophenoxyacetic acid; NO = beta-naphthoxyacetic acid; NA = alpha-naphthaleneacetic acid; IA = indoleacetic acid; CIS = cinnamic acid.

molecules. Consequently, with the aid of the data of table I auxin activity was plotted in terms of undissociated molecules. The results are given as the solid lines in figure 1.

In order to make certain that the results obtained were not due to some inherent characteristics of the pea test, experiments with four auxins were repeated using pea stem sections for testing material. The results of these tests are shown in figure 2. A comparison between the results obtained from curvatures of split pea stems (fig. 1) and those from elongation of isolated sections of the pea stem shows indeed that the curves for the various auxins are almost identical for the two tests. Both the position of the curves as

well as their slopes are identical regardless of test method. An exception is indoleacetic acid, which has a curve which is considerably steeper in the pea test than in the section test.

The data of figures 1 and 2 were compiled from averages from individual tests extending over a period of several months. In order to ascertain that the difference in slope of the IA curves is real, IA and 2,4-D were tested simultaneously in both pea and section tests. The results obtained (fig. 3) confirm the conclusions drawn from a comparison between figures 1 and 2. The shape of the concentration curve of IA is indeed dependent upon the type of test; the 2,4-D concentration curve is independent of the type of test.

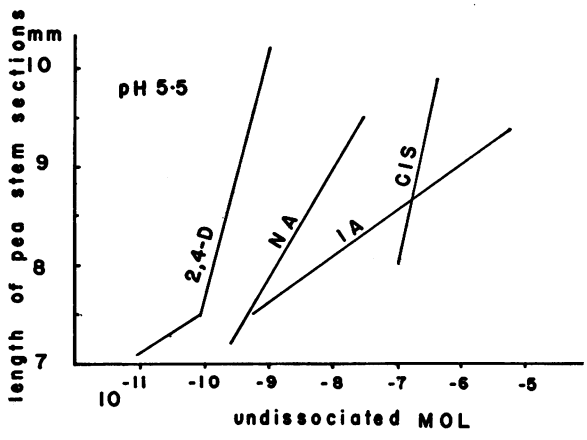

FIG. 2. Increase in straight growth of pea stem sections by four different auxins as a function of the concentrations of undissociated auxin molecules. Initial section length 5.4 mm.; duration of test, 16 hours. The abscissa is drawn at the growth level occurring in the buffer alone. Standard error of the mean, 0.1 mm. or less.

Further analysis showed that the difference in IA concentration curve between the split pea and the stem section test is not due to the small volume of solution employed in the section test, as variation of this factor did not materially affect the slope of the curve. The effect is probably due to the nature of the pea test in which the curvature of the stem halves results from a difference in elongation between the inside and the outside portions of the stems. As was already pointed out at an earlier date (**25**), low concentrations of indoleacetic acid cause considerable growth of both the inside and the outside of the stem halves, thereby concealing a growth effect of IA at low concentrations. This effect results in a concentration curve for the pea test which appears steeper than it really should be on the basis of promotion of straight growth (fig. 3 A). The section test, therefore, is a more reliable test for elongation effects than the pea test.

From the concentration curves of figures 1 and 2 the following conclusions may be drawn: The activity of the various auxins differs greatly. The

most active of the auxins tested are 2,4-D and 2,4,5-T. The slopes of the concentration curves differ greatly. 2,4-D and cis-cinnamic acid have steep slopes, while naphthaleneacetic acid and indoleacetic acid have flatter lines. The significance of these two points will be discussed below.

Discussion

From the original data of ZIMMERMAN (**29**) one might conclude that 2,4-D is not more active than IA or NA, as all three of these compounds have the concentration of 0.0015% as the lower limit for activity in causing an auxin curvature in the tomato petiole. Indeed, this agrees with the dotted curves (data not corrected for dissociation) of figure 1. However,

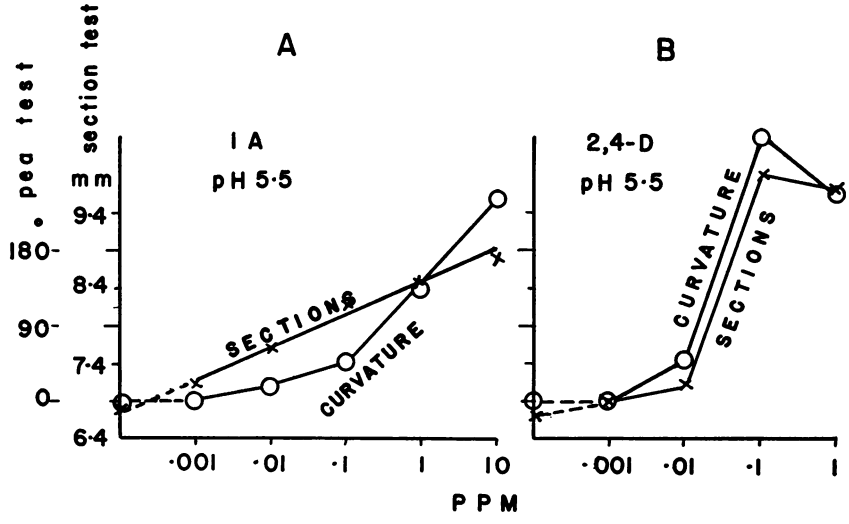

FIG. 3. Direct comparison of section and pea tests. A. With indoleacetic acid. Averages of three tests each run in triplicate. B. With 2,4-D. Averages of one test run in triplicate. In low concentrations of IA the pea test curvature lags behind the growth effect as measured by the section test.

if the concentration/activity curves are corrected for dissociation (solid curves of figures 1 and 2) it becomes at once apparent that the activity of 2,4-D is higher than that of IA or NA. At no point does the 2,4-D curve for undissociated molecules cross any other auxin curve. The compound 2,4,5-T is considered here together with 2,4-D, having a similar activity in the pea test. Over its entire range, therefore, 2,4-D is more active than any of the other auxins. This fact clearly invalidates D. M. Bonner's earlier thesis (**3**) that on the basis of undissociated molecules all auxins have the same activity. The untenability of this thesis was also discussed by AUDUS (**1**).

In another publication (**24**) it was pointed out that 2,4-D exceeded other auxins in balancing the anti-auxin effect of a given amount of trans-cinnamic acid. There it was shown that one undissociated molecule of 2,4-D had

an effect equivalent to 10 of NA, 100 of cis-cinnamic acid, and 500 of IA. It was concluded that this fact might reflect the affinity of these auxins for the cytoplasmic protein with which they are thought to combine. In its greater activity, undissociated molecule for undissociated molecule, one has one point in which 2,4-D differs from the other auxins.

The importance of a high auxin activity for success as a herbicide is also illustrated for beta-naphthoxyacetic acid (NO). In the activity diagram of figure 1 its position is well to the right of that of 2,4-D. For equal auxin effects at least 10 times higher NO than 2,4-D concentrations are required. This fact explains McNew and Hoffmann's evaluation (**12**): "Although the beta-naphthoxyacetic acid is a recognized growth regulant, with strong ability to induce formative effects, it does not operate effectively as a herbicide at low dosage."

If the solid lines of figures 1 and 2 are once more compared, it will be observed that due to diverging auxin curves differences in activity between the auxins are not necessarily constant, but that between certain auxins these differences increase with increasing concentration. Thus, in figure 2 a comparison between the 2,4-D and NA curves shows that to produce a section length of 7.5 mm. a NA concentration is required which is five times higher than that of 2,4-D. To produce a section length of 8.5 mm. a 10 times higher NA concentration is required, while for a section length of 9.5 mm. a 15 times higher NA concentration is needed.

In the same figure 2 a comparison between the 2,4-D and IA curves shows that to produce a section length of 7.5 mm. a five times higher IA than 2,4-D concentration is needed. For this small auxin effect this is the same difference in activity as was found between NA and 2,4-D. In order to increase the length of the pea stem sections to 8.5 mm. it requires an IA concentration which is 130 times higher than that of 2,4-D. For a still greater auxin effect, a growth promotion to 9.5 mm., a comparison of the curves will now show that it takes an IA concentration which is 10,000 times larger than that of 2,4-D. In other words, one undissociated molecule of 2,4-D will do the work of 10,000 undissociated molecules of the native auxin indoleacetic acid.

In figures 1 and 2 in which the ordinate reads pea test curvature and length of stem sections, the curves may not be extrapolated further upward because of the well known optimum curve expressing the relation between auxin effect and auxin concentration (**22**, p. 440). However, when the ordinate is made to read "auxin effect" instead of "length of sections" or "pea test curvature," it would seem allowable to extrapolate the 2,4-D and IA curves upward. When this is done, it will be clear that when auxin effects are reached which are herbicidal, it will take 100,000 to 1,000,000 or perhaps even more molecules of undissociated IA to do the work of only one undissociated 2,4-D molecule. For NA intermediate figures may be expected. This effect of the diverging auxin curves is another aspect in which 2,4-D

differs from the other auxins, and may well constitute another reason for its great activity as a herbicide.

Having established two principal physiological differences between 2,4-D and other auxins, one might next ask how auxins of high activity and in high concentration bring about the death of a plant. The literature quoted in the introduction, as well as many additional papers, is a clear indication that under the influence of auxins the metabolism of the plant changes.

Evidence now on hand suggests that the reason for the lethal action of 2,4-D may be found in this changed metabolism. It is well possible that metabolites which normally are produced in small harmless quantities are produced in the changed metabolism in larger, toxic quantities. FULTS and JOHNSON (**6**) have demonstrated this with scopoletin. This compound, a coumarin derivative, is normally present in small quantities (see also **7**). Under the influence of 2,4-D much larger quantities accumulate. Coumarin and many of its derivatives are phytotoxic and thus Fults and Johnson suggested that the increased concentration of scopoletin is the direct cause of the phytotoxic action of 2,4-D. This way of thinking makes understandable the earlier observations by AUDUS and QUASTEL (**2**) who studied the effect of coumarin: "With respect to the inhibition of root-growth, one feature of the activity of coumarin is its broad similarity to the action of 2,4-dichlorophenoxyacetic acid."

Reasoning along similar lines has also yielded a clue for the selective action of 2,4-D. It is well established that 2,4-D is a selective herbicide which affects broad-leaved plants more than grasses. How this selective action is brought about has long puzzled plant physiologists. It has recently become known that beta-methyl umbelliferone, another derivative of coumarin, is more phytotoxic to broad-leaved plants than to grasses (**10**). Umbelliferone, like scopoletin, is a normal plant metabolite. Both are demonstrable in the plant by their fluorescence in UV radiation. These facts suggest that accumulation of coumarin derivatives under the influence of 2,4-D might well explain the selective herbicidal effect of this auxin.

Another aspect also may be considered here briefly. In an earlier publication (**24**) it was shown that trans-cinnamic acid, also a naturally occurring coumarin derivative, is an anti-auxin. In relatively low concentrations such as 15 p.p.m. the anti-auxin effect of this compound could be completely reversed by auxins. However, in higher concentrations such as 30 p.p.m., it was noticed that trans-cinnamic acid displayed symptoms of phytotoxicity, but only in the presence of relatively high concentrations of auxins (such as 10 p.p.m. of naphthaleneacetic acid). It therefore appears as if such relatively high auxin concentrations make the tissue of the plant more susceptible to damage by naturally occurring metabolites. This also is probably another aspect of the same general phenomenon, the drastic change in the plant's normal metabolism under influence of auxins.

Summary

The herbicidal action of 2,4-D is considered to consist of two steps: (1) the very high activity of 2,4-D as an auxin, and (2) the metabolic changes brought about by this auxin activity. It is in the first step that 2,4-D differs from the other, "non-herbicidal," auxins. It is a quantitative rather than a qualitative difference. It is in the second step that the phytocidal effects take place.

The differences between 2,4-D and "non-herbicidal" auxins are on the basis of undissociated molecules: (1) a considerably greater auxin activity at all concentrations, and (2) an increasingly greater relative activity with increasing auxin concentration. Thus in the pea stem section test an increase in length of about 10% over that of the control is brought about by a concentration of undissociated molecules of indoleacetic acid which is about five times higher than that of 2,4-D. But in order to bring about a 35% increase in the length of the stem sections it takes a concentration of IA which is 10,000 times greater than that of 2,4-D. With this in mind, it is conceivable that in the range of herbicidal effects one undissociated molecule of 2,4-D may have the activity equivalent to 1 million or more undissociated molecules of indoleacetic acid.

Based on data available in the literature it was reasoned that auxins bring about a change in the plant's metabolism. Abnormally high auxin concentrations might conceivably lead to abnormal accumulations of metabolites, such as coumarin derivatives. Because of its very high auxin activity 2,4-D can be expected to bring about such metabolic changes to a larger degree than other, "non-herbicidal" auxins. One of these coumarin derivatives, β-methyl umbelliferone, has been shown to be more toxic to broad-leaved plants than to grasses. Thus, a 2,4-D-induced accumulation of metabolites, among which coumarin derivatives suggest themselves at the present state of knowledge, is capable of explaining both the phytotoxicity and the selectivity of this auxin herbicide.

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SHELL OIL COMPANY
AGRICULTURAL LABORATORY
MODESTO, CALIFORNIA

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