

tions, and three had four treatments. The remainder had 4-18 inhalations, the highest number being reserved for the chronic bronchitis and chronic sinusitis. The range of duration of treatments for the patients with diseases of the lower respiratory tract was 10-18 treatments, administered in from 4 to 6 days.

It is singular that up to the present time no sensitivity reaction to the penicillin has been noted. With the vapor aerosol method, transient fever, dyspnea, or dermatitis has occurred in 4-20 per cent of the cases, depending upon the concentration of the drug used.

Penicillin assays of urine and blood were determined by the Flemming modification of the Wright slide cell technique. Values ranged from .03 to 1.92 units/cc. Blood-level curves would indicate a slow absorption, the maximum level being obtained 3-3½ hours after the inhalation.

This new method of administering penicillin appears to offer more effectiveness than other aerosol methods in ridding the upper respiratory tract of gram-positive bacteria. Appreciable blood levels would indicate the protracted effective absorption offered by this method, and the possible use of inhalation treatment for systemic conditions other than those of the respiratory tract. Although the method would appear to have a definite value in initiating the process of ridding the respiratory tract of pathogens, it may be necessary to supplement the process with other medicaments. In other instances its value may be adjunctive rather than primary.

Formaldehyde in Plant Collecting

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Recently Schultes has published an intelligent and much-needed discussion of the use of formaldehyde in the preparation of herbarium specimens, recommending its use to prevent disarticulation, molding, and decay when immediate drying is impractical (*Rev. Fac. Agron.* (Medellín, Colombia), 1946, **6**, 46-52; *Rhodora*, 1947, **49**, 54-60). His development of this method resulted from a conversation with Paul H. Allen, who has for some years employed alcohol and formaldehyde in the preparation of his excellent specimens of Panamanian plants.

Since I was present at the conversation referred to and have independently developed a somewhat different formaldehyde technique, it seems worth while to supplement Schultes' papers with an account of some of the possible variations and certain minor drawbacks, as well as to publish the method where it will reach a wider audience.

Allen's original method was to spray the freshly pressed specimens, using a common Flit gun, with a mixture of 70 per cent alcohol and enough formaldehyde to give it a strong odor. I tried this, but found that the specimens still molded and deteriorated to a certain extent, especially when final drying was delayed for a considerable time. Spraying with a Flit gun had the additional disadvantage of practically pickling the collector as well as his plants.

Much experimenting finally resulted in a mixture that seems to work under most conditions and has one big advantage over the straight formaldehyde-water solution advocated by Schultes. This mixture consists of approximately 1 part each of concentrated (40 per cent) formaldehyde, 95 per cent alcohol

and water. If 70 per cent alcohol only is available, as in many small towns, I commonly use 1 part formaldehyde and 2 parts alcohol. The alcohol gives the mixture much better wetting properties than those of a straight water solution. Not only does it readily wet even waxy leaves and those with a prominent coating of hair or scales, but it also penetrates much more quickly and thoroughly.

After I had suffered an atmosphere of formaldehyde spray from a Flit gun for several trips, Norman C. Fassett suggested applying it with a small paint brush. This method was much more effective and lacked the unpleasant features. I had already discarded the dipping method as rather ineffective and clumsy at best, and completely unsuited to plants that tended to be limp. Of the three methods, use of a soft, 2-inch paint brush is by far the most satisfactory. H. H. Bartlett (in a letter) has suggested a rather different method which consists essentially of tying a fair-sized bundle of specimens together in folded newspapers, standing them on end, and pouring a sufficient amount of the solution described above into the bundle to wet it.

It is usually suggested that after the application of formaldehyde the bundles of specimens be wrapped in oilcloth, waxed paper, ploidfilm, double canvas, or other material to retard evaporation. If specimens are to be mailed, this is doubtless necessary, since otherwise they would probably not be accepted by postal authorities. However, I have found that there are no evident bad effects if they are left simply wrapped in either wrapping paper or several thicknesses of newsprint. The solution largely evaporates off after killing the tissues of the plants as well as the spores of such fungi as are likely to cause trouble. The dead tissues lose their water much more readily than do living ones, and drying goes on to a certain extent even in the bundles. Subsequent drying over heat also takes place much more rapidly for this same reason.

Those to whom the color of the finished specimens is of importance will not find any of the methods employing either formaldehyde or alcohol very satisfactory, since the specimens are practically always either discolored or bleached. However, few collections from wet tropical regions, prepared by any method, have been preserved in anything like their original colors; furthermore, a few years in the atmosphere of the large cities in which our herbaria are unfortunately located soon makes the original color unimportant. Colors, if of any importance, should always be recorded on the labels.

I regard the formaldehyde method, not as something that should supersede the ordinary method of drying the plants over heat after a few hours in a tight press, but as a supplementary method for use in places where ordinary drying is difficult and with material that is not satisfactorily prepared by the ordinary methods. Leaving the material in a wet condition for any considerable length of time cannot have a good effect and probably results in a certain amount of brittleness. However as Schultes very effectively points out, many groups of plants that make traditionally bad specimens give very gratifying results with this technique. One has only to look into the covers of almost any genus of tropical mimosoid legumes to be convinced that improved methods are in order. Certain of these which were not dried for three weeks after gathering show no tendency whatever to lose their leaves. The formaldehyde apparently completely and instantaneously stops the formation of abscission layers. It might be well to try this technique on such conifers as *Tsuga* and *Picea* and on the almost mature cones of

Abies, of which I have seen few satisfactory specimens because of the dropping of leaves and cone scales on drying.

The tissue-killing effect of this solution also lightens immeasurably the burden of preparing material of such difficult plants as Crassulaceae, Portulacaceae, Aizoaceae, and other fleshy groups, even in a dry climate.

Direct Introduction of Chemical Substances Into Herbaceous Plants¹

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During studies concerned with the effect of 2,4-D upon plants the need for a means of introducing chemicals into any desired part of herbaceous plants, without either injuring the plant itself or taking up too much of the experimenter's time, became obvious. The principal methods of treatment hitherto used have major drawbacks.

The solution to be tested is placed upon the upper surface of a leaf blade either in the form of a drop or in a small disk of filter paper (2, 3). Also, pellets containing mixtures of a paste-like carrier and growth regulators have been used (1). Necessarily, these treatments are limited to those organs of the plant which, by virtue of their shape, support a drop of liquid or a disk of moist filter paper. They are not feasible for application to erect or inclined parts or to surfaces of a repellent or impermeable nature. The injection method (4) is not practicable with herbaceous plants in large-scale experiments because it is too time consuming; also, the inner turgor of the tissues usually prevents penetration of additional fluids from outside, and the cells are readily injured or ruptured when even a minimum of liquid is forced into them.

A good deal of knowledge is still lacking with regard to the fundamental facts about the mechanism of growth, metabolic systems, and correlations between physiological and morphological developments in plants. This ignorance may be due to inadequate means employed in tracing and analyzing various phases of reactions which take place in the intact growing plant.

Attention is called here to a technical device used by Winifred O. Roberts (5) in diagnosing mineral deficiencies in leaves, and also developed independently by the author for experiments with plant growth regulators. The fact that essentially the same method was conceived by researchers of different countries seems to confirm the need for, and the versatility of, the technique.

Needles formed from florist steel wire No. 30 are threaded with 2-3 inches of white mending cotton. The thread is soaked to saturation in the solution to be tested and drawn through the desired part of the plant. The needle is then severed from the thread, the latter being left inside the tissue (Fig. 1). If a continuous supply of a substance is desired, the end of a longer thread may be left immersed in the solution, thus serving as a wick. Powdered substances may be introduced similarly by making them adherent to a slightly moistened thread. If the

exact amount of introduced substance is to be determined, the thread may be dried after soaking and the differences in weight of the treated and untreated threads calculated for any given length. It has been observed that a thread impregnated with a solution of 2,4-D, dried, and then introduced is equally effective.

There was no instance of a detectable effect from puncturing the tissues with the needle or introducing a thread soaked in distilled water. In order to avoid contact of the plant sap with metal, the wire may be replaced by a plastic or glass-fiber

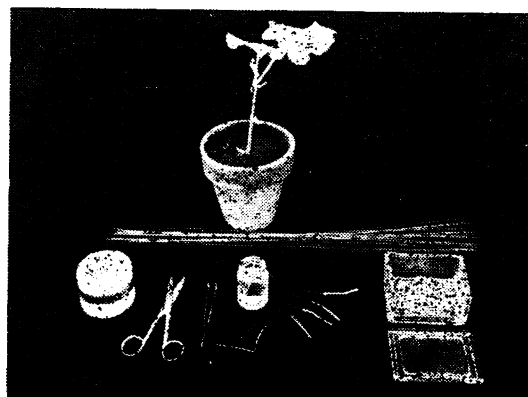


FIG. 1. Thread introduced in the first node of a bean seedling (above), and tools used in preparing threaded needles (below): wire, mending cotton, scissors, flat forceps, and glass bottle for soaking threads. Square glass jar (right) is used for storage of threaded needles.

material. The time factor involved in using this method for experimental purposes is negligible. An unskilled worker can prepare 60-80 needles per hour, and the treatment of 150 plants requires about one hour.

The "thread method" already has been used successfully in experiments conducted to test (1) the responsiveness of specific morphological parts of bean plants to 2,4-D, (2) the transport of 2,4-D within the plant, (3) the effect of 2,4-D in relation to acid and alkaline systems of plants, and (4) the pH in the growing plant, by using threads impregnated with indicator solutions. In some of the experiments several thread tests involving various time intervals between the single treatments, have been used on the same plant. A detailed report of these studies will be published elsewhere.

This method seems to be useful in cases where (1) specific areas of a plant cannot be reached by applying a drop, (2) minimum amounts of a substance are to be introduced, and (3) the capacity of specific tissues is to be tested relative to absorption, accumulation, or transport of metabolic or artificially introduced substances. The method offers innumerable possibilities of experimental tests with normal and diseased plants and may facilitate new discoveries of both morphological and physiological correlations and the fundamental mechanism of growth.

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