what remote relationship between the Equisetineae and the most primitive Ferns.

Both from its size and from the many sporophytes produced from it, the gametophyte of *Equisetum debile* is strongly reminiscent of certain liverworts, especially Anthoceros.

THE EFFECT OF SMALL AMOUNTS OF CHEMICALS IN INCREASING THE LIFE ACTIVITIES OF PLANTS

BY F. E. DENNY

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH

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Many chemicals in numerous ways increase the rate of growth of plants. But I desire to exclude from this discussion those that are ordinarily regarded as fertilizing elements or those which furnish building material, or yield energy to the plants by oxidation. The substances to which reference is made are those that act in a manner not now well understood, which are not regarded as being utilized directly as food and which produce results seemingly out of proportion to the energy they could furnish even if they were absorbed and oxidized. I must further restrict the discussion mainly to those chemicals with which I have had experimental experience.

The influence of small amounts of chemicals in increasing the life activities of plants may be illustrated by the experiments on the coloration of citrus fruits by the use of ethylene. Lemons and oranges may be commercially mature and yet have considerable amounts of green color in the rind. If such fruits after being harvested are stored under favorable conditions, the green color will disappear and will be replaced by the desired yellow or golden color.

It had been known for a long time that heating the storage rooms with kerosene stoves reduced the time required for coloration to about one-fourth the usual time. It was thought that this increased rate of coloring was due to the conditions of temperature and humidity within the heated rooms, but Sievers and True¹ showed that the results were produced mainly by the gaseous combustion products from the kerosene stoves. My experiments,^{2,3,4} in 1922 and 1923 indicated that the effective gas in causing these changes was ethylene (C_2H_4).

Hence it was found possible to dispense with the kerosene stoves and to substitute for them the pure gas itself. Since ethylene was obtainable as a compressed gas in cylinders at a reasonable price its use was found to be commercially possible. The new method has been quite

generally adopted in practical work in California and to a certain extent in Florida.

Surprisingly low concentrations of ethylene added to the air surrounding the fruit are able to bring about hastened coloration. If precautions are taken to prevent the loss of ethylene by diffusion, the process is successful with 1 part ethylene to 10,000 parts of air, 1 part in 100,000 or even 1 part in 1,000,000. In practical operation a somewhat higher original concentration is used in order to take care of losses of gas, but it has not been necessary to use a greater concentration than about 1 cubic foot of ethylene to about 5000 cubic feet of air.

A study of the respiration of lemons exposed to these low concentrations of ethylene showed that the life processes of the fruit were greatly increased.⁵ The rate of production of carbon dioxide was doubled or even trebled in 48 hours by exposure to air containing ethylene in concentrations of 1 part in 1000, 1 part in 10,000 or less. These experiments were interesting in showing that after the respiration of a given lot of fruit had been increased by the use of ethylene, a discontinuance of the exposure to ethylene led to a fall in the previously high rate. The rate did not fall to the original value before the ethylene was applied, but the results indicate that the effect of the ethylene was not to start a series of reactions that could proceed in its absence, but suggested that the continued presence of ethylene was necessary to maintain this high respiration rate.

The mechanism of this great increase in respiration is not known at present. We should consider first whether treatment with ethylene makes available in the tissue additional amounts of oxidizable substances. E. M. Harvey⁶ who made the first measurements of the effect of ethylene upon the metabolism of the plant showed in 1915 that as a result of exposure of sweet-pea seedlings to ethylene the proportion of the simpler forms of sugars, and of amino acids and amides, was increased. These results were confirmed in certain respects by the later work of Rosa¹⁴ with tomatoes, and more recently by that of Regeimbal and R. B. Harvey.⁷ But in the experiments of E. M. Harvey this increase in these forms of chemicals did not, in general, lead to an increase in respiration; decreases were observed in all cases except at the shortest period of exposure. It appears, therefore, that other factors in addition to the amount of oxidizable materials are involved.

It has been proposed that the rate of production of carbon dioxide in respiration is related: to oxidizing enzymes such as oxidase, peroxidase and catalase;⁸ to reducing enzymes such as dehydrase, Wieland;⁹ to the activity of the dipeptide, glutathione, Hopkins;¹⁰ to the presence of pigments such as cytochrome, Keilin;¹¹ to the catalytic activity of iron, Warburg.¹² But we do not yet know how such small concentrations of ethylene can so modify the behavior of these substances as to cause such a large

increase in the rate of respiration. It may be necessary to consider that the primary effect of the ethylene is upon the protoplasm itself, and that the coöperation of these factors in increasing the output of carbon dioxide is secondary.

A further possibility in this connection may be related to the discovery of the anti-oxidizing substances by Moreau and Dufraisie,¹³ who found chemicals that were able to retard oxidation; if there are oxidation retarders in plant tissue it is possible that ethylene may modify the action of such rate-controlling substances either directly or through its effect on the living substance.

Returning now to the experiments on the coloring of citrus fruits, it was found that high concentrations of ethylene, too high temperatures, too low temperatures and lack of oxygen prevented or retarded coloring. That is, conditions favorable for the life processes of the fruit were also favorable for coloring. These facts and those obtained in measuring the increased respiration of the treated fruits indicated that the effect of the ethylene was to hasten the processes that normally occur in the fruit after detachment from the tree, so as to bring about in a short time changes which, in the absence of ethylene, required a longer time for completion. They show that the ethylene is acting in a physiological and not in a directly chemical manner, that by some method not now understood the general level of life activity within the tissues of the plant is raised.

Subsequent experiments by others have shown that these stimulative effects of ethylene are not restricted to citrus fruits. Thus Rosa¹⁴ showed that the coloration of tomatoes was hastened by ethylene; during this process the acidity of the fruits was decreased and the proportion of soluble nitrogenous compounds was increased. Rosa¹⁵ also found that the rest period of dormant buds in the potato could be shortened by suitable treatment with ethylene. Using methods similar to those developed in California for citrus fruits, R. B. Harvey¹⁶ reported favorable results in the blanching of celery. Wallace¹⁷ was able to force the development of dormant buds on apples and other species; he also noted the renewed growth of lenticle tissue with the formation of excrescences.

There is evidence in the older experiments, in which the toxicity of ethylene for plants was mainly investigated, that the life activities at least of certain tissues or groups of cells were increased by ethylene; thus the swelling of stems, observed by Knight and Crocker,¹⁸ the enlargement of cells and increased cell divisions in roots, noted by E. M. Harvey and Rose,¹⁹ the proliferations of tissue in lenticles and leaf-scars described by Doubt,²⁰ can be regarded as being the results of a renewal of growth or increased life processes of the cells in these regions of the plant body.

Further illustrations of stimulative chemical effects are found in the results of treatments designed to break the rest period of Irish potatoes.

Freshly harvested tubers, if replanted under conditions favorable for growth, do not sprout at once but require a period of after-ripening. The depth of dormancy varies with the variety but is generally two months or more.

The experiments of McCallum²¹ in 1909 showed that exposure of the potatoes to the vapors of such chemicals as ethyl bromide, ethylene dichloride, carbon tetrachloride, ammonia, bromine and gasoline would induce early sprouting. Rosa²² in 1923 obtained sprout-forcing effects with solutions of sodium nitrate.

I extended these results^{23,24} by testing the effects of a large number of chemicals (at this time about 240) at different concentrations and with various periods of treatment. McCallum's results with the first three chemicals in the list above, and Rosa's with sodium nitrate were corroborated and in addition the following chemicals were found to induce sprouting in advance of untreated tubers: ethylene chlorhydrin, the thiocyanates of sodium, potassium or ammonium, thiourea, carbon bisulphide, trichlorethylene, dichlorethylene, ethyl iodide and xylol.

With the Bliss Triumph variety especially favorable results were obtained with ethylene chlorhydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$). A suitable method consists in dipping the tubers (cut into pieces ready for planting) into a solution consisting of about 30 to 40 cc. of 40% ethylene chlorhydrin per liter of water, removing the dipped potatoes and storing them 24 to 48 hours in a closed container. The vapors of the chlorhydrin come in contact with the dormant potato buds and in some manner induce prompt sprouting. The gain in time of sprouting depends on the stage of dormancy of the potato at the time of treatment but varies usually from 2 to 6 weeks.

Other varieties such as Irish Cobbler and Green Mountain responded in a somewhat better manner to treatment with thiocyanate solutions. Soaking the cut tubers for 1 hour in a 1 to 2% solution of thiocyanate (sodium, potassium or ammonium) broke the rest period and caused prompt sprouting.

Further experiments showed that the dormancy of the buds of woody twigs of apple, grape, plum, lilac and locust could be forced into growth in the autumn and early winter by exposure to low concentrations of ethylene chlorhydrin, acetaldehyde and ethylene dichloride. The gain in time of budding of grape and apple cuttings was about 2 months. Experiments have been undertaken to determine whether these results can be applied in a commercial manner for forcing early blooming of certain potted plants which are favorites in the florist's trade but which have a long dormant period and are slow in coming into bloom.

An interesting point in connection with these experiments is that so many different kinds of chemicals were able to induce sprout or bud development. An examination of the list of chemicals shows no evident relation between the effectiveness of a chemical and its molecular grouping.

When we come to consider the question how these chemicals can cause this increase in activity and renewal of growth, we find that the first difficulty relates to our lack of knowledge of the causes of dormancy or retardation in growth. Johanssen,²⁵ the pioneer worker in this field, shows that lack of growth in these organs cannot be due to a deficiency in the ordinary nutrients such as sugar. In fact, Hooker²⁶ has suggested that repression of growth is correlated not with a deficiency but with an accumulation of carbohydrate.

If food supply were the principal factor we should expect that a gradual change in stored food would take place during natural after-ripening. The experiments of Appleman,²⁷ however, showed, at least for potato, that no significant change in the starch, sugar or nitrogenous compounds took place during the period preceding the renewal of growth. There is little evidence in my experiments that the chemicals used were acting in a nutritive way. The fact that so many different chemicals were able to produce the same or a similar result shows that the action here must be indirect, that the chemicals used did not furnish food or energy, but caused some internal physiological condition which, in a manner not now understood, led to a renewal of growth.

An important factor in these results as well as in many of the results obtained by previous workers is that forcing appears to be related to incipient injury. If the concentration of chemical is great enough the tissue is injured or killed. But at concentrations of chemical and periods of exposure just below the point of injury, stimulation of the tissue into renewed activity occurs. It may be that this slight injury to the tissue changes the semi-permeable character of the plant membranes (as suggested by Coville,²⁸ in the case of plants subjected to low temperatures), weakening the membranes, and allowing greater rates of diffusion of enzymes, or food material. An increased permeability to oxygen may also be important since, according to Appleman,²⁷ oxygen supply to the tissue was found to be a critical factor in his experiments with potato.

Suggestive evidence on this point has also been provided by Carrel and Baker²⁹ who found that the substance in embryonic tissue which markedly stimulated the multiplication of isolated cells was a product of the partial hydrolysis of a protein. Neither the protein itself nor the products of its complete hydrolysis were effective, but when the hydrolysis of the protein was continued for about 3.5 hours the resulting liquid containing proteoses and other products of partial hydrolysis caused rapid multiplication of the cells. These results may throw some light on the experiments in forcing the buds of potatoes and other plants. It is possible that the slight injury that is produced causes a partial decomposition of constituents of the tissue resulting in the release of growth-promoting substances which start up renewed cell division and bud growth.

The enzyme activity of the treated plants should be studied especially with the view of determining the effect of the treatment upon the hydrolytic processes occurring in the tissues. The types of reaction that are favored by the treatment, the nature of the substances formed and their effect upon growth should give valuable information regarding the mechanism of the response.

In conclusion: I have described a number of experiments in which favorable results have been obtained in increasing the life activities of plants by chemical treatments. The chemicals that were found to cause this stimulation varied greatly in chemical character, so that no evident relation could be found between the type of chemical used and the result obtained. In most cases it did not seem possible that the substance was used by the plant directly as food, and in no case could it have supplied sufficient energy to account for the resulting increased activity. The mode of action of these chemicals, the details of the methods by which plant processes, previously inhibited or retarded, are induced to start again at a quickened rate are not now understood. I discussed the results from the viewpoint of certain theories regarding life activities in plants but it must be understood that the suggestions made do little more than indicate the sort of experimental work that may be needed to test the hypotheses that have been proposed.

¹ Sievers and True, *U. S. Dept. Agr. Bur. Plant Ind. Bull.*, **232**, 1912.

² See *California Citrograph*, Riverside, Calif., for March, 1923, and December, 1923.

³ Denny, *J. Agric. Res.*, **27**, 1924 (757).

⁴ See U. S. Patent 1,475,938, December 4, 1923.

⁵ Denny, *Bot. Gaz.*, **77**, 1924 (322).

⁶ Harvey, E. M., *Bot. Gaz.*, **60**, 1915 (193).

⁷ Regeimbal and R. B. Harvey, *J. Amer. Chem. Soc.*, **49**, 1927 (1117).

⁸ See Kostytshew, *Pflanzenatmung*, Berlin, 1924; and Appleman, *Maryland Agric. Exp. Sta. Bull.*, **191**, 1915.

⁹ Wieland, *Ber. d. chem. Ges.*, **47**, 1914 (2085).

¹⁰ Hopkins, *J. Biol. Chem.*, **54**, 1922 (527).

¹¹ Keilin, *Proc. Roy. Soc., Lond.*, **B98**, 1926 (312).

¹² Warburg, *Science*, **61**, 1925 (575).

¹³ Moreau and Dufraisse, *Compt. rend. soc. biol.*, **86**, 1922 (321).

¹⁴ Rosa, *Proc. Amer. Soc. Hort. Sci.*, 1925 (315).

¹⁵ Rosa, *Potato News Bull.*, **2**, 1925 (363).

¹⁶ Harvey, R. B., *Minnesota Agric. Exp. Sta. Bull.*, **222**, 1925.

¹⁷ Wallace, *Bull. Torrey Bot. Club*, **53**, 1926 (385).

¹⁸ Knight and Crocker, *Bot. Gaz.*, **55**, 1913 (337).

¹⁹ Harvey, E. M., and Rose, *Bot. Gaz.*, **60**, 1915 (27).

²⁰ Doubt, *Bot. Gaz.*, **63**, 1917 (209).

²¹ McCallum, *Ann. Rept. Arizona Agric. Exp. Sta.*, 1909 (584).

²² Rosa, *Proc. Amer. Soc. Hort. Sci.*, 1923 (180).

²³ Denny, *Amer. J. Bot.*, **13**, 1926 (118).

²⁴ *Ibid.*, **13**, 1926 (386).

²⁵ Johannsen, *Das Aether—Verfahren beim Frühtreiben*, Jena, 1906.

²⁶ Hooker, *Proc. Nat. Acad. Sci.*, 11, 1925 (710).

²⁷ Appleman, *Bot. Gaz.*, 61, 1916 (265).

²⁸ Coville, *J. Agric. Res.*, 20, 1920 (151).

²⁹ Carrel and Baker, *J. Exp. Med.*, 44, 1926 (503).

THE ARCHITECTURAL EVOLUTION OF PUEBLO BONITO

BY NEIL M. JUDD

U. S. NATIONAL MUSEUM, WASHINGTON, D. C.

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Pueblo Bonito is a prehistoric Indian village in northwestern New Mexico. Since 1921 annual explorations have been pursued there by the National Geographic Society. As a result the village has been entirely laid bare and much of its ancient history recovered. From this archeological record, we judge Pueblo Bonito to have been abandoned approximately one thousand years ago. The Bonitians had no beasts of burden; no metal tools.

On the present occasion we are to concern ourselves solely with the architectural development of Pueblo Bonito and shall have nothing to say regarding those remarkable cultural achievements that made it the most influential settlement in the southwestern United States in pre-Columbian times.

Pueblo Bonito covers over three acres of ground; it is semi-circular in plan, its straight south side being 518 feet long. There are nearly three hundred rooms on its ground floor; portions of four story walls still stand. In its heyday, Pueblo Bonito included approximately eight hundred rooms and sheltered between twelve and fifteen hundred individuals. These were farmers; they cultivated maize, beans and squash in neighboring fields.

The population of Pueblo Bonito included two distinct peoples. These were entirely unlike despite the basic similarity of their respective cultures. Their architecture was different; the product of their minor industries differed. They doubtless spoke different languages. But they dwelled together here, in apparent harmony, for a long period of time.

The first group, the actual founders of Pueblo Bonito, lived there several generations before the second group came in to dominate the settlement and create for it a prestige, the influence of which was felt as far away as the Pacific Coast and even the valleys of central Mexico. The first group produced a single type of masonry; the second, being more progressive, created three types.