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Agriculture Handbook No. 126

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TEST METHODS

with plant-regulating chemicals

By JOHN W. MITCHELL • GEORGE A. LIVINGSTON • PAUL C. MARTH



UNITED STATES DEPARTMENT OF AGRICULTURE

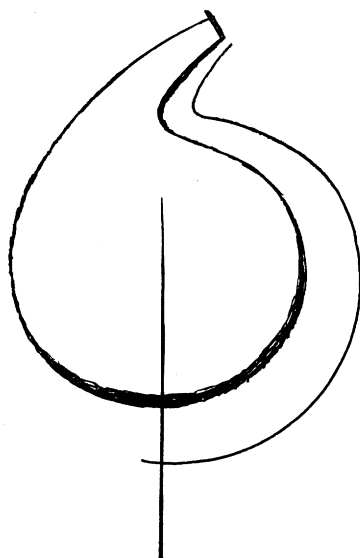
TEST METHODS

with plant-regulating chemicals

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UNITED STATES DEPARTMENT OF AGRICULTURE

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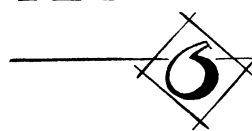
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TEST METHODS



with plant-regulating chemicals

INTRODUCTION

Organic compounds are being used in increasing amounts to control the growth and behavior of crop and ornamental plants. Not only have many different compounds been found to possess growth-regulating properties, but various kinds of plants and plant parts have been found to respond in widely different ways to these interesting and useful chemicals. For example, 2,4,5-trichlorophenoxyacetic acid can be used to retard the formation of an abscission zone in the stem of an apple. Applied to a rhododendron cutting, it stimulates root formation. 2,4-Dichlorophenoxyacetic acid often suppresses growth of pods when applied to snap bean plants. In contrast, 4-chlorophenoxyacetic acid has been used experimentally to accelerate growth of snap bean pods, to increase their succulence, and their vitamin C content.

Plant scientists are searching for and finding not only a variety of new regulating compounds, but also new ways in which plants can be made to behave through the use of these chemicals. In this publication an attempt has been made to make the methods used by these scientists easily available, and thus facilitate research of this kind.

Many useful methods for studying plant-regulating properties of compounds have been devised. These methods result from the efforts of many scientists working on a variety of research problems in various countries. Without drawing the published methods together, it is difficult to fully utilize them in teaching and research since their publication occurs in a wide variety of journals. Furthermore, some of the methods have not yet been published.

It is hoped that this compilation will be useful in several ways: First, by affording those experienced in this field of research an opportunity to compare the ways in which scientists have

tested chemicals for plant-regulating properties, and how they have recorded and evaluated plant responses obtained with these compounds. This information should lead to the development of even more suitable methods. Secondly, the compilation will afford the experienced worker and the novice in this field ready access to many of the methods that have been used. Third, it may aid in the teaching of college courses where laboratory exercises are used to demonstrate the effects of regulating chemicals. Some of the methods described may also be useful as laboratory exercises in the teaching of high school courses in biology and plant science.

The compilation is divided into three parts. The first section includes methods designed to detect regulating properties of organic compounds and measure the response of plants to these compounds. The second section includes methods designed to detect and, in some instances, to measure the amounts of regulating compounds present in plants, in water, and to some extent in animals. The third section describes pictorially some techniques that are of general use in experimenting with plant-regulating compounds.

The methods are described step by step in the manner of a cookbook with the hope that they will be readily understandable and easy to use. Descriptive titles have been assigned to each method indicating the use suggested by the author. In some instances additional uses have been suggested by those compiling the methods. A few well-known and very useful tests, such as the oat coleoptile test, have not been included since these tests are widely used and descriptions of them are easily obtainable. It is hoped that scientists will find the methods described in the present compilation useful in many other ways besides those suggested here.

PART I. METHODS OF APPLYING REGULATING COMPOUNDS AND OF STUDYING RESPONSES THEY INDUCE

Abscission

APPLE PETIOLE TEST

Method based on research by:

L. J. Edgerton; L. J. Edgerton and M. B. Hoffman.

Description of test

The rate of abscission of petioles of debladed leaves on apple water sprouts following treatment with various chemicals is used as an indication of the effectiveness of the compounds in retarding harvest drop of apples.

This test can also be used to evaluate the effectiveness of plant regulators, other compounds, or combinations of them, in accelerating or reducing the rate of leaf abscission.

Suggested plant material

Rapidly growing vegetative branches or shoots (commonly called water sprouts) from the main branches or trunks of apple trees (*Malus sylvestris* Mill., also called *Pyrus malus* L.)—McIntosh, Winesap, Stayman, or other varieties.

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Hand-sprayer (250 ml.) or nasal atomizer (15–30 ml.)
25–200 mg. of each compound to be evaluated
500 mg. of alpha-naphthalene-acetic acid or its sodium salt for standard treatment
Solvent such as 95% ethanol
Wetting agent such as Santomerse, Dreft, or Tween 20

Preparation and selection of plant material

In midsummer tag apple trees that have pro-

duced an abundance of water sprouts during the current season. New sprout growth can be induced to develop 1 to 2 months earlier than usual by selecting experimental trees prior to winter pruning and cutting back the existing water sprouts, leaving stubs 1–3 in. (3–8 cm.) in length. These stubs will then produce long, vegetative shoots early during the next growing season. One- to two-year-old potted trees may be used during winter and spring in the greenhouse. Select shoots of uniform diameter, each having 15 to 25 leaves.

Method of conducting test

Prepare the spray solutions by dissolving the desired weight of the chemical to be evaluated in a minimum of solvent (1–2 ml. of 95% ethanol); add enough wetting agent to make a final concentration of 0.02–0.2% of the wetting agent. Pour this mixture, while stirring, into sufficient tap water to make a final concentration of 10–30 p.p.m. of the chemical being tested. Apply the spray solution with the hand sprayer to 5 or 6 water sprouts, thoroughly wetting the leaf blades, petioles, and twigs. Within 2 to 3 days after treatment remove 10 or more leaf blades from each shoot, leaving the entire petioles attached to the twigs. To make the results more nearly uniform, do not use the 4 or 5 basal leaves or the young, partially expanded leaves near the tip of the shoots. Remove blades from 10 or more leaves on comparable unsprayed sprouts of each test tree and designate these as controls.

Method of taking results

Within 10 to 11 days after treatment, and at frequent intervals thereafter for the duration of the test, count the petioles that remain attached in each experimental lot. The final percentage of petioles attached is an index of the effectiveness of the compound when used for retarding fruit abscission.

Suggested standard treatment for comparison

10, 20, or 30 p.p.m. concentration of alpha-naphthaleneacetic acid or its sodium salt.

Edgerton, L. J. A Method for Evaluating the Effectiveness of Growth Substances in Delaying Apple Abscission. Amer. Soc. Hort. Sci. Proc. 49: 42-44. 1947.
Edgerton, L. J. and Hoffman, M. B. The Effect of Some Growth Substances on Leaf Petiole Abscission and Preharvest Fruit Drop of Several Apple Varieties. Amer. Soc. Hort. Sci. Proc. 62: 159-166. 1953.

ATTACHED PETIOLE TEST

Method based on research by:

J. W. Mitchell, P. Marsh, and R. Bender.

Description of test

The test compound is placed on debled petioles, and a known pressure is then applied to the petioles each day to determine the length of time required for the petioles to abscise compared with that for untreated petioles.

Suggested plant material

Young cotton plants (*Gossypium hirsutum* L.), Deltapine variety, and young bean plants (*Phaseolus vulgaris* L.) of the Pinto or other available varieties.

Apparatus, chemicals, and other materials

Constant-pressure apparatus
(see fig.1)
Razor blades or sharp knife
Lanolin
Endothal acid, monohydrate,
1-2 g.
Phenylmercuric acetate 1-2 g.
Analytical balance or pharmaceutical balance
Vials
Medical applicator sticks
or toothpicks
Tween 20
Compounds to be evaluated as
growth regulators

Preparation and selection of plant material

Plant cotton seeds in composted soil contained in small clay pots, using several seeds per pot. After the plants have developed cotyledons about 1.5 inches across (3-5 cm.), and the first true leaf is beginning to expand from the terminal bud (10-12 days after planting), select a

group of test plants of uniform size and thin them to one plant per pot. Arrange the plants uniformly in rows of 5 plants each on a greenhouse bench that is evenly illuminated. Remove the blade of one cotyledon of each plant by severing the petiole where it joins the blade.

Method of conducting test

Dissolve 12.5 mg. of the compound to be tested in approximately 0.25 g. of Tween 20 (7 drops from a 10 ml. pipette); then add 1 g. of melted lanolin. Stir the mixture thoroughly. Dilute one-half of this 1% mixture with an equal amount of the Tween 20-lanolin carrier, and continue the dilution process with the mixtures obtained until a suitable range of dosage levels has been obtained.

Treat the debled petioles of one row of plants by applying with disposable applicator sticks a portion of the 1% mixture about the size of a wheat seed as a band about 2 mm. wide around the cotyledonary stump of each plant. Apply the paste 2-3 mm. from the stems. Apply equal portions of the pastes containing progressively smaller amounts of the test compound to debled petioles in corresponding rows in a similar manner.

To determine when the petioles readily abscise, apply pressure once each day in a downward direction against the upper surface of each debled cotyledonary petiole. Accomplish this by pressing the thin metal spring of the pressure applicator against the petiole until tension equal to approximately 5 g. develops, at which time the light on the tension applicator will flash (fig. 1). Always apply pressure to the petiole at a predetermined distance (8-10 mm.) from the stem. The pressure applicator may be calibrated prior to use by pressing the spring on a balance with a 5 g. weight on the opposite pan.

Method of taking results

Record the number of petioles that abscise each day and calculate the number that fall from the treated plants in terms of the number that fall from plants treated with the Tween 20-lanolin carrier alone.

Alternative method

Use young bean plants in the same manner except remove one primary leaf blade of each plant, leaving the petiole attached. Select plants with primary leaves that are approximately 2-3 in. (3-5 cm.) wide, and with the first trifoliate leaf just beginning to unfold from the terminal bud.

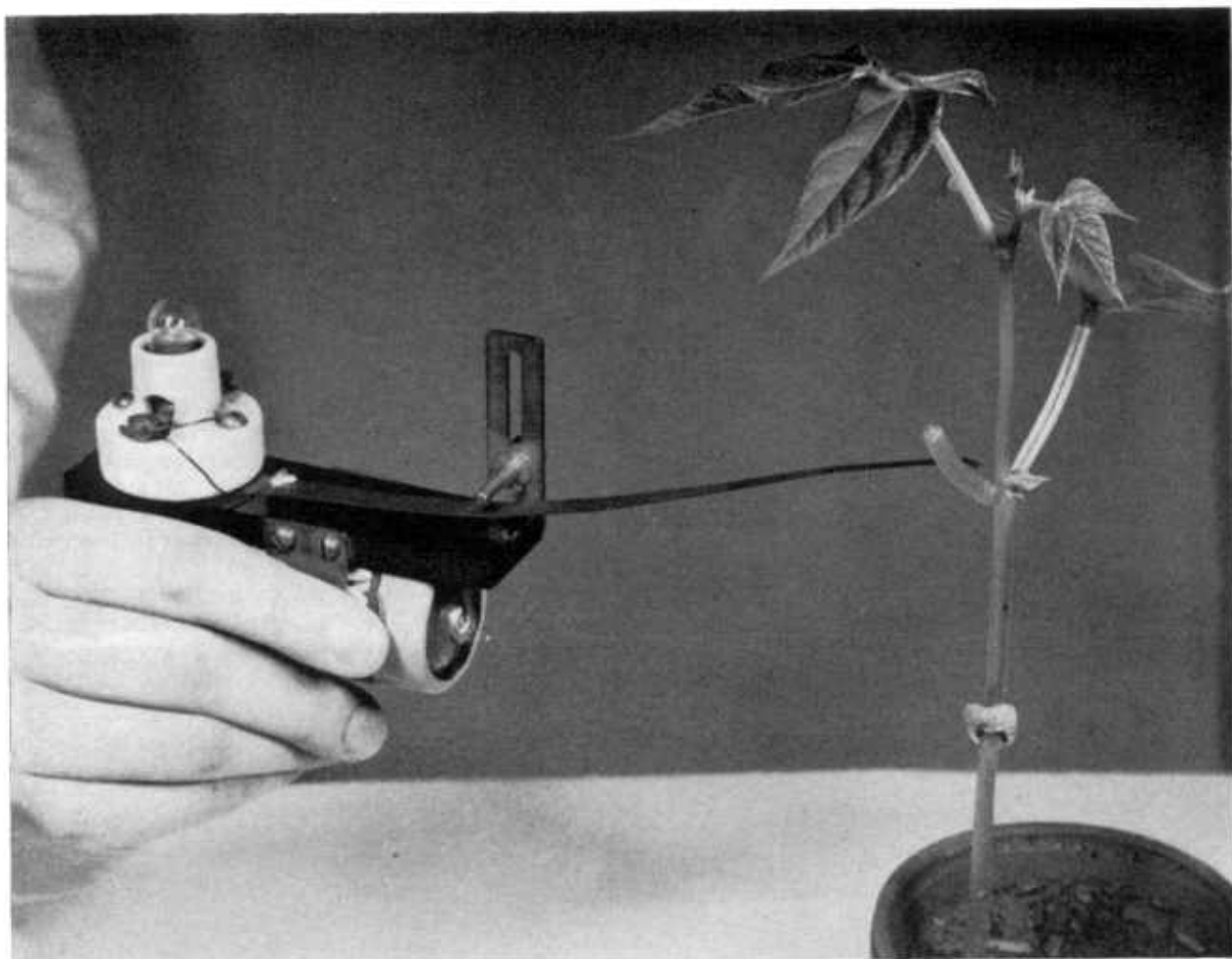


Figure 1. This pressure apparatus is used to determine whether or not a chemical will make leaves fall from a plant. First, remove the blade from one leaf on each of several plants, leaving the petioles attached. Apply the chemical to some of the debladed petioles. Use the apparatus daily to apply a measured amount of pressure to both the treated and the untreated petioles, as illustrated. Place the end of the spring and adjustable pin make contact and the light bulb is activated; then quickly release the pressure. Determine if treated petioles fall sooner or later than those not treated, or at the same time.

(NEG. PN-292)

Suggested standard treatment for comparison

Endothal acid monohydrate, phenylmercuric acetate, or naphthyl maleimide for cotton; endothal acid monohydrate, or phenylmercuric acetate for bean plants.

Mitchell, J. W., Marsh, P. and Bender, R. Unpublished data.

EXPLANT TEST

Method based on research by:

F. T. Addicott, R. S. Lynch, G. A. Livingston, and J. K. Hunter.

Description of test

Small sections of leaves (explants) containing an abscission zone are treated with compounds, and the effect on abscission observed.

Suggested plant material

Citrus leaves or leaves of other kinds of plants that have abscission zones near a leaf blade.

Apparatus, chemicals, and other materials

Scalpel
Forceps
Filter paper

Petri dishes
 Dark chamber maintained at
 about 77° F. (25° C.)
 Analytical balance or pharmaceutical balance
 Closed bell jar or desiccator
 0.5–1 g. of each compound to be
 evaluated
 For standard treatment:
 ethylene gas or
 0.5–1 g. 3-indoleacetic acid
 Agar

Preparation and selection of plant material

Excise for use as test material section of leaves containing the abscission zone and the adjacent tissues (see fig. 2.) These sections are called explants. Trim off all but the midrib of the blade portion of the leaves in preparing the explants. If the petioles are winged, trim the wing from them. Always leave a longer piece of tissue, consistently either distal

or proximal to the abscission zone, so that identification of the blade or petiole tissue can be made readily. Prepare 10 of these explants for each concentration of each treatment, and an additional 20 or 40 to serve as controls. After cutting, and before treatment, store the explants on moist paper in a Petri dish. In preparation of the explants, utilize leaves that have attained their full size and hard texture but still have a dark green color.

Method of conducting test

Prepare 4% agar in water, pour it into Petri dishes, and allow it to cool and set. The depth of the agar in the dish should be about $\frac{1}{4}$ in. (6 mm.). After the agar has set, remove a strip of it, about 1 in. (3 cm.) wide, along the diameter of the plate, leaving a narrow bridge of agar across one end to hold the agar in place and to act as a marker (see fig. 2).

Prepare 10–25 ml. of each concentration of

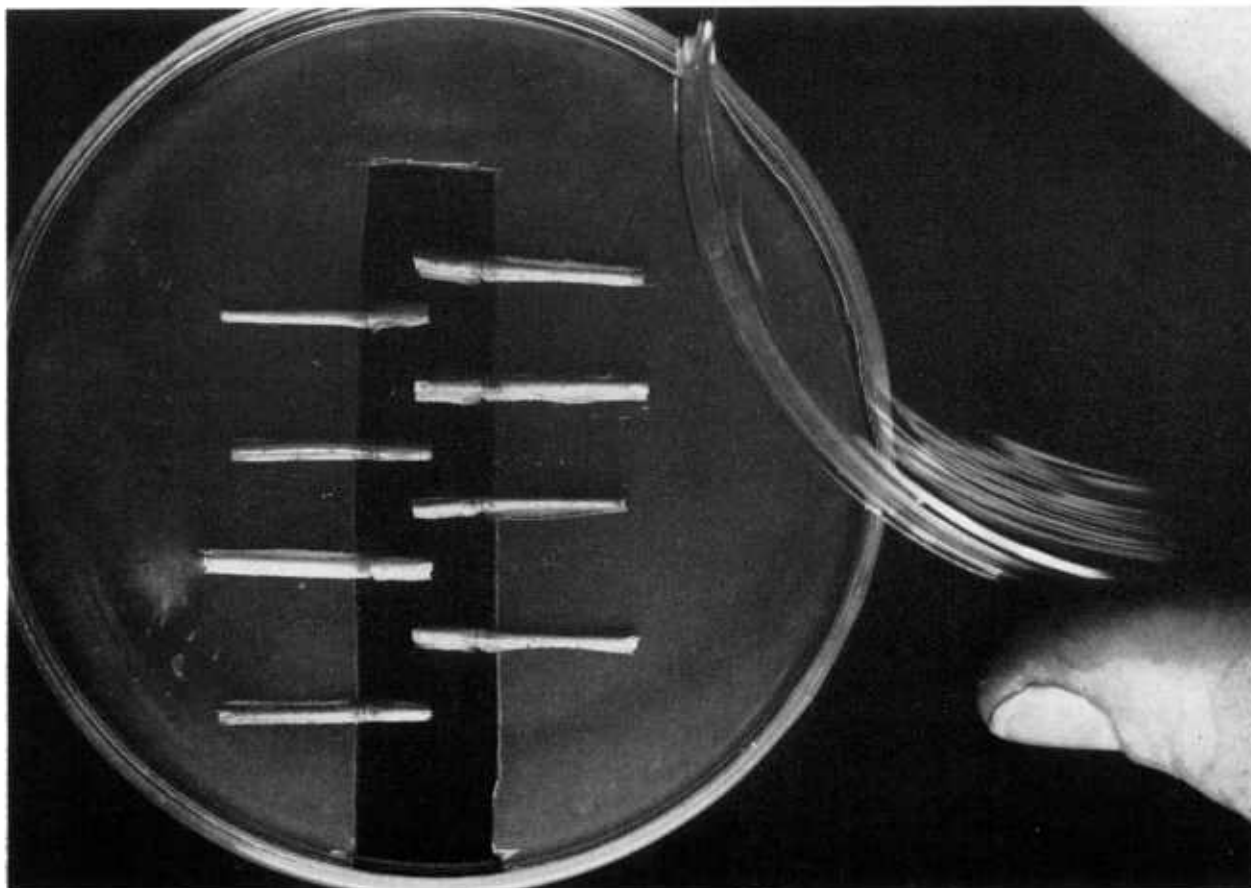


Figure 2. This test is used to learn whether or not a chemical makes leaves drop. Cut segments of petioles from orange or bean leaves, including the abscission zone in each section, as illustrated. Treat some of the segments with the compound; then place the treated sections along with untreated sections on agar, as illustrated. Replace the lid of the Petri dish and observe how long it takes abscission to occur in the treated and untreated segments.

(NEG. PN-293)

each chemical to be tested. The suggested concentration range is 1–1,000 p.p.m. Treat explants (10 per treatment) by immersing them in the test solution for 30 to 60 seconds. Remove the treated explants from the solution and mount them on the agar of a Petri dish so that the shorter part and the abscission zone overhang the central channel cut in the agar. The compound may also be administered by including it in the agar support or by application of small droplets to the cut petiole or blade surface of the explant.

Prepare dishes containing untreated explants to serve as controls. Keep the Petri dishes containing the explants in the dark at about 77° F. (25° C.) except for the short daily period of examination.

Method of taking results

Observe for evidence of abscission by examining the explants with the dishes closed. To determine if abscission is complete but not evident, raise the dish cover and lift the explants slightly by pressing the tips of forceps upward under their free ends. The period during which the explants are kept under observation is usually 6 or 7 days. With some experience, various "stages" of abscission can be identified. Maintain daily records, listing the percentage of abscission in each treatment and, if desired, the "stage" of abscission which each explant exhibits at each time of observation.

Suggested standard treatment for comparison

To initiate or accelerate abscission, place Petri dishes containing the explants inside an air-tight container and add ethylene to make a concentration of 20–50 p.p.m. Use 0.001–100 p.p.m. of 3-indoleacetic acid (30–60 seconds dip) as a standard for delay of abscission.

Addicott, F. T., Lynch, R. S., Livingston, G. A., and Hunter, J. K. A Method for the Study of Foliar Abscission In Vitro. *Plant Physiology* 24 (3): 537–539. 1949.

LANOLIN PASTE TEST

Method based on research by:

P. C. Marth, W. H. Preston, Jr., and J. W. Mitchell.

Description of test and some other uses

The abscission of apple fruits following application of a chemical in lanolin paste around the

stem of the individual fruits prior to the usual harvest date indicates the effectiveness of the chemical in retarding fruit abscission.

With slight modifications this test is applicable to studies of abscission rates of a wide variety of fruits.

Suggested plant material

Apple trees (*Malus sylvestris* Mill., also called *Pyrus malus* L.) bearing an abundance of fruit readily accessible from the ground — Grimes, McIntosh, York, or other varieties.

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Small shell vials with stoppers
Wooden board 6–8 in. wide x
1–2 ft. long x 1 in. thick
15 x 30 x 3 cm.) having 1/2 in. (1 cm.)
diameter holes drilled 3/4 in. (2 cm.) deep
and 2 in. (5 cm.) apart for carrying
the vials in the field
Toothpicks or disposable wooden
applicators without cotton
Small labels with string or wire attached
25–100 mg. of each compound to be evaluated
0.5 g. of alpha-naphthaleneacetic acid
for standard treatment
Ethanol
Lanolin
Tween 20

Preparation and selection of plant material

Select individual fruits that are readily accessible from the ground and located around the perimeter of each tree used for the experiment. The fruits should be of uniform size and free of blemishes. Make the fruit selection and apply the treatments about 30 days prior to the estimated, or usual, harvest date for the variety.

Method of conducting test

Prepare lanolin-Tween 20 mixtures containing 0.1 and 1% concentrations of the respective compounds. To prepare the 1% mixture, place 25 mg. of the compound to be tested in a vial and add 14 drops of Tween 20. Stir to dissolve the chemical, and add 2 g. of lanolin. Melt the lanolin by placing the vial in warm water (not over 55° C.) for a few minutes. Remove the vial and stir the mixture thoroughly until it reaches room temperature and becomes semi-solid. To prepare the 0.1% mixture, weigh 0.2 g. of the 1% mixture previously prepared

and mix this with 1.8 g. of the lanolin-Tween 20 carrier (the carrier made by mixing 2 g. of lanolin with 14 drops of Tween 20).

Apply a small portion, the size of a wheat seed, of the mixture as a band about 3 mm. wide around the stem of each of 10 fruits near the junction of stem and branch. As each fruit is treated, attach a label designating the treatment. Repeat, using an additional 10 fruits for each mixture. Fruits used for an individual mixture should be distributed evenly around the perimeter of the tree. Remove all untreated fruits except 10 which are left as controls.

Method of taking results

One week after treatment and at weekly or bi-weekly intervals thereafter (depending upon the prevalence of wind or hail), record the number of fruits remaining attached in each treatment.

Record data regarding advance or retardation in maturity, color changes, cracking, or other responses for 30 days or more after the usual harvest date for the variety. The percentage of treated fruits remaining attached throughout the course of the experiment compared with the percentage of untreated fruits remaining attached serves as a measure for evaluating the effect of individual compounds on fruit abscission.

Suggested standard treatment for comparison

Lanolin paste mixture containing either 0.1 or 1% alpha-naphthaleneacetic acid.

Marth, P. C., Preston, W. H., Jr., and Mitchell, J. W. Relative Effectiveness of the Mono-, Di- and Tri-Chlorophenoxyacetic Acids in Retarding the Abscission of Mature Apples. *Bot. Gaz.* 117: 51-55. 1955.

Absorption and Translocation

BEAN ROOT TEST

Method based on research by:

T. J. Muzik.

Description of test and some other uses

Test plants are arranged with one-half of their root systems in the test solution and the other half in tap water; then the roots that have been in the test solution are cut off at different time intervals. The resulting growth modifications or injurious effects of the chemicals can be observed, and the rapidity of absorption and translocation of the test compound by the roots can be determined.

This method can be utilized to study the effects of environmental factors, such as light, temperature, hydrogen ion concentration, mineral nutrition, and oxygen supply, on the ability of roots to absorb and translocate regulating chemicals.

Suggested plant material

Plants with root systems that can be arranged in two portions of about the same size — beans (*Phaseolus vulgaris* L.) or similar plants.

Apparatus, chemicals, and other materials

Clay pots 3-6 in. (8-15 cm.)
Composted soil

200- to 500-ml. glass containers
Supports to hold a plant with one-half of its root system in one container, the other half in another container
Analytical balance or pharmaceutical balance
Compressed air for aerating nutrient solutions
Greenhouse space with controlled temperature, humidity, and light intensity, or devices for recording temperature, humidity, and light intensity
2-5 g. of each compound to be evaluated
3-(para-Chlorophenyl)-1,1-dimethylurea for standard treatment
For nutrient solution:
124 g. of $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$
83 g. of $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$
68 g. of KH_2PO_4
85 g. of $\text{Ca}(\text{NO}_3)_2$

Preparation and selection of plant material

Grow bean plants in soil or other suitable medium. After the plants have developed one partially expanded trifoliate leaf, select the desired number of plants of uniform size and carefully wash them free of soil. Support the plants with their roots in nutrient solution aerated by means of compressed air, and allow them to grow 3 to 5 days to condition them for the test. For the nutrient solution, use stock solutions of MgSO_4 , CaCl_2 , KH_2PO_4 , and $\text{Ca}(\text{NO}_3)_2$ made by dissolving the quantities listed above, each in a separate liter of tap water. To 500 ml. of tap water add 9 ml. each of the MgSO_4 , CaCl_2 , and

KH_2PO_4 stock solutions, and 18 ml. of the $\text{Ca}(\text{NO}_3)_2$ stock solution. Add sufficient water to make 1 liter of nutrient solution.

Method of conducting test

Arrange pairs of containers for the test plants and fill one container of each pair with a water solution of the compound to be tested (25–200 p.p.m.). Fill the remaining container of each pair with tap water. For the control plants fill both containers with tap water.

Select plants of uniform size and vigor from those placed in nutrient solution earlier. Support each test plant over a pair of containers and arrange the roots so that one-half of the root system is immersed in the test solution and the other half in tap water. Arrange control plants with each half of the root system growing in tap water. In this manner, use 6 to 12 plants for each test solution and an equal number of plants with their roots in tap water for untreated controls. Allow the plants to remain in a greenhouse and record the prevailing temperature, humidity, and light intensity, or grow them under controlled conditions.

After 30 minutes, cut from one-third of the treated plants the roots immersed in the test solution but leave on these plants the roots growing in tap water. Also cut one-half of the roots from each of one-third of the control plants. After another 30 minutes repeat the procedure, using the second third of the treated and control plants. Finally, repeat the procedure after an additional 30 minutes, using the remaining treated and control plants. Replace the tap water with nutrient solution that is continuously aerated, and allow the treated and untreated plants to grow for a week or two with their remaining roots in the nutrient solution.

Method of taking results

Epinasty, formative effects (leaf modification), discoloration of leaves, or gall formation observed on the treated plants, but not on untreated controls, indicate absorption and translocation of the test compound. In addition, the time required for this to occur through the roots can be determined by comparing the treated and untreated plants which have had their roots removed at different intervals.

Suggested standard treatment for comparison

25–200 p.p.m. of 3-(para-chlorophenyl)-1,1-dimethylurea.

Unpublished method submitted by Thomas J. Muzik, Federal Experiment Station, Mayaguez, Puerto Rico.

BEAN STEM CURVATURE TEST

Method based on research by:

A. S. Crafts; B. E. Day; J. W. Mitchell, W. M. Dugger, Jr., and H. G. Gauch.

Description of test and some other uses

The compound to be tested is placed on one leaf of a plant; then absorption and translocation of the compound (or of a metabolite of it) are evaluated on the basis of stem curvature that develops. This test is applicable only to compounds that induce or retard cell elongation.

The test may be used under controlled conditions to compare the relative effectiveness of different compounds that are known to cause stem curvatures. With some modifications the test can also be used to detect the effects of adjuvants on absorption and translocation of a regulator, or metabolite of it, that induces stem curvature at a distance from the area to which the regulator is applied.

Suggested plant material

Young bean plants (*Phaseolus vulgaris* L.)—Black Valentine, Red Kidney, Pinto, or other varieties that germinate and grow uniformly.

Apparatus, chemicals, and other materials

Clay pots 3–6 in. (8–10 cm.)
Composted soil
Greenhouse space maintained
at about 75°–85° F. (24°–29° C.)
Analytical balance or pharmaceutical balance
Several 0.1-ml. pipettes graduated in
0.01-ml. divisions
250 ml. beakers for preparing solutions
Glass applicator (about 15 cm. long)
with one end drawn out to make a narrow
portion of about 2-mm. diameter and
7-cm. length
Facial tissue or paper towels
Source of fluorescent white light
Protractor
Adjustable bevel square
About 100 mg. of each compound to be
evaluated
100 mg. of 2,4-dichlorophenoxyacetic acid
for standard treatment
Tween 20, Glim, Joy, or other solvent
of the liquid detergent type
Distilled water

Preparation and selection of plant material

Plant 3 or 4 seeds per pot in composted soil. After the plants have developed partially expanded but still wrinkled primary leaves, and the trifoliate leaves are still tightly folded in the terminal buds, select one plant in each pot for similarity of size. Sever the stems of the remaining plants in each pot at the soil level and discard them. Place the selected plants in a well-lighted greenhouse with the pots widely spaced so that the plants will receive adequate illumination. As soon as the primary leaves of the majority of the plants have expanded so that they are no longer wrinkled, usually about 48 hours after the first selection, reselect the plants for size and uniformity, and place them in rows of 8 to 10 plants each. Water the soil surrounding the roots of each plant before conducting the test.



Method of conducting test

Dissolve in Tween 20 or other suitable solvent a weighed portion of the compound to be tested and add this mixture to warm, distilled water so that 0.02 ml. of the final mixture will contain 1–5 μ g. of the chemical and sufficient Tween 20 to make a final concentration of 0.1% of this solvent.

Fill a pipette with the solution; then drain exactly 0.02 ml. out so that it adheres as a drop on the tip of the pipette. Touch the tip of the pipette near one edge of one primary leaf blade and move the tip across the leaf, thus applying the mixture as a narrow band extending from one side of the leaf blade to the other (fig. 3, upper photo). Immediately support the blade by pressing a folded pad of facial tissue or paper towel under the leaf, place the applicator in the mixture, and move the applicator slowly toward the tip of the leaf, thus spreading the liquid evenly over the surface of the leaf blade (except for an area near the end to which the petiole is attached, fig. 3, lower photo.) Apply other 0.02 ml. portions to one primary leaf of each of the remaining plants in the row.

Make certain that the treated leaves are oriented on the same side of each row. Arrange as controls a comparable row of untreated plants, and a third row treated similarly with water and Tween 20, or other solvent. Allow the plants (8 to 10 per treatment) to grow in a greenhouse, or place them in the fluorescent light so that they receive illumination of at least 700-foot-candle intensity.

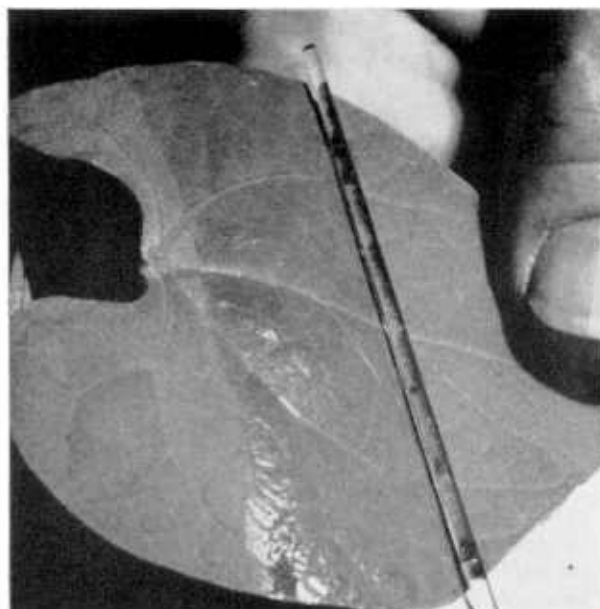


Figure 3. An easy method of applying minute, measured amounts of growth-regulating chemicals to leaves. Dissolve a measured amount of the regulating compound in water and add a wetting agent. Apply a measured volume of the mixture as a band across the upper surface of a young leaf (upper photo); then, resting the leaf on a folded paper towel, spread the mixture evenly over the entire upper surface of the leaf with a glass rod (lower photo). (NEG. PN-294 A AND B)

Method of taking results

Observe the plants at 30-minute or hourly intervals. Measure the angle of stem curvature

resulting from the presence of the chemical by lining up one leg of the adjustable square with the hypocotyl and lower half of the first internode, and adjusting the other leg of the square so that it is in line with the upper half of the first internode and second internode above the curved section of stem. Compare average readings from the control plants with those from plants that received the compounds being tested. The method is applicable to compounds which induce or retard cell elongation.

Suggested standard treatment for comparison

1.0 μ g. of 2,4-dichlorophenoxyacetic acid on one primary leaf of each of 8 to 10 plants.

Crafts, A. S. *Herbicides, Their Absorption and Translocation*. Agr. and Food Chem. 1: 51-55. 1953.

Day, B. E. The Absorption and Translocation of 2,4-Dichlorophenoxyacetic Acid by Bean Plants. *Plant Phys.* 27: 143-152. 1952.

Mitchell, J. W., Dugger, W. M., Jr., and Gauch, H. G. Increased Translocation of Plant Growth-Modifying Substances Due to Application of Boron. *Sci.* 118: 354-355. 1953.

Angle and Elongation of Branches

LANOLIN TEST

Method based on research by:

A. P. Preston and H. W. B. Barlow; L. Ver-
ner.

Description of test

A piece of flexible tubing is filled with paste containing the test chemical, slipped onto the stump of a decapitated branch of a young tree, and the effects on branch-trunk angle and growth of the branch are noted.

Suggested plant material

Young trees in whip stage (no lateral branches) or trees with 1-year-old lateral shoots—apple (*Malus sylvestris* Mill., also called *Pyrus malus* L.), pear (*Pyrus communis* L.), plum (*Prunus* spp.), willow (*Salix* spp.), or others.

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Several ft. (about 1 m.) of $\frac{1}{4}$ -in. (6-mm.)
flexible rubber tubing or sheets of thin,
transparent celluloid
Protractor
Test tubes
1 g. of each compound to be evaluated
About 2 g. of indolebutyric acid for
standard treatment
Lanolin

Preparation and selection of plant material

Select trees of uniform size and stage of development. Use 10 trees for each compound or concentration to be tested.

Method of conducting test

Prepare lanolin mixtures, 3 g. of lanolin per

mixture, containing an amount of the chemicals to be tested covering a range of 0.1-1% of the weight of lanolin used. Apply lanolin mixtures either to the unbranched trunk or to a vigorous 1-year-old lateral shoot. Cut off the terminal portion of the shoot to be treated, usually a segment 4-8 in. (10-20 cm.) in length. Cut off a $1\frac{1}{2}$ -in. (4-cm.) length of the rubber tubing and half-fill it with the paste containing the compound to be tested. Slip the end of the tube containing the paste over the decapitated shoot for a distance of about $\frac{1}{2}$ in. (1 cm.). Double the empty end of tubing over and tie it in this position with a string to protect the lanolin mixture. Repeat this procedure, using different shoots with lanolin alone and also with lanolin plus 3-indolebutyric acid (0.1%), and designate these as a control and a standard treatment.

In an alternate method, flexible sheet plastic or similar material is employed in place of the rubber tube. Cut strips of the flexible material, 8 x 6 cm.; wrap a strip around the end of each cut branch, and secure it with a rubber band. Melt the lanolin mixture and put about 5 ml. of it into the tube formed by the strip. Slip a test tube over the open end of the tube to protect the lanolin.

Method of taking results

After the lateral shoots on the controls have attained an average length of 6-8 in. (15-20 cm.), determine the average length of the first 3 or 4 lateral shoots nearest the treated portion on each tree. Compare this average with the averages obtained from trees treated with the 3-indolebutyric acid mixture, and with lanolin alone.

Measure with a protractor the angle formed by the trunk of each plant and the first 3 or 4 lateral shoots nearest the treated portion. Compare these measurements with similar measurements on plants given the standard treatment, and on control plants.

Suggested standard treatment for comparison

A 0.1% lanolin mixture of 3-indolebutyric acid.

Preston, A. P., and Barlow, H. W. B. The Use of

Growth Substances to Widen Crotch Angles. East Malling Res. Sta. Ann. Rept. 1950: 76-79 (pub. 1951).

Verner, L. Effect of a Plant Growth Substance on Crotch Angles in Young Apple Trees. Amer. Soc. Hort. Sci. Proc. 36: 415-422. 1939.

Verner, L. Unpublished method. University of Idaho, Moscow, Idaho.

Cell Division

CAMBIUM TEST ¹

Method based on research by:

W. C. Cooper, S. Tayloe, and N. Maxwell;
W. C. Cooper, A. Peynado, and G. Otey.

Description of test

A knife cut is made through the bark in a ring around the trunk of young evergreen fruit trees, and growth-regulating chemicals are applied to test for inhibition of cambial activity. The degree of tightness of the bark that develops subsequent to treatment indicates the amount of reduction in cambial activity.

Suggested plant material

Young semi-tropical evergreen fruit trees—orange (*Citrus sinensis* [L.] Osbeck), grapefruit (*Citrus paradisi* Macfad.), avocado (*Persea americana* Mill., also called *Persea gratissima* Gaertn.), or others.

Apparatus, chemicals, and other materials

Pocket knife
Analytical balance or pharmaceutical balance
Small vials
Applicator sticks with a wad of cotton on one end
2 mg. of each compound to be evaluated
50% ethanol for compounds insoluble in water
Distilled water

Preparation and selection of plant material

Use seedlings or budded trees about 2 years old, preferably growing in the nursery row. Select trees with trunks free of lateral branches

¹ This test is useful in selecting chemicals that might temporarily reduce vegetative growth of semi-tropical fruit trees and thus increase their resistance to low temperatures.

and with bark that peels readily. Prepare the trees for testing by making a knife cut just through the bark and extending as a ring around the trunk at a point about 6 in. (15 cm.) from the ground.

Method of conducting test

The 2-mg. sample of each chemical to be tested is placed in a separate small vial and dissolved in 2 ml. of distilled water, or in 2 ml. of 50% ethanol if the compound is not water soluble. Using a separate applicator for each solution, moisten the cotton with the test solution and swab over the knife-cut ring and over the adjacent trunk surface for a distance of 1 in. (3 cm.) above and below the ring. Use a separate lot of trees (3 to 6) for each solution.

Method of taking results

At weekly intervals following the treatment, estimate cambial activity by determining the ease with which bark can be peeled from the trees. In this test make two parallel longitudinal cuts through the bark about 1/2 in. (1 cm.) long and about 1/8 in. (3 mm.) apart. Join these cuts at the bottom by a transverse cut. Insert a knife blade under the transverse cut, and peel the bark from the sapwood by pulling in an upward direction. The force required to remove the bark is given a rating. The bark-peeling tests should be made both above and below the ring and both within and outside the treated area.

Suggested standard treatment for comparison

No standard treatment suggested.

Cooper, W. C., Tayloe, S., and Maxwell, N. Preliminary Studies on Cold Hardiness in Citrus as Related to Cambial Activity and Bud Growth. Rio Grande Valley Hort. Inst. Ann. Proc. 9: 1-15. 1955.

Cooper, W. C., Peynado, A., and Otey, G. Effects of Plant Regulators on Dormancy, Cold Hardiness, and Leaf Form of Grapefruit Trees. Amer. Soc. Hort. Sci. Proc. 66: 100-110. 1955.

Cell Elongation

BEAN HYPOCOTYL TEST

Method based on research by:

R. L. Weintraub, J. W. Brown, J. A. Throne,
and J. N. Yeatman.

Description of test

A single droplet containing a known amount of a growth regulator is placed on one side of a decapitated bean hypocotyl and after 5 hours the degree of curvature is measured and compared with curvature induced by a standard compound such as 2,4-dichlorophenoxyacetic acid.

Suggested plant material

Young bean plants (*Phaseolus vulgaris* L.)—Black Valentine, Pinto, Red Kidney, or other varieties.

Apparatus, chemicals, and other materials

Vermiculite (No. 3)
Galvanized iron pans, 20 in. wide x 26 in. long x 3 in. deep (51 x 66 x 8 cm.) perforated for drainage
Light-tight room at $87^{\circ} \pm 1^{\circ}$ F. (31° C.) with relative humidity of $60 \pm 10\%$
40-w. red fluorescent lamps
Sharp knife or razor blade
Board $\frac{3}{4} \times \frac{3}{4} \times 17$ in. (2 x 2 x 43 cm.) with row of V-shaped notches along one edge
0.25-ml. tuberculin-type glass syringe
No. 27 stainless-steel hypodermic needle with end cut off at right angle
Micrometer caliper with anvil removed and frame modified to hold syringe securely or dial-type micrometer modified to hold syringe, syringe-micrometer assembly calibrated to 0.0001-ml. units with mercury
60-w. Mazda lamp with Wratten OA filter
Pan 4 in. wide x 16 in. long x 2 in. deep (10 x 41 x 5 cm.)
Bromide photographic paper
Movable-arm protractor
Analytical balance or pharmaceutical balance
Chemicals to be evaluated for cell-elongating activity
Relatively pure 2,4-dichlorophenoxyacetic acid for standard treatment
95% ethanol
Photographic developer
Petroleum jelly

Preparation and selection of plant material

Germinate the seeds and grow the seedlings in pans containing vermiculite until the plants are uniformly 8–13 cm. tall. This requires about 4 days, and is done in a light-tight room at $87^{\circ} \pm 1^{\circ}$ F. (31° C.) with relative humidity of $60 \pm 10\%$. Suspend red fluorescent lamps (40-w.) 4–8 ft. (1–2 m.) above the developing plants to induce straight stem growth.

Remove plants from vermiculite carefully so as not to cause serious root damage. Select plants of uniform size and, with a sharp knife, remove tops at the base of the hypocotyl crook. Arrange 20 decapitated plants so that a portion of the hypocotyl just above the roots is in a notch of the board support. Place a dab of petroleum jelly over each to hold it in place. Rest the ends of the board on the edges of the shallow pan so that the roots are submerged in tap water for 2 hours prior to the test.

Method of conducting test

Dissolve the chemical to be tested for cell-elongating activity in 95% ethanol. This solution should contain 0.065 μ g. of the chemical per 0.0015 ml. of alcohol if comparison is to be made with the cell-elongating effect of 2,4-dichlorophenoxyacetic acid. Load the syringe with the growth-regulator-ethanol solution and apply a single 0.0015-ml. droplet to one side of each bean stem 5 mm. below the cut surface. Treat each of the 20 stems per board; then repeat the operation for each dosage level of each compound to be tested. Always apply the treatment in the same relative position on each stem. Leave one lot of stems untreated and treat another with the solvent only (95% ethanol) for comparison. Use a 60-watt Mazda lamp fitted with a Wratten OA filter to furnish light during application of treatments and for a 5-hour test period thereafter.

Method of taking results

Five hours after treatment, excise the 20 hypocotyl stumps just above the support and lay them on a sheet of glass over a piece of bromide paper. Orient the stumps so that the treated side is always on the left. Briefly expose them to a white light from a source directly above to produce a shadowgraph. Develop the bromide paper and measure the angle of curvature of the hypocotyl stumps with the protractor. The average degree of curvature of treated stumps in comparison with that of the un-

treated stumps indicates the relative cell-elongating property of the chemical.

Suggested standard treatment for comparison

2,4-dichlorophenoxyacetic acid applied in 95% ethanol so that each 0.0015-ml. droplet contains 0.065 μ g. of the acid.

Weintraub, R. L., Brown, J. W., Throne, J. A., and Yeatman, J. N. A Method for Measuring Cell-Elongation-Promoting Activity of Plant-Growth Regulators. Amer. Jour. Bot. 38: 435-440. 1951.

EPIDERMAL ROOT-CELL TEST

Method based on research by:

Hans Burström; Kersin Lexander.

Description of test and some other uses

Germinated wheat seeds are grown in a nutrient solution containing the chemical being tested, and its effect on the elongation of epidermal cells is observed with a microscope.

This test can be adapted for the study of environmental factors that influence the ability of root cells to absorb and respond to plant-regulating chemicals.

Suggested plant material

Roots of young wheat plants (*Triticum aestivum* L., also called *T. sativum* Lam. and *T. vulgare* Vill.)—varieties of Durham or soft red winter types.

Apparatus, chemicals, and other materials

Petri dishes
Filter paper
Pipettes
Tweezers
Sterilizing oven
Autoclave
50-ml. conical flasks
Constant-temperature chamber or room controlled at about 72° F. (22° C.)
Analytical balance or pharmaceutical balance
Microscope with ocular micrometer
Slides
Cover glasses
Room relatively free of dust
2 mg. of each compound to be evaluated for its effect on cell elongation
3-Indoleacetic acid for standard treatment
Formaldehyde
Hydrogen peroxide

Nutrient solution containing the following concentrations of compounds computed on an anhydrous basis:

KNO_3 ($2.0 \times 10^{-4}\text{M}$)
 KH_2PO_4 ($3.0 \times 10^{-4}\text{M}$)
 $\text{Ca}(\text{NO}_3)_2$ ($4.0 \times 10^{-4}\text{M}$)
 MgSO_4 ($2.0 \times 10^{-4}\text{M}$)
 MnCl_2 ($8.0 \times 10^{-6}\text{M}$)
 ZnSO_4 ($6.2 \times 10^{-6}\text{M}$)
 H_3BO_3 ($3.2 \times 10^{-7}\text{M}$)
 H_2MoO_4 ($1.24 \times 10^{-6}\text{M}$)
 $\text{Fe}_2(\text{SO}_4)_3$ ($2.5 \times 10^{-6}\text{M}$)

Copper-free distilled water

Preparation and selection of plant material

Sterilize seeds and all solutions, equipment, etc., as follows: soak seeds in 0.1% formaldehyde solution for 15–20 minutes; then rinse well with sterile distilled water, and dry. Heat Petri dishes, pipettes, and tweezers in oven at about 300° F. (150° C.) for 2 hours. Autoclave nutrient solution for 10 minutes and distilled copper-free water for 20 minutes at 20-lb. pressure per sq. in. (9 kg. per 6.5 sq. cm.) at 250°–260° F. (121°–127° C.).

Place 25 seeds in each of 12 flasks and cover them with 0.1% hydrogen peroxide. Stopper the flasks and allow them to stand at room temperature, about 65°–68° F. (18°–20° C.), for 6 hours.

Rinse seeds four times with sterile distilled water. Place them in Petri dishes, with embryos up, on filter paper moistened with sterile distilled water. Put dishes in darkened constant-temperature room at about 72° F. (22° C.) for 2 days for the seeds to develop primary roots about 1–2 cm. long.

Method of conducting test

Prepare growth-regulator test solutions 10 times the final desired concentrations (10^{-8} – 10^{-3}M) by dissolving the regulators in sterile distilled water. A range of three concentrations can thus be prepared for each compound under study.

Add 4.5 ml. of nutrient solution to each of 24 flasks; stopper, and autoclave for 10 minutes. Add 0.5 ml. of one growth regulator to each of 5 of these flasks. Repeat with the other two concentrations of this growth regulator. Finally, add 0.5 ml. of sterile distilled water to 5 control flasks. Select about 90 wheat plants for uniformity of root length. Place one pair of plants in each of the 20 flasks. Use the remaining 4 flasks, each representing a control and one concentration of the growth regulator under study, for measuring pH, and then discard them. Measure the root length of about

50 of the remaining plants in millimeters. Place the flasks containing the sterile plants in the darkened constant-temperature room for 24 hours.

Method of taking results

Record the pH of the solutions and the final root length of plants given the 4 treatments. Determine root growth by subtracting initial root length from final root length. Cut sections about 5 mm. long from the newly formed part of 20 roots that received one treatment. Gently squeeze the sections between a slide and a cover glass. Keep the sections continuously bathed in the nutrient solution. Measure under a microscope the lengths of at least 240 fully elongated epidermal cells from the different sections. Measure in the same way cells from sections of roots given the other treatments. Compare average lengths of cells of treated roots with those of the control.

Suggested standard treatment for comparison

10^{-8} — 10^{-3} M concentration of 3-indoleacetic acid.

Burström, Hans. Studies on Growth and Metabolism of Roots. IX. Cell Elongation and Water Absorption. *Physiol. Plant.* (Copenhagen) 6: 2, 262–276. 1953.

Lexander, Kersin. Growth-Regulating Substances in Roots of Wheat. *Physiol. Plant.* (Copenhagen) 6: 2, 406–411. 1953.

PEA STEM SECTION TEST

Method based on research by:

G. S. Christiansen and K. V. Thimann.

Description of test

The effect of a compound on stem elongation is measured by floating sections from etiolated pea stems on the surface of a solution containing the compound, and measuring the response in terms of increase in length or weight of the pieces.

Suggested plant material

Etiolated stems of peas (*Pisum sativum* L.).

Apparatus, chemicals, and other materials

Dark room with temperature controlled at about 80°–85° F. (27°–29° C.) and

humidity controlled at 85–90%
Analytical balance or pharmaceutical balance
Glass or metal containers to hold sand in which to germinate seeds
Corning Signal Red filter
Quartz sand
Razor blades mounted 20 mm. apart on a block of wood
500-ml. beakers
Filter paper for blotting water from seeds
Petri dishes
Sodium hydroxide for adjusting pH of solutions and M/1000 potassium chloride
1 g. of 3-indoleacetic acid
250–500 mg. of each compound to be tested

Preparation and selection of plant material

Soak the seeds in tap water for 6 to 8 hours; plant them in sand moistened with tap water, and allow the plants to grow in darkness. All observations and manipulations should be made in a minimum of light from an incandescent lamp passed through the Corning filter. After 7 days, select 30 to 50 plants of uniform size from which to make stem sections.

Method of conducting test

Do not use plants that have developed fourth internodes longer than 3 mm. Cut 20-mm. sections (exactly measured) from the third internodes. Immediately place the sections in tap water, wash, and divide them into groups of 15 sections each. Blot dry with filter paper and weigh the groups separately to the nearest mg. Keep sections moistened.

Prepare aqueous solutions (25–100 ml.) of the compounds to be tested, adjusting their pH to 6.0 with the NaOH, and add the KCl to each to avoid variation in potassium content of the solutions. Store the solutions in a refrigerator if they are to be used repeatedly and make up new ones every few weeks if they are to be used for an extended period. Concentrations ranging from 0.1 to 2.0 mg. per liter of distilled water are suggested.

Place the solution containing the compound to be tested in a Petri dish and float a weighed group of 15 sections on the surface of the liquid for a period of 24 hours in darkness.

Method of taking results

At the end of the 24-hour growth period remove the sections, blot with filter paper, weigh them as a group to the nearest mg., and measure their length individually to the nearest mm. Compare the weight increase and average length increase of these sections (from each

concentration of the test chemical) with comparable measurements of sections treated with distilled water only, and others treated with distilled water containing different concentrations of 3-indoleacetic acid.

500–1,000 mg. of 3-indoleacetic acid for standard treatment
Sodium hydroxide
Potassium chloride
Distilled water

Suggested standard treatment for comparison

Solution containing 0.1, 0.5, 1.0, and 2.0 mg. 3-indoleacetic acid per liter.

Christiansen, G. S., and Thimann, K. V. The Metabolism of Stem Tissue During Growth and Its Inhibition. I. Carbohydrates. Arch. Biochem. 25: 230–247. 1950.

Emendations submitted by K. V. Thimann.

SPLIT PEA STEM TEST

Method based on research by:

Martha Kent and W. A. Gortner; K. V. Thimann and C. L. Schneider; F. W. Went and K. V. Thimann.

Description of test and some other uses

The angle of curvature that develops when split pea stems are immersed in an aqueous solution of a compound indicates the influence of the compound on cell elongation.

This test has been carefully standardized and widely used in the measurement of cell-elongating properties of synthetically prepared regulators and regulating compounds obtained from plant material.

Suggested plant material

Stem sections of etiolated pea plants (*Pisum sativum* L.)—Alaska variety.

Apparatus, chemicals, and other materials

Quartz sand
Completely darkened room with temperature controlled at 80°–85° F. (27°–29° C.)
Ruby glass incandescent lamp
Time clock
Razor blades
Analytical balance or pharmaceutical balance
Petri dishes (10 cm.)
Several 500-ml. beakers
Protractor
Photographic paper and developer, if shadowgraphs are to be made to record stem curvatures
200–500 mg. of each compound to be evaluated

Preparation and selection of plant material

Soak pea seeds in water for 6 hours; then plant them in moist sand in a container which allows for drainage, and keep them in complete darkness. After 7 days the plants should have developed 2 nodes, each bearing a scale, and one nearer the top bearing a leaf. Using diffused white light (about 5- to 10-foot candles), select plants that have developed between this leaf and the terminal bud an internode less than 5 mm. in length.

The sensitivity of the test plants can be increased by exposing them to light. This can be accomplished in either of two ways: First, 32 hours prior to the start of the tests, expose the plants for 4 hours to light from a 60-w. ruby glass incandescent lamp, 2 ft. (61 cm.) away; second, expose the plants for 10 minutes of every 12 hours during the period in which they are grown in sand to light from a 25-w. ruby glass incandescent lamp 2 ft. (61 cm.) away. Control the exposure time by means of a time clock and make the exposures in late afternoon and early morning. Cut off the top of each of 5–8 plants 5 mm. from the terminal bud and discard these parts. Split the stems centrally lengthwise with a razor blade for a distance of about 3 cm. Cut off the split sections a few millimeters below the split portion and wash the split sections for several hours in tap water.

Method of conducting test

Prepare several dilutions (distilled water) of the compound to be tested (10^{-7} – 10^{-3} M) and place 10 ml. of each dilution in a separate Petri dish. These solutions should not be more acid than pH 4. Transfer 6 or 8 of the split sections to each dish and leave them for 6 to 24 hours in darkness. Curvature will be complete at the end of 6 hours and will not change.

In an alternative test, split oat coleoptiles are used in place of split pea stems. Grow the plants (Victory variety), as described, for 76 hours. Decapitate the coleoptiles at a distance of 2–4 mm. from their tips, and split each one lengthwise with a razor blade for a distance of 20 mm. from the cut surface. Cut the coleoptile off a few millimeters below the split and wash the split sections in tap water. Discard the seeds and roots.

Place not more than 8 of the sections in 20 ml. of the solution to be tested. Use Petri

dishes to contain the test solution and adjust its pH to 5.5 with sodium hydroxide. Add 0.001 M potassium chloride solution, which is known to increase slightly the effect of auxin.

Method of taking results

Measure the angles A on each section with the protractor (fig. 4) and average the measurements. If curves are needed to compare observations, plot the average values against the logarithms of the concentration. A straight line will be obtained within certain limits of concentration. Use the slope of the line to compare the activity of different compounds by the formula:

$$a = K \log \frac{C}{C_0}$$

Where a is the mean observed curvature
C the concentration (moles per liter)
C₀ the concentration at which the curvature is 0
K the slope of the line

Use at least 3 points to characterize the slope of the line.

If split coleoptile sections are used, measure the angles that develop during the 24-hour period immediately following the immersion of the sections in the test solution and compare the activity of different compounds.

Suggested standard treatment for comparison

10⁻⁷, 10⁻⁶ and 10⁻⁵ M 3-indoleacetic acid.

Kent, Martha, and Gortner, W. A. Effect of Pre-illumination of the Seedlings to Red Light. Bot. Gaz. 112: 307-311. March 1951.

Thimann, K. V., and Schneider, C. L. Differential Growth in Plant Tissues. Amer. Jour. Bot. 25: 627-641. 1938.

Went, F. W., and Thimann, K. V. Phytohormones. Macmillan Co. New York. 1937.

WHEAT COLEOPTILE SECTION TEST

Method based on research by:

F. Wightman.

Description of test

The effects of compounds on cell elongation are measured by floating sections of wheat coleoptiles on aqueous solutions of the chemical and comparing the length of these with the length of sections floated on distilled water.

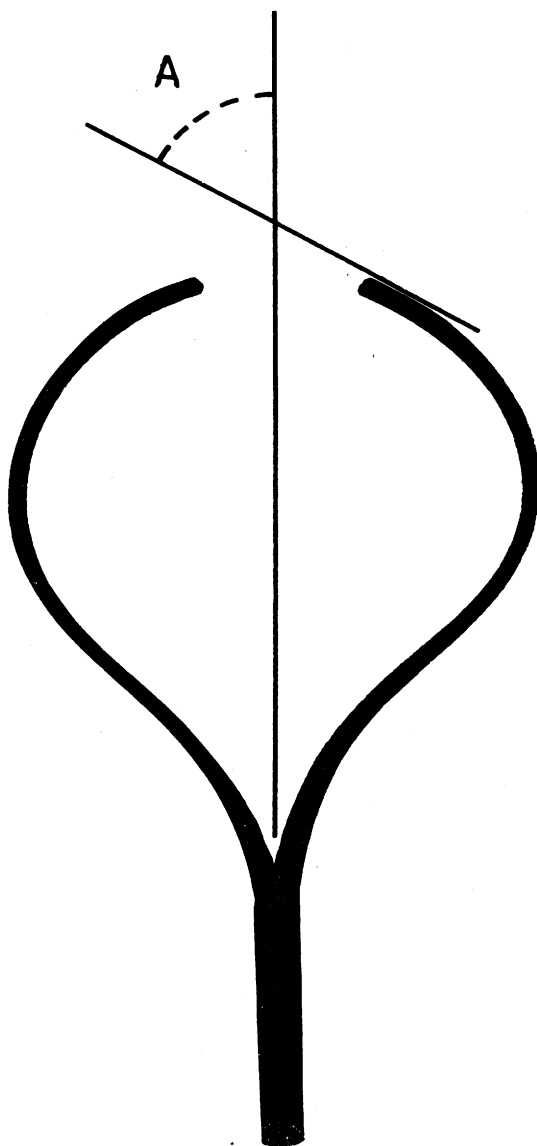


Figure 4. Shadowgraph of a split section of pea stem showing curvature that developed upon exposure to a growth-regulating chemical, and method of measuring the angle of curvature. Lay the stem sections on photographic paper (protect the paper from moisture with a piece of glass), expose the sections and paper to light, and develop the image on the paper using usual photographic methods. Draw a line through the center of each stem and bisect this line with another line parallel with the tip of the curved, split portion of the section. Measure the angle A with a protractor. Draw a similar line using the opposite segment, and measure the angle that corresponds to A. Most regulating compounds having cell-elongating properties induce a decrease in the angle indicated compared with that formed by the section when placed in distilled water. The angle measurement illustrated is one of several that have been utilized. For a general discussion of angle measurements and their relation to the amount and kind of regulating chemical used, see references listed at the end of this method.

(NEG. PN-295)

Suggested plant material

Germinating seeds of wheat (*Triticum aestivum* L., also called *T. sativum* Lam., and *T. vulgare* Vill.)—Eclipse variety.

Apparatus, chemicals, and other materials

Filter paper (10 cm.)
Petri dishes (10 cm.)
Table space in a room with the temperature controlled at 76°–78° F. (24°–26° C.)
Constant humidity boxes with glass covers
Kodak Ruby Signal light filter or other similar filter
Razor blades
Analytical balance or pharmaceutical balance
250–500 mg. of each compound to be evaluated
500–1,000 mg. of sodium 2,4-dichlorophenoxyacetate for standard treatment
Distilled water

Preparation and selection of plant material

Place wheat seeds with embryos facing upward on moist filter paper in open Petri dishes (30–40 seeds per dish) and leave them for 72 hours in a humid atmosphere at 76°–78° F. (24°–26° C.). Maintain a humid atmosphere (80–85% relative humidity) around the seeds by placing the germination dishes in constant humidity boxes. During the first 60 hours expose the seeds to light from an incandescent lamp (25-w.) passed through the red filter. Place lamp about 2 ft. (61 cm.) from the seeds. Allow subsequent growth of the plants to take place in darkness. About 72 hours after placement of the seeds on the moistened paper, select plants with coleoptiles 17–18 mm. long. With

a razor blade, or with 2 razor blades fastened on a wooden block 15 mm. apart, cut one section exactly 15 mm. long from each coleoptile. Place the sections in tap water.

Method of conducting test

Prepare solutions representing a dilution series of the compound to be tested by dissolving different amounts of it in distilled water; suggested range of concentrations 0.01, 0.1, 1, 10, and 100 p.p.m. Place about 20 ml. of each concentration in respective Petri dishes and float 15 coleoptile sections on the surface of the liquid in each dish. Allow the sections to grow for 24 hours in darkness at 76°–78° F. (24°–26° C.). Float additional sections on distilled water and use these as controls.

Method of taking results

Remove the sections and blot dry. Measure each section to the nearest millimeter. Compare the average elongation of sections in the various solutions with that of sections in distilled water. Differences in the average length of control and treated sections indicate the effect of the compound on cell elongation.

Suggested standard treatment for comparison

Solution containing 1 p.p.m. of sodium salt of 2,4-dichlorophenoxyacetic acid.

Method submitted by F. Wightman, Agricultural Research Council Unit on Plant Growth Substances and Systemic Fungicides, Wye College, University of London, London, England.

Cell Growth

OVARY TISSUE CULTURE TEST

Method based on research by:

J. P. Nitsch.

Description of test

Excised ovaries of various plants are grown *in vitro* on synthetic nutrient medium. Regulating or other chemicals are added, and their effects on growth of the fruits are determined.

Suggested plant material

Commercial varieties of crop plants—tomato (*Lycopersicum esculentum* Mill.), bean (*Phase-*

olus vulgaris L.), tobacco, (*Nicotiana tabacum* L.), strawberry (*Fragaria* spp.), cucumber (*Cucumis sativus* L.), common varieties in flower.

Apparatus, chemicals, and other materials

Scalpel
Table space, relatively dust free for use in ovary preparations, transfers, etc.
Jars
Petri dishes
Filter paper
Test tubes
Forceps
Non-absorbent cotton for test tube plugs
Room exposed to indirect sunlight with tem-

peratures of about 73° F. (23° C.) and night temperatures of about 63° F. (17° C.)
 Calcium hypochlorite (5%, C. P. in water)
 Salts for nutrient solution:

1 g. of $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$
 1 g. of KNO_3
 1 g. of $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$
 1 g. of KH_2PO_4

Chemicals for trace-element solution:

5 ml. of H_2SO_4 , sp. gr. 1.83
 5 g. of $\text{MnSO}_4 \cdot 4 \text{H}_2\text{O}$
 1 g. of $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$
 1 g. of H_3BO_3
 1 g. of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$
 1 g. of $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$

Ferric citrate solution:

10 g. of $\text{FeC}_6\text{O}_5\text{H}_7 \cdot 5 \text{H}_2\text{O}$

Paraffin

Water redistilled in Pyrex

Agar

100 g. sucrose

Analytical balance or pharmaceutical balance

Trace-element solution

Component	Amount per liter of final solution
H_2SO_4 , sp. gr. 1.83	0.5 ml.
$\text{MnSO}_4 \cdot 4 \text{H}_2\text{O}$	3,000 mg.
$\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$	500 mg.
H_3BO_3	500 mg.
$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	25 mg.
$\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$	25 mg.
Add sufficient water redistilled in Pyrex to make 1 liter of final solution.	

Ferric citrate solution

10 g. of $\text{FeC}_6\text{O}_5\text{H}_7 \cdot 5 \text{H}_2\text{O}$ diluted to 1 liter of water redistilled in Pyrex.

Preparation and selection of plant material

Use healthy greenhouse plants. At flowering, hand-pollinate strawberry and cucumber flowers in order to insure pollination. Shake tomato, bean, and tobacco plants occasionally to insure pollination.

Cut the flowers from the plants 2 days after pollination and dip the cut ends into melted paraffin to seal them. Place these flowers into sterile jars and conduct all subsequent operations in a room free from dust, using **sterile utensils and materials**.

Pour a decanted or filtered 5% calcium hypochlorite solution into the jar containing the flowers. After about 10 minutes, pour the calcium hypochlorite solution off and wash twice with sterile water. Transfer the flowers to Petri dishes containing a double thickness of filter paper to absorb the excess water.

Method of conducting test

Prepare the following solutions:

Mineral salt solution

Salt	Amount per liter of final solution
$\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$	500 mg.
KNO_3	125 mg.
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	125 mg.
KH_2PO_4	125 mg.

Add sufficient water redistilled in Pyrex to make 1 liter of final solution.

To 1 liter of the mineral salt solution add 1 ml. of the ferric citrate solution, 1 ml. of the trace-element solution, and 50 g. of sucrose.

Divide the medium into separate aliquots and add required amounts of the test chemicals. Three treatments and a control, each having 10 replicates, can be run by dividing 1 liter of nutrient medium into four 250-ml. portions and supplying each ovary with 25 ml. of nutrient medium in separate tubes. To support the ovaries, use filter-paper discs having a diameter of about twice that of the tubes. Make a hole in the center of each disc slightly larger than the diameter of the pedicel. Form a paper cup with each disc by pressing it against the edge of a tube. Push one cup inside of each tube, edges downward, so that it stays at least 2 inches above the level of the liquid. Plug the tubes with nonabsorbent cotton. Autoclave the tubes 20 minutes at 15 lbs. pressure. When planting the sterile ovaries, push the paper down with the forceps until the pedicel reaches the liquid.

A variant of this technique uses no filter paper but solidifies the media with 1% agar. Suggested concentrations of the chemical to be tested are 0.001-100 mg. per liter in serial dilutions.

Due to possible breakdown at high temperatures it is sometimes advisable to avoid autoclaving solutions that contain organic compounds. Addition of test chemicals aseptically to the nutrient medium can be done as follows: Autoclave the four 250-ml. portions of nutrient medium mentioned previously and the plugged tubes containing the filter-paper discs, separately. Sterilize a metal spoon by dipping it into alcohol, and allow it to dry. Weigh the required amount of test chemical, place it in the spoon, and add sufficient ethyl alcohol to barely cover the compound. Allow the alcohol to evaporate; then immerse the bowl of the spoon containing the chemical in one of the sterile portions of nutrient medium and stir until the compound

is uniformly dispersed. Remove the cotton plugs and the filter-paper discs from the tubes with sterile forceps and pour approximately 25 ml. of the mixture directly into each tube. Replace the filter-paper discs and cotton plugs.

Cut off the stamens, petals, two-thirds of the sepals, and most of the pedicel from the sterilized flowers. Cut the pedicel off above the abscission zone (at least 1 mm. below base of flower) to eliminate the abscission zone and prevent the abscission phenomenon from interfering with the growth of the ovary. Care must be taken with tomatoes to avoid any film of liquid remaining between the sepals and the ovary, since this seems to cause the ovary to turn brown and die. Support the sterile, trimmed flower on the disc of filter paper provided with a central hole so that only the pedicel extends into the solution.

Place the tubes containing the ovaries on shelves where they are exposed to indirect sunlight in a room with a day temperature of about 73° F. (23° C.) and a night temperature of about 63° F. (17° C.).

Method of taking results

At intervals measure length and diameter with a small translucent metric rule through the walls of the test tubes. At conclusion of experiment, remove each ovary and accurately measure it. Prepare a table of length and diameter dimensions with corresponding volumes for each specific variety of plant. Use these data for comparing growth rates of treated and untreated fruits.

Suggested standard treatment for comparison

No standard treatment suggested.

Nitsch, J. P. Growth and Development *in vitro* of Excised Ovaries. Amer. Jour. Bot. 38 (7): 566-577. 1951.

Nitsch, J. P. Test Tube Fruits: A New Technique in Fruit Physiology. Report of the Thirteenth International Hort. Congress, pp. 263-266. 1952.

ROOT TISSUE CULTURE TEST

Method based on research by

Philip R. White.

Description of test

Root tips from tomato seedlings or cuttings cultured *in vitro* are treated with various regulators or other chemicals, and their effect on growth of root cells is determined.

Suggested plant material

Ripe tomato fruits, or tomato plants (*Lycopersicon esculentum* Mill.) from which cuttings can be made.

Apparatus, chemicals, and other materials

Scalpel
Forceps
Petri dishes
Filter paper (No. 1 Whatman)
Dark chamber maintained at room temperature of 70°-75° F. (21°-24° C.)
Wide-mouth glass jars
Blotting paper
Heavy, paraffin-coated cardboard
Analytical balance or pharmaceutical balance
10-liter bottle
2,500-ml. Pyrex beakers
Test tubes
Rubber test tube stoppers
Refrigerator space
125-ml. Erlenmeyer flasks
Absorbent cotton
Gauze
50-ml. beakers or paper cups
Autoclave
Salts for nutrient solution:
25 g. of $\text{Ca}(\text{NO}_3)_2$
25 g. of Na_2SO_4
10 g. of KCl
2 g. of NaH_2PO_4
1 g. of MnSO_4
1 g. of ZnSO_4
1 g. of H_3BO_3
1 g. of KI
50 g. of MgSO_4
Vitamins:
0.5-1 g. of glycine
0.5 g. of nicotinic acid
0.5 g. of thiamine
0.5 g. of pyridoxine
Sugar-iron solution:
50 g. of C.P. sucrose
1 g. of $\text{Fe}_2(\text{SO}_4)_3$
Compounds to be evaluated as growth regulators
Sodium hypochlorite solution, such as laundry bleach
Distilled water

Preparation and selection of plant material

Seed Method: Select sound, ripe tomato fruits; wash and dry them carefully. *Sterile equipment and aseptic conditions should be utilized throughout the experiment.* With a

scalpel, divide the fruit into quarters by making cuts into the fruit deep enough so that it can be broken open easily. Start the incisions about $\frac{1}{2}$ in. (1 cm.) from the stem and cut to the stylar end. Break open the fruit and expose the seeds carefully so that the seeds do not come in contact with fingers or with the outer, nonsterile surface of the fruit. Remove 25 mature seeds that have not been touched with the scalpel, with sterile forceps, and place them in 5 Petri dishes (5 seeds per dish) fitted with filter paper moistened with the sterile water. Keep the Petri dishes in a dark place maintained at a temperature of about 70°–75° F. (21°–24° C.) for a few days until the seeds germinate and the roots reach a length of 2–3 cm.

Cutting Method: Grow tomato plants in a greenhouse or field plot. Take 6 straight stem cuttings about 20 cm. long from a healthy tomato plant and remove the leaves. If all these cuttings are from the same plant, genetic variability will be reduced. Wash thoroughly with the 5% hypochlorite disinfectant diluted 1 to 10 parts of water. Rinse with sterile water and shake off the surplus. Line the wide-mouth glass jars with sterile blotting paper and add sterile water to a depth of about 3 cm. Cover each jar with a sheet of heavy, paraffin-coated cardboard a little larger than the mouth of the jar. Make a hole in the cardboard and insert the basal end of a tomato cutting through it so that about 15 cm. of stem extends into the moist air above the water surface. Keep the jars containing the cuttings in a dark place at a temperature of about 70°–75° F. (21°–24° C.) for about 10 days, or until new roots develop to a length of 3–5 cm.

Method of conducting test

Prepare a stock salt solution, using the best grades of analytical chemicals, by dissolving 20 g. of $\text{Ca}(\text{NO}_3)_2$, 20 g. of Na_2SO_4 , 8 g. of KCl , 1.65 g. of NaH_2PO_4 , 0.45 g. of MnSO_4 , 0.15 g. of ZnSO_4 , 0.15 g. of H_3BO_3 and 0.075 g. of KI one at a time in 8 liters of water, redistilled in Pyrex. Also use water redistilled in Pyrex in preparing the following solutions. If hydrated salts are used, it is essential to make weight corrections. Dissolve 36 g. of MgSO_4 separately in 2 liters of water. Mix the two solutions slowly in a 10-liter bottle. Store this stock solution, which is 10 times the concentration needed, in the dark or in a black bottle.

Prepare a second stock solution of vitamins by dissolving 300 mg. of glycine, 50 mg. of nicotinic acid, 10 mg. of thiamine, and 10 mg. of pyridoxine in 100 ml. of water. This solution is 100 times the concentration needed.

Pour 20 ml. aliquots of this vitamin stock solution into test tubes, stopper, and store in a refrigerator.

Sucrose is used as a carbon source for the tissue cultures. Dissolve 40 g. of C. P. sucrose in 1 liter of water. Dissolve 10 mg. of $\text{Fe}_2(\text{SO}_4)_3$ in 100 ml. of water, discard half the quantity, and add the remainder to the sugar solution. (Diluting and discarding half the ferric sulfate solution eliminates the necessity of weighing accurately a quantity of the salt less than 10 mg.)

Mix the sugar-ferric sulfate solution with 200 ml. of the stock salt solution and 20 ml. of the vitamin stock solution, and add sufficient water to make 1,600 ml. Distribute 40 ml. of this complete nutrient solution to each of the desired number of the 125-ml. Erlenmeyer flasks. Plug each flask with cotton covered with gauze and cap with a 50-ml. beaker or small paper cup to keep out dust while allowing air exchange. After being autoclaved (15 lbs. pressure per sq. in. for 20 minutes) and allowed to cool, the nutrient in each flask is ready for the addition of 10 ml. of sterile distilled water containing the test compound at 5 times the final concentration desired. For control flasks, add 10 ml. of the sterile water.

Select 10 roots, 2–3 cm. long, from the germinated seeds; sever them with a sharp scalpel, and transfer each root to a flask of nutrient. In order to obtain a sufficient number of root tips from a single source, culture these roots for a week; then cut 1-cm. tips from the main root and well-established branch roots, using scissors. With a sterile bacteriological loop, insert each root tip into a culture flask containing fresh nutrient. If rooted cuttings are used, cut 1-cm. root tips and insert into culture flasks in the same manner. To reduce the possibility of genetic effects influencing the results, use root tips derived originally from a single seed or from cuttings from a single plant. Or, if a larger amount of material is needed for a more extensive experiment, label and distribute such genetically uniform roots so that one or more is used for each treatment.

Treat the excised roots with plant regulators by adding these in the required amounts aseptically (i.e., using sterile water and utensils) to the nutrients as described above. A range of final concentrations from 10^{-13} to 10^{-1} g. per liter, prepared by serial dilution is suggested. Do not autoclave the aqueous solutions of regulating chemicals. Some regulating chemicals are relatively insoluble in water in the acid, ester, or amide form. Employ a salt form of these compounds to facilitate their use in this method. Use a group of untreated solutions, each brought to a volume of 50 ml. with sterile distilled water as controls. Place the culture

flasks in a dark place at 70°–75° F. (21°–24° C.).

Method of taking results

Since roots grown in culture do not undergo secondary thickening, and therefore the diameter remains constant to within a few millimeters of the growing point, length measurements are an accurate measure of volume. Without removing the root from the culture flask, make day-by-day measurements from the outside using a flexible celluloid rule, either by bending the rule to approximate the root curvature or by washing the root up onto the wall of the flask so that it lies straight. Also measure growth at the end of the week by removing the root tissue and obtaining wet or dry weights.

Suggested standard treatment for comparison

No standard treatment suggested.

White, Philip R. *The Cultivation of Animal and Plant Cells*. 239 pp. Ronald Press Co., New York. 1954.

STEM TISSUE CULTURE TEST

Method based on research by

A. C. Hildebrandt and A. J. Riker; B. E. Struckmeyer, A. C. Hildebrandt, and A. J. Riker; P. R. White.

Description of test

Squares of phloem tissue from willow are grown on nutrient agar containing regulating chemicals and effects of the chemicals on growth and tissue development determined.

Suggested plant material

Young willow tree (*Salix nigra* L. or *S. caprea* L.).

Apparatus, chemicals, and other materials

Sharp knife
Scalpel
Tweezers
Cork or soft pine board
5-liter bottle
125-ml. Erlenmeyer flasks
Test tubes
Rubber test tube stoppers
Refrigerator space
Cotton

Gauze
50-ml. beakers or paper cups
Autoclave
Chemicals for complete nutrient solution (see page 18)
Compounds to be evaluated as growth regulators
Agar (Difco "Noble" brand)
Distilled water
Analytical balance or pharmaceutical balance

Preparation and selection of plant material

Cut from the young willow tree a piece of branch or stem about 1 ft. (30 cm.) long and 3–10 mm. in diameter. Take the branch into the laboratory. Surface-sterilize by first scrubbing the surface with soap and water. Dip the branch into 95% alcohol several times and burn the alcohol off each time after the branch is dipped.

Method of conducting test

Prepare a standard salt solution and a vitamin stock solution, as described on page 19, but use only half the volume of water. Use sterile water redistilled in Pyrex for all solutions. Dissolve 40 g. of C. P. sucrose in 400 ml. of water. Dissolve 10 mg. of $\text{Fe}_2(\text{SO}_4)_3$ in 100 ml. of water, discard half the quantity, and add the remaining 50 ml. to the sugar solution. Prepare a 1% agar solution by dissolving 10 g. of Difco "Noble" agar (or its equivalent in thoroughly leached agar) in 1 liter of hot water. Combine the agar, the sugar-ferric sulfate solution, 100 ml. of stock salt solution, and 10 ml. of vitamin stock solution, and add sufficient water to make 1,600 ml. Mix together thoroughly and divide the hot nutrient-agar solution into aliquots, each containing 60 ml., and autoclave. To a 60-ml. aliquot of the hot agar solution, add 15 ml. of warm aqueous solution of the test compound. This solution should be prepared with sterile distilled water in sterile utensils and should be 5 times the desired final concentration. It is desirable to use salt forms of the growth regulator. While the agar-chemical mixture is still hot, distribute equal volumes of it among 5 sterile test tubes. Repeat, using other concentrations and regulators, and prepare one set, using distilled water in place of the regulator, as controls. A range of concentrations of the regulator from 10^{-13} – 10^{-1} g. per liter is suggested. Plug the test tubes with cotton, and cap them with 50-ml. beakers or paper cups to keep out dust. If the nutrient-agar medium does not solidify satisfactorily on cooling, adjust the acidity to a pH not lower than 5.5 with 0.1 N sodium hydroxide.

Lay the sterile branch on a sterile block and, with a sterile scalpel, make a series of transverse cuts through the branch, about 1 mm. apart. In this way, obtain 10 or 15 branch sections or discs. If the branch is 5 mm. or more in diameter, divide the discs into several sectors. If a smaller branch is used, merely cut the discs in half. Cut off and discard outer cortical tissue (bark) and much of the wood. The remaining blocks should be rectangular with the long axes tangential to the original surface and transversed by the cambium.

After the nutrient agar has cooled and solidified, transfer one of the prepared blocks to each tube using sterile tweezers. Push the blocks part way into the agar so that they are left half exposed and half submerged. Stopper the tubes with cotton and set them aside in the dark at a room temperature of 70°–75° F. (21°–24° C.).

Method of taking results

After 4 to 6 weeks, compare the growth of tissue on nutrient agar containing various concentrations of growth regulators with growth on nutrient agar alone. Proliferation should

occur at or just above the agar level, the new growth spreading out over the agar in the control tubes. Study the effects of the growth regulators on development of the treated tissue during a 4- to 6-weeks' observation period. After this period, remove the samples of tissue grown on nutrient agar and on nutrient agar plus regulating chemical, and compare their wet or dry weights. In addition, anatomical effects, such as total number of cells, number of tracheal elements per unit area, and gross morphological effects, should be recorded.

Suggested standard treatment

No standard treatment suggested.

- Hildebrandt, A. C., and Riker, A. J. Influence of Some Growth-Regulating Substances on Sunflower and Tobacco Tissue *in vitro*. Amer. Jour. Bot. 34: 421–427. 1947.
 Struckmeyer, B. E., Hildebrandt, A. C., and Riker, A. J. Histological Effects of Growth-Regulating Substances on Sunflower Tissue of Crown-Gall Origin Growth *in vitro*. Amer. Jour. Bot. 36: 491–495. 1949.
 White, P. R. The Cultivation of Animal and Plant Cells. 239 pp. Ronald Press Co., New York. 1954.

Effect of One Compound on the Absorption and Translocation of Another

BEAN STEM CURVATURE TEST

Method based on research by:

A. S. Crafts; J. W. Mitchell, W. M. Dugger, Jr., and H. G. Gauch.

Description of test

The magnitude of stem curvature induced by a known amount of a growth-modifying substance is compared with that induced by the same amount of the substance plus an adjuvant.

Suggested plant material

See page 7.

Apparatus, chemicals, and other materials

See page 7.

Compounds to be tested as adjuvants are also required.

Preparation and selection of plant material

See page 8.

Method of conducting test

Using the bean stem curvature test, page 8, determine the amount of ammonium 2,4-dichlorophenoxyacetate or other water-soluble salts of the acid required to induce a stem curvature of about 5°–10° in 2½–3 hours. In these preliminary tests, dissolve the salt directly in distilled water and use a concentration range that includes 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 µg. of the salt per 0.02 ml. of solution.

After the threshold concentration has been determined, prepare 500 ml. of this concentration. Divide it into the required number of 50-ml. portions. Reserve one portion to be applied as a control, and mix each adjuvant to be tested with a 50-ml. portion. Apply to test plants as described on page 8.

Method of taking results

Determine the effect of the adjuvants on the magnitude of stem curvature induced in 2½ to 3 hours by comparing the average curvature developed by plants treated with the salt alone with that developed by plants treated with the salt plus the adjuvant.

Suggested standard treatment for comparison

0.8, 0.9, or 1.0 μ g. of ammonium 2,4-dichlorophenoxyacetate or other water-soluble salt applied in distilled water alone.

Exudation of Regulators from Roots

BEAN TEST

Method based on research by:

W. H. Preston, Jr., J. W. Mitchell, and W. Reeve.

Description of test

Two young plants, one treated by placing a compound on its stem or leaves, are grown in soil contained in a pot. Formative or other effects that become apparent as the untreated plants develop indicate transfer of the compound from one plant to the other through the root systems.

Suggested plant material

Young herbaceous dicotyledonous plants known to respond morphologically to the compounds to be tested—snap bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.), squash (*Cucurbita* spp.), sunflower (*Helianthus annuus* L.).

Apparatus, chemicals, and other materials

- Clay pots 3, 4, or 5 in. (8, 10, or 13 cm.)
- Composted soil
- Greenhouse space maintained at 70°–85° F. (21°–29° C.), slightly cooler at night
- Shell vials (about 10-ml. capacity)
- Analytical balance or pharmaceutical balance
- Small glass or wooden rods used as applicators (1- to 2-mm. diameter, 5–10 cm. long)
- 250–500 mg. of each compound to be evaluated
- Alpha-methoxyphenylacetic acid for standard treatment²
- About 50 ml. of Tween 20
- About 50 g. of lanolin
- 2–400 ml. beakers
- Source of compressed air

Preparation and selection of plant material

Plant 3–4 seeds per pot in composted soil if a single species is used. If two species are

Crafts, A. S. *Herbicides, Their Absorption and Translocation*. Agr. and Food Chem. 1: 51–55. 1953.
 Mitchell, J. W., Dugger, W. M., Jr., and Gauch, H. G. *Increased Translocation of Plant Growth-Modifying Substances Due to Application of Boron*. Sci. 118: 354–355. 1953.

employed, plant 3–4 seeds of each per pot. After germination, allow the plants to grow until the first leaf formed above the cotyledons is partially expanded. While the next leaves are still tightly folded in the terminal buds, select in each pot two vigorously growing plants that are the same species or of two different species. Discard all plants except these two by severing their stems near the soil level. Arrange 3–5 pots (each containing a pair of plants) in a row to be used for each compound tested.

Method of conducting test

Prepare a 1% lanolin mixture of the compound being tested, as described on pages 5–6. With an applicator, apply a portion of the paste about the size of a wheat seed to one plant in each pot in one row. Leave the other plant in each pot untreated. Apply the paste as a band 2–3 mm. wide around the first internode of each plant midway between the first and the second node (or around the upper portion of the hypocotyl if cucumber or sunflower is used). Allow the treated and untreated plants to grow in a greenhouse for a week or more.

For an alternative method, carefully wash the roots of at least three of the young plants free from soil. Support the plants with their roots immersed in about 200 ml. of tap water in the beaker. Aerate the water by passing compressed air as fine bubbles through it. Treat the stems of all of the plants as described. Arrange a second set of plants in another beaker of water without treatment as controls. Allow all of the plants to grow for 3 days, replacing any water lost. Then remove and discard all of the plants. Introduce an equal number of young untreated plants and allow them to grow for several days with their roots in the continuously aerated water that previously supported growth of the treated and untreated plants.

Method of taking results

In the case of plants with their roots in soil contained in the same pot, observe in the un-

²Obtainable from The Monroe Chemical Company, P.O. Box 388, Rockland, Mass.

treated plants any growth modification such as deviation in size or shape of newly expanded leaves (formative effects). Make a similar comparison for the alternative test using aerated water.

Supplementary test — Determination of the path of movement of the compound

In the case of volatile compounds there is a possibility that the compound may pass from the treated plant to the untreated one through air. To determine the path of movement, select 5 pots of test plants as previously described with 2 plants of uniform size growing in each. Also select 10 other plants, each growing individually in a pot. Cut out 10 squares (5 cm.) of cardboard and punch 2 holes, 1 cm. in diameter and 15 mm. apart, in each square. Make a narrow slit from the edge of the cardboard to each hole. Using the pots containing 2 plants each, slip the cardboard around the stem of each plant so that the stems protrude through the holes and are thus held about 15 mm. apart.

Place the pots containing individual plants in pairs and slip a cardboard piece around the stems of each pair of plants in the same manner.

Apply the compound to be tested to the stem of 1 plant of each pair. Allow the plants to grow in a greenhouse until the next 2 leaves are partially expanded. Leaf modification in both plants with their roots in separate pots indicates that the compound moved through the air. Modification of leaf shape in both plants growing in soil contained in the same pot, but not in the untreated plants of pairs growing with their roots in separate pots, indicates that the compound passed from one plant to the other through their root systems.

Suggested standard treatment for comparison

Alpha-methoxyphenylacetic acid, 1% lanolin-Tween 20 paste.

Preston, W. H., Jr., Mitchell, J. W., and Reeve, W. Movement of Alpha-methoxyphenylacetic Acid from One Plant to Another Through Their Root Systems. Science 119: 437-438. 1954.

Form or Growth Modification

BEAN LEAF TEST

Method based on research by:

J. W. Brown and R. L. Weintraub.

Description of test and some other uses

The growth-regulating chemical in a small droplet applied to the terminal bud of bean plants suppresses the vegetative growth of the young leaves, which serves as an index of growth-suppressing activity.

With slight modification, this test can be used for studying the effect of regulating chemicals on the formation of abscission layers near the terminal buds of succulent plants.

Suggested plant material

Young bean plants (*Phaseolus vulgaris* L.)
—Black Valentine, Red Kidney, Bountiful varieties.

Apparatus, chemicals, and other materials

Composted soil
4-in. (10-cm.) clay pots or
other suitable containers in
which to grow plants

Greenhouse or, for more precise experiments, use an artificially lighted room (about 700-foot candles) equipped with temperature and humidity controls

Analytical balance or pharmaceutical balance
5-lambda pipette

Photographic paper, printer, and developer

200 mg. of each compound to be evaluated

200 mg. of 2,4-dichlorophenoxyacetic acid for standard treatment

95% ethanol

Tween 20 or a similar wetting agent

Sulfuric acid

Preparation and selection of plant material

Plant 3 or 4 bean seeds in soil in each of the containers and grow the plants under greenhouse conditions. If the plants are grown under controlled environmental conditions, employ a day length of approximately 14-16 hours, and a minimum of 700-foot candles of light, a day temperature of about 75° F. (24° C.), a night temperature of about 72° F. (22° C.) and, if possible, a relative humidity of 50-70%. Select test plants for uniformity (1 plant per

pot) after 6–8 days when the second internode is 3–7 mm. in length. Cut off and discard all other plants.

Method of conducting test

Dissolve a weighed amount of chemical to be tested in a measured volume of solvent mixture composed of 95% ethanol and 1% Tween 20. With the lambda pipette, apply a 5-lambda droplet to the terminal bud of each of 10 to 20 test plants. Repeat the procedure, using additional plants for each desired dosage level. Apply equal amounts of the solvent mixture to additional plants for comparison, and leave others untreated for controls. Replace all plants in the greenhouse or under the uniform, controlled environmental conditions described until the control plants have developed a partially expanded, trifoliate leaf (5–13 days).

Method of taking results

At the end of this growth period, remove the first trifoliate leaf from each plant, and determine its fresh weight and area. Leaf area may be obtained by tracing the outline of the leaf on paper and cutting out the image. Weigh a piece of the paper that is exactly 10 cm. square. Determine the weight of the leaf image and calculate its area from the known weight of 1 square cm. of paper. A contact shadowgraph of the leaf made on photographic paper can be substituted for the tracing as used above. Calculate the effect of the chemical on leaf area as percentage increase or decrease in comparison with controls. Record any other differences between leaves on treated and control plants.

Suggested standard treatment for comparison

Use standard dose of 0.015 μ g. of 2,4-dichlorophenoxyacetic acid per 5-lambda droplet per plant. This induces approximately 48% suppression in leaf area.

Brown, J. W., and Weintraub, R. L. A Leaf-repression Method for Evaluation of Formative Activity of Plant Growth-Regulating Chemicals. *Bot. Gaz.* 111: 448–456. 1950.

LANOLIN PASTE TEST

Method based on research by:

P. C. Marth, W. H. Preston, Jr., and J. W. Mitchell.

Description of test

Compounds are applied in a paste carrier to stems of young plants, and the effect on their subsequent growth and development is observed.

Suggested plant material

Young herbaceous plants—bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), sunflower (*Helianthus annuus* L.).

Apparatus, chemicals, and other materials

4-in. (10-cm.) clay pots
Composted soil
Greenhouse space with temperature controlled at 75°–85° F. (24°–29° C.)
Shell vials (about 10-ml. capacity)
Wooden applicators that can be used once and discarded (obtainable without cotton from drug- or hospital-supply store)
Analytical balance or pharmaceutical balance
100–200 mg. of each chemical to be evaluated
For standard treatment:
50 mg. of 2,4-dichlorophenoxyacetic acid, or 50 mg. of (4-hydroxy-5-isopropyl-2-methylphenyl) trimethyl ammonium chloride, 1-piperidine-carboxylate (Amo-1618), or 50 mg. of 3-indoleacetic acid
Tween 20
Lanolin

Preparation and selection of plant material

Plant 3 or 4 seeds of one of the species per pot in the composted soil. Germinate the seeds at about 85° F. (29° C.) and as soon as the plants appear above the soil, reduce the temperature 5°–10° F. (9°–18° C.). After the plants are erect and about 2–3 in. (5–8 cm.) tall, select one in each pot that is of average size. Pinch the remaining plants off at the soil level and discard them.

Method of conducting test

Arrange each species of plant in a group with 3 to 5 plants per row. Prepare lanolin paste as described on page 5. With an applicator stick, apply a portion of the paste about the size of a wheat seed in the form of a band 3–6

mm. wide around the first internode midway between the first and second nodes of each bean plant in one row. Use the same procedure if cucumber and sunflower plants are employed except place the band on the hypocotyls of these plants just below the cotyledons. Allow the plants to grow for 7–12 days along with several rows of untreated plants. Additional plants should be treated in a similar manner with the lanolin-Tween 20 paste alone to study the effect of the carriers.

As a modification, using additional plants, apply an equal amount of the paste containing the chemical to be tested to an area about 4 mm. wide and 10 mm. long on the upper surface of one primary leaf of each bean plant and on the upper surface of the first true leaf of the cucumber and sunflower plants. For the most effective treatment, cover an area starting at the junction of the petiole and leaf blade and extending toward the tip of the blade. Cover the midrib and an area extending about 2 mm. on each side of it with the paste.

Method of taking results

For bean and sunflower plants, measure the terminal growth that occurs during the week following treatment by placing the end of a ruler at the node immediately above the treated section of the stem and determining the distance from this point to the tip of the terminal bud. Measure the untreated plants in a similar manner. In the case of cucumber plants, place the end of the ruler at the cotyledonary node and measure the distance from this point to the tip of the first true leaf, since during the test period the measurable growth occurs almost entirely in this leaf. Measure corresponding control plants in the manner described.

Average the measurements in each test and compute the percentage of inhibition or stimulation of terminal growth of bean and sunflower and leaf growth of cucumber by the following formula:

$$\frac{\text{Untreated control}^{\text{a}} - \text{minus treated}}{\text{Untreated control}} \times 100 = \begin{array}{l} \% \text{ inhibition} \\ \text{(plus value) or} \\ \text{stimulation} \\ \text{(minus value)} \end{array}$$

During the test, note any differences in the shape of leaves, stems, or other parts that develop, the development of galls indicating cell enlargement or cell proliferation, initiation of roots, color changes, and other modifications of

growth that result from presence of the chemical in the plant. Note the position of the affected parts in relation to the treated area as an indication of transportability of the compound.

Suggested standard treatment for comparison

On the basis of knowledge gained from the literature or previous experience, select several compounds known to be relatively effective in inducing specific responses, such as 2,4-dichlorophenoxyacetic acid for gall formation, translocation, and inhibition of terminal growth; Amo-1618 for suppression of stem growth; 3-indoleacetic acid for gall formation and induction of root primordia on stems. Apply these to the selected test plants as examples of compounds that are relatively effective in inducing these various types of growth modifications.

Marth, P. C., Preston, W. H., Jr., and Mitchell, J. W. Growth-Controlling Effects of Some Quaternary Ammonium Compounds on Various Species of Plants. *Bot. Gaz.* 115: 200–204. 1953.

UNDERGROUND GROWTH TEST

Method based on research by:

T. J. Muzik and H. J. Cruzado.

Description of test

Plants are grown in soil in boxes constructed with glass sides in order to observe the effects of chemicals on growth and development of the underground parts without disturbing the plants.

Suggested plant material

Plants or plant parts whose underground growth is to be observed, such as seeds, stem pieces, root pieces, bulbs, corms, tubers, and rhizomes.

Apparatus, chemicals, and other materials

Wooden or metal boxes 9 in. wide at the top x 30 in. long x 17 in. deep (23 x 76 x 43 cm.) with glass sides slanting inward at an angle of about 60° and with removable wooden, tar paper, metal, or cloth shutters to exclude light

Composted soil

Greenhouse or other space suitable for growing test plants

^a Control plants treated as described with the lanolin-Tween 20 paste alone should also be included if the effect of such substances on the test plants is not known.

Small hand sprayer

1-5 g. of each compound to be evaluated

For standard treatment:

1-2 g. of 2,4-dichlorophenoxyacetic acid, maleic hydrazide, or 3-indolebutyric acid depending upon type of response to be studied

Tween 20 or other suitable solvents and wetting agents for preparing and applying sprays

Lanolin

Analytical balance or pharmaceutical balance

Preparation and selection of plant material

Select uniform plant parts such as seeds, stem pieces, chains of nutgrass nutlets (*Cyperus rotundus*), rhizomes, and bindweed (*Convolvulus arvensis* L.), or Canada thistle (*Cirsium arvense* Scop.), and plant them in composted soil against the sloping glass sides of the boxes.

Method of conducting test

Apply the chemical to be tested to the plant parts before planting, after emergence, or to the soil surface before emergence. Use a foliage spray application (1,000 p.p.m. suggested), a dust or spray application (range of 0.1-16 lb. per acre suggested) (1 and 7 kg. per 4,047 sq.

m.), or as a paste (0.1-1% in lanolin) applied to the leaves or stems covering areas of 1-4 sq. cm. Leave some entire boxes of plants untreated as controls. Maintain the plants at optimum moisture, light, and temperature conditions for growth of the test species employed.

Method of taking results

Observe the underground parts of treated and untreated plants periodically by removing the shutters. Periodically, record data on root initiation and elongation, death of roots, rate of tuber formation, bud or stem growth, discoloration, and other responses. Comparison of these results with those from the control plants indicates the effectiveness of the compounds for inducing the type of response involved.

Suggested standard treatment for comparison

For standard treatment, compound used will depend upon type of plant response involved. For example, growth suppression, 2,4-dichlorophenoxyacetic acid, maleic hydrazide; root initiation on stem or root pieces, 3-indolebutyric acid.

Muzik, T. J., and H. J. Cruzado. The Effect of 2,4-D on Sprout Formation in *Cyperus Rotundus*. Amer. Jour. Bot. 40: 507-512. 1953.

Fruit Set**AEROSOL METHOD****Method based on research by:**

L. D. Goodhue; C. L. Hamner, and H. A. Schomer; P. C. Marth, and E. M. Meader.

Description of test

Regulating or other chemicals are applied to plants or plant parts in the form of mists produced by use of liquefied gases.

Suggested plant material

Tomato plants (*Lycopersicum esculentum* L.) that have developed two open flowers in the first cluster.

Apparatus, chemicals, and other materials

Test-tube-type apparatus (fig. 5) for small-scale experiments or screening tests

Metal (steel) containers of 0.5-1 lb. (.23-.45 kg.) capacity developed for applying insecticidal liquefied gas aerosol mixtures and fitted with 1/2-in. (1.27 cm.) refrigerant valves
Flexible rubber pressure tubing for transferring liquefied gases
Brass fittings for connecting valves and tubing
Analytical balance or pharmaceutical balance
Suction pump or air vacuum line
50-mm. metal funnel soldered into 1/4-in. (.5 cm.) threaded brass valve fitting
Spring scales or balance with a capacity of 1,000 g.
Spray nozzles (standard oil burner nozzles or capillary metal tube soldered into 1/4-in. [.5-cm.] thread fitting)
25 mg.-1 g. of each compound to be tested
5.0 g. beta-naphthoxyacetic acid for standard treatment
Dimethyl ether
100-200 ml. of solvents such as cyclohexanone, acetone, or carbitol that are

miscible with liquefied gases and which will dissolve chemicals to be tested
5-25 lb. (2.3-11.3 kg.) supply tank of methyl chloride (boiling point 10.5° F.

[-11.9° C.]), or dichlorodifluoromethane (Freon) (boiling point 21.7° F. [-6° C.]) or dimethyl ether (boiling point 9.6° F. [-12.4° C.])

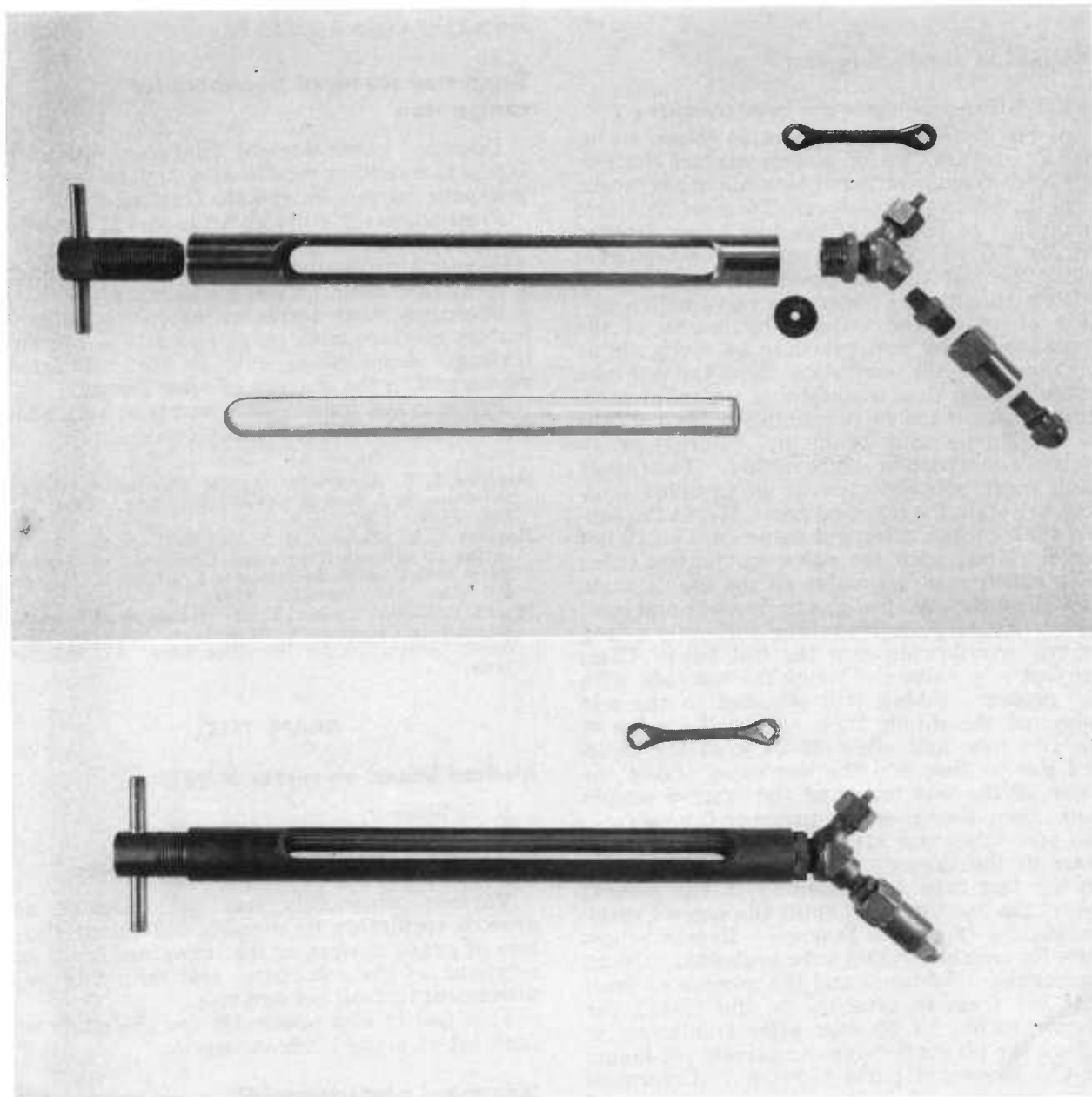


Figure 5. Views of unassembled and assembled glass-walled aerosol bomb used in experimentally preparing aerosol mixtures of regulating chemicals.

(NEG. PN-206 A.B.)

Preparation and selection of plant material

Tomato plants (*Lycopersicon esculentum* L.) of varieties such as Marglobe, Globe, Rutgers, or others that can readily be grown in a greenhouse.

Method of conducting test

Preparing and loading aerosol mixtures

A. For test-tube-type apparatus shown in figure 5: Prepare 25 g. of aerosol mixture containing a 1% concentration of beta-naphthoxyacetic acid as follows: Weigh out 2.5 g. of this acid and place in bottom of the test tube; dissolve this in 2.5 ml. of cyclohexanone (or acetone); place the test tube in holder and seal tightly with basal plug. Connect the valve with a suction pump or vacuum line by means of the pressure tubing and evacuate as much air as possible from the test tube. Close the test tube valve tightly, thus maintaining a partial vacuum. Connect the valve opening of the test tube to the supply tank of methyl chloride or dimethyl ether with pressure tubing. The supply tank must be supported in an inverted position to obtain the liquefied gas. Warm the supply tank gently, using hot water or a small hot plate. Now, open the valve on the test tube; then slowly open the valve on the supply tank, and allow the liquefied gas to flow out and completely fill the pressure tubing with only a drop or two overflowing into the test tube. Close the test tube valve and weigh the test tube with the pressure tubing still attached to the test tube and the supply tank. Open the valve of the test tube and allow 22–25 g. of the liquefied gas to flow into the test tube. Close the valve of the test tube and the reserve supply tank; then disconnect the pressure tubing from the test tube, and attach the spray nozzle in place of the tubing. Mark level of the liquid on the test tube for reloading in the future; invert the test tube, and apply the aerosol spray to clusters of tomato flowers. Repeat procedure for each compound to be evaluated. Count the number of flowers and the number of fruit that set (remain attached to the plant) per cluster within 14–20 days after treatment, or before the plants become excessively pot bound or the above-soil parts crowded. Determine the percentage of fruit set by the treated and the untreated clusters.

B. For large (0.5–1.0 lb. or larger) aerosol cylinders: Evacuate the air from the cylinder with a suction pump; close valve; attach funnel to valve and fill with solvent plant-regulator solution prepared as in "A" above; open valve and allow partial vacuum to suck contents of the funnel into cylinder (retain as much partial

vacuum as possible within the cylinder), close the valve, and load with liquefied gas, as in "A" above. Conduct test with tomato plants as described. Do not use Freon as most acids are not soluble in it. Freon can be used for dispersing esters and other oil-soluble compounds since they are soluble in it.

Suggested standard treatment for comparison

Dimethyl ether aerosol mixture containing 1% beta-naphthoxyacetic acid and 10% cyclohexanone solvent for tomato fruit-set test.

Precautions: Methyl chloride gas is toxic to animals and humans, and should not be inhaled. As a safety precaution, prepare mixtures under a ventilated hood, or use a gas mask.

Dimethyl ether forms an explosive, inflammatory mixture with air at about 18% concentration. Load tubes only in well-ventilated rooms and in the absence of open flames.

Freon is non-inflammable and relatively non-toxic to plants and animals.

Goodhue, L. D. Insecticidal Aerosol Production. Spray Solutions in Liquefied Gases. Ind. Eng. Chem. 34: 1456–1459. 1942.

Hamner, C. L., Schomer, H. A., and Marth, P. C. Application of Growth-Regulating Chemicals in Aerosol Form with Special Reference to Fruit-Set in Tomato. Bot. Gaz. 106: 108–123. 1944.

Marth, P. C. and Meader, E. M. Influence of Growth-Regulating Chemicals on Blackberry Fruit Development. Amer. Soc. for Hort. Sci. Proc. 45: 293–299. 1944.

GRAPE TEST

Method based on research by:

R. J. Weaver.

Description of test and some other uses

Various compounds may be evaluated as growth regulators by dipping individual clusters of grape flowers or the immature fruits in solutions of the compound and recording improvement in fruit set and size.

This test is also applicable for the study of fruit set of many kinds of berries.

Suggested plant material

Grape vines bearing numerous clusters of flower buds—Black Corinth, White Corinth (*Vitis vinifera* L.), or other varieties that ordinarily set relatively few berries.

Apparatus, chemicals, and other materials

Containers of suitable size, such as discarded

but clean 1-qt. (.95-liter) tin cans, to hold solutions so that the flower clusters can be dipped without bruising or damaging them
 Analytical balance or pharmaceutical balance
 Small labels with string or wire attached
 25–100 mg. of each compound to be evaluated
 About 0.5 g. of para-chlorophenoxyacetic acid for standard treatment
 Wetting agent such as Dreft, Tween 20, Santomerse
 Solvent such as 95% ethanol

Preparation and selection of plant material

Select clusters of uniform size having about the same number of flowers that have attained the same stage of development. Tag the selected clusters and prepare to apply treatments when the flowers are in full bloom or within 10 days thereafter.

Method of conducting test

Prepare aqueous solutions of each compound at concentrations of 5, 15, and 50 p.p.m. by first dissolving the required amount of the regulator in 0.5–1 ml. of alcohol; then adding the alcoholic solution to the required amount of water. Add sufficient wetting agent to make a final concentration of 0.05–0.1% of the detergent.

Dip the clusters of flowers when they are in full bloom or within 10 days thereafter. Use 10 clusters per treatment and leave comparable labeled clusters untreated for controls. Additional clusters should be dipped in a solution containing the solvents and the wetting agent to determine the effect of these constituents.

Method of taking results

Determine the percentage of fruit set by first counting the number of flowers immediately after dipping them. After the fruits are relatively mature, count the number of berries present. Calculate the percentage of fruits that developed from the flowers. Compare this figure with that of the controls.

Suggested standard treatment for comparison

5, 15, and 50 p.p.m. of para-chlorophenoxyacetic acid.

Weaver, R. J. Response of Certain Varieties of Grapes to Plant Growth Regulators. Bot. Gaz. 113: 75–85. 1951.

TOMATO TEST

Method based on research by:

D. D. Hemphill; F. S. Howlett; A. E. Murneek, S. H. Wittwer, and D. D. Hemphill; R. H. Roberts and B. E. Struckmeyer.

Description of test and some other uses

Compounds are applied in liquid or paste carriers to flower clusters of greenhouse plants, and the effectiveness of the substances in preventing flower or fruit drop is evaluated.

This method is also adapted to studies of the effect of regulating compounds on seed development and the morphological development of fruits. It is also useful in studying the effect of regulators on the number of flowers produced.

Suggested plant material

Tomato plants (*Lycopersicum esculentum* L.) of varieties such as Marglobe, Globe, Rutgers, or others that can be readily grown in a greenhouse.

Apparatus, chemicals, and other materials

Clay pots 4–10 in. (10–23 cm.) or discarded 1 qt. (.95 liter) tin cans with perforated bottoms

Composted soil

Space in greenhouse maintained at about 70°–80° F. (21°–27° C.) by day and at 60°–70° F. (15°–21° C.) by night

Small vials (20–25 ml.)

Clean containers of 100–200 ml. capacity

Toothpicks or wooden medicinal applicators or a nasal spray atomizer (15–30 ml.)

Analytical balance or pharmaceutical balance
 25–100 mg. of each compound to be evaluated

About 0.5 g. para-chlorophenoxyacetic acid
 Lanolin

Solvents such as ethanol, methanol

Wetting agents such as Tween 20, Carbowax 1500

Preparation and selection of plant material

A. For tests of limited duration (14–20 days):

Grow the plants in 4- to 6-in. (10- to 15-cm.) pots set side by side in rows 4 inches (10 cm.) apart, and select those of uniform size which have developed two open flowers on the first cluster.

- B. For prolonged tests involving maturation of the fruit (35–45 days):** Grow the plants in 10- to 12-inch (25- to 30-cm.) pots, or grow them in ground beds. In either case select uniform plants that have developed 2 open flowers in the first cluster. The plants should be at least 12 in. (30 cm.) apart, in rows 2 ft. (61 cm.) apart.

Method of conducting test

- A. Paste Method:** Prepare a lanolin-Tween 20 paste containing 1% of the chemical (see pages 5, 6), and apply a narrow band of the mixture around the stalk (peduncle) of the first flower cluster and about 1–2 cm. from the main stem of the plant. To obtain reliable data, apply one treatment to an individual row of plants extending across the bench or soil bed. Repeat the procedure using additional treatments and corresponding rows. Leave an additional row of plants untreated to serve as controls. When all of the treatments have been applied and the control row designated, an experimental block has been completed. Repeat the procedure making a second, third, and fourth block, randomizing the relative positions of each treatment within each block.
- B. Spray Method:** Prepare aqueous mixtures to obtain a concentration range of 10, 20, 40, or 80 p.p.m. of the compound to be tested (see pages 8, 28–29). Use the above experimental plan and spray the mixture on the flower cluster with a nasal atomizer until the liquid drips from each flower. Spray flowers of control plants in a similar way, using water and the co-solvent only.

Method of taking results

- A. Plants used in tests of limited duration:** Count the number of flowers and the number of fruit that set (remain attached to the plant) per cluster within 14 to 20 days after treatment, or before the plants become excessively pot bound or the above-soil parts crowded. Determine the percentage of fruit that set. Cut the green fruits midway and at right angles to their axes, and record the relative amount of gelatinous pulp and the relative numbers of seeds present.
- B. Plants used in prolonged tests:** Record data as described above and, in addition, record the number of days required for the fruit to develop a pink or red color.

If needed, use the second and third flower clusters on each plant to repeat the earlier treatments. Plants in ground beds may develop 5 or 6 flower clusters that are suitable for treatment. In all instances, apply only one concentration of one chemical to a plant.

Suggested standard treatment for comparison

A paste of 0.1% lanolin-Tween 20 or a spray mixture containing 30 p.p.m. of para-chlorophenoxyacetic acid.

- Hemphill, D. D. The Effects of Plant Growth-Regulating Substances on Flower Bud Development and Set. Mo. Agr. Expt. Sta. Res. Bul. 434. 1949.
- Howlett, F. S. Experiments Concerning the Practicability of Certain Chemicals as a Means of Inducing Fruit Setting in the Tomato. Amer. Soc. Hort. Sci. Proc. 37: 886–890. 1939.
- Murneek, A. E., Wittwer, S. H., and Hemphill, D. D. Supplementary "Hormone" Sprays for Greenhouse Tomatoes. Amer. Soc. Hort. Sci. Proc. 45: 371–381. 1944.
- Roberts, R. H. and Struckmeyer, B. E. The Use of Sprays To Set Greenhouse Tomatoes. Amer. Soc. Hort. Sci. Proc. 44: 417–427. 1944.

Fruit Size

GRAPE TEST

Method based on research by:

R. J. Weaver.

Description of test and some other uses

Various compounds may be evaluated as growth regulators by dipping individual clusters of grape flowers or the immature fruits in solutions of the compound, and recording improvement in fruit size.

This test is useful in studying the effect of plant regulators on the growth of a variety of different kinds of fruits. It is also a useful means of studying the effect of regulating chemicals on the production of parthenocarpic fruit, such as tomatoes and blackberries.

Suggested plant material

Grape vines bearing numerous clusters of flower buds—Thompson Seedless, Monukka, Sultana (*Vitis vinifera* L.), or other varieties that set numerous fruits.

Apparatus, chemicals, and other materials

See page 28.

Preparation and selection of plant material

Select clusters of uniform size having about the same number of flowers. Tag these to be used after the flowers are fully open and before any berries have abscised.

Method of conducting test

Prepare aqueous solutions of each compound at concentrations of 5, 15, and 50 p.p.m. using alcohol as a solvent, and a wetting agent (see page 29). Dip the flower clusters in the solutions soon after the flowers are fully open. Use 10 clusters per treatment and leave comparable labeled clusters untreated for controls. Additional clusters should be dipped in a solution

containing the solvents and wetting agent to determine the effect of these constituents.

Method of taking results

Determine the effect of treatment on berry size by removing the berries from the plants after they are mature, and obtaining the fresh weight of 200 fruits selected at random from each treatment. Compare this weight with the results of similar measurements using control fruits. Note any difference in time of maturity or other effects due to treatments.

Suggested standard treatment for comparison

5, 15, and 50 p.p.m. of para-chlorophenoxy-acetic acid.

Weaver, R. J. Response of Certain Varieties of Grapes to Plant Growth-Regulators. Bot. Gaz. 113: 75-85. 1951.

Fruit Thinning**APPLE BLOSSOM TEST****Method based on research by:**

C. P. Harley, H. H. Moon, and L. O. Regeimbal.

Description of test and some other uses

By applying regulating chemicals to selected limbs of trees when in blossom, the compounds are evaluated as a means of thinning the fruit.

By modifying the dosage, the method may be useful in entirely removing undesirable fruits of such trees as the Gingko, mulberry, and hickory without injury to the trees.

Suggested plant material

Apple trees (*Malus sylvestris* Mill., also called *Pyrus malus* L.), with the majority of the blossoms fully open and with a few petals falling—Jonathan, Wealthy, Golden Delicious, Grimes Golden, Baldwin, or other varieties.

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
For whole-tree experiments use standard orchard-spray equipment such as is used for applying insecticidal or fungicidal sprays under high pressure (400-800 lb. pressure per sq. in. [181-363 kg. per 6.5 sq. cm.])

Sprayers having a large tank divided into several 50- or 100-gal. (189- or 379-liter) compartments facilitate the work
3-gal. (11-liter) hand-pressure sprayer can be used if individual limbs are to be sprayed

Hand tally registers (2 or 3 per person) needed to record data

5-10 g. of each compound to be evaluated

10-20 g. of alpha-naphthalene-acetamide for standard treatment

Tween 20 or other wetting agent

Preparation and selection of plant material

For whole-tree experiments, select and tag trees of uniform size that have produced about the same number of flowers per tree. Five trees are suggested for each treatment.

If individual limbs are to be used, select on comparable trees outer limbs of about the same size and bearing about the same number of blossoms at the desired stage of development. Apply each treatment to a single limb selected at random from each of five or more trees. In this way several treatments can be used on each tree.

Method of conducting test

Prepare aqueous sprays containing several concentrations of each chemical (see page 29) (10, 30, and 60 p.p.m. are suggested). Apply sprays within a few days after about 75 per-

cent of the petals have fallen, so as to wet thoroughly all parts of the tree or branch. About 1 gal. (3.8 liters) for each year of age is required for spraying entire trees. Leave unsprayed trees or branches as controls, and hand-thin other trees or branches so as to have branches or trees thinned an optimum amount for comparison.

Method of taking results

Determine the number of fruits thinned chemically after June drop has occurred. With the aid of two tally registers, one in each hand, record separately the total number of spurs that blossomed and the number that set fruits. Spurs from which fruits have dropped are easily identified by the pedicel scars. Use a third tally register to record vegetative spurs if desired. Compare the number of spurs that bear fruits with the number from which fruits have fallen. Compare ratios thus obtained with those of the untreated and the hand-thinned controls.

Suggested standard treatment for comparison

50 p.p.m. spray concentration of alpha-naphthalene-acetamide.

Harley, C. P., Moon, H. H., and Regeimbal, L. O. A Modified Method of Obtaining Fruit-Set Records for Apples. Amer. Soc. Hort. Sci. Proc. 63: 146-148. 1954.

PEACH AND PRUNE FRUIT TEST

Method based on research by:

P. C. Marth and V. E. Prince.

Description of test and some other uses

A method of evaluating the fruit-thinning effects of chemicals applied as sprays to limbs or entire trees is described.

By modifying the dosage used, these methods may be useful in entirely removing undesirable fruits of such trees as Gingko, mulberry, and hickory, without injury to the trees.

Suggested plant material

Peach or plum trees bearing young fruits (30-45 days after full bloom)—Elberta, Hale-

haven, Hiley, or other varieties of peach (*Prunus persica* [L.] Batsch) or Stanley, Italian, or German varieties of plum (*Prunus domestica* L.).

Apparatus, chemicals, and other materials

Same as on page 31 except that 50-100 g. of isopropyl N-(3-chlorophenyl) carbamate are required for standard treatment.

Preparation and selection of plant material

See page 31.

Method of conducting test

Prepare aqueous sprays containing several concentrations of each chemical (see page 29) (125, 250, and 500 p.p.m. suggested). Apply sprays about 30-45 days after full bloom or 10-20 days after shucks (dried flower parts) have fallen. Wet all parts of the tree or branch thoroughly. At the time the fruits are sprayed they should be about 1-2 cm. in diameter. About 1 gal. (3.8 liters) for each year of age is required for spraying entire trees. Leave unsprayed trees or branches as controls; also hand-thin other trees or branches so as to leave an optimum number of fruits for further comparison.

Method of taking results

Within a day or two after applying the sprays, determine the number of fruits that remain attached. After June drop has occurred, determine the number of fruits thinned by comparing the number on sprayed, unsprayed, and hand-thinned branches or trees. At the usual harvest date record the number, size, the degree of coloration of fruits, and the effect of treatment on time of maturation. Compare these data to evaluate effectiveness of the chemical treatment.

Suggested standard treatment for comparison

Spray concentration of isopropyl N-(3-chlorophenyl) carbamate, 200 p.p.m.

Marth, P. C., and Prince, V. E. Effect of 3-Chloroisopropyl-N-phenyl Carbamate on Abscission of Young Fruits of Peach. Science 117: 497-498. 1953.

Growth Inhibition or Stimulation

VEGETATIVE SPROUT TEST

Method based on research by:

P. C. Marth and E. S. Schultz; P. C. Marth.

Description of test and some other uses

Pieces of carrots, potato tubers, and small onions are dipped in a solution of the test compound and placed in darkness. Measurement of sprouts indicates the effectiveness of the compound as a sprout inhibitor.

This test may also be adapted for a study of the growth and development of buds on any vegetative reproductive organ.

Suggested plant material

Unsprouted vegetable storage organs such as carrots (*Daucus carota* L.), onions (*Allium cepa* L.), and potato tubers (*Solanum tuberosum* L.) that have experienced a rest period.

Apparatus, chemicals, and other materials

1/2-in. (1-cm.) cutting spoon such as is used for making melon balls for salad

Analytical balance or pharmaceutical balance

Sphagnum, peat moss, or fine quartz sand

Petri dishes or other shallow containers about 4 in. (10 cm.) in diameter

Space in darkened room with temperature controlled at 65°–75° F. (18°–24° C.)

0.1–0.5 g. of each compound to be evaluated

0.5 g. of methyl ester of alpha-naphthalene-acetic acid for standard treatment

Preparation and selection of plant material

Carrot: Select carrots of about medium size for uniformity. Sever petioles so that about

1/2 in. (1 cm.) of each petiole remains attached to each root. Remove and discard the lower part of the root saving about 1 in. (2.5 cm.) of the upper portion. Place the pieces in humid air at 40°–50° F. (4°–10° C.) for 3–4 days or until they become suberized.

Onion: Small onions about 1/2 in. (1 cm.) in diameter and of uniform shape are prepared for treatment by removing the loose outer scales.

Potato tuber: Select tubers of medium size and, with a cutting spoon, remove pieces of the tubers so that each piece contains an eye (fig. 6). Place the pieces in humid air at 40°–50° F. (4°–10° C.) for several days until they become suberized.

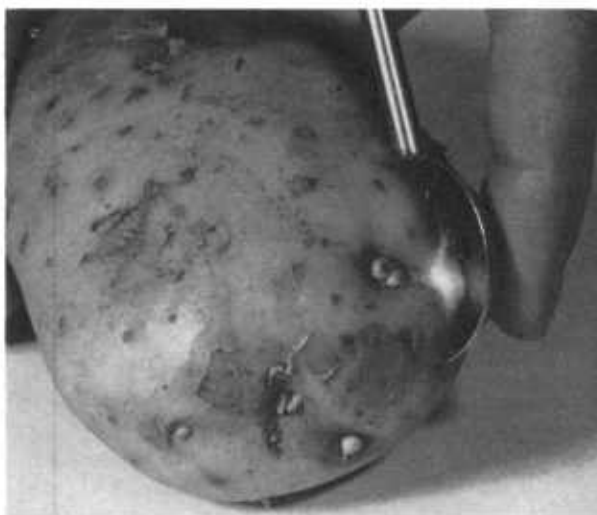
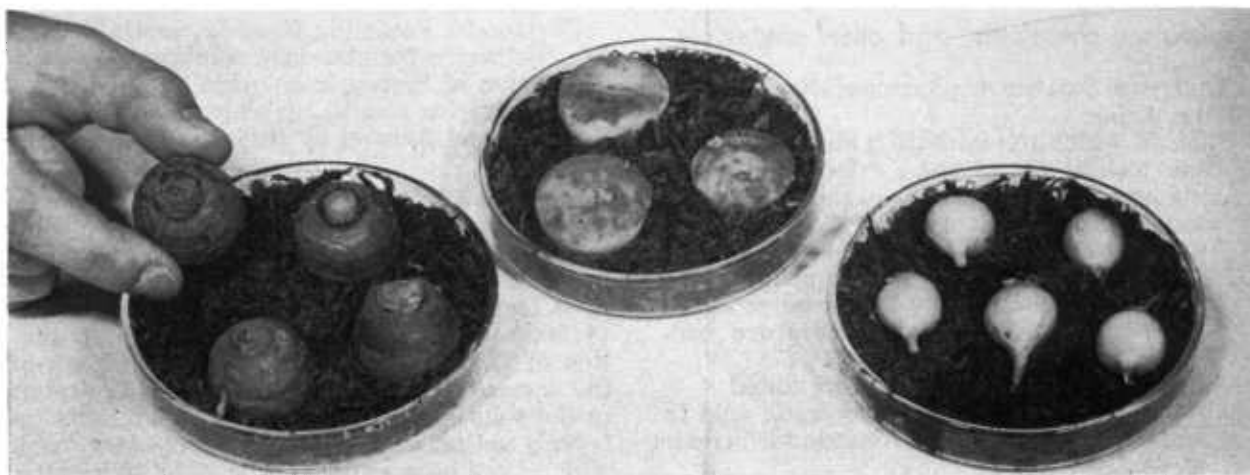


Figure 6. A melon ball cutting spoon provides a means of easily removing buds from potato tubers. (above). Cut around each eye with the instrument and place the pieces along with small anian sets and pieces of carrot roots on a moistened sphagnum mass contained in a Petri dish (below). (NEG. PN-297 A, B)



Method of conducting test

Select 10 of the prepared carrot pieces. Dip these momentarily in a tap-water mixture containing the desired amount of the chemical being tested (1,000 p.p.m. suggested). Allow pieces to drain, and place equal numbers of them upright on the moist sphagnum, peat moss, or sand contained in dishes about 4 in. (10 cm.) in diameter and without covers (fig. 6). Dip comparable batches of carrot pieces in tap water and place them on sphagnum or sand in the manner described to serve as controls. Keep the sphagnum or sand moist, and allow the plant material to grow in darkness at room temperature, 65°–75° F. (18°–24° C.).

Apply the chemical to the onion sets and the potato pieces, and culture them in the same manner as described.

Method of taking results

After the controls have developed a measurable amount of new vegetative growth, measure the length of each sprout and measure the fresh weight of the detached sprouts. Compare these measurements with similar ones for the treated material.

Suggested standard treatment for comparison

1,000 p.p.m. of methyl ester of alpha-naphthaleneacetic acid.

Marth, P. C., and Schultz, E. S. Effect of Growth Regulators on Sprouting of Stored Table Stock Potatoes and on Waste Piles for Control of Diseases. *Amer. Potato Jour.* 27: 23–32. 1950.

Marth, P. C. Unpublished data, Plant Industry Station, Beltsville, Md.

Growth Modification (Aquatic Plant)

STERILE NUTRIENT TEST

Method based on research by:

L. G. Nickell and A. C. Finley.

Description of test

Lemna minor L. (duckweed) is grown under aseptic and rigidly controlled conditions in a nutrient solution. Regulating chemicals (including antibiotics) are added to the nutrient, and their effect on growth of the plant studied.

Suggested plant material

Duckweed plants (*Lemna minor* L.).

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Petri dishes

Tank in which to maintain a stock supply of the test plant. (*Lemna minor* will grow vigorously through the year in dilute nutrient solutions at a pH of 5–6 or in tap water over a small amount of sandy soil.)

Table space in a room with constant light intensity (700- to 1,000-foot candles for 12 hr. per day) and the temperature controlled at 75°–80° F. (24°–27° C.)

0.2 g. of each compound to be evaluated

0.5–1 g. of 2,4-dichlorophenoxyacetic acid in water-soluble salt form for standard treatment

Basic nutrient medium containing the ingredients listed:

KNO ₃	0.002M	Thiamine	100γ/1.
Ca(NO ₃) ₂	0.003M	Pyridoxine	800γ/1.
KH ₂ PO ₄	0.001M	Nicotinamide	800γ/1.
MgSO ₄	0.001M	B	0.1 p.p.m.
CaCl ₂	0.003M	Mn	0.1 p.p.m.
KCl	0.002M	Zn	0.3 p.p.m.
MgCl ₂	0.001M	Cu	0.1 p.p.m.
Sucrose	2%	Mo	0.1 p.p.m.
		Fe	0.5 p.p.m.

Dilute sodium hydroxide and hydrochloric acid solutions

Glass or other type filters with which solutions can be filtered free of contaminating organisms

Suction flasks with adapters for filters
Erlenmeyer flasks (125 ml.)

Tweezers

Cotton

Bacitracin, Penicillin G, or isonicotinic hydrazide for standard solutions

Solution of mercuric chloride (1:2000 of water) for sterilizing plants

O.T. Clear Aerosol or comparable detergent

Preparation and selection of plant material

Select a vigorously growing supply of *Lemna*. Sterilize this material by immersing rosettes (3–4 fronds) in the mercuric chloride solution (1:2000 water). Add one drop of a 1:20 dilution of O.T. Clear Aerosol to each 100 ml. of the mercuric solution. Place several rosettes in the solution and remove one at 5-minute intervals so that a series of treated rosettes result which have been exposed from 5 to 30 minutes

to the mercuric chloride mixture. As each rosette is removed, rinse several times in sterile water and finally place it in a flask containing sterile nutrient medium. Allow all of the plants to grow in the flasks for a week to determine which are aseptic. Uncontaminated cultures are used for further experimentation.

Method of conducting test

The pH of the basic medium should be about 5.0. Adjust any variation from this with dilute acid or alkali solutions. Prepare 50–100 ml. of an aqueous stock solution of the regulator to be tested so that it contains 300 p.p.m. of the chemical. Remove contaminating organisms in this solution by passing it through the sintered glass filter. Place 50 ml. of the sterile nutrient medium aseptically into each of 5 flasks of 125 ml. capacity. Introduce one sterile duckweed plant into each flask and designate these as controls. Repeat the procedure, but before placing a plant in the nutrient, add a sufficient volume of the sterile stock solution containing the regulating chemical to make a final concentration of 1 p.p.m. Prepare other concentrations of the test chemical in additional aliquots of the nutrient (5, 10, 20, 30 p.p.m. etc.). For each concentration level, use at least 5 replicate flasks. Place one of the sterile, selected, duckweed plants in each flask aseptically. Stopper the flasks with cotton and place them under controlled environmental conditions, 22.5° C. with light from white or day-lite fluorescent tubes having a constant intensity of at least 700-foot candles.

Method of taking results

After the required period of growth (1–2 wks.), remove the test plants, blot them free of water, for each flask record the number of plants and the number of fronds on each, and immediately obtain the fresh weight of all plants from each flask, weighing to the nearest milligram. Compare any increase or decrease in growth of control and treated plants in terms of number of plants, fronds, and average weight per plant.

Suggested standard treatment for comparison

Bacitracin, Penicillin G, or isonicotinic hydrazide at 20 p.p.m. concentration.

Nickell, L. G., and Finlay, A. C. Growth Modifiers, Antibiotics, and Their Effect on Plant Growth. Agr. and Food Chemistry 2: 17–182. 1954.

WATER CULTURE TEST

Method based on research by:

H. R. Offord.

Description of test

An aquatic plant, duckweed (*Lemna minor* L.), is grown in water containing a regulating compound, and the growth-modifying effect of the chemical is observed.

Suggested plant material

Duckweed plants (*Lemna minor* L.).

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Petri dishes

Tank in which to maintain a stock supply of the test plant. (*Lemna minor* will grow vigorously through the year in dilute nutrient solutions at a pH of 5–6 or in tap water over a small amount of sandy soil.)

Table space in a room with constant light intensity (700- to 1,000-foot candles for 12 hr. per day) and the temperature controlled at 75°–80° F. (24°–27° C.)

0.2 g. of each compound to be evaluated

0.5–1 g. of 2,4-dichlorophenoxyacetic acid in water-soluble salt form for standard treatment

Dilute solution of potassium hydroxide

Dilute solution of sulfuric acid

Preparation and selection of plant material

Select relatively large, uniformly green plants that are growing vigorously.

Method of conducting test

Prepare tap water solutions (150 ml.) of each compound in a range of concentrations (0.001–1,000 p.p.m. suggested). Place 50 ml. of each concentration in each of 3 Petri dishes. Place the same number of *Lemna* plants in each test solution, using 10 or more for each. Include some dishes that contain plants in tap water as controls. Record the number of plants, the total number of fronds, and the time of immersion for each culture. Using dilute sulfuric acid or potassium hydroxide, adjust the pH of all solutions to approximately the same level (about 5–6), since excess acid or alkali exerts a toxic action on *Lemna*. Maintain the tem-

perature of the solutions at 75°–80° F. (24°–27° C.), and the light intensity constant so that tests carried out at different times of the year can be compared.

Method of taking results

At regular intervals, record any visible differences in the plants such as change in color, presence or absence of vegetative division, and presence or absence of new fronds. Evaluate the relative effectiveness of each concentration of each compound tested, as follows:

Marked inhibitory effect: Color change becomes steadily more apparent until a uniform discoloration of the fronds is evident. The typical color of fronds in this category is reddish brown to pale yellow or white. There is no change in total area of fronds or in number of plants.

Moderate inhibitory effect: Plants divide and form single or double fronds before discoloration becomes noticeable. Most or all of the plants split into single fronds. Chlorosis

occurs slowly, but uniformly, on all fronds, and uniform discoloration results. Typical color of the plants is pale or yellowish green. There is no change in the total area of the fronds.

Slight inhibitory effect: Symptoms appear slowly. Single fronds from parent plants may be unevenly discolored, but fronds of the parent plants remain fairly green. No change is apparent in the total area of the fronds.

No inhibitory effect: Plants are divided into single or double fronds and are dark green in color. There is an apparent increase in the number and in the total estimated area of the fronds.

Suggested standard treatment for comparison

Determine by trial the minimum concentration of 2,4-dichlorophenoxyacetic acid which induces *marked inhibitory effect*. No standard suggested for stimulation of growth.

Offord, H. R. Rapid Estimation of the Phytocidal Action of Chemicals. Science 103: 474–476. 1946.

Growth Regulation

FIELD TEST

Method based on research by:

J. R. Havis; P. C. Marth and R. E. Wester.

Description of test

Test chemicals are sprayed on soil in which seeds have been planted or on leaves and stems of plants growing under field conditions, and their effect on growth and productivity of the plants is measured.

Suggested plant material

Pieces of stem of sugarcane (*Saccharum officinarum* L.) such as are ordinarily used for planting this crop, or seeds of crop plants such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L., also called *T. sativum* Lam. and *T. vulgare* Vill.), bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* [L.] Merr.), lima bean (*Phaseolus lunatus* L.), pea (*Pisum sativum* L.), or others.

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Knapsack sprayer, to be operated at 30 lb.

pressure per sq. in. (14 kg. per 6 sq. cm.)
5–50 g. of each compound to be evaluated as growth regulator

5 g. of 2,4,5-trichlorophenoxyacetic acid for standard treatment

Solvent for preparing concentrated solutions of chemicals to be evaluated (alcohol, acetone, or other organic solvent)

Preparation and selection of plant material

Plant seeds of crop plants or uniform stem pieces of sugarcane in the usual manner. For tall-growing crops such as corn or sugarcane, use four or more plots of 1/100 acre (40 sq. m.) for each chemical or concentration tested. Space the plants 18–24 in. (46–60 cm.) apart in rows 40–42 in. (101–106 cm.) apart. For short-growing crops such as soybeans and peas, space the plants 6 to 8 in. apart in rows 30–36 in. (76–91 cm.) apart. Use two or more replications of rows 5–10 ft. (1.5–3 m.) long for each chemical or concentration tested. It is sometimes advantageous to select uniform plants 2 ft. (60 cm.) or more apart; mark these and use 10 of them for each chemical or concentration tested.

Method of conducting test

For pre-emergence application, apply the test

chemicals within a few days after planting, using suggested rates of 1, 2, 4, and 16 lb. per acre (0.5, 1, 2, and 7 kg. per 4,047 sq. m.). For post-emergence treatment, apply the sprays (0.001–1,000 p.p.m.) at any selected stage of development after emergence.

Method of taking results

Obtain growth and yield data by making linear measurements of at least 10 individual plants in each treatment during the growing period. Also obtain yield of fruits or seeds as they mature. In pre-emergence experiments,

plants near the center of each plot should be measured.

Suggested standard treatment for comparison

Aqueous sprays (0.001–1,000 p.p.m. of 2,4,5-trichlorophenoxyacetic acid or 2,4-dichlorophenoxyacetic acid) if applied post-emergence, or 1, 2, 4, and 16 lbs. per acre (0.5, 1, 2, and 7 kg. per 4,047 sq. m.) if applied pre-emergence.

Havis, J. R. Effect of 2,4-D Sprays on the Growth of Sugarcane. *Weeds* 2: 148–154. 1953.

Marth, P. C., and Wester, R. E. Effect of 2,4,5-Trichlorophenoxy-Acetic Acid on Flowering and Vegetative Growth of Fordhook 242 Bush Lima Beans. *Amer. Soc. Hort. Sci. Proc.* 63: 325–328. 1954.

Root Growth

AGAR SLANT TEST

Method based on research by:

R. L. Jones, T. P. Metcalfe, and W. A. Sexton.

Description of test and some other uses

Seeds of rape and oats are germinated on agar slants containing a test chemical, and the root growth studied.

This method is also useful in studying the root growth of a wide variety of other kinds of germinating seeds.

Suggested plant material

Seeds of a crop and a weed plant, such as oat (*Avena sativa* L.) and rape (*Brassica napus* L.).

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance

Test tubes $\frac{1}{2}$ x 6 in. (1 x 15 cm.)

Test tube racks

Light-proof chamber at room temperature of 70°–75° F. (21°–24° C.)

About 0.1 g. of each compound to be evaluated
For standard treatment:

250–500 mg. of 2,4-dichlorophenoxyacetic acid

250–500 mg. of isopropyl-N-phenylcarbamate

Agar

Preparation and selection of plant material

Sort the seeds, eliminating any that are shriveled or diseased.

Method of conducting test

Prepare about 4% agar in water in an amount sufficient to provide 500 ml. of agar mixture for each concentration of each chemical to be evaluated plus 1,500 ml. to be utilized for an untreated control and for standard treatments with 2,4-dichlorophenoxyacetic acid and isopropyl-N-phenylcarbamate. Dissolve 50 mg. of each test compound in 500 ml. portions of the melted agar mixture.

Pour the agar-water and agar-water-chemical mixtures into separate, labeled test tubes, 10 ml. per tube. Slant the tubes so that the agar extends about 5 in. (13 cm.) up the side of the tube and allow the agar to solidify with the tube held in this position. Approximately 46 tubes of each agar-water-chemical medium plus an equal number of plain agar-water tubes will then be available for testing. Use at least 12 replicates of each treatment for statistically reliable results when high concentrations of the chemicals, such as 50 and 100 p.p.m., are employed. Eighteen replicates are sufficient for lower concentrations.

Using half the test tubes of each treatment, place two oat seeds on the agar near the top of the slant in each tube. Removal of the husks (glumes) facilitates germination. Place 3 rape seeds on the agar near the top of the slant of each of the remaining test tubes. It may be necessary to apply a slight pressure to partially embed the seeds in the agar to hold them in place.

Place the tubes in an upright position and store them in racks in a dark chamber at room temperature of 70°–75° F. (21°–24° C.) for the duration of the test. Maintain a high relative humidity in the storage chamber to prevent the agar from drying out and to favor uniform growth of the plants. Drying of the

agar must be avoided in order to prevent a corresponding increase in concentration of the test chemical.

Method of taking results

After 5–7 days, or when the rape roots in the untreated control tubes have attained a length of 5–6 cm., measure and record the lengths of the rape roots in each tube. Eight to 10 days after initiation of the test, or when the oat roots in the untreated control tubes have attained a length of 6–7 cm., measure and record the lengths of the oat roots in each tube. Also record the number of seeds in each tube that failed to germinate.

Calculate the average length of the roots in the control tubes, but do not include the zero root growth of the non-germinated seeds in the calculation of these averages. Determine the percentage of growth that the roots of each plant in each treated tube exhibit as compared with the average root length of the untreated control plants.

If one-half or more of the test plants of one species exhibit 50 per cent or greater reduction in root growth in a treatment, the applied chemical is considered to be *very active* under the conditions of the test. If one-half or more of the test plants of one species exhibit 20–50% reduction in root growth in a treatment, the applied chemical is considered to be *active* under the conditions of the test. If less than one-half of the test plants of one species exhibit greater than 20 per cent reduction in root growth in a treatment, the applied chemical is considered to be *inactive* under the conditions of the test. If a compound is shown to be active or very active at the initial concentrations tested, conduct tests at lower concentrations and repeat until the minimum dosage required to produce an effect classified as *active* is determined.

Suggested standard treatment for comparison

Use 2,4-dichlorophenoxyacetic acid at 0.1 p.p.m. concentration as a standard for rape, and isopropyl-N-phenylcarbamate at 0.1 p.p.m. as a standard for oats. Isopropyl-N-phenylcarbamate and 2,4-dichlorophenoxyacetic acid at 0.1 p.p.m. are rated as *very active* by this test when applied to oat and rape plants as described.

Jones, R. L., Metcalfe, T. P., and Sexton, W. A. The Relationship Between the Constitution and the Effect of Chemical Compounds on Plant Growth. I. 2-Phenoxyethylamine Derivatives. *Biochem. Jour.* 45: 143–149. 1949.

CONE CONTAINER TEST

Method based on research by:

T. K. Pavlychenko.

Description of test and some other uses

Seeds of barley or other grass plants are dipped in dilute concentrations of a regulating chemical and germinated in soil held in cone-shaped containers. Growth of roots and other parts of the plants is studied in detail by removing the containers and washing away the soil from some of the plants at intervals as the plants mature.

This method can be modified to study the effect of application of different regulating chemicals to either the tops of the plants or to the soil in which the plants are grown.

Suggested plant material

Barley seeds (*Hordeum sativum* L.), well matured (stored for 8–9 months after harvest), or other grass seeds.

Apparatus, chemicals, and other materials

Cone-shaped galvanized iron cylinders, 8 in. diameter at top, 10 in. at bottom, 24 in. or more high (20, 25, 61 cm.) fitted with perforated wooden or metal removable bottoms

Analytical balance or pharmaceutical balance
Sandy loam soil free from undecomposed organic material, uniformly mixed and well packed into cone-shaped cylinders
Greenhouse maintained at about 65°–80° F. (18°–27° C.)

Beakers or other containers in which to soak the seed

50–100 mg. of each chemical to be evaluated for root growth effects

1–5 g. alpha-naphthaleneacetamide for standard treatment

About 100 ml. 95% ethanol

Preparation and selection of plant material

Discard any diseased, broken, or malformed seeds.

Method of conducting test

Dip a number of seeds in aqueous mixtures of each plant regulator at each of three dosage levels: 0.002, 0.004, and 0.006 per cent are suggested. The chemicals are dispersed in water

by the aid of a small amount of ethanol. The alcohol concentration in the final mixture should not exceed 0.1 per cent. Plant 3 seeds in duplicate cylinders for each chemical and each concentration level. Repeat so as to obtain duplicate sets that can be examined after 3 weeks, 6 weeks, and at maturity. Three days after emergence, remove all but the one most vigorous plant in each cylinder. Grow plants in a cool 68°–70° F. (20°–21° C.) greenhouse for 2 weeks; then increase the temperature to 75°–85° F. (24°–29° C.) thereafter. Dip seeds in water only and grow them in the same manner for controls.

Method of taking results

Record time of emergence and the height of the plants at 3-day intervals. Harvest a duplicate lot of plants from each treatment and also from the controls at the end of 3 weeks, 6 weeks, and at maturity, tapping the cylinders and then lifting them off over the tops of the plants. Separate roots from tops and record the number, fresh and dry weight of leaves and stems, and the number of tillers. Carefully remove roots from soil by soaking in water the cylinders of soil containing them and washing away the remainder of the soil with a stream of water. Record depth of root penetration, number and length of roots, and then the dry weight. Compare all of these measurements with those of the controls.

Suggested standard treatment for comparison

Seeds soaked in 0.002, 0.004, and 0.006% alpha-naphthaleneacetamide.

Unpublished method proposed by T. K. Pavlychenko, American Chemical Paint Co., Ambler, Pa.

IMPREGNATED FILTER PAPER TEST

Method based on research by:

J. W. Brown.

Description of test

Root growth of germinating seeds on filter paper treated with the test chemicals is observed.

Suggested plant material

Wide variety of seeds—morning glory (*Ipomoea* spp.), sunflower (*Helianthus annuus* L.),

pigweed (*Amaranthus* spp.), crabgrass (*Digitaria* spp.), mustard (*Brassica* spp.), wheat (*Triticum aestivum* L., also called *T. sativum* Lam. and *T. vulgare* Vill.), pea (*Pisum sativum* L.), bean (*Phaseolus vulgaris* L.), buckwheat (*Fagopyrum esculentum* Moench), cotton (*Gossypium hirsutum* L.), cucumber (*Cucumis sativus* L.), barley (*Hordeum sativum* L.), corn (*Zea mays* L.), oat (*Avena sativa* L.), rice (*Oryza sativa* L.), and rye (*Secale cereale* L.) have been used.

Apparatus, chemicals, and other materials

Analytical balance or pharmaceutical balance
Pressure-sensitive tape with adhesive on both sides (Scotch brand No. 400, 1/2 in. [13 mm.] wide)

Filter paper (Whatman No. 1) 3 x 9 in. or 3 x 18 in. (8 x 23 or 8 x 46 cm.) depending on seed size

Filter paper strips 1/2 in. (1 cm.) wide with 1/4-in. (6-mm.) holes punched with their centers 1/2–1 in. (1–3 cm.) apart. For large seeds such as bean, pea, sunflower, and corn use holes 1 in. apart; for small seeds, 1/2 in. apart

Paper drinking cups 4 in. (10 cm.) tall

Metal trays 19 in. wide x 22 in. long x 6 in. deep (48 x 56 x 15 cm.) with false perforated bottoms 1 in. (3 cm.) above the solid bottom of the tray

Glass plates large enough to cover completely the top of the metal trays

Constant-temperature chamber maintained at about 82° F. (28° C.)

10 mg. of each compound to be evaluated

0.5–1 g. of 2,4-dichlorophenoxyacetic acid for standard treatment

Acetone

Preparation and selection of plant material

Sort the various kinds of seeds, eliminating any that are shriveled or diseased.

Method of conducting test

Dissolve 10 mg. of the test chemical in 5 ml. of 100% acetone; then dilute to 1 liter with tap water, making a 10 p.p.m. solution. Make 1 and 0.1 p.p.m. solutions by diluting aliquots of this original solution with a 0.5% concentration of acetone in water.

Place the pressure-sensitive tape along one edge of the large piece of filter paper, extending the tape 1/2 in. (1 cm.) past the edge of the large paper. Place the filter paper strip with the evenly spaced holes on top of the tape.

Make the length of paper strip such that it will leave fifteen ¼-in. (6-mm.) discs of adhesive exposed. Place 15 seeds of the first species to be tested on the exposed discs, one seed to a disc. Roll the filter paper with the seeds inside and hold the roll in place with the exposed pressure-sensitive tape. Repeat using additional species.

Stand the rolls upright in the paper cups with the part containing the seeds uppermost. Pour 70 ml. of a concentration of one chemical over rolls containing seeds of each plant species to be tested. Repeat with other concentrations and chemicals. On one set of rolls containing seeds pour an equal volume of a 0.5% concentration of acetone in water to serve as controls.

Place the cups containing the treated and control rolls in the metal trays filled with water to a depth of ½ in. (1 cm.) beneath the perforated false bottom. Cover the trays with the glass plates to maintain a high relative humidity, and place the trays in a chamber maintained at about 82° F. (28° C.).

Method of taking results

After 72–96 hours (depending upon the germination rate of the species) open the seed rolls, and measure the length of the primary roots to the nearest centimeter. The average length of the 10 longest roots is used for computing the percent inhibition of root elongation:

$$\frac{\text{Average control root length (acetone-water solution) minus average treated root length}}{\text{Average control root length (acetone-water solution)}} \times 100 = \text{percent inhibition}$$

In case the root elongation is obviously affected by some factor other than the test compound, such as profuse fungal or bacterial contamination, or if the control roots average less than 5 cm. long, the test is rerun.

Suggested standard treatment for comparison

1 p.p.m. 2,4-dichlorophenoxyacetic acid in tap water containing a concentration of 0.5% acetone.

Brown, J. W. Two Tests for Herbicide Screening. Northeastern Weed Control Conference Proc. (Sup.) 7: 71–74. 1953.

NUTRIENT MIST TEST

Method based on research by:

B. T. P. Barker; W. Carter; L. J. Klotz; M. C. Vyvyan and G. F. Trowell.

Description of test and some other uses

Trees are grown with their roots in a closed chamber containing air filled with a fine mist of nutrient solution in which the chemical being tested has been dissolved or suspended, and effects of the regulating chemical on growth of the roots are observed.

This method can be adapted for the study of growth responses of many different kinds of plants.

Suggested plant material

Young trees—maple (*Acer* spp.), pine (*Pinus* spp.), oak (*Quercus* spp.), apple (*Malus sylvestris* Mill., also called *Pyrus malus* L.), peach (*Prunus persica* [L.] Batsch), and *Citrus*.

Apparatus, chemicals, and other materials

Mist chamber ⁴

Analytical balance or pharmaceutical balance
5 g. of each compound to be evaluated

Nutrient solution

Optional materials:

lanolin

solvent, such as 95% ethanol

wetting agent, such as Tween 20

Preparation and selection of plant material

Select young trees at a uniform stage of development and growing at approximately the same rate.

Method of conducting test

Support trees (2 to 6 of each species) so that their roots are suspended in the chamber containing air filled with a mist of nutrient solution. Set up duplicate groups of trees for each additional compound or concentration to be tested. Add the plant regulator or other chemical to be tested directly to the feed tank containing the nutrient solution (concentrations of 0.01–10 p.p.m. suggested). Maintain comparable sets of trees with their roots in the nutrient mist only and designate these as controls.

As an alternative method, the chemical to be tested can be applied to the exposed tops of the trees as over-all aqueous sprays (1–1,000 p.p.m. suggested) or in a lanolin-paste mixture as a narrow band on the stem (100–10,000 p.p.m. suggested).

If regulating chemicals are to be applied uniformly to all the trees, several trees may be

⁴ Construction of various types of mist chambers is described in articles listed at the end of this method.

grown in the same chamber. The size of the chamber may be varied from approximately 1-liter capacity to room size, or large enough to permit a person to enter.

Method of taking results

After roots of the controls have developed a measurable amount of new growth, measure and record the length of all of the roots, and record the number of those living and dead on both treated and control trees.

Suggested standard treatment for comparison

No standard treatment suggested.

- Barker, B. T. P. Studies of Root Development. Long Ashton Res. Sta. Ann. Rpt. 1921: 9-20.
 Carter, W. A Method of Growing Plants in Water Vapor to Facilitate Examination of Roots. *Phytopathology* 32: 623-625. 1942.
 Klotz, L. J. A Simplified Method of Growing Plants with Roots in Nutrient Vapors. *Phytopathology* 34: 507-508. 1944.
 Vyvyan, M. C., and Trowell, G. F. A Method of Growing Trees with Their Roots in a Nutrient Mist. East Malling Res. Sta. Ann. Rpt. 1952: 95-98. (Pub. 1953.)

Root Induction

STEM-CUTTING TEST

Method based on research by:

J. W. Mitchell and P. C. Marth; H. B. Tukey; J. S. Wells.

Description of test and some other uses

Cuttings are soaked, dusted, or dipped in mixtures containing chemicals, and the effect on root initiation and growth determined.

Compounds that affect top growth can also be applied separately or simultaneously with root-inducing substances.

Suggested plant material

Pieces of stems of woody plants such as privet (*Ligustrum* spp.), yew (*Taxus* spp.), osmanthus (*Osmanthus* spp.), and holly (*Ilex* spp.), or of herbaceous plants such as chrysanthemum (*Chrysanthemum* spp.), geranium (*Geranium* spp.), lantana (*Lantana* spp.), and carnation (*Dianthus caryophyllus* L.).

Apparatus, chemicals, and other materials

Knife with sharp, thin blade 4-6 in. (10-15 cm.) long

Analytical balance or pharmaceutical balance
 Containers of suitable size to hold cuttings upright in solutions

Propagating case with top or sides made of translucent material, with temperature controlled at 65°-70° F. (18°-21° C.) and humidity controlled at 75-95%

Perlite or fine sand washed free of organic matter, clay, or other foreign material
 Clay pots 3 in. (8 cm.) in diameter
 Composted soil
 100-200 mg. of each compound to be evaluated
 500 mg. of 3-indolebutyric acid or alpha-naphthaleneacetamide
 Solvent such as 95% ethanol
 1 lb. (0.5 kg.) of dust carrier such as talc, fuller's earth, or finely ground clay

Preparation and selection of plant material

Cuttings of woody plants: Select terminal shoots during late summer or early fall, or a few weeks after vegetative growth has ceased. The leaves should be fully developed and the wood hard. Plant species vary with regard to the best time of year that cuttings should be taken in order to obtain optimum results. Remove the cuttings from the plants with a sharp knife, making one cut on a slant and at the base of the current season's growth. Cuttings 4-6 in. long (10-15 cm.) or pieces including 3 to 6 nodes are then made from these branches. Make the basal cut immediately below a node and the uppermost cut just above a node. Remove the lower leaves, but let 3 or 4 of the uppermost leaves remain attached. Using rubber bands, fasten cuttings in bundles of 10 to 20 with stems parallel and bases even. Do not allow cuttings to dry out or the leaves to wilt at any time during these preparations.

Cuttings from herbaceous plants: Stock plants may be maintained under greenhouse or outdoor conditions so that succulent new shoots are available for cutting material throughout the year. Prepare bundles of cuttings for treatment in the same manner as with woody plant material.

Method of conducting test

Three methods of treating cuttings have proved suitable for this test:

Soaking method: Prepare aqueous solutions of each compound to be tested in concentrations of 25, 50, and 100 p.p.m. Stand a bundle of cuttings in each dilution so that the bases of the cuttings are covered with the solution to a depth of about 1 in. (3 cm.). Vary the size of the container to suit the size of the batch of cuttings, and do not allow the leaves to come in contact with the solution. After 2 to 4 hours in diffused light, remove the cuttings and insert the basal ends of the stems to a depth of 2 to 3 in. (5-8 cm.) in the rooting medium contained within the propagating chamber. Pack the moist rooting medium firmly around each stem and water thoroughly. Keep the medium moistened throughout the test (fig. 7).

Figure 7. Steps to follow in testing the effect of regulating chemicals on the initiation of roots by stem cuttings.



After preparing a dust mixture containing a known amount of the test compound, moisten the end of the cutting with tap water; then roll the wet end in the dust. Tap the cutting lightly to remove loose dust.



The chemical can also be applied by soaking the cuttings in a water mixture of the compound. To accomplish this, stand the cuttings in a mixture containing a known amount of the regulator so that the basal ends are immersed to a depth of 1 to 1½ inches. Place the container with the cuttings in diffused light and allow the cuttings to stand in the mixture for 3 to 6 hours.



Insert the cuttings in a trench made in moist building sand (or other suitable inert material) to a depth of about ⅓ of their length. Press the sand tightly around the bases of the cuttings. Shade the cuttings and keep the sand moistened with water but not overly wet during the next 2 to 4 weeks. If possible, keep the temperature below 70°-75° F. (21°-24° C.).



Examine several of the cuttings at intervals to determine whether roots have formed.

(NEG. PN-298 A-D)

Dust method: Succulent cuttings are usually treated with a mixture of 1 part of regulating chemical to 1,000–5,000 parts of dust carrier; woody cuttings usually require a stronger dust mixture—1 part regulator in 500–750 parts of dust carrier. To prepare dust mixtures, first dissolve the desired weighed amount of the compound in a volatile solvent such as ethanol, then add the proportionate weighed amount of the dust carrier. The volume of solvent should be sufficient to make a thin paste of the regulator-dust mixture. Stir the entire mixture thoroughly, then allow the solvent to evaporate at room temperature, with occasional stirring during this process.

Treat each cutting individually by rolling about 1 in. (3 cm.) of the basal part in the dust mixture so that a thin coating of dust adheres to it. Tap off any loose dust and set the treated portion of the cutting in the rooting medium.

Do not rub the dust from the cuttings when inserting them in the medium; place in a trench wide enough so that the dust remains on the stem.

Dip method: Prepare a solution of each regulating chemical, using 50–95% ethanol. Vary the concentration of each compound in a range of 100–500 p.p.m. for succulent cuttings and 500–1,000 p.p.m. for woody cuttings. Dip the bases of one bundle of cuttings to a depth of 1 in. (3 cm.) in one dilution of a compound, and insert the treated portion of these cuttings individually in the rooting medium. Repeat, using other bundles of cuttings for each concentration of each test chemical.

Method of taking results

After 10–14 days in the case of succulent cuttings, or 14–30 days in the case of woody ones, carefully loosen the rooting medium around the cuttings and remove each one individually from the medium. Sort them into the following categories: (a) Those that are dead, (b) those not rooted, (c) those with a few short roots, (d) those with a moderate number and fairly long roots, (e) those with many relatively thick, long roots. Note and record the number of cuttings in each category. Also note any unusual characteristics of roots in each category. Pot the cuttings, using soil, and observe their subsequent growth.

Suggested standard treatment for comparison

Soak method, using 3 concentrations of 3-indolebutyric acid or alpha-naphthaleneacetamide (25, 50, and 100 p.p.m.) for 2–4 hours.

Mitchell, J. W., and Marth, P. C. *Growth Regulators*. Univ. of Chicago Press, Chicago, Ill. 129 pp. 1947.
 Tukey, H. B., editor. *Plant Regulators in Agriculture*. John Wiley and Sons, Inc., New York. 269 pp. 1954.
 Wells, J. S. *Plant Propagation Practices*. Macmillan Co., New York. 344 pp. 1955.

Seed Formation

LANOLIN PASTE TEST

Method based on research by:

S. L. Emsweller and N. W. Stuart; R. E. Wester and P. C. Marth.

Description of test

A mixture of lanolin and a regulator is ap-

plied to a wound on the petal base or on the pedicel, and the effect on seed development is observed.

Suggested plant material

Species of *Lilium* such as *L. longiflorum* (Creole, Croft, or Ace clones), bush lima beans (*Phaseolus lunatus* L.), Fordhook, Early Market, or Peerless varieties, or other plants difficult to hybridize.

Apparatus, chemicals, and other materials

Clay pots 6–10 in. (15–25 cm.)

Composted soil

Space in greenhouse maintained at about 70°–85° F. (21°–24° C.) by day and 60°–75° F. (18°–21° C.) by night

Small vials (25–50 ml.) or other containers
Analytical balance or pharmaceutical balance
250–500 mg. of each compound to be evaluated

1 g. each of alpha-naphthaleneacetamide,
para-chlorophenoxyacetic acid and 3-indolebutyric acid for standard treatment

Lanolin

Tween 20

Preparation and selection of plant material

Grow the plants, known to be difficult to cross, under usual greenhouse conditions.

Method of conducting test

Prepare a range of concentrations of the chemical to be tested in lanolin, as described on page 5.

Lilies: Immediately before hand pollinating the flowers, separate one “petal” (i.e., one member of the outer whorl of the perianth) from the receptacle, and cover the injured part of the receptacle with lanolin-regulator mixture.

Lima beans: Immediately after cross-pollina-

tion has been completed, scratch the base of the flower stalk (pedicel) lightly with a pin or dissecting needle. The wound should be made close to the juncture of the pedicel and peduncle. With a pin or dissecting needle, place a small portion of the lanolin-regulator mixture on this wounded surface. Pollinate additional flowers as controls.

Method of taking results

Observe and record the number of fruits and their rate of development. When fruits are mature, remove the seeds and record the number of mature and immature ones. Determine the viability of the seeds.

Suggested standard treatment for comparison

For lima beans, make a lanolin mixture containing sufficient indolebutyric acid to make a concentration of 0.1%, and a second lanolin mixture containing sufficient para-chlorophenoxyacetic acid to make a 0.1% mixture. Combine portions of these two mixtures in a ratio of 4 parts of the indolebutyric mixture to one part of the phenoxyacetic acid mixture.

Emsweller, S. L., and Stuart, N. W. Use of Growth-Regulating Substances to Overcome Incompatibilities in *Lilium*. Amer. Soc. Hort. Sci. Proc. 51: 581–589. 1948.

Wester, R. E., and Marth, P. C. Some Effects of a Growth Regulator Mixture in Controlled Cross-Pollination of Lima Bean. Amer. Soc. Hort. Sci. Proc. 53: 315–318. 1949.

PART II. METHODS OF DETECTING REGULATING COMPOUNDS

Detection of Plant-Growth Regulators in Animals

BEAN STEM TEST

Method based on research by:

J. W. Mitchell, R. E. Hodgson, and C. F. Gaetjens.

Description of test and some other uses

Regulating chemicals are extracted from animal tissues, fluids, secretions, or excrement. The extract is applied to stems of young bean plants, and elongation indicates the presence of plant-regulating compounds in the extract.

The method described here has been used to estimate the amount of 2,4-dichlorophenoxyacetic acid (or its salts) in the blood of an animal to which this compound was fed. The method has also been used to detect the presence of this chemical in the animal's tissues and milk. Naturally occurring, cell-elongating compounds present in urine and feces can also be detected through the use of this method.

Suggested plant material

Young bean plants (*Phaseolus vulgaris* L.) of the Black Valentine or Pinto variety.

Apparatus, chemicals, and other materials

Millimeter rule
Pipette, 0.1 ml. capacity graduated in 0.01 ml.
Fluorescent lights that will supply 800-foot candles of light intensity at leaf surface
Wiley Mill or suitable means of grinding samples to 60 mesh
Glass containers for holding samples
Analytical balance or pharmaceutical balance
1-2 g. of sodium or ammonium salt of 2,4-dichlorophenoxyacetic acid
Centrifuge
Composted soil

Preparation and selection of plant material

Germinate bean seeds in composted soil contained in 3-in. (8-cm.) clay pots, using several seeds per pot. Allow the plants to grow in a

well-illuminated greenhouse until they are about 3.5 in. (9 cm.) tall. If the temperature is maintained near 80° F. (27° C.) during germination, and adequate sunlight is available, about 5 or 6 days will be required for the plants to produce sturdy stems and reach the required height. Select plants having first internodes that do not vary more than 1 mm. in length from the average of the entire group selected. The average internode length of the selected plants should, whenever possible, be 13 mm. but may in some cases be 12 or 14 mm. Select a sufficient number of plants so that 10 can be used for each treatment. Arrange the plants under the fluorescent lights making certain that the lights extend beyond the edge of the area covered by the plants so as to obtain light, solely from the artificial source, of relatively uniform intensity throughout. Number each plant, and measure to the nearest mm. the length of each first internode. Assign treatments in the form of randomized blocks.

Method of conducting test

Prepare four aqueous solutions of sodium 2,4-dichlorophenoxyacetate, the first containing 60 p.p.m. of the salt (6 mg. salt in 100 ml. water), the second, third, and fourth containing 30, 15, and 7.5 p.p.m., respectively, of the salt. Make these by diluting the more concentrated solutions with water.

In detecting the regulator in blood, obtain blood serum from animals that have not injected the chemical under test, and also serum from animals that have been fed the test compound. Measure out 5 portions of the "control" serum, each of 3 ml. volume. Prepare standard growth-regulator-serum mixtures by adding none of the aqueous salt solutions to the first control serum aliquot, 1 ml. of the 60 p.p.m. solution to the second aliquot of serum, 1 ml. of the 30 p.p.m. solution to the third aliquot of serum, continuing until each concentration of salt has been added to a corresponding aliquot of "control" serum. Measure out several 3-ml. portions of serum from the animal which was fed the growth regulator.

Evaporate separately all of the serum samples on a steam bath. Grind the residues quantitatively through a 60-mesh screen and

extract each sample for 15 minutes with 5 ml. of hot distilled water. Filter the mixtures and wash the residues with hot water. Evaporate the filtrates on a steam bath just to dryness, cool the receptacles, and wash the residues obtained into centrifuge tubes using 1 ml. of water for each, and centrifuge for 5 to 10 minutes. Decant the centrifugate and apply 0.01 ml. of each preparation to each of 10 plants. Place the measured serum preparation on the first internode of each plant as a narrow band (approximately 2 mm. wide) extending around the internode midway between the first and second node. Deliver the measured volume directly from the 0.1 ml. pipette onto the stem. Illuminate the plants, using only the artificial light source and 12 hours of light followed by 12 hours of darkness.

Method of taking results

Twenty-four hours after treatment, measure the length of each first internode to the nearest mm. Calculate the elongation that occurred

during the period. Compare the elongation of plants which received serum from untreated animals with that of plants which received serum from the treated animals. Determine if the differences are statistically significant. Draw a standard curve showing internode elongation induced by the known amounts of 2,4-dichlorophenoxyacetate when added to serum from the untreated animal. Using this standard curve, express the amount of elongation obtained with serum from the treated animal in terms of parts per million of 2,4-dichlorophenoxyacetate equivalent.

Use this method for measuring the amount of the salt in animal organs or in milk.

Suggested standard treatment for comparison

Sodium or ammonium salt of 2,4-dichlorophenoxyacetic acid used as described above.

Mitchell, J. W., Hodgson, R. E. and Gaetjens, C. F. Tolerance of Farm Animals to Feed Containing 2,4-Dichlorophenoxyacetic Acid. *Jour. Anim. Sci.* 5: 226-233. 1946.

Penetration of Soil by Regulators

SEED TEST

Method based on research by:

P. J. Linder.

Description of test and some other uses

Chemicals are applied to the surface of soil contained in glass tubes. Soil samples are collected as thin layers from various depths and bioassayed for the presence of the chemical.

This test is useful in studying the effect of simulated rainfall on the movement of regulating chemicals through soil, the effect of various adjuvants, types of soil, and the physical structure of the soil on the rate of movement of these compounds through soil. It also can be adapted to the study of persistence of regulating chemicals in soil as affected by factors such as temperature, moisture, duration of exposure, and microbial content of the soil.

Suggested plant material

Seeds of mustard (*Brassica* spp.) or other small-seeded plants known to be sensitive to the compounds to be tested.

Apparatus, chemicals, and other materials

Straight glass tubes $1\frac{1}{4}$ in.
(3 cm.) in diameter x $5\frac{1}{2}$ in.
(14 cm.) in length

Cork stoppers to plug one end of the glass tubes

Fertile soil passed through a screen with $\frac{1}{4}$ -in. (0.6-cm.) openings

Cork stoppers with a maximum diameter slightly less than the inside diameter of the glass tubes

$\frac{1}{4}$ -in. (0.6-cm.) dowel 1 ft. (30 cm.) long

Analytical balance or pharmaceutical balance

Hand-sprayer capable of distributing a test solution accurately over a given area

Razor blade

Shallow tin or plastic cups or screw-type jar lids of about $2\frac{1}{4}$ -in. (6-cm.) diameter

Dark chamber maintained at about 80° F. (27° C.) with a high relative humidity

About 5 g. of each compound to be tested

Preparation and selection of plant material

Sort the seeds, eliminating any that are shrunken or defective.

Method of conducting test

Tightly stopper one end of the glass tube.

Pour 75 g. of air-dry soil into the stoppered tube. Then place a loose-fitting cork on top of the soil. Place the end of the dowel against the loose-fitting cork and subject the other end of the dowel to a pressure of 2 kg. for the purpose of compressing the soil a measured amount.

Remove the tightly fitting cork stopper from the tube and, by pressing the dowel against the loose-fitting stopper, force the soil upward in the tube until the surface is flush with the open end of the tube. Mark off an area of about 1 sq. yd. (1 sq. m.) on the floor. If need be, cover the area with disposable paper or other material. Stand the tubes to be used for one treatment on end within the marked area so that the flush and exposed soil surfaces are uppermost.

Apply the compound to be evaluated evenly over the entire marked area including the exposed soil surfaces. This can be done by spraying the mixture back and forth over the entire area, first in one direction, and then in a direction at right angles to this, so that the area is covered uniformly with the mixture.

Using separate sets of tubes of soil, various amounts of the chemical can be tested. To determine the amount of each chemical to be used, carry out preliminary experiments with each compound to find the dosage range necessary to inhibit shoot extension above the soil from 0 to 100 percent. Use 10 soil tubes for each concentration of each chemical, and treat 5 tubes with only water and solvent, if a solvent was used, to serve as solvent-treated controls.

To test for the presence of the applied compound, collect a soil sample by using the dowel and loosely fitting stopper to push the column out through the open end of the cylinder for a distance of 3 mm.; then slice off the ejected portion with a razor blade. In this way it is possible to collect a surface layer 3 mm. or more in thickness, or any lower layer of soil desired.

To obtain a sufficient volume of soil for

assaying, collect comparable samples from 5 replicates and combine and mix them. Divide the composite sample into 3 equal parts. Place each soil sample in the shallow tin or plastic cup. Sow seeds of the test plants uniformly in the soil. Add uniformly sufficient water to bring each soil sample to optimum moisture content for seed germination. Store the cups containing the soil and seeds in a dark, moist place at about 80° F. (27° C.).

Method of taking results

Measure the height (or fresh weight) of the plants in the various soil samples, including the prepared standards and the solvent-treated control. Compare the average amount of growth with the solvent-treated controls and with that of the plants grown in cups containing soil samples to which have been applied known concentrations of the test chemical.

The soil in the tubes can be (1) assayed, as described, shortly after chemical application to determine the rate and depth of penetration of the chemical, or (2) stored under controlled moisture, temperature, and other conditions before being assayed to determine the effect of these on persistence of the chemical, or (3) subjected to simulated rainfall for leaching studies, etc., before the assay. The prepared series of standards may be used also to determine the amount of chemical present in field plots which have been treated with a test chemical.

Suggested standard treatment for comparison

No standard treatment suggested.

Linder, P. J. Movement and Persistence of Herbicides Following Their Application to the Soil Surface. Northeastern Weed Control Conference Proc. 6: 7-11. January 1952.

Residue of Regulators in Soil

REPEATED PLANT TEST

Method based on research by:

A. J. Loustalot and R. Ferrer; W. W. Robbins, A. S. Crafts, and R. N. Raynor.

Description of test and some other uses

Several successive crops of plants are grown in soil treated once with a chemical in order to determine how long the chemical remains in

the soil in sufficient amounts to affect plant growth.

This test is convenient for testing the effect of cultivation or mixing of the chemical with the surface or with lower layers of the soil, and for studying the effect of environmental conditions on the persistence of the chemical in soil.

Suggested plant material

Use plants known to be highly sensitive to

the compound under test. If the sensitivity of plants to the chemical is not known, select a number of plant species and determine useful ones on the basis of their sensitivity to direct application of the chemical to be tested.

Apparatus, chemicals, and other materials

Soil to be tested

Unperforated small containers

(Clean, used No. 2 tin cans have the advantage over clay pots of not absorbing any of the applied chemicals.)

Analytical balance or pharmaceutical balance

Greenhouse space

About 2-3 g. of each compound to be evaluated

Preparation and selection of plant material

Sort the seeds, and eliminate any that are defective.

Method of conducting test

The amount of the chemical used in these tests will depend on the specific compound tested. (A mixture of 0.25 mg. of a chemical with 500 g. of a clay loam soil is equivalent to 1 lb. [0.5 kg.] of the chemical applied to an acre [4,047 sq. m.] of clay loam soil and mixed to a depth of 7 in. [18 cm.])

Insoluble compounds, or compounds that are dry and of small particle size, can be dry-mixed with the soil before placing the chemical-soil mixtures in the containers. Use a total of 500 g. of soil per container.

To estimate the amount of moisture to be maintained in each container of soil, punch 10 to 12 holes in the bottom of one can with a 10-penny nail. Place 500 g. of soil in the can and record the total weight of can plus soil. Immerse the can in water to a distance of 1 in. (3 cm.) and maintain the water at this level until the surface of the soil becomes moist. Remove the can containing the soil and allow it to drain 10 to 15 minutes. Record the weight of the can, water, and soil as the weight indicating the amount of moisture to be maintained. In conducting the experiment, add enough water each day or two to maintain this weight. Subtract the weight of the can plus soil from the weight of the can, soil, and moisture to obtain the initial weight of water to be added. To distribute the chemical throughout the soil in each container, dissolve the required amount of soluble compound in the above-determined weight of water and pour this evenly over the surface of the soil.

An alternative method that provides even distribution of the chemical throughout any soil can be used with any compound that is soluble in water or suitable organic solvents (acetone, alcohol, etc.). Dissolve a measured amount of the test compound in 6 ml. of a suitable solvent (addition of a wetting agent, such as 0.4 ml. of Tween 20, may be advantageous). Add 20 g. of fine, clean quartz sand to this solution and stir to make a slurry. Dry this mixture at room temperature. Add the chemically-coated sand to enough soil to make a total of 500 g. and mix it thoroughly before placing the treated soil in the container.

After the seeds have been planted, bring the soil samples to the correct moisture level. The number of seeds to be planted in each can will depend on the size of the plant.

Grow the test plants for 30 days. Then measure the height of the plants in the treated and untreated containers, cut off the plants at the soil level, and determine the fresh weight of the tops. Return the tops to the cans where they were originally grown and, after a 30-day drying period, pulverize and replace the soil, putting the now dry tops beneath the soil. Re-plant the soil and bring it to the correct moisture level. Six crops a year can be grown in this manner in order to determine the residual effects of the compounds.

A variation of this test is as follows: Treat the soil with the test chemical as above but do not plant all of the containers at once. Store some of the containers for $\frac{1}{2}$, 1, and 2 months under different conditions. Conditions that can be altered easily are soil temperature and moisture content. Combinations of these conditions can be used, but it is strongly suggested that the conditions selected closely approximate the normal variation found in the field.

Method of taking results

The weight and height measurements taken after each successive growth period are compared. Comparative studies of residual effects can be made following use of one chemical and many soil types, or several chemicals in a single or a few soil types.

Suggested standard treatment for comparison

No standard treatment suggested.

- Loustalot, A. J., and Ferrer, R. Studies on the Persistence and Movement of Sodium Trichloroacetate in the Soil. *Agronomy Jour.* 42: 323-327. 1950.
Robbins, W. W., Crafts, A. S., and Raynor, R. N. *Weed Control*, 1st Edition, 503 pp. McGraw, Hill Book Co., New York. 1942.

Translocation of Radioactively Tagged Compounds

ISOTOPE COUNTER TEST

Method based on research by:

John W. Mitchell and Paul J. Linder.

Description of test

Translocation of radioactively tagged molecules is measured by placing a tagged compound on one part of a plant and measuring radioactivity that results from the movement of the compound, metabolites, or degradation products of it to other parts of the plant. Radioactivity in dried, ground, plant tissue is measured directly. Since self-absorption is involved, the method is only reliable for comparison of radioactivity in comparable tissues ground to approximately the same particle size.

Suggested plant material

Any kind of terrestrial plant.

Apparatus, chemicals, and other materials

Conventional Geiger tube
Q-gas counter (or counter of similar sensitivity if carbon tagged compounds are used)
Wiley mill
Scaler
Lead tube for shield
Remote control applicator (fig. 8)
Brass rings (inside diameter 25 mm., outside diameter 31 mm., 3 mm. high)
Cellophane tape, 1.5 in. wide (3 cm.)
Analytical balance or pharmaceutical balance
Radioactively tagged plant regulator
Lanolin
Tween 20

Preparation and selection of plant material

The following method is designed for use in studying translocation from leaves of plants such as bean (*Phaseolus vulgaris* L.), tomato (*Lycopersicum esculentum* L.), or other plants commonly grown in a greenhouse, but it can be modified for application to any part of a terrestrial plant. Plant seeds in soil contained in clay pots and allow several plants to grow in each pot until the primary leaves or the first secondary leaves have become photosynthetically active and capable of supplying photosynthate to other parts of the plants. Thin the

plants to one in each pot, retaining only plants of uniform size.

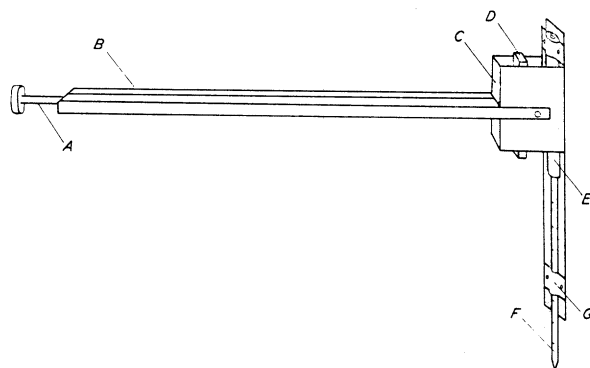


Figure 8. Diagram of a remote-control applicator useful for applying small volumes of solutions containing radioactive regulating chemicals to leaves and stems of plants. Support an aluminum rod (A), approximately 65 cm. long and 5 mm. in diameter, by means of two 13-mm. aluminum angles (B). Thread one end of the rod and screw it through a plate into a metal box (C) so that it rests against a movable plate (D). Turn the rod to press the plate (D) against the piece of soft rubber tubing (E) which is held in place by clamps. Close the upper end of the tubing with a glass plug. Insert a 0.1 ml. pipette (F) in the lower end of the tubing, and fasten the pipette with a clamp (G). Turn the rod and expel the air from the pipette; then replace it with a solution containing the radioactive growth regulator. Force a measured portion of the solution (0.005, 0.01, or 0.02 ml.) out of the pipette by turning the rod, and apply the drop by touching the tip of the pipette to the surface of a plant. When necessary, use the tip of the pipette to spread the liquid evenly over the plant surface.

(NEG. PN-299)

Method of conducting test

Prepare an aqueous mixture which contains 0.1% of Tween 20 (or other suitable cosolvent) and 10 μ g. of the radioactive plant regulator per 0.01 ml. This may be easily accomplished by using the proper precautions⁵ and weighing the required number of mg. of the radioactive substance into a vial, adding the required amount of distilled water containing the cosolvent. By means of a short piece of glass rod 3–5 mm. in diameter, crush all crystals of the growth regulator and stir until the very fine pieces dissolve.

Use a short piece of rubber tubing (6–7 mm. inside diameter) and stamp a ring of lanolin on the upper surface of the leaf enclosing an area in the desired position (fig. 15). By means of the remote control applicator, place 0.01 ml. of

⁵ Experiments using radioactive materials should not be undertaken without a thorough knowledge of the hazards involved.

the mixture within the lanolin ring. If the entire upper surface of the leaf is to be covered with the mixture, use the method described on page 8. Replicate treatment with any one tagged compound at least five times, using an equal number of comparable plants.

After application of the radioactive regulator, place the plants in a well-illuminated greenhouse, or if necessary, illuminate them by means of fluorescent tubes so that they receive at least 700-foot candles of light at the leaf surface. After the desired length of time (from a few hours to several days) remove the treated leaves very carefully and place them in a paper bag to be discarded. Wash the remainder of the plants free of soil, divide them into roots, stems, etc. Dry each part in a well-ventilated oven and grind each sample to 40 mesh, exerting care that one sample does not become contaminated by another.

The size and density of the tissue particles that make up a sample of ground plant material control, to some extent, the amount of self-absorption of radioactivity that occurs within the sample. It is difficult to grind to comparable particle size tissues from plants grown under widely different environmental conditions, or tissues from different parts of plants grown under comparable conditions. The amount of fiber and secondary thickening that develops in stems and leaves is dependent to some extent upon the environmental conditions to which the plants are subjected. Plants grown in relatively high light intensity often contain proportionally more fiber than do those grown in shade and the former are, therefore, more difficult to grind to a small particle size. More mature parts of stems generally contain proportionally more fiber than do relatively immature parts of stems, and samples of the former are, therefore, difficult to grind into particles of relatively small size. Care must be exercised in the utilization of the present method so that self-absorption, due to differences in size and density of particles, does not introduce significant errors.

Method of taking results

To determine the radioactivity in a sample, place a brass ring on the tacky surface of the cellophane and trim off the cellophane flush with the outside of the ring; then determine the weight of the ring and cellophane. Thoroughly mix the powdered sample and place a small portion of it on the tacky surface of the cellophane. By gently tapping the ring, roll the powdered tissue back and forth until the entire surface is evenly covered. Shake the excess of tissue into its original container and

weigh the ring, cellophane, and adhering tissue to determine the amount of tissue used to measure the radioactivity. Place the ring in the holder of the counter and determine the number of counts per minute that emanate from the sample. Calculate net counts per milligram per minute by subtracting background counts.

Suggested standard treatment for comparison

Ten $\mu\text{g.}$ of 2,4-dichloro-5-iodophenoxyacetic acid tagged with I^{131} , one of its salts, or 2,4-dichlorophenoxyacetic acid tagged with C^{14} .

Mitchell, John W., and Linder, Paul J. Some Methods Used in Tracing Radioactive Growth-Regulating Substances in Plants. *Bot. Gaz.* 112: 126-129. 1950.

RADIOAUTOGRAPH TEST

Method based on research by:

A. S. Crafts.

Description of test

Radioactive 2,4-dichlorophenoxyacetic acid is placed on a leaf of the plant. The plant is later dried and stored adjacent to an X-ray film which is then developed. The autographic outline thus obtained is registered with an outline of the plant on Ozalid paper, and the presence of the radioactivity in different parts of the plant observed.

Suggested plant material

Succulent plants that can be readily obtained in the field or grown in a greenhouse.

Apparatus, chemicals, and other materials

- Darkened room
- Oven for drying plants
- Waxed paper
- Dry ice
- Dry sheets of newsprint
- Blotters of a size that will cover the entire plant
- Kodak No-screen X-ray film
- Aluminum foil
- Empty metal cartridge cases to be used as light-tight containers (obtainable from army supply stores) or other suitable light-tight boxes
- Analytical balance or pharmaceutical balance
- Ozalid paper

2,4-Dichlorophenoxyacetic acid labeled in the carboxyl position with carbon ¹⁴ and having a specific activity of at least 1 microcurie per mg.

Developer and fixer for use with X-ray film
Wetting agent such as Tween 20, or other detergents

Preparation and selection of plant material

Select plants that suit the objectives of the test, generally plants that are growing vigorously and that have developed suitable leaves located in the same relative position on each plant. Use only leaves that have reached a stage in their development at which they are capable of supplying photosynthate to other parts of the plant. Very young, rapidly expanding leaves do not translocate regulating compounds to other parts of the plant.

Method of conducting test

Prepare mixtures containing the radioactive regulator as described ⁶ (see page 49). Apply from 1–10 μ g. of the labeled compound to one leaf of several plants to be used as replications. After 2 or 3 days (or any other desired period of time), wash the plants free of soil and place them between sheets of waxed paper, then between blocks of dry ice. The plants will freeze solid within 2 minutes. After 5 minutes, remove the plants and place them between clean, dry sheets of newsprint; and place these, in turn, between blotters. Dry the

blotters, papers, and plants in a well-ventilated oven at 70°–80° C. for a period of 10 to 12 hours. Place one of the dry plants on a sheet of the X-ray film (in a darkened room) and cover this with a blotter to which has been clipped a sheet of aluminum foil of equal size. Place another blotter on top of the "pile", then another sheet of film, and continue to stack the plants and sheets until each plant is next to a sheet of film. Bind all together between suitable light-weight boards and place the entire "pile" in a cartridge case. Store the "piles" for a period of 4 weeks at room temperature; then develop the film and make an Ozalid print of each plant. Mark each autograph and Ozalid print so that they can be readily identified.

Method of taking results

Place an autograph over the corresponding Ozalid print and register the images. Compare visually the autographic image developed from the different plants and record any differences in translocation of radioactive substances.

Suggested standard treatment for comparison

Ten μ g. of 2-4-dichlorophenoxyacetic acid ($C^{14}OOH$) applied in an aqueous mixture containing 0.1% Tween 20 or other wetting agent. Specific activity of acid, 1 microcurie per mg.

Unpublished method suggested by A. S. Crafts, Calif. Agr. Expt. Sta.

Volatility⁷ of Plant Regulators

DISPOSABLE CONTAINER TEST

Method based on research by:

P. C. Marth and J. W. Mitchell.

Description of test

This test is useful for detecting volatility of plant-regulating compounds that induce easily detectable growth responses. It can be used to detect or measure evaporation of regulating chemicals from surfaces of plants previously treated with these compounds, or from surfaces of other materials to which these substances have been applied.

Suggested plant material

Determine a suitable test plant by placing a

small amount of the chemical to be tested on several kinds of young plants; suggested are snap bean (*Phaseolus vulgaris* L.), buckwheat (*Fagopyrum esculentum* Moench), cucumber (*Cucumis sativus* L.), and tomato (*Lycopersicon esculentum* Mill.).

Apparatus, chemicals, and other materials

3-in. (8-cm.) clay or paper pots or clean discarded tin cans of about 1-lb. (454-g.) capacity

Analytical balance or pharmaceutical balance
Greenhouse space maintained at 75°–85° F.

⁶A thorough understanding of hazards involved must first be obtained.

⁷Carelessness in the use or disposal of highly effective or volatile, regulating chemicals can result in contamination of an entire laboratory and greenhouse, or loss of valuable plants and use of space contaminated.

(24°–29° C.) for growing test plants
Table or bench space in darkened room maintained at 70°–90° F. (27°–32° C.) for conducting tests

Several 1- and 5-ml. pipettes

20–30 ml. vials with tightly-fitted stoppers or screw caps

Filter paper No. 1 or other absorbent paper discs 7–9 cm. in diameter

Cellophane bags 4 in. (10 cm.) square and 16 in. (41 cm.) tall

Adhesive cloth, plastic tape, paper clips or electric iron for sealing cellophane bags

95% ethanol or acetone for solvent

0.1–1 g. of each pure compound to be tested

1–2 g. of the butyl or isopropyl ester of 2,4-dichlorophenoxyacetic acid for standard treatment

Preparation and selection of plant material

Plant several seeds of bean, tomato, or other test plant in soil contained in disposable pots or containers. Employ optimum greenhouse conditions of light, temperature, and moisture so as to produce rapidly growing seedlings for the tests. Select bean plants that have primary leaves that measure 3 cm. across, and with trifoliate leaves still folded in the terminal buds (about 5–7 days old); or tomato plants 2.5–3 in. (6.4–7.6 cm.) tall (about 25 days old). Thin the plants, leaving a single plant of uniform size in each pot.

Method of conducting test

Prepare an alcoholic or acetone solution of the pure chemical by placing a weighed amount of it in a vial and adding sufficient 95% ethanol or acetone to give the desired concentration. For example, weigh 5 mg. of the chemical into a vial and add 25 ml. of solvent to obtain a solution containing 0.2 mg. of the compound per ml.

Impregnate a filter paper or other suitable paper disc with 1 ml. of the alcoholic mixture, and allow the solvent to evaporate completely (5–30 minutes). Use 3 or more replications for each compound tested.

Place a pot containing the selected test plant upright in the cellophane bag. Fasten the impregnated filter paper with a small piece of cellophane tape inside the bag and about 2 in. (5 cm.) from the top. Do not allow the impregnated filter paper to come in contact with the plant during the test. Close the open ends of the cellophane bags and fold them downward at a distance of about 1 in. (2.5 cm.); then seal with a hot iron.

Expose the plants to the vapor from the test chemical for the desired length of time (24–72 hr.) and temperature; then remove, and allow them to remain in a greenhouse for later observation. Dispose of the plants, filter paper containing the chemical, bags, etc., so as to avoid contamination of room or greenhouse.

Method of taking results

The response of the plant (leaf and stem curvature) is due to two separate factors; first, the ability of the chemical to induce the response when in direct contact with the plant; and secondly, the rate that the chemical evaporates from the paper and comes in contact with the plant. Since this method is based both on the biological effectiveness of the compound and its ability to evaporate, the method cannot be used to measure either of these factors separately.

Immediately before removal of the plants from the bags, record the degree of curvature, comparing the plants with a diagram or photograph illustrating typical plants that have responded slightly, moderately, or to a marked degree. For convenience, assign these categories numerical values such as 0, 1, 2, 3, and 4. Carefully dispose of all contaminated materials including plants exposed to the vapors.

Suggested standard treatment for comparison

A filter paper disc that is impregnated with 0.2 mg. of butyl ester of 2,4-dichlorophenoxyacetic acid and the plants exposed to the vapors for 48 hr. at 80°–90° F. (27°–32° C.).

Marth, P. C., and Mitchell, J. W. Comparative Volatility of Various Forms of 2,4-D. *Bot. Gaz.* 110: 632–636. 1949.

GERMINATING SEED TEST

Method based on research by:

W. P. Anderson.

Description of test

Relative volatility of regulating compounds is determined by exposing germinating seeds to vapors of these chemicals in closed containers.

Suggested plant material

Cucumber, buckwheat, corn, or other seeds.

Apparatus, chemicals, and other materials

Petri dishes about 15 cm. diameter x 2 cm. deep and 5 cm. diameter x 2 cm. deep
Filter paper (No. 1 Whatman) to fit the Petri dishes

Forceps

Wide-mouth moisture- and air-tight containers of 1- to 2-pint size (0.5-1 liter)

Constant-temperature box or room controlled at 70°-75° F. (21°-24° C.)

Analytical balance or pharmaceutical balance
250-500 mg. of each compound to be evaluated

Highly-volatile solvent such as acetone, ether, or ethanol

Compounds known to have a relatively high degree of vapor activity, such as isopropyl ester of 2,4-dichlorophenoxyacetic acid and isopropyl N-(3-chlorophenyl) carbamate for standard treatment

Preparation and selection of plant material

Germinate seeds in the large Petri dish containing moistened filter paper at a temperature of about 70°-75° F. (21°-24° C.). When the seed coat has cracked and the tip of the radical is visible through this opening, select 5 seeds at a uniform stage of germination. Place these on moistened filter paper in the small Petri dish and then place the dish in the air-tight container. In the case of cucumber and buckwheat seeds, this stage is reached 17 and 20 hours, respectively, after placing the seeds in moist Petri dishes.

Method of conducting test

Impregnate a disc of filter paper (5 cm. diam-

eter) with 50 mg. of the chemical to be tested, dissolved in highly volatile solvent. After evaporating the solvent, place the filter paper in the air-tight container in such a manner as to avoid direct contact with the Petri dish containing the germinating seeds. Close the container tightly and place it in the constant-temperature box along with others for the same test. The period of exposure may vary from a few to 48 hours or more, depending upon the type of response to be measured and the relative rates of evaporation of the chemicals.

At the end of the designated period, remove the Petri dishes from the containers, cover them, and allow the seedlings to grow for several more days; observe variations in growth caused by the treatment.

Method of taking results

Make visual comparisons of the response of the seedlings, noting increase or reduction in length of the root hair zone, and the relative number of hairs, increase or decrease of branching along the tap root, and the relative lengths of the tap and branch roots; also abnormalities occurring in the growth of the hypocotyl.

Suggested standard treatment for comparison

Compounds known to have a relatively high degree of vapor activity, such as isopropyl ester of 2,4-dichlorophenoxyacetic acid and isopropyl N-(3-chlorophenyl) carbamate.

Unpublished method submitted by W. P. Anderson, American Chemical Paint Company, Ambler, Pa.

Detection of Regulators in Water**ROOT GROWTH TEST****Method based on research by:**

D. Ready and V. O. Grant.

Description of test and some other uses

Small amounts of 2,4-dichlorophenoxyacetic acid in aqueous solutions are detected by the inhibiting effect on growth of primary roots of cucumber.

This test may also be adapted for the detection of other compounds in water solution provided the roots of the test plant used are suffi-

ciently sensitive to the chemical being tested.

Suggested plant material

Cucumber seeds (*Cucumis sativus* L.).

Apparatus, chemicals, and other materials

Petri dishes 6 in. (15 cm.) in diameter

Filter paper

Analytical balance or pharmaceutical balance
Darkened constant-temperature room maintained at about 82° F. (28° C.)

1 g. of 2,4-dichlorophenoxyacetic acid for standard treatment

Distilled water

Preparation and selection of plant material

Use seeds having a high percentage of germination; sort them, and discard those that are defective.

Method of conducting test

Place 15 cucumber seeds on filter paper in the bottom of each Petri dish. Using 5 Petri dishes for each concentration level of each solution to be tested, moisten the paper in each dish with 15 ml. of the test solution. A range of 15 concentrations varying in uniform steps from 0.001 p.p.m. to 10 p.p.m. is suggested. Cover the Petri dishes and place them in a darkened constant-temperature room maintained at about 82° F. (28° C.). Prepare 5 dishes using distilled water instead of the test solution and maintain in the same manner for controls.

Method of taking results

After 96 hours, measure the length of the primary root of each seedling in each dish to the nearest millimeter. Compare these root measurements with those of the control seedlings and the seedlings treated with a known range of concentrations of 2,4-dichlorophenoxyacetic acid. The amount of reduction in length of the primary roots indicates the amount of the plant regulator in the test solution.

Suggested standard treatment for comparison

2,4-Dichlorophenoxyacetic acid at concentrations of 0.001, 0.005, 0.025, 0.050, 0.075, 0.10, 0.25, 0.50, 0.75, 1, 2.5, 5, 7.5, and 10 p.p.m.

Ready, D. and Grant, V. O. A Rapid Sensitive Method for Determination of Low Concentrations of 2,4-Dichlorophenoxyacetic Acid in Aqueous Solution. *Bot. Gaz.* 99: 39-44. 1947.

PART III. TECHNIQUES IN GENERAL USE

Some of the techniques developed by various scientists are of general use in studying the responses of plants to regulating chemicals. Several of these techniques are described on the following pages with the belief that they may be of particular interest to those who are undertaking work with regulating chemicals for the first time. No attempt is made to credit authors of these techniques since these methods are widely known and used, and because of the difficulty of determining the exact origin of each technique.



FIGURE 9.

(NEG. PN-300)

Lanolin as a carrier for regulating chemicals

Lanolin is widely used as a carrier for regulating chemicals (fig. 9). Applied in small amounts, this semi-solid, fatty substance does not injure plants. It makes close contact with the plant surface, and regulators, when mixed with lanolin, diffuse readily from a thin layer

of the paste into the plant. Place a weighed amount of lanolin in a vial and immerse the vial in warm water to melt the lanolin. Weigh out the finely powdered or liquid regulator and stir it thoroughly into the lanolin until the mixture becomes semi-solid. Apply the mixture to stems or leaves with a toothpick or other disposable applicator.

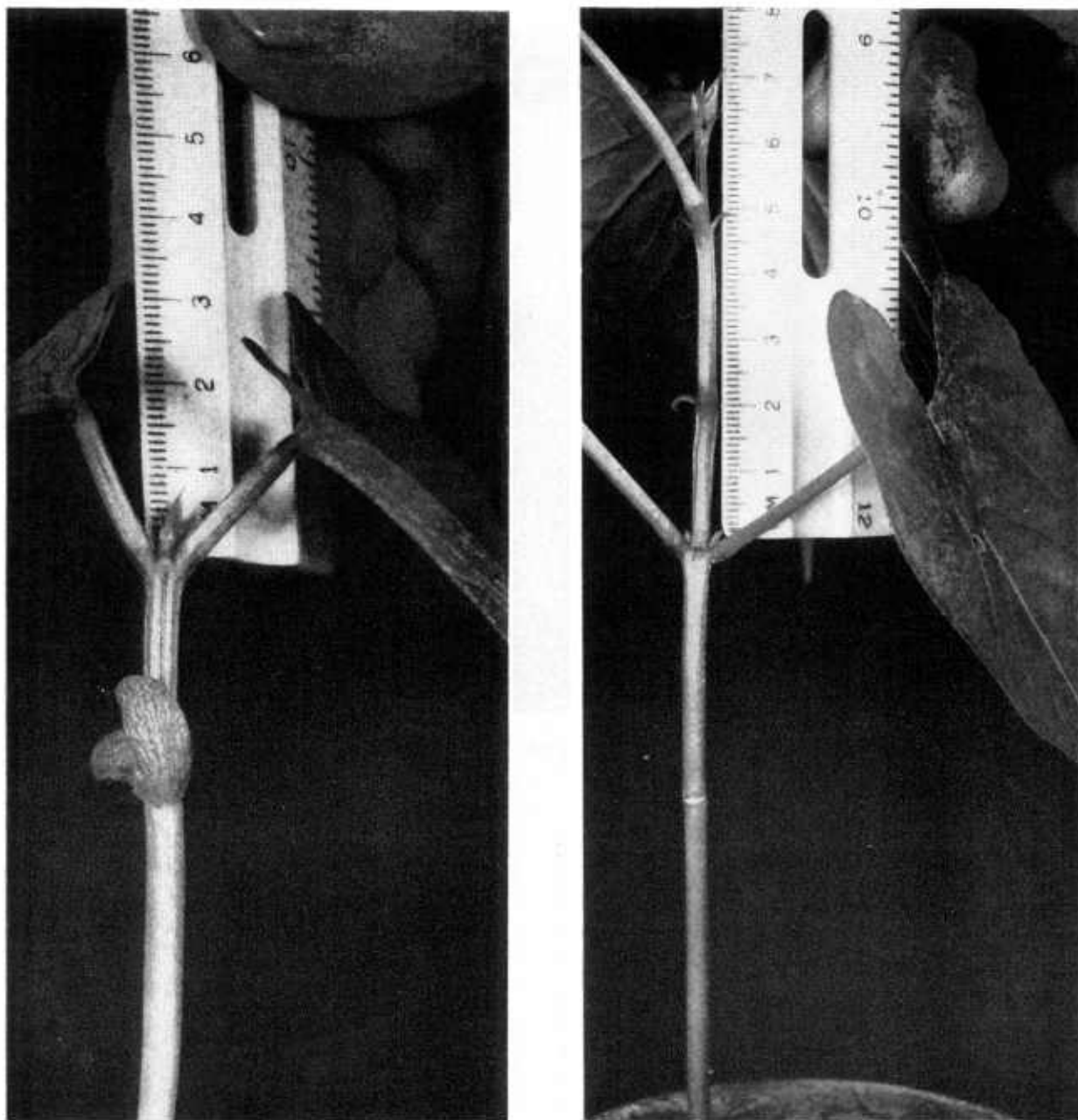


FIGURE 10.

(NEG. PN 308 A, B)

Terminal growth measurements

Many regulating chemicals cause an increase or a decrease in the rate of growth of the main stems of plants. The effect of a compound on the length of the stem is therefore one means of evaluating the regulating properties of the chemical.

Apply the chemical in a suitable carrier (lanolin or aqueous mixture) to bean plants, for

example, that have developed primary leaves which are partially expanded (3.5–5 cm. across) and when the trifoliate leaves are still folded in the terminal bud (fig. 10, left). Measure the length of the terminal bud from second node to tip of bud at the time of treatment. Measure from the same node (second) to the tip of the terminal bud about 1 week after the treatment (fig. 10, right), and compare growth with comparable measurements of untreated plants.



FIGURE 11.

(NEG. PN-302)

Convenient way to measure effect of regulating chemicals on root growth

Place a disc of blotting paper^{*} impregnated with a water mixture of the regulating chemical and another impregnated with tap or distilled water in separate Petri dishes. Place several cucumber seeds of uniform size on the surface of each paper. Store the closed dishes at room temperature in darkness for 24 hours; then measure growth of the roots repeatedly during the following 2 or 3 days with the dishes closed (fig. 11). Compare growth of the roots on the chemically treated papers with that of roots on the papers impregnated with water.

A device that delivers measured amounts of a regulating chemical

This microinjector delivers measured vol-

umes of lanolin paste containing a regulating chemical (fig. 12, above). Prepare a mixture of lanolin and the regulating chemical. While the mixture is melted, draw the syringe about one-third full; then allow the mixture in the syringe to solidify. Fasten the syringe in the clamp and operate the lever until a uniform amount of the paste is delivered with each stroke. Collect portions of desired volume (regulated by the number of strokes used) on the end of a toothpick or other disposable applicator (fig. 12, below) and apply the paste quantitatively to leaves or stems. Determine the weight of paste delivered per stroke by first weighing a piece of aluminum foil, then collecting on the foil the amount of paste ejected after one stroke, and reweighing the paste and foil. Repeat the procedure 5 to 10 times; then calculate the average number of micrograms of growth-regulating chemical delivered per stroke.

Construction of this microinjector is described by C. C. Roan and Shizuko Maeda in

^{*} Ordinary blotting papers often contain chemicals which inhibit root growth. Blotting paper especially prepared for seed tests such as that described can be obtained from the Standard Paper Manufacturing Company, Richmond, Va.

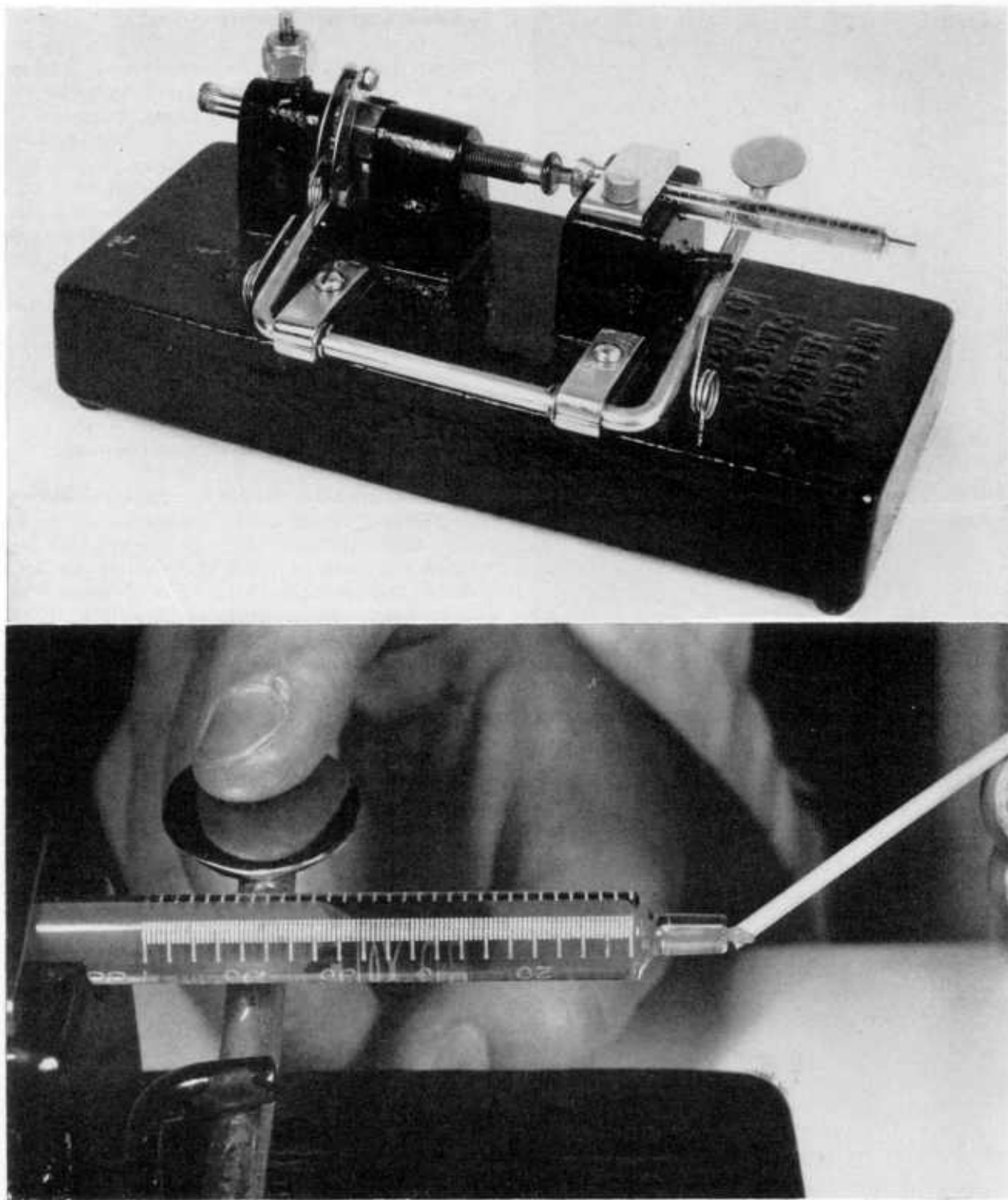


FIGURE 12.

(NEG. PN-303 A, B)

"A Microdevice for Rapid Application of Toxicants to Individual Insects," publication No. ET-306 of the former Bureau of Entomology

and Plant Quarantine, United States Department of Agriculture, 1953.



FIGURE 13.

(NEG. PN-309)

Simulated spray-droplets

Some investigators use a small glass loop to place droplets of relatively uniform size on leaves. With the aid of a flame, draw out a glass rod so that it forms a thin thread about 0.5–0.75 mm. in diameter. Break the thread of glass and with a very little heat bend the end of the small rod so that a loop 2–3 mm. in diameter is formed. Dip the loop into an aqueous mixture of the regulating chemical. Transfer the liquid that adheres to the loop onto the surface of a leaf by touching the loop to the desired area (fig. 13). Droplets of different size and various patterns of distribution can be obtained to simulate spray-droplets.

Formative effects of regulating chemicals

Some regulating compounds cause plants to change their usual pattern of growth, especially their pattern of leaf growth. Very minute amounts of such compounds as 2,4-dichlorophenoxyacetic acid on such plants as cotton, bean, and grape often result in relatively small leaves with narrow curled blades and sometimes enlarged veins (fig. 14; leaf from treated plant, right).

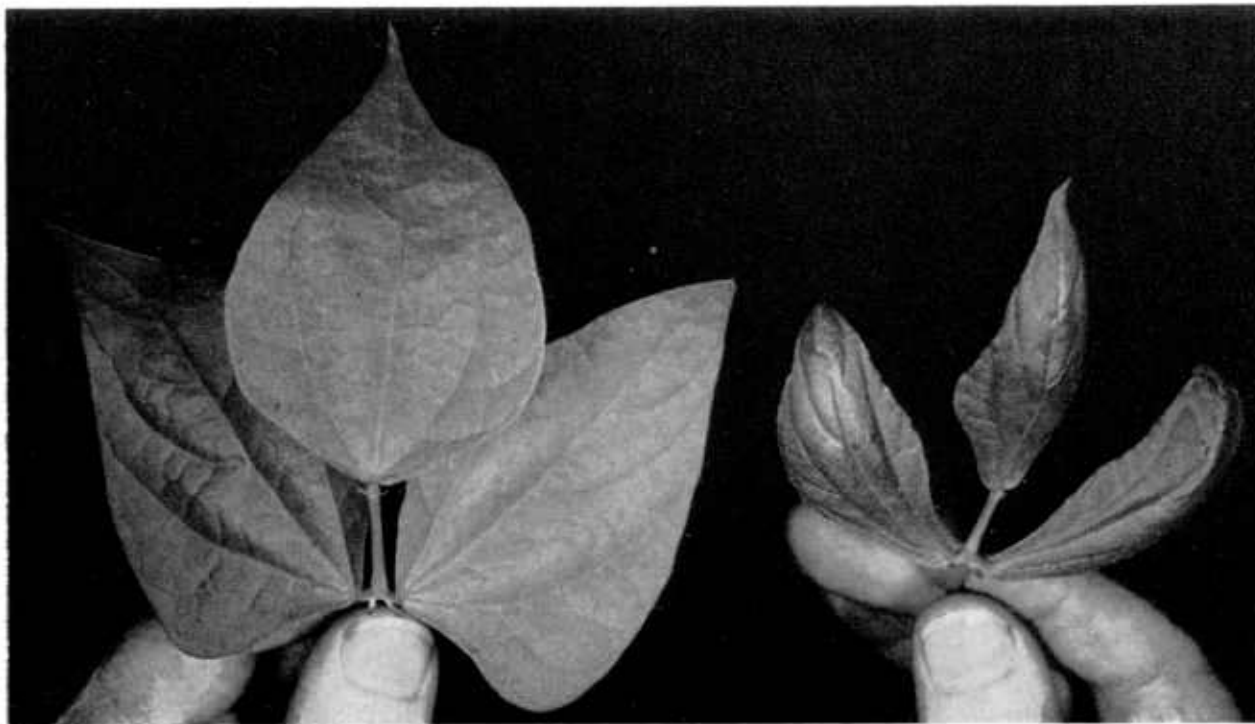


FIGURE 14.

(NEG. PN-305)

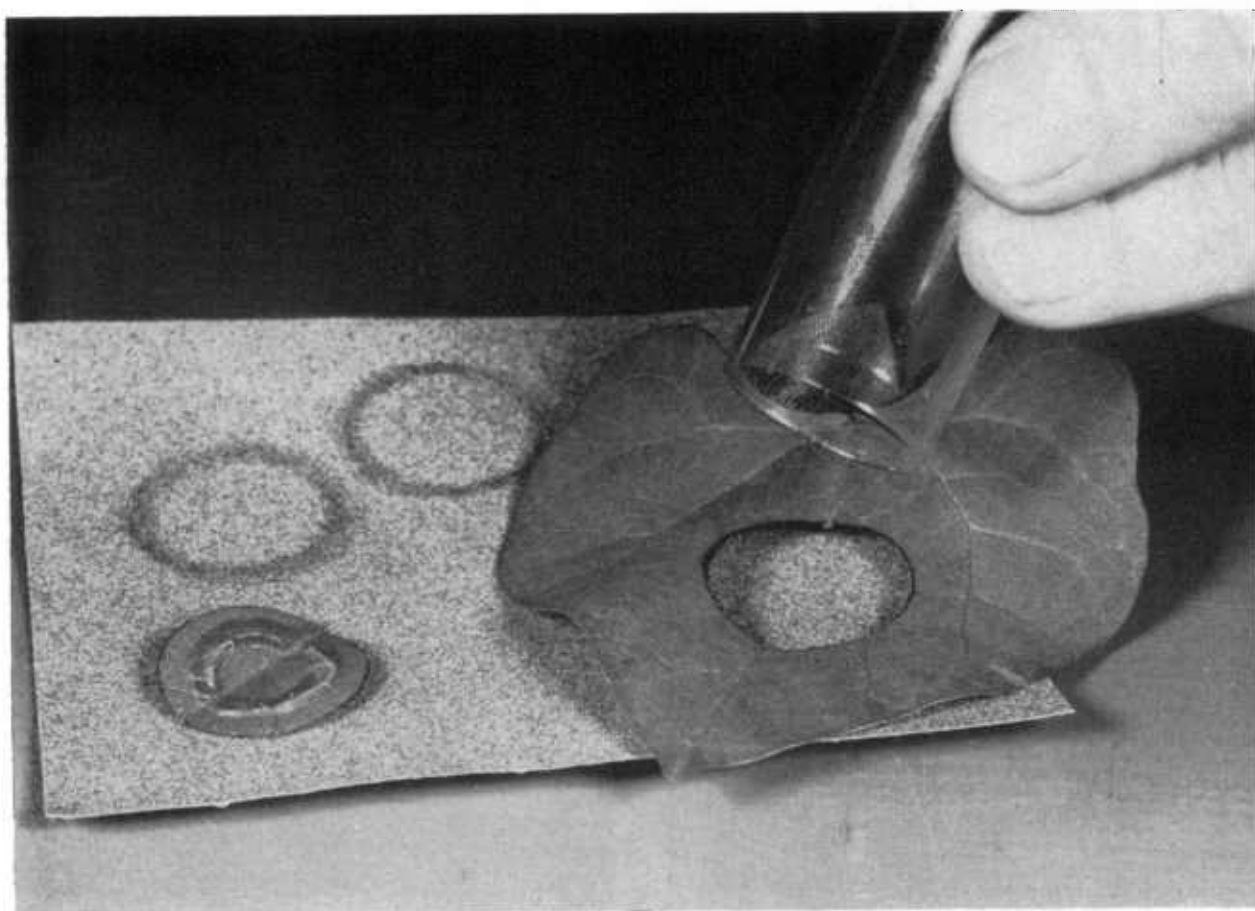


FIGURE 15.

(NEG. PN-307)

Simplified method of separating areas of leaves treated with a regulating chemical from the remaining untreated portions of the leaf

In studying the movement of radioactively tagged regulating chemicals in leaves, it is often desirable to remove the treated portion of a leaf so that the uncontaminated portion which remains can be assayed for radioactivity. The following simplified technique is useful since the equipment required is inexpensive and, if accidentally contaminated, it can be discarded, thus minimizing the danger of contaminating untreated portions of the leaf. Place the detached leaf on a piece of sandpaper (C weight,

3/0-120) or emery cloth. Place the open end of a vial over the treated portion of the leaf in such a way that the inside edge of the vial does not come in contact with the radioactive material on the leaf. Turn the vial back and forth with slight pressure and thus cut out and remove the treated area of the leaf (fig. 15). Treated portions of several leaves can be removed without contamination of untreated portions by using only 1 vial and 1 piece of abrasive material and then discarding the equipment.

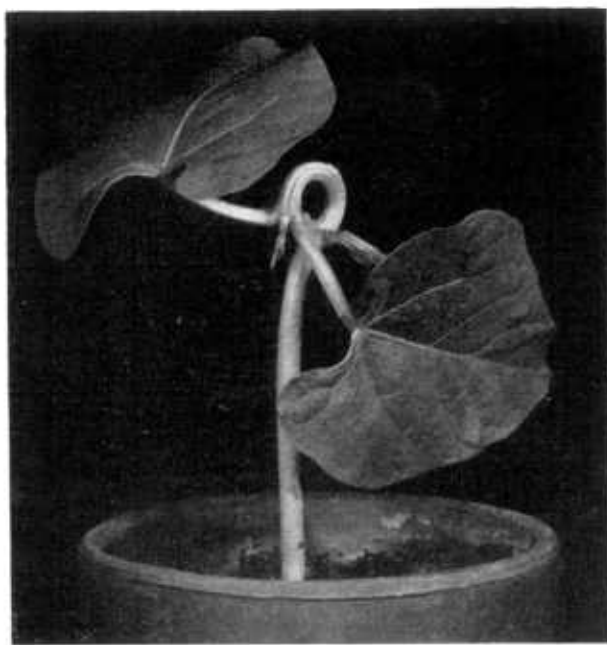


FIGURE 16. (NEG. PN-301 A, B)

Highly sensitive test plants

Some plants respond quickly to regulating chemicals. Young rapidly growing plants are generally more sensitive to these chemicals than are more mature plants. Young bean, cucumber, and sunflower plants are among the more

sensitive, and these are especially useful since they can be quickly and easily grown to a size that is useful for tests with regulators. Plant the seeds in composted soil; keep the soil uniformly moist and at a temperature of not less than 80° F. (about 26° C.) until the seedlings appear above the surface of the soil; then reduce the night temperature to 70°–75° F. (about 21°–24° C.). About 5 to 8 days are usually required for such plants to grow to a useful size. Application of the regulator in a suitable carrier, such as lanolin, to the stem or leaves of these test plants (fig. 16, above) will often induce a marked growth response within a few hours or during the following over-night period (fig. 16, below).



FIGURE 17. (NEG. PN-304)

An easy method of impregnating dust carriers with regulating chemicals

Weigh the desired amount of carrier (diatomaceous earth or other finely ground inert material) and place in a container. Dissolve the required amount of regulating chemical in a sufficient amount of ethyl alcohol to make a slurry when combined with the dust carrier. Combine the alcoholic solution with the dust (fig. 17). Stir the slurry thoroughly and allow the alcohol to evaporate at room temperature. Mix the impregnated dust thoroughly before it is applied to plants.

Useful concentrations of regulating chemicals in dust carriers often range from 500 to 5000 parts of the chemical in 1,000,000 parts of carrier. Effectiveness of some regulators applied in talc dust has been slightly enhanced experimentally by the addition of small amounts of hygroscopic agents, such as glycerine, to the alcoholic mixture, and its final incorporation in the dust preparation. The amount of hygroscopic agent used should not be so great as to interfere with the dustability of the preparation.

Application of a measured amount of regulating chemical to a known area of leaf surface

It is sometimes necessary to apply measured amounts of a regulating chemical to a limited area on a leaf, especially when radioactively tagged regulators are used. Slip a short piece of rubber tubing of appropriate diameter over the end of a cork borer of suitable size, leaving about 1 cm. of tubing extending beyond the end of the cork borer. Calculate the inside cross-sectional area of the rubber tube. Spread lanolin over the surface of a glass plate to the depth of about 1–2 mm. and press the end of the rubber tubing in the lanolin layer; then gently stamp a ring of the lanolin on the leaf surface (fig. 18, above). Fill a 0.1 ml. pipette (graduated in 0.01 ml. portions) with the aqueous mixture of the regulating chemical to be used, touch the tip of the pipette against a piece of glass or other hard surface to remove excess mixture, then allow 0.01 ml. of the mixture to drain from the pipette so that the measured volume remains suspended at the end of the pipette. Touch the end of the pipette to the area enclosed by the lanolin ring and move the tip gently over the area until the measured volume of liquid is spread evenly over the enclosed surface (fig. 18, below). Aqueous mixtures of regulating chemicals spread over leaf surfaces most readily when a surfactant such as Tween 20 or Triton X 100 is present in the mixture at a concentration of 0.05 or 0.1 percent.

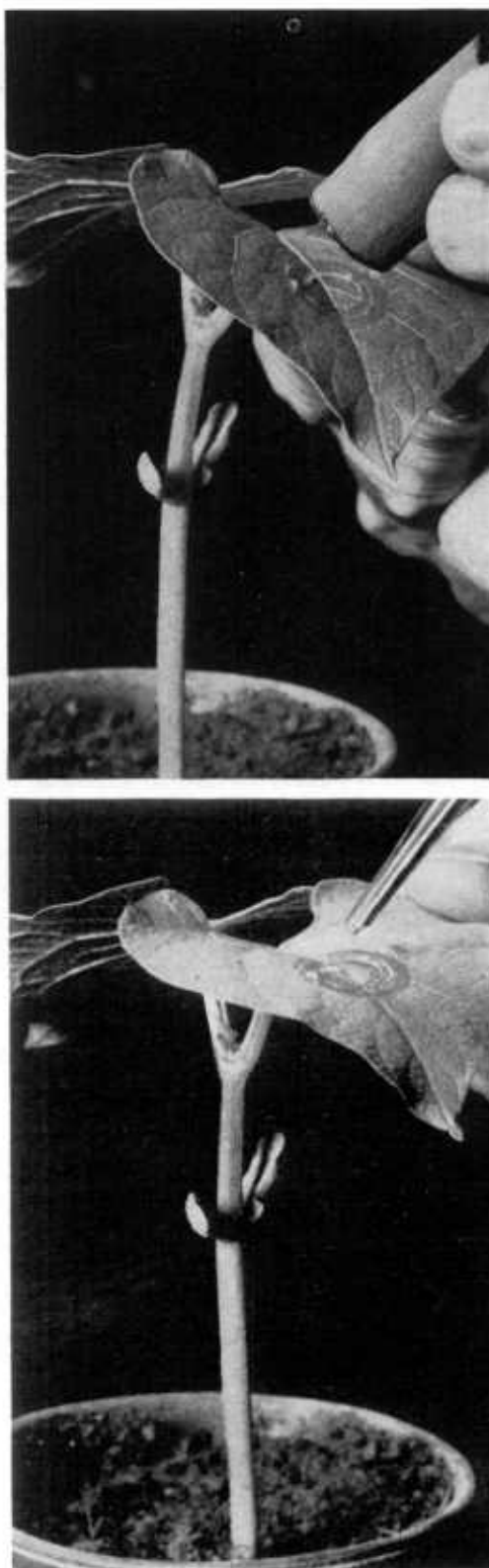


FIGURE 18.

(NEG. PN-306 A. 11)

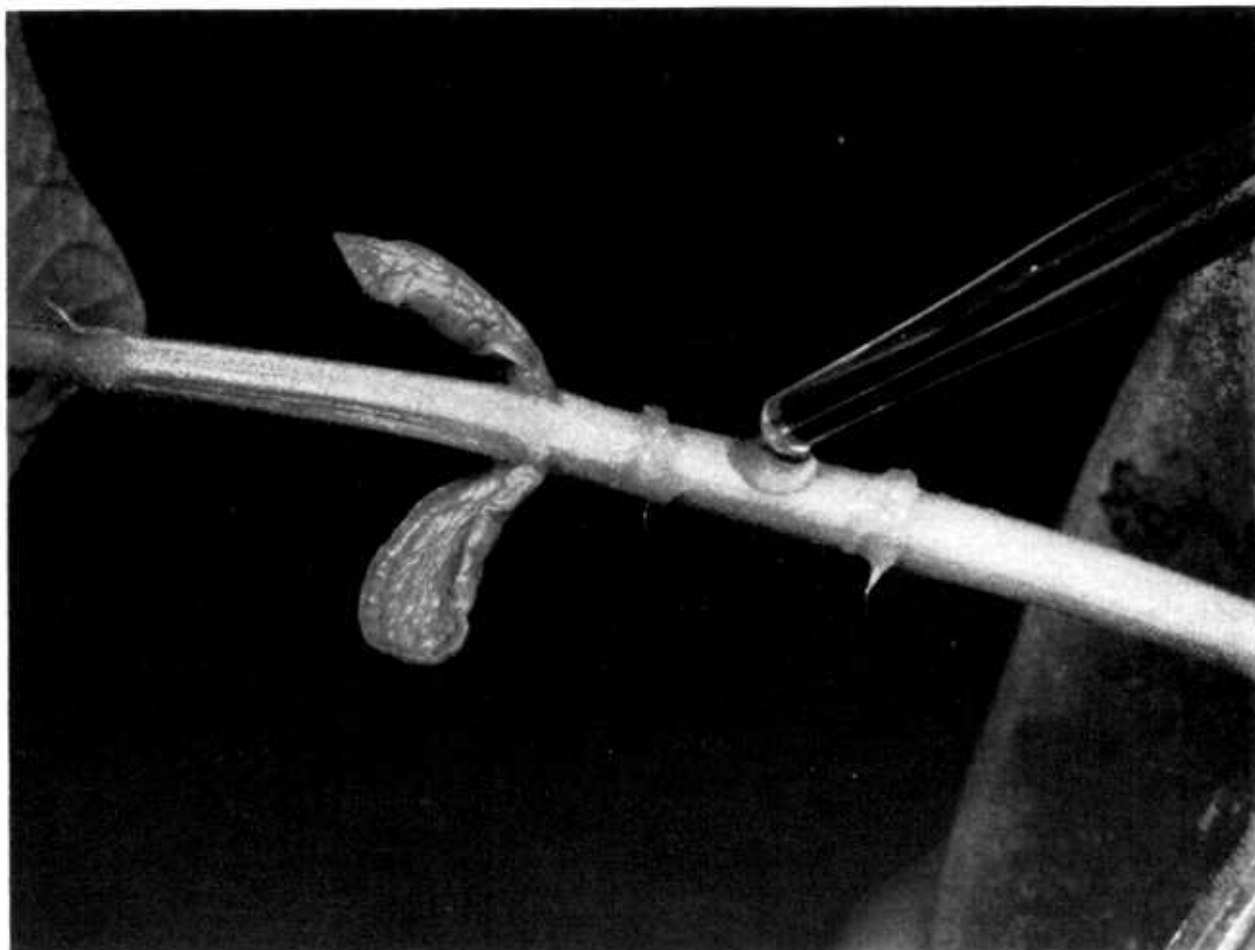


FIGURE 19.

(NEG. PN-370)

Convenient method of applying aqueous mixtures of regulating chemicals quantitatively to stems

Apply two narrow lanolin bands about 1 to 2 cm. apart and extending around the stem of the selected plant. Allow 0.01 ml. of the aqueous mixture containing the regulating chemical to flow from a 0.1 ml. pipette so that the measured drop clings to the tip of the pipette. Hold

the stem in a horizontal position and transfer the drop from the pipette to the stem surface between the lanolin bands (fig. 19). Revolve the plant and spread the mixture over the surface between the lanolin bands with the tip of the pipette. Keep the stem in a horizontal position and revolve the plant at frequent intervals so that the liquid remains evenly spread over the surface while it dries. Return the plant to an upright position and record growth effects that develop.

SUPPLEMENTARY INFORMATION

Sources of Plant-Regulating Compounds

Compounds mentioned in this compilation can be obtained from chemical supply houses such as the following:

Eastman Kodak Co.,
Chemical Division,
Rochester 4, N. Y.

E. H. Sargent Co.,
4647 West Foster Avenue,
Chicago 30, Ill.

Fisher Scientific Co.,
1458 North Lamon Avenue,
Chicago 51, Ill.

Will Corporation of Maryland,
5 North Haven Avenue,
Baltimore, Md.

Measures and Equivalents

WEIGHTS

- 1 kilogram (kg.) = 1,000 grams (g.) = 2.2 pounds (lb.)
- 1 gram (g.) = 1,000 milligrams (mg.) = 0.35 ounce avoirdupois (oz.)
- 1 milligram (mg.) = 1,000 micrograms (γ or gamma)
- 1 pound (lb.) = 16 ounces avoirdupois = 453.6 grams or 0.45 kilogram
- 1 ounce avoirdupois (oz.) = 28.35 grams
- 1 pint (pt.) of water weighs approximately 1 pound
- 1 gallon (gal.) of water weighs approximately 8.34 pounds

VOLUMES

- 1 liter (l.) = 1,000 milliliters (ml.) = 1.057 fluid quarts (qt.)
- 1 milliliter (ml.) or cubic centimeter (cc.) = 0.034 fluid ounce
- 1 milliliter or cubic centimeter of water weighs 1 gram
- 1 liter of water weighs 1 kilogram
- 1 gallon (gal.) = 4 quarts = 3.785 liters
- 1 quart (qt.) = 2 pints = 0.946 liter
- 1 pint (pt.) = 16 fluid ounces = 0.473 liter
- 1 fluid ounce (oz.) = 29.6 milliliters or cubic centimeters

- 1 gallon (gal.) = 16 cups = 128 fluid ounces
- 1 quart (qt.) = 4 cups = 32 fluid ounces = 64 tablespoons
- 1 pint (pt.) = 2 cups = 16 fluid ounces = 32 tablespoons
- 1 cup = 8 fluid ounces = 16 tablespoons = 48 teaspoons
- 1 fluid ounce (oz.) = 2 tablespoons = 6 teaspoons
- 1 tablespoon (tbsp.) = 3 teaspoons (tsp.)

While it is impracticable to provide a complete list of dealers, this partial list is furnished for your information, with the understanding that no discrimination is intended and no guarantee of reliability implied.

LENGTHS

- 1 mile (mi.) = 5,280 feet = 1.609 kilometers
- 1 rod (rd.) = 16.5 feet = 5.029 meters
- 1 yard (yd.) = 3 feet = 0.914 meter
- 1 foot (ft.) = 12 inches = 0.305 meter
- 1 inch (in.) = 2.540 centimeters
- 1 kilometer (km.) = 1,000 meters = 0.621 mile
- 1 meter (m.) = 100 centimeters = 3.281 feet
- 1 centimeter (cm.) = 10 millimeters = 0.394 inch
- 1 millimeter (mm.) = 0.039 inch = 1,000 microns
- 1 micron (μ .) = 1,000 millimicrons = 0.0000394 inch
- 1 millimicron (m μ .) = 10 Angström units

- 1 square kilometer (sq. km.) = 1,000,000 square meters = 0.386 square mile
- 1 square meter (sq. m.) = 10,000 square centimeters = 10.764 square feet
- 1 square centimeter (sq. cm.) = 100 square millimeters = 0.155 square inch
- 1 square millimeter (sq. mm.) = 0.002 square inch

AREAS

- 1 square mile (sq. mi.) = 640 acres = 2.590 square kilometers
- 1 acre (A.) = 160 square rods = 4046.873 square meters = 43,560 square feet
- 1 square rod (sq. rd.) = 30.25 square yards = 25.293 square meters
- 1 square yard (sq. yd.) = 9 square feet = 0.836 square meter
- 1 square foot (sq. ft.) = 144 square inches = 0.93 square meter
- 1 square inch (sq. in.) = 6.452 square centimeters

Temperature Conversions

Short-cut method of converting from Centigrade to Fahrenheit degrees

Double the Centigrade reading, subtract 10% of this value, and add 32.⁹

Conventional method of converting from Centigrade to Fahrenheit degrees

Multiply the Centigrade reading by nine-fifths and add 32.

$$(^{\circ}\text{C.} \times 9/5) + 32 = ^{\circ}\text{F.}$$

Conventional method of converting from Fahrenheit to Centigrade degrees

Subtract 32 from the Fahrenheit reading and multiply by five-ninths.

$$(^{\circ}\text{F.} - 32) \times 5/9 = ^{\circ}\text{C.}$$

Methods of Making Nutrient Solutions

If plants are to be grown only for a period of 2 to 3 weeks in nutrient solution, a suitable nutrient can be prepared by adding only macro-nutrients to tap water. If the plants are to be grown for a longer period, however, or if distilled water is used, it is generally necessary to add micro-nutrients.

Nutrient solution made with tap water

Prepare a 1/2-molar stock solution of each of the following macro-nutrients in separate containers using tap water:

KH_2PO_4 (68.07 g. per liter)
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (123.25 g. per liter)
 $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (109.54 g. per liter)
 NaNO_3 (42.50 g. per liter)

To make the nutrient solution, add the macro-nutrients from the stock solutions in the following amounts to 50 liters of tap water:

156 ml. of 1/2-molar solution of KH_2PO_4
 581 ml. of 1/2-molar solution of MgSO_4
 • $7\text{H}_2\text{O}$
 306 ml. of 1/2-molar solution of CaCl_2
 • $6\text{H}_2\text{O}$
 375 ml. of 1/2-molar solution of NaNO_3

Nutrient solution made with distilled water

Prepare a 1/2-molar stock solution of each of the following macro-nutrients in separate containers using distilled water:

KH_2PO_4 (68.07 g. per liter)
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (123.25 g. per liter)
 $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (109.54 g. per liter)
 NaNO_3 (42.50 g. per liter)

In preparing the macro-nutrient solution, add the macro-nutrient from the stock solutions in the following amounts to 50 liters of distilled water:

156 ml. of 1/2-molar solution of KH_2PO_4
 581 ml. of 1/2-molar solution of MgSO_4
 • $7\text{H}_2\text{O}$
 306 ml. of 1/2-molar solution of CaCl_2
 • $6\text{H}_2\text{O}$
 375 ml. of 1/2-molar solution of NaNO_3

Prepare micro-nutrient stock solutions:

A. Add the following micro-nutrients to 2 liters of distilled water:

5.72 g. of H_3BO_4
 3.62 g. of MnCl_2
 0.44 g. of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
 0.16 g. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
 0.18 g. of H_2MoO_4

Add 1 cc. of the micro-nutrient stock solution per liter of prepared macro-nutrient solution.

B. Add 1 ml. of 0.5% ferric citrate per liter of macro-nutrient solution.

⁹ Reprinted by permission from Chemical and Engineering News, Nov. 21, 1949.

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Compounds are applied in a paste carrier to stems of young plants, and the effect on their subsequent growth and development is observed	24		
Underground growth test		Root growth	
Plants are grown in soil in boxes constructed with glass sides in order to observe the effects of chemicals on growth and development of the underground parts without disturbing the plants	25	Agar slant test	
		Seeds of rape and oats are germinated on agar slants containing a test chemical, and the root growth studied	37
Fruit set		Cone container test	
Aerosol method		Seeds of barley or other grass plants are dipped in dilute concentrations of a regulating chemical and germinated in soil held in cone-shaped containers. Growth of roots and other parts of the plants is studied in detail by removing the containers and washing away the soil from some of the plants at intervals as the plants mature	38
Regulating or other chemicals are applied to plants or plant parts in the form of mists produced by use of liquefied gases	26	Impregnated-filter-paper test	
Grape test		Root growth of germinating seeds on filter paper treated with the test chemicals is observed	39
Various compounds may be evaluated as growth regulators by dipping individual clusters of grape flowers or the immature fruits in solutions of the compound and recording improvement in fruit set and size	28	Nutrient mist test	
Tomato test		Trees are grown with their roots in a closed chamber containing air filled with a fine mist of nutrient solution in which the chemical being tested has been dissolved or suspended, and effects of the regulating chemical on growth of the roots are observed	40
Compounds are applied in liquid or paste carriers to flower clusters of greenhouse plants and the effectiveness of the substances in preventing flower or fruit drop is evaluated	29	Root induction	
Fruit size		Stem-cutting test	
Grape test		Cuttings are soaked, dusted, or dipped in mixtures containing chemicals, and the effect on root initiation and growth determined	41
Various compounds may be evaluated as growth regulators by dipping individual clusters of grape flowers or the immature fruits in solutions of the compound and recording improvement in fruit size	30	Seed formation	
Fruit thinning		Lanolin paste test	
Apple blossom test		A mixture of lanolin and a regulator is applied to a wound on the petal base or on the pedicel, and the effect on seed development is observed	43
By applying regulating chemicals to selected limbs of trees when in blossom, the compounds are evaluated as a means of thinning the fruit	31	Translocation of radioactively tagged regulators	
Peach and prune fruit test		Isotope counter test	
A method of evaluating the fruit-thinning effects of chemicals applied as sprays to limbs or entire trees is described	32	Translocation of radioactively tagged molecules is measured by placing a tagged compound on one part of a plant and measuring radio activity that results from the movement of the compound, metabolites, or degradation products of it to other parts of the plant	49
Growth inhibition or stimulation		Radioautograph test	
Vegetative sprout test		Radioactive 2,4-dichlorophenoxyacetic acid is placed on a leaf of a plant, the plant later dried and stored adjacent to an X-ray film, which is then developed. The autographic outline thus obtained is registered with an outline of the plant on Ozalid paper and the presence of the radioactivity in different parts of the plant observed	50
Pieces of carrots, potato tubers, and small onions are dipped in a solution of the test compound and placed in darkness. Measurement of sprouts indicates the effectiveness of the compound as a sprout inhibitor	33	Volatility of plant regulators	
Growth modification (aquatic plant)		Disposable container test	
Sterile nutrient test		This test is useful for detecting volatility of plant-regulating compounds that induce easily detectable growth responses. It can be used to detect or measure evaporation of regulating chemicals from surfaces of plants previously treated with these compounds or from surfaces of other materials to which these substances have been applied	51
<i>Lemna minor</i> L. (duckweed) is grown under aseptic and rigidly controlled conditions in a nutrient solution. Regulating chemicals (including antibiotics) are added to the nutrient, and their effect on growth of the plants studied	35	Germinating seed test	
Water culture test		Relative volatility of regulating compounds is determined by exposing germinating seeds to vapors of these chemicals in closed containers	52
An aquatic plant (<i>Lemna minor</i> L.) is grown in water containing a regulating compound, and the growth-modifying effect of the chemical observed	36	Water contaminated with regulator	
Growth regulation		Root-growth test	
Field test		Small amounts of 2,4-dichlorophenoxyacetic acid in aqueous solutions are detected by the inhibiting effect on growth of primary roots of cucumber	53
Test chemicals are sprayed on soil in which seeds have been planted, or on leaves and stems of plants growing under field conditions, and their effect on growth and productivity of the plants is measured	46		
Penetration of soil by regulators			
Seed test			
Chemicals are applied to the surface of soil contained in glass tubes. Soil samples are collected as thin layers from various depths and bioassayed for the presence of the chemical	46		
Residue of regulators in soil			
Repeated plant test			