older racemes there were empty pollen sacs and pollen could be squeezed from others. The fourth series (17 August), at the time pollen shed was starting, showed the same general symptoms by 28 August as the third treatment except that the proportion of surviving pollen sacs and the extent of shedding was greater. Though the third and fourth treatments clearly caused reduction in the amount of pollen shed, they were too late to prevent it completely. That the pollen shed from these late treatments was not altogether normal was indicated by their vacuolate condition, similar to the effect of 2,4-D on bindweed pollen (*6*). No test was made, however, of their allergenic properties.

These experiments indicated that the safest procedure would be to treat plants before the involucres are open. Limited trials with lower concentrations and more extensive experience with other weeds have indicated that concentrations lower than 1,000 ppm might be equally effective in stopping development of the flowering parts, though the response would probably be slower.

A preliminary trial was also made of a new method of treatment which shows great practical possibilities in the large-scale application of chemical agents to plants. On 6 August a test of 2,4-D with a fog machine[2] was made, in which a 1-per cent solution in SAE 30 oil was "fogged" across an area of mixed weeds at the rate of about 1 to 2 gallons per acre from the back of a truck moving 3 or 4 miles per hour. Despite unfavorable atmospheric conditions, cool and damp with updrafts, telemorphic effects were visible on ragweed up to 100 feet from the machine within 24 hours. A week later severe symptoms were found throughout the first 50 feet, and many plants were already dead in the first 10 feet. The response was at least as rapid and apparently as effective in preventing pollen shed as the water spray. Because of the possible advantage of more highly oil-soluble compounds in fog application, the ethyl ester of 2,4-D was prepared and tested on a small scale. Dimethyl ether aerosols gave responses on ragweed similar to 2,4-D.

### Conclusion

Water sprays of 2,4-D at 1,000 ppm at sufficiently early stages of flower development were shown to be effective in preventing pollen shed, though further work may show that lower concentrations or rates of application would be sufficient. A preliminary trial with a fog machine showed excellent possibilities, and with further experiments to determine the best carrier, concentration, and technic of fog application it promises to be a more economical method than sprays. Apart from any differences in amounts of 2,4-D required in the two methods, the fog machine would have the advantages (1) of avoiding the large volumes of water necessary in spray methods, (2) of swifter application, and (3) of requiring considerably lower cost in equipment. This new technic of application, combined with relatively low cost, favorable selectivity of herbicidal action (*3*) and low toxicity (*5*), make 2,4-D a promising herbicide for ragweed control. It is hoped that these experiments will encourage others to test 2,4-D on a larger scale.

[2] Made available through the courtesy of the Todd Shipyards Corporation.

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## Rapid Estimation of the Phytocidal Action of Chemicals[1]


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Recent reports on the herbicidal action of substituted phenoxy compounds (*1, 3, 5*)[2] have stimulated investigations of organic compounds which are essentially new to workers doing research on weed-control problems. The large number of organic compounds which might legitimately be selected for test as herbicides, together with the high cost and limited supplies of many of these chemicals, require that preliminary tests of their toxicity be simple, rapid, and economical. The utilization of *Lemna minor* L. (smaller duckweed) as a test plant fulfills most of these requirements.

The procedures here proposed should provide direct evidence of the effectiveness of chemicals for the destruction of aquatic plants—a matter of widespread interest and of economic importance. Tests with duckweed, however, will not necessarily indicate the magnitude of the toxic action of an unproved herbicide on a terrestrial plant. For investigators concerned with

[1] Work conducted for the purpose of improving methods for the chemical eradication of *Ribes* to control white-pine blister rust, *Cronartium ribicola* Fisher. Greenhouse and laboratory facilities, used by the Bureau of Entomology and Plant Quarantine at the University of California, Berkeley, are maintained in cooperation with the College of Agriculture through its Department of Forestry.

[2] The following features of 2,4-dichlorophenoxyacetic acid, a substituted phenoxy compound, differentiate it from compounds previously recognized as effective herbicides: (1) very low solubility in water, (2) telemorphic action, (3) effective killing action of small dosages, and (4) selectivity of toxic action. Data on the action of 2,4-dichlorophenoxyacetic acid as a differential herbicide are given by P. C. Marth and J. W. Mitchell (*Bot. Gaz.*, 1944, **106**, 224–232).

terrestrial plants, the proposed tests should provide useful data on the comparative phytocidal action of a group of new chemicals and, in particular, should indicate the relative toxicity of low concentrations of new and proved herbicides. In insecticidal as well as herbicidal investigations, there is considerable interest in recognition of the phytocidal action of low concentrations of chemicals. Data from tests with duckweed should facilitate evaluation of potential foliage damage by new chemicals, once preliminary standards have been established by which correlations can be made between reactions of duckweed and the particular foliage or plant under consideration. Tests with duckweed, or any other test plant in the laboratory or greenhouse, should be considered as preliminary to final evaluation of the herbicide under field conditions, and for the particular weed under investigation.

Aquatic plants such as *Nitella, Valonia, Chlorella,* and *Lemna* have been used extensively as test plants for many types of physiologic studies. *Nitella clavata* (Bertero) A. Br. has been used specifically to study the toxic action of the herbicide, sodium chlorate (*6*), and *Lemna minor* for investigations of the herbicidal properties of the oxyhalogens (*4*). More recently, growth stimulation by low concentrations of ammonium sulfamate (*2*) has been shown by the rate of increase in numbers of *Lemna minor.*

*Lemna minor,* a monocotyledon, is a chlorophyllose, floating aquatic plant which reproduces aggressively by vegetative means. The upper surface of its leaf-like stems, or fronds, is uniformly bright green. Large numbers of test plants can be maintained in small space in simple, inexpensive containers. Plants do well in dilute nutrient solution at a pH of about 5–6 or in tap water over a small amount of sandy soil, and can be grown readily at all times of the year. For ease of handling and convenience of observation, *Lemna minor* has recently been used in this laboratory in preference to other plants for rapid estimation of the toxic action of expensive chemicals. *Spirodela polyrhiza* was used for the same purpose, but the normal variegated color of its fronds interfered with observation of early symptoms of injury.

In preliminary tests of new herbicides made by the writer during an 18-month period, the rate and character of damage to *Lemna minor* were used as measures of toxicity. Four different, clearly detectable, and replicable reactions of *Lemna minor* were observed in the course of toxicity tests with herbicides in concentrations not immediately destructive to the tender plants. These reactions may be described as follows:

(a) Damage progresses steadily, as shown by chlorosis and discoloration of fronds. The end result is a generally uniform discoloration of the fronds. Plants are not split into components or frond combinations smaller than those originally present. The typical color of injured fronds is reddish brown to pale yellow or albino. There is no change in the total area of the fronds.

(b) Plants divide to form single or double fronds before discoloration becomes noticeable. Subsequent chlorosis takes place slowly, but uniformly, on all fronds. The end result is a generally uniform discoloration of fronds. Most or all of the plants are split into single fronds. The typical color of injured plants is a pale or yellowish green. There is no change in the total area of the fronds.

(c) Damage is slow and varies among the test plants. Single fronds break from the parent plant, and these separated fronds may be damaged while the fronds of the parent plant remain fairly green and normal in appearance. The end result is an uneven discoloration of fronds. There is no apparent change in total area of the fronds.

(d) Plants divide into single or double fronds without evidence of injury. Some stimulation of growth and regeneration is evident from the increase in the number and the total (estimated) area of the fronds. Fronds remain dark green in color and healthy until bacterial action, lack of nutrient, or protracted contact with the chemical solution causes injury.

In comparing the toxicity of an organic compound, such as 2,4-dichlorophenoxyacetic acid, with a series of its homologues or analogues, the following procedure is recommended: Prepare standard test solutions of the 2,4-D which provide a range of concentration from that which is rapidly toxic (1,000 ppm damages within a few hours) to that which is relatively nontoxic (10 ppm exerts no significant damage for several days). Prepare solutions of the new herbicides in concentration equivalent to the standard. Measure 50 ml. of the several test solutions into Petri dishes. Three replications are adequate for most purposes. Place the same number (10 or more) of duckweed plants in each test solution (including a control of tap water or a dilute nutrient solution of conventional composition), taking care to select vigorous, healthy plants of a uniformly green color. The number of fronds per plant need not be the same for all tests. For each culture, record the number of plants, the total number of fronds, and the time of immersion, so that comparisons between number of plants and fronds in the control and the several test solutions can be made at regular intervals. Unless the pH effect is the immediate object of the tests, the pH of the test solutions should be approximately the same, because excess acid or alkali exerts a definite toxic action on duckweed. The temperature of the solutions and the light intensity should also be generally comparable for all tests. Under these conditions the rate at which damage occurs and the character of the damage, as previously described, will provide an estimate of toxicity of equivalent weights of the standard and the new herbicides.

Attention is called to the convenience and economy in the use of *Lemna minor* for estimating the phytocidal action of chemicals where a large number of tests are needed and where cost and availability of chemicals are important considerations. This plant may be useful as physiological test material in assaying the potency of commercial preparations of weed killers, particularly those containing organic poisons not easily determined by conventional methods of chemical analysis. Results of toxicity tests on duckweed should be directly applicable to practical problems in the control of aquatic plants.

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## Tributyl Phosphate as a Solvent for Preparing Concentrated and Oil-miscible Solutions of 2,4-Dichlorophenoxyacetic Acid and Similar Substances[1]

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Concentrated solutions of many difficultly-soluble growth-regulating compounds for use in oil solutions may be prepared by the use of tributyl phosphate.

During the past few years much attention has been given to the use of 2,4-dichlorophenoxyacetic acid as a plant growth regulator and weed killer. For practical use in sprays, this compound, and others of a similar nature, must be used with a diluent or carrier. This acid is only slightly soluble in water, and attempts to dissolve it directly in inexpensive mineral oils, such as kerosene and fuel oil, have been unsuccessful. In an effort to find a suitable co-solvent of 2,4-dichlorophenoxyacetic acid for use in mineral oils, over 50 of the more common organic solvents were tested, and of these only tributyl phosphate proved satisfactory. The others were found to be unsuitable either by reason of insufficient solvent power, immiscibility with oil, or undesirable volatility. The co-solvent ability of tributyl phosphate in this combination is a critical property of this compound, since closely related substances such as triethyl phosphate and tricresyl phosphate are unsatisfactory.

At ordinary temperatures tributyl phosphate will dissolve up to about 36 per cent, by weight, of 2,4-dichlorophenoxyacetic acid. A range of from 5 to 36 per cent of the compound dissolved in tributyl phosphate was found convenient and useful for subsequent dilution with mineral oils. Best results have been obtained with solutions in which the ultimate concentration of 2,4-dichlorophenoxyacetic acid was from 0.5 to 5.0 per cent by weight after dilution of the tributyl phosphate solution with kerosene or low-grade fuel oil.

The solutions of 2,4-dichlorophenoxyacetic acid and tributyl phosphate are stable at ordinary temperatures, have no heat of solution when dissolved in mineral oils, and are miscible with them in all proportions. In addition, tributyl phosphate causes local burning of plant tissues at points of direct contact, which, for herbicidal purposes, may be desirable. There is evidence that greater inhibition, per unit weight of compound, is produced in some broad-leaved plants when 2,4-dichlorophenoxyacetic acid is applied in oil solutions containing tributyl phosphate than when the acid is applied in the form of aqueous solutions. It is not known whether the increased inhibitory effectiveness of such solutions is due to the contact injury produced by tributyl phosphate.

Tributyl phosphate also has been found to be capable of dissolving large amounts of 2,4,5-trichlorophenoxyacetic acid, para-chlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid and other substituted phenoxyacetic acids. In general, 2,4-dichlorophenoxyacetic acid can be replaced with equivalent amounts of one of the three compounds named above. This solvent likewise is useful in preparing concentrated solutions of mixtures of such compounds for use as such or in oil solutions.

[1] Studies conducted at Camp Detrick from January to September 1945, under the direction of Dr. A. G. Norman.

## Treatment of Muck and Manure with 2,4-Dichlorophenoxyacetic Acid to Inhibit Germination of Weed Seeds[1]

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Herbicidal sprays containing 2,4-dichlorophenoxyacetic acid have been used to destroy noxious plants (*2, 3*). Aqueous sprays at 1,000 ppm are selective in

[1] Journal Article 787 (N.S.) from the Michigan Agricultural Experiment Station, accepted for publication December 1945.