PROCEEDINGS

of the

Krome Memorial Institute

EIGHTH ANNUAL SESSION

Orange Court Hotel, Orlando, Florida, April 17, 1941

DR. H. S. WOLFE, Gainesville, Vice-President and Chairman

INDUCED FLOWERING OF PINEAPPLES UNDER FLORIDA CONDITIONS

WILLIAM C. COOPER and PHILIP C. REESE

Bureau of Plant Industry, United States Department of Agriculture, Orlando, Florida

Introduction

Knowledge of the physiology of floral initiation in herbaceous plants has come mainly through studies of the photoperiodic responses in plants. Results of these studies suggest the possibility that, under certain photoperiods, the leaves manufacture a flower-forming hormone which moves to various parts of the plant and results in floral initiation. It remains to be established, however, that the flowering response is actually controlled by a specific chemical substance. The present investigation deals with the effects of certain unsaturated hydrocarbons, such as ethlyene and acetylene, on floral initiation in the pineapple. It is not claimed that ethylene is a specific flower-forming hormone, but ethylene is rather looked upon as another tool with which to discover additional information on factors involved in floral initiation.

Review of Literature

For many years pineapple growers, first in the Azores and later in Puerto Rico, have used wood smoke to induce pineapple plants to flower prematurely. One of the constituents of wook smoke is ethylene gas, and it was found by Rodriguez (1932) that this gas does cause flowering of pineapple plants. Having taken Rodriguez's findings as a starting point for our investigations on the problem here in Florida, his results are briefly summarized here. He placed pineapple slips in a closed room and applied ethylene gas in two different concentrations - 1 part gas to 80 parts of air and 1 part gas to 200 parts of air. The gas was applied daily at 8 a. m. and again at 6 p. m. for a period of one week. The room was ventilated for about thirty minutes before each new application of gas. The slips were planted in August and flowered three months later, approximately six months before untreated plants flowered. Both concentrations of gas, 1:80 and 1:200, were equally effective. Ripe fruits were picked from the treated plants six months after treatment. The fruits were in general undersized and of no commercial

value, but this was to be expected from such small plants.

Researches conducted in Hawaii have shown that acetylene gas is also effective in inducing flowering in pineapples and that it is both cheaper and more convenient to use under field conditions, since it can be dissolved in water and applied in that form. This method of application has been adopted in Hawaii (Collins 1935) and is recommended for use in Queensland, Australia (Lewcock 1937).

Still another method of induced flowering practiced in Hawaii consists in applying a small quantity of solid calcium carbide in the center of the individual plants near the growing point. The carbide reacts with moisture caught by the leaf bases in the heart of the plant, or with moisture from the air. The reaction produces acetylene which in turn induces flower formation. This method is reportedly used with great success in Puerto Rico (Schapple 1940) and Panama (Lindsey 1939).

The present work deals mainly with the use of ethylene in inducing flowering in pineapples here in Florida. The acetylene water and carbide methods, however, have also been tested and results are compared with those of the ethylene method.

Description of Plants

The experimental work was conducted on plants of the Abachi variety grown in the field at the Flatwoods Plantation, Lake Worth, Florida (1). Most of the vlants used were started from crowns because *rowns* of this variety grow faster than slips. Crowns planted in August developed into large plants with about 20 mature active leaves by the following July. Treatments to induce flowering were started in July and were continued on through August, September and October. Untreated plants continued to grow vegetatively until late November when formation

1. The writers are indebted to Mr. O. R. Winchester, Flatwoods Plantation, Lake Worth, Florida, for his cooperation in furnishing plants for this investigation and in helping in many ways with the experiments.

of new leaves stopped and flower formation started.

Results and Discussion

Ethylene Treatment in the Field. In preliminary field experiments the plants were covered with a tarpaulin and soil thrown up over the edges to seal it to the ground. Ethylene was introduced to this enclosed space in an initial charge sufficient to bring the concentration of gas in the air up to 1 part gas to 500 parts of air. The tarpaulin was left over the plants for 24 hours and then removed. No flowering resulted from this treatment. Likewise, when gas was introduced at six-hour intervals for a period of 36 hours no flowering took place. However, when ethylene was introduced by a continuous flow of minute amounts of the gas for 24 hours, nearly 100 percent of the plants were induced to bloom (Plates 1 and 2).

The method of introducing a continuous flow of gas is referred to as the "trickle system" and was developed by Winston, Wright and Wooten (1933) for introducing ethylene into orange coloring rooms. The flow of gas from the cylinder was first reduced to a fraction of a pound pressure by a reducing valve and bubbler. This apparatus is shown in Plate 3. With this equipment it is possible to obtain rates of delivery ranging from 1 to 100 bubbles (10 bubbles under conditions of these tests equaled about 1 cc. gas at atmospheric pressure) of gas per minute.

For further investigation on the use of ethylene, an experimental unit was constructed of light weight cypress framing, covered with oilcloth. The frame was made in seven sections (15'x12'x4') that could be placed end to end to cover a bed of 375 plants. Ethylene was introduced into this tent at seven different points and the rate of flow was kept constant at each point by the bubbling method. Each bubbler, therefore, supplied gas for approximately 53 plants.

By means of this unit separate beds of plants were treated in October, 1939 for 24-hour periods with bubbling rates of 10, 20, 40, 80, and 100 bubbles per minute. Nearly 100 percent of flowering was induced by each treatment while no flowering was observed on untreated plants. The minimum effective treatment under the conditions of these tests, therefore, was below 10 bubbles per minute per 53 plants.

The oilcloth covering used on this experimental unit did not withstand use and had to be discarded after about one month's use. In later experiments conducted during the summer of 1940, muslin was substituted for oilcloth. This material was found to be easy to handle on a large scale, and has held up well with use in the field under practical grower's conditions (Winchester 1941).

Plants covered with a muslin canopy were treated for 6-, 9-, 12-, and 24-hour periods with a 40-bubble per minute (per 72 plants) flow of gas during the months of July, August, September, and October, 1940. Nearly 100 percent of flowering was obtained from all 12- and 24-hour treatments (Table 1). Six-hour treatments never introduced flowering, while nine-hour treatments were only partially effective.

A comparison of the percentage of bloom induced by the ethylene gas, acetylene water, and calcium carbide methods is shown in Table 1. The acetylene water method gave results about equal to those of the ethylene method. In these experiments the acetylene water technique consisted in pouring approximately one-fourth pint of a saturated solution of acetylene into the heart of each plant. The saturation of the water with the gas is not readily obtained unless the gas is confined in an enclosed space under pressure. In these small scale experiments the saturated solution was prepared by placing a

Table 1. Induced flowering from ethylene gas, acetylene water, and calcium carbide methods of treatment in the field.

Date of treatment	Kind of treatment	No. of plants treated	Percentage flowering
1940 July 23			······································
	Ethylene gas (1) 6 hours	72	0
	Ethylene gas 12 hours	72	92.
	Ethylene gas 24 hours	72	87.
14 A.	Acetylene water	80	97.
	Calcium carbide	144	29.
Aug. 17		•	
-	Ethylene gas (1) 6 hours	72	0
	Ethylene gas 9 hours	72	0
•	Ethylene gas 12 hours	72.	100.
	Ethylene gas 24 hours	72	100.
	Acetylene water	82	95.
	Calcium carbide	32	47.
Sept. 17	•	······································	
	Ethylene gas (1) 9 hours	72	67.
	Ethylene gas 12 hours	72	87.
	Ethylene gas 24 hours	72	98.
	Acetylene water	48	100.
	Calcium carbide	48	56.
Oct. 17			
	Ethylene gas (1) 6 hours	72	0
	Ethylene gas 9 hours		50.
	Ethylene gas 12 hours	36	100.
	Acetylene water	32	70
	Calcium carbide	40	65.

(1). 40 bubbles per minute, or approximately 4 cc. per minute.

weighed^{*} quantity of carbide into a pressure cooker nearly filled with water. The lid was clamped on and a pressure of 15 pounds was allowed to develop while shaking the cooker. Lewcock (1937) and Winchester (1941) describe a method of preparing the solution on a large scale.

A point to consider in the use of the acetylene water treatment under Florida conditions is that rains immediately following the treatment wash out the acetylene solution and reduce its effectiveness. On the other hand, the ethylene gas treatments were found to be effective in rainy or in clear weather. when heavy rains occur nearly every day, as they did in September 1940, the ethylene is more likely to prove satisfactory.

The calcium carbide method, which consists in applying small pieces of solid carbide into the center of the plant, did not give as good results under the conditions of these tests as those which were obtained with either the ethylene gas or acetylene water methods. The percentage of bloom obtained varied from 29 in July to 65 in October (Table 1). This treatment usually killed several leaves in the heart of the plant.

Description of initiation and development of inflorescence following ethylene treatment. The first morphological evidence of a change from the vegetative to the flowering state in the pineapple is indicated by a marked increase in the area of the apical meristem (Kerns, Collins, and Kim 1934). When pineapple plants are treated with ethylene this change which culminates in flowering may be induced months before the natural flowering of untreated plants. In order to determine the time required for the initiation of the inflorescence and to study subsequent stages of development, treated plants were dissected at varying periods. The apical meristems were imbedded in paraffin, cut in longitudinal sections 10 microns thick, mounted and stained. Microscopic examination of such sections reveals that the change from the vegetative to the flowering stage of development occurs in a relatively short time. Leaf formation ceases abruptly and the inflorescence is initiated.

The evidence is presented in Plate 4. Untreated plants have a small apical meristem. There is scarcely perceptible enlargement of this region three to four days after the ethylene treatment (Figs. 2, 3), but the sixth day the widening has become definitely evident (Fig. 4). By the eighth day the bracts that subtend the first row of flowers appear as bulges at the margin of the meristem (Fig. 5); and by the tenth to fourteenth day the bracts have enlarged further and flower primordia appear in the axes of the bracts (Figs. 6, 7, 10). By the twentieth day the first rows of flowers of the inflorescence are forming (Fig. 8). The sepals, petals, stamen, and pistils which were formed in sequence may be distinguished in the first formed flowers in the inflorescence 44 days after treatment (Figs. 9, 11). Untreated control plants killed at the end of the 44-day period and sectioned show that the apical meristem region had remained unchanged and was still producing leaves (Fig. 12).

If, after ten days or two weeks following the ethylene treatment, the leaves are carefully removed one by one from the stem, the inflorescence can be detected without the aid of a microscope and the labor of preparing slides. A small hemispherical swelling at the tip of the stem is produced by the differentiating inflorescence. The small cluster of bracts are characteristically shorter than vegetative leaves and always one of the first formed bracts is folded back upon itself in a reverse curve. The presence of this folded bract may be relied upon as definite evidence that the floral differentiation has begun. The end of an untreated stem will remain flat.

Plate 5 shows a series of such stems from which the leaves were removed. From left to right are: the control-untreated, and treated plants 2, 4, 8, 14, and 20 days after treatment. By the third week the peduncle

^{*}Theoretically 32.3 gms. calcium carbide will produce sufficient acetylene to saturate 6 liters of water and bring 2 liters of air above the water to 15 lbs. pressure.

which supports the inflorescence usually shows considerable elongation.

Plants treated with ethylene in July and August usually show the first sign of the inflorescence without removing the leaves about 30 days after treatment. The bases of the youngest leaves are pushed laterally by the developing inflorescence which causes the apex of these leaves to bend inward giving a pinched effect. Ten days later the inflorescence has grown enough to expose the flower buds and red leaves attached to the peduncle.

Elongation of the internodal regions in the peduncle raises the inflorescence to a height of approximately 35 cm. by the end of two months. Then anthesis begins, the flowers opening in acropetal succession over a two weeks' period. A simultaneous development of the ovaries causes an enlargement of the inflorescence. The fruit develops from the inflorescence and consists of the ovaries and the fused adjacent parts, bracts, sepals, etc., of many flowers, and is technically a multiple fruit.

The speed with which the plant goes through these stages depends upon the time of year of treatment (Table 2). The time September and October. These differences in speed of development are in general attributed to temperature differences during the developing period. July treated plants had a longer period of warm weather in which to develop than did the plants treated at later dates. So even though plants may be treated at monthly intervals the harvest intervals of these plants during the winter and spring may be longer than a month. An unseasonal cold spell in late February of this year delayed for nearly two weeks the ripening of the August 15 treated fruit.

Minimum Effective Gas Concentration and Length of Treatment

This phase of the investigation was conducted in the laboratory where facilities were available for controlling temperature and gas concentration during the treatment. Mature plants used for this work were dug from the field at the Flatwoods Plantation, planted in boxes, and transported to Orlando.

The treating chamber, located in an airconditioned room, consisted of a nearly airtight plywood box (3'x3'x2') large enough to hold six plants. After sealing the plants in this box a known amount of ethylene was

Date of treatment	Showing red in heart of plant		Ripe fruit	
	Date.	No. days from treatment	Date	No. months from Treatment
July 24 Aug. 17	Aug. 30 Sept. 28	38 42	Jan. 10 Mar. 3	5 ½ 6 ½
Sept. 17	Nov. 1	42 45	Apr. 15	7
Oct. 17 Untreated (2	Dec. 12) Jan. 20	56 61	May 15 (1) June 15 (1)	7 6 ½

Table 2. Development of inflorescence and fruit of pineapples following ethylene treatment at different times of year 1940.

 Estimate based on performance observed for 1939-40 crop.
 Differentiation of inflorescence of untreated plants began about November 20. This date is used as a starting point for calculations on time required for development of untreated paints.

required to "show red" in the heart of the plant was 18 days longer for October treatments than for July treatments. The interval between treatment and harvest was $5\frac{1}{2}$ months for July treatments, $6\frac{1}{2}$ months for August, and an estimate of 7 months for introduced to bring the ethylene concentration to the desired point for the test. Then in order to maintain this concentration for the duration of the test an ethylene-air mixture of the same ethylene concentration was circulated through the box at the rate of

Winchester--Observations on Growing Pineapples on Flatwoods Plantation



Figure 1. Continuing the cover across second bed of pineapples. Sand is shoveled onto the edge of the cover to hold it in place.

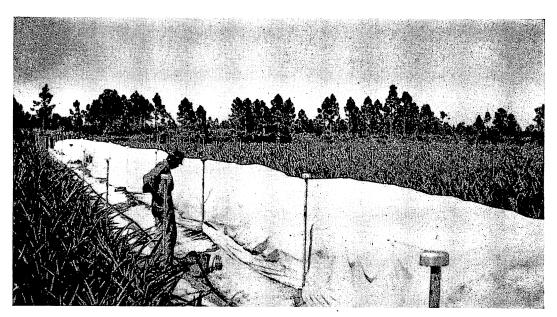


Figure 2. Muslin cover over one bed pineapples. Garden hose laid in ditch and bubblers ready to attach. Note bucket of water for testing connections.

Winchester--Observations on Growing Pineapples on Flatwoods Plantation



Figure 3. Showing treated and untreated beds. Bed on left treated with ethylene September 1940. Bed on right untreated.

one complete change of air per hour.

Three series of tests were conducted with this experimental set-up in late October, 1940. In the first series the temperature was maintained at 25°C., and the plants treated with an ethylene-air mixture of 1 to 1000 for 0, 4, 6, 8, and 10 hours. The 6-, 8-, and 10-hour treatments induced 100 percent of flowering, while no flowering occurred on 4-hour treatments and untreated controls. Apparently . six hours is approximately the minimum period of exposure required to cause floral initiation with a gas concentration of 1 to 1000. A treatment for this period was 100 percent effective, while a treatment for 2 hours less time was wholly ineffective.

A second and similar series of tests was conducted except the temperature of the room was kept at 15°C instead of 25°C. The results were the same as at 25°C.

In the third series the temperature was maintained at 25°C. while the concentration of ethylene in the ethylene-air mixture was varied. Mixtures of 1 to 1,000, 1 to 10,000, 1 to 25,000, and 1 to 100,000 were tested for 6- and 12-hour periods. For 6 hour treatments a 1:1,000 mixture induced 100 percent of flowering; 1:10,000 and 1:25,000, 33 percent of flowering; and 1:100,000, no flowering. For 12-hour treatments, however, all ethylene-air mixtures induced nearly 100 percent of flowering. Evidently the lower concentration of gas the longer the treatment required for floral initiation.

Influence of leaves. It has been shown that large plants containing 20 or more mature active leaves may be induced to flower by the application of ethylene under the experimental conditions. Smaller plants with only a few leaves, however, may also be induced to flower by the same ethylene treatment (Plate 6). In some instances plants flowered due to the ethylene treatment when all mature leaves except one were removed. Never-the-less, at least one, or a part of one mature active leaf is necessary to induce flowering, because plants from which all green leaves were removed before treatment failed to initiate an inflorescence. The mutilation of the plant in this instance does not account for its failure to bloom because plants which had all green leaves removed immediately after a 24-hour ethylene treatment always bloomed. Apparently the presence of a leaf or leaves during the treatment is essential for floral initiation.

These results indicate that the ethylene stimulus is received by the leaves and not directly by the growing point, and that the stimulus, resulting in floral initiation, is transported from these leaves to the growing point rather rapidly because removing the leaves immediately after a 24-hour treatment did not prevent flower formation. For an elucidation of the mechanism involved between reception of the ethylene stimulus by the leaf and actual floral initiation at the growing point, further work is necessary.

Leaves not only influence floral initiation but also profoundly influence later development of the inflorescence and fruit. The inflorescences on defoliated plants were always very small and were slow in developing. Furthermore, the fruits produced on small plants with few leaves were not nearly as large as those produced on large plants (Plate 6). Although only a small leaf area is essential for floral initiation, a much larger leaf area is required to produce a normal size fruit. It may be that minute amounts of substances in the nature of hormones are required for floral initiation while much larger quantities of other substances classed as foods are needed for the development of marketable sized fruit.

Literature Cited

- Collins, J. L. 1935. Further notes on gas treatment of plants to induce flowering and the possibility of preventing "hold over" plants by an adaptation of this method. Pineapple News 9(4): 78-79.
- Kerns, K. R., J. L. Collins and H. Kim. 1936.
 Developmental studies of the pineapple
 Ananas comosus (1) Merr. I. Origin and growth of leaves and inflorescence. New Phytol. 35: 305-317.
- Lewcock, H. K. 1937. The use of acetylene to induce flowering in pineapple plants. Queensland Agric. Jour. 1937: 532-543.

Lindsay, W. R. 1939. Annual report of the

Canal Zone Experiment Gardens 1939. 23-24.

- Roderiquez, A. G. 1932. Influence of smoke and acetylene on the fruiting of the pineapple (Ananas sativus). Jour. Dept. Agric. Puerto Rico 26: 5-18.
- Schapple, N. A. 1940. Personal correspondence.
- Winchester, O. R. 1941. Observations on growing pineapples at Flatwoods plantation. Proc. Fla. State Hort. Soc., present issue.
- Winston, J. R., R. C. Wright and J. F. Wooten. 1933. Process for coloring fruits and vegetables. U. S. Patent No. 1,920,540.

OBSERVATIONS ON GROWING PINEAPPLES AT FLATWOODS PLANTATION

By O. R. WINCHESTER, Boynton, Florida

Mr. Chairman, Ladies and Gentlemen:

You have just heard a very interesting paper by Dr. Cooper on "Induced Flowering of Pineapples" in which he described the results of experimental work. I would like to tell you something of the same thing but from the viewpoint of the commercial producer. Although we have been inducing flowering in pineapples for over a year, it still seems a bit miraculous to me that by application of certain chemicals in relatively small amounts, a pineapple plant is caused to produce a bloom and fruit identical in every way to natural bloom and fruit.

The importance of this ability to cause the pineapple plant to bloom can not be over emphasized. Although in past years we have produced large well flavored fruit, we were limited by the very short harvest season of about four to five weeks beginning about June 15th. The harvest comes with such a rush and ends so quickly that there is no chance to develop markets. By the time the housewives begin to ask for our pineapples the harvest is finished. The prices received for our fruit during this short season are never satisfactory --- often below the cost of production. On the other hand, the small amount of fruit which ripens at other times, sells readily and usually at good prices. We have tried various methods such as planting at different seasons in the year, fertilizing

with various materials, in an attempt to cause the pineapple plant to fruit at time other than the usual harvest season. None of these methods are of any value.

Today we use two of the methods of inducing bloom described by Dr. Cooper. We call these two methods, the acetylene-water treatment and the ethylene gas treatment. We have not found the carbide treatment, also described by Dr. Cooper, of any value.

First I would like to describe the acetylene water treatment as used on a large scale. In this treatment, water, in which acetylene gas is dissolved, is poured into the heart of the plant. We use about one-half pint per plant. We prepared the water solution of acetylene in a large steel pressure tank which holds 600 gals. of water. This we mounted on a truck for hauling to the field. Attached to this tank is a 1½ inch centrifugal pump run by a half horsepower electric motor. The acetylene gas was introduced on the intake side of the pump so that the gas was beaten into the water as it passed thru the pump. We used acetylene gas compressed in heavy steel cylinders and released thru reduction valves of the same type used with welding apparatus. We found that when pressure was built up to about ten pounds in the 600 gal. tank, the water would be well saturated with acetylene gas.

We applied this water solution of acety-