THE USE OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE AS A PLANT GROWTH REGULATOR

By

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A THESIS

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Paul Rood

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INTRODUCTION

The isolation of indoleacetic acid, and the discovery that it was one of the most important natural occurring auxins regulating the growth of plants has stimulated research on a large variety of chemical compounds which exhibit similar activity. Slight changes in the structure of these compounds frequently alter the physiological responses of plants or the agricultural uses for the compound.

One of these growth regulating chemicals, 2,4-dichlorophenoxyacetic acid is so much more active than the natural auxin that it is used extensively in weed control. An even more effective growth regulator for the control of woody perennials was developed by substituting an additional chlorine atom in the number five position of the ring making 2,4,5-trichlorophenoxyacetic acid. By omitting the chlorine atom in the ortho position of 2,4-dichlorophenoxyacetic acid still another compound, para-chlorophenoxyacetic acid was synthesized which was less toxic and capable of inducing parthenocarpic development of tomato flowers.

Naphthaleneacetic acid is used in thinning tree fruits soon after blossom time, and in delaying the preharvest drop of apples in the fall. Other compounds will increase the color of fruit and hasten its ripening. Indolebutyric acid, and many of these other compounds are useful in the rooting of cuttings.

More recently many other compounds with quite different structures have been found to show growth regulating activity. Some of these such as maleic hydrazide, the benzoic acid derivatives, and trans cinnamic acid have been called anti-auxins. Maleic hydrazide is used in preventing the sprouting of carrots, onions, and potatoes. It influences the development of the seed stalk of celery and causes a delay in the blossoming of raspberries. It has also been used as an inhibitor of plant growth and as a weed killer. Trans cinnamic acid and the benzoic acids have been used chiefly in physiological investigations.

A multitude of practical applications have been found for plant growth regulators in many phases of agriculture; however, the biochemical mechanism by which these compounds affect the physiology of the plant is not entirely clear. Studies with growth regulators have frequently yielded valuable fundamental knowledge of normal plant metabolism. It is very difficult to predict just what the structure of a compound should be for it to be most effective for a certain purpose, but by empirically testing different compounds, new growth regulators can be found.

The investigations described in this thesis were conducted to explore many of the practical applications as well as the fundamental physiological effects of a new plant growth regulator, 4-phthalimido-2,6-dimethylpyrimidine.

same as those given a preliminary test by Hoffmann and Smith and were synthesized and furnished by Naugatuck Division of the United States Rubber Company with which they are associated. Phthalic acid was tested by Thompson (94) in the gigantic screening program of the U. S. Army Chemical Corps and classified by him as one of the least promising compounds.

If the activity of 4-phthalimido-2,6-dimethylprimidine is primarily due to the pyrimidine nucleus, it is one of the first synthetic compounds of this type to be a growth regulator.

The review of literature relevant to the varied horticultural practices or physiological problems in which these growth regulators were used will be discussed with the appropriate experiment.

EXPERIMENTS ON THE SETTING OF TOMATO FRUITS

Review of Literature

The first plant which was stimulated with a synthetic growth regulator to develop parthenocarpic fruit was the tomato. This was achieved by Gustafson (30) in 1935, and since that time many different compounds have been tested (31, 90, 107) on many different plants. This early work has been reviewed by Gustafson (32) and Avery (6). More recently Hoffmann and Smith (38) have reported that a group of N-aryl phthalamic acids are capable of setting fruit on Bonny Best tomato plants. The concentrations required to set fruit, to inhibit fruit set, and to produce formative effects on the leaves varied with the substituents on the aryl group. The minimum concentration which was effective in setting fruit was 63 ppm. They also report that the N-aryl phthalimides also behave similarly, but do not mention any specifically or give any data. The compound used in these experiments, 4-phthalimido-2,6dimethylpyrimidine belongs to this class of chemical compounds.

The ability of growth regulators to set tomatoes has afforded a solution to two practical problems. One is the setting of greenhouse tomatoes during the cloudy winter months, and the second is the setting of tomatoes in the field when the night temperatures are low. Tomatoes grown in the greenhouse during the winter in some areas do not receive enough sunlight to develop normally. Burk (16) found that the stigmas of Bonny Best tomatoes grown under low light intensities and short days, protruded beyond the stamens making self pollination difficult, while the pistils of tomatoes receiving more light were about the same height as the stamens. Howlett (39) confirmed this and in another paper (40) reported that morphologically normal pollen produced by tomato plants low in carbohydrates failed to germinate. Pollen produced by plants still more deficient in carbohydrate frequently degenerated during its development. This degeneration usually occurred following the liberation of the microspore from the tetrad but sometimes occurred later during its maturation up to the time of the mitotic division of its nucleus. Howlett utilized the classification of Kraus and Krabill (48) who had found that tomato plants grown on soils high in nitrogen, or under conditions unfavorable for photosynthesis, tended to make rapid vegetative growth, be spindly, and did not set fruit well. Plants growing in warm, humid, greenhouses in cloudy winter weather often grow this way. Withrow (105) has shown that the yield of greenhouse tomatoes can be increased by supplying additional light to the young seedlings. Wittwer (106) observed that the low light intensity during the setting and development of the fall crop of greenhouse tomatoes was accompanied by a much lower yield as compared with the yield of the spring crop of tomatoes grown when the light intensity was greater.

The last three investigators mentioned and many others (65, 68, 77, 88, 112) have attempted to increase the set and yield of greenhouse tomatoes with growth regulators. This has proven practical and para-chlorophenoxyacetic acid and B-naphthoxyacetic acid have been found to be the best compounds. The fruit set with these compounds frequently softens prematurely, has a green pulp around the seeds which may give the fruit a scalded or blotchy appearance to the skin which also takes on a glossy, water soaked appearance which decreases its market value (41, 42).

The influence of various normal metabolites and environmental conditions, upon the ability of para-chlorophenoxyacetic acid to set excised flowers of a male sterile line of John Baer tomatoes was studied by Leopold and Scott (52). They found asparagine and glutamine gave the greatest increase in set and that adenine, guanine, thiourea, urea, pyridoxine (vitamin B_6), and thiamin (vitamin B_1) increased the number of flowers which set in about the order in which they are mentioned. It is noteworthy that thiamin, which has a pyrimidine nucleus, ranks low on this list, so the activity of 4-phthalimido-2,6-dimethylpyrimidine in setting fruit is probably not due to the similarity of its structure with that of thiamin. They also found that sugars, the organic acids of the dicarboxylic acid cycle, ascorbic acid, glutathione, amino acids, and inorganic sources of nitrogen also increased fruit set. The importance of an adequate supply of nitrogen containing compounds is evident. The optimum temperature in these experiments was about 68 degrees Fahrenheit with very poor fruit set occurring at 50 degrees. They report that abscissing flowers are still capable of being set. They also state that the failure of some flowers to develop which do not absciss is due to the lack of nutritive materials and not to a lack of sufficient auxin. The methods employed in this study are somewhat arbitrary and the small number of flowers used in each experiment probably do not give too reliable estimates. Still this is the most complete study of its kind and the trends indicated are probably valid. In another study Zalik, Hobbs, and Leopold (111) reported that tomato fruits set by applying para-chlorophenoxyacetic acid to the joint or abscission zone of the pedicel, were puffy, having large grooves in the carpel wall. If the night temperature is below 58 degrees Fahrenheit tomatoes will not set well. This has been the experience of Wittwer (108) in Michigan, of Went and Cosper (102) in California, and of Went (101) under the controlled conditions of his air conditioned greenhouses. Smith and Cochran (83) studied the effect of temperature on the germination and tube growth of tomato pollen. They found the optimum temperature for pollen germination was between 70 and 85 degrees Fahrenheit and that pollen tube growth was best at 70 degrees and very poor at 50 degrees. Even under optimum conditions the pollen tube required about two and a half to three days to grow the length of the style, and if it was too cold for good growth the pollen tube never reached the ovule.

In a recent study, Osborne (70) reports that low temperatures from 50 to 63 degrees Fahrenheit are capable of inducing parthenocarpic development of unpollinated tomato flowers. This may have been a factor in some of the early fruit setting experiments.

Greenhouse Experiment

Methods and Materials

This experiment was conducted to determine if 4-phthalimido-2,6-dimethylpyrimidine was capable of setting parthenocarpic tomatoes of higher quality than those produced by the other growth regulators.

Early in November of 1951, Rutgers tomato seeds were planted in a flat. They were transplanted to three-inch pots and later to six-inch pots. During January while the plants grew from five inches to a foot high, they received additional light from fluorescent lights used on a neighboring bench. This amounted to about six hours in addition to the normal day length of eleven hours. This was a longer period of light per day, for more days, at a later stage of development of the plant, but a lower light intensity than that used by Withrow (103).

The plants were growing in a soil composed of two parts of Hillsdale loam, one part of a raw peat, and one part of sand. This soil mixture had a pH of 7.1. Using the active Spurway methods (86), the nitrogen tested 80 pounds per acre, the phosphorus tested 13 pounds per acre, and potassium tested 384 pounds per acre. The pH was near optimum, however the soil lacked sufficient nitrogen, and was severely deficient in enought phosphorus for optimum plant growth. The amount of potassium was more than sufficient for good growth. About a half a gram of mono-calcium phosphate was placed in holes in the soil of each pot at intervals throughout the growing season to correct this deficiency. Ammonium nitrate was dissolved in water and the plants were watered with this solution periodically. On one occasion some of the solution was accidentally applied to the lower leaves which developed necrotic spots but the plants outgrew this in a couple of weeks. In the middle of February the plants were transplanted to twelve-inch pots and tied to stakes. The plants were trained up on strings when they outgrew the stakes and the suckers were kept pruned out. As the plants grew, they became pot bound and fertilizers were applied as described earlier. The plants sometimes showed nitrogen and phosphorus deficiency symptoms, although tissue tests for these elements indicated a moderate supply at all times.

In order to prevent normal self-pollination and fruit set the flowers of the first four hands were emasculated just before anthesis during March and April. Every three or four days, the flowers were dipped in solutions of

10, 25, 50, or 100 ppm of 4-phthalimido-2,6-dimethylpyrimidine. With few exceptions, all of the hands on a plant received the same treatment. The flowers of one group of plants used as controls were emasculated but not set.

Fresh solutions were carefully made up at least every two weeks to avoid any natural decomposition. All of the compounds used in these experiments, including 4-phthalimido-2,6-dimethylpyrimidine are crystalline solids in their pure state at room temperature. They were dried in a desiccator over calcium chloride, for at least 24 hours before weighing as recommended by Gortner (29) who found the failure to do this could easily result in errors of 25 percent because of water of crystallization. Enough distilled water was added to make a stock solution of 1000 ppm and this was shaken until the compound went into solution. The density of water at room temperature was corrected for, so it was felt that the concentration of the solution was accurate to three significant numbers on a weight per weight basis. The solutions of other concentrations were made by diluting the stock solutions.

All of the glassware was kept very clean by soaking it in activated charcoal for a day or longer to absorb any growth regulator present, as described by Lucas and Hamner (54) and then carefully washed with soap and water and rinsed with distilled water.

The tomato plants receiving the different treatments were randomized on the bench. The dates of emasculation and the dates of dipping to set the fruit were recorded on a tag as shown in Figure 1. When the fruit matured its weight was recorded, it was cut open and inspected for seeds, or abnormalities, and the number of days between emasculation and ripening of the fruit was recorded.

Results

The 4-phthalimido-2,6-dimethylpyrimidine did induce the parthenocarpic development of tomato fruits. The 100 ppm concentration was superior to all others and the 50 ppm concentration was about half as effective as 100 ppm in causing parthenocarpic development on tomatoes as shown in Table I. The 25 ppm concentration was capable of setting fruit, however, 10 ppm was too low a concentration for effective fruit set. The fruit set with 4-phthalimido-2,6-dimethylpyrimidine required approximately as much time to develop to a red tipe condition as did the control fruit. The parthenocarpic fruits were just as large as the pollinated fruit of the control treatment and had excellent color. The fruits were of normal shape except that some were slightly pointed at the blossom end and the style persisted on some as is shown in Figure 2.

The dipping of the flowers in this solution seemed to make more of them absciss as shown in Table I. The 50 ppm treatments appeared to delay the process of abscission however. These factors are of little importance in tomato growing but indicate that the compound might be useful in thinning tree fruits.

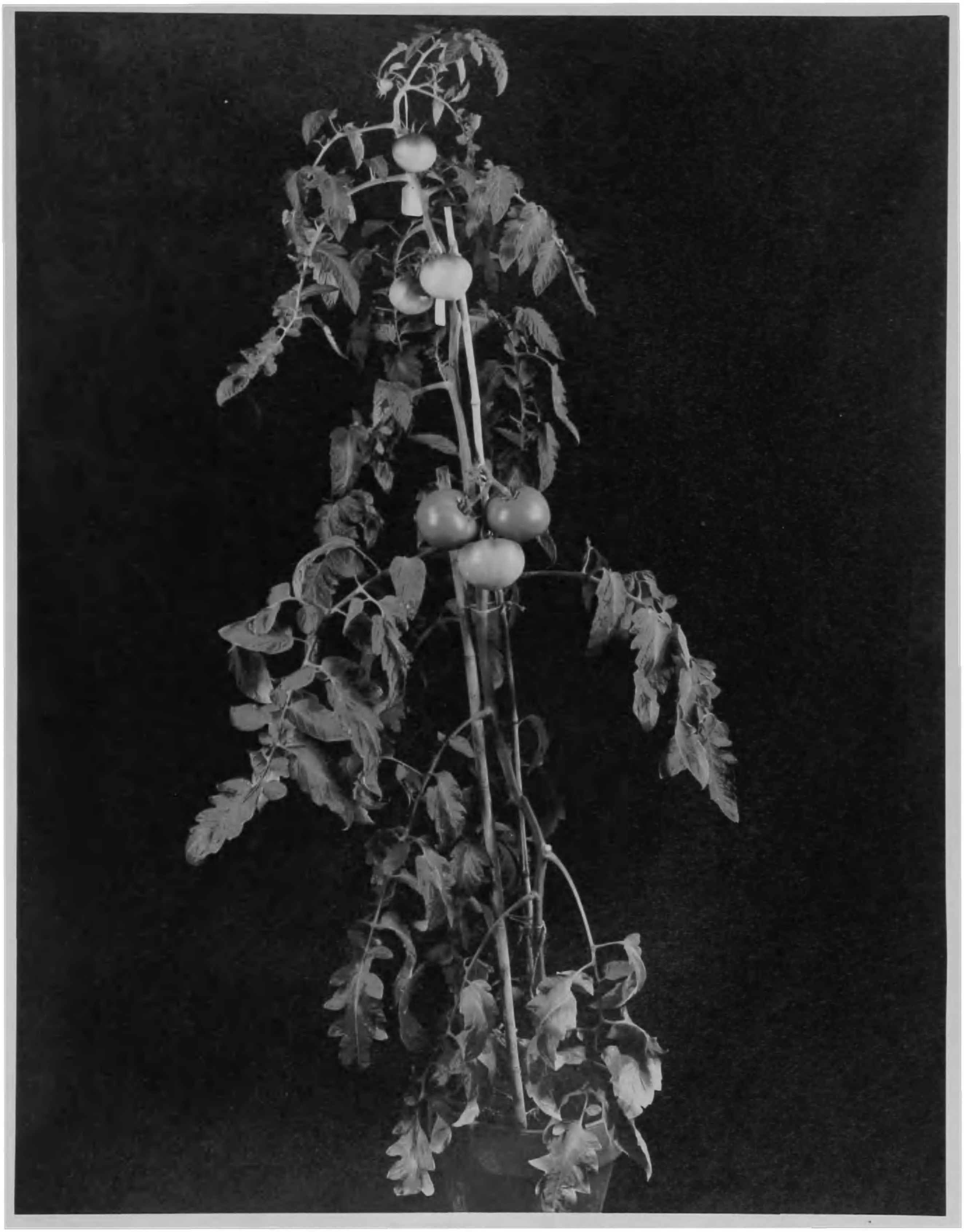


Figure 1. A tomato plant with fruit set with 100 ppm of 4-phthalimido-2,6-dimethylpyrimidine.

TABLE I

EFFECTIVENESS OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE IN STIMULATING THE PARTHENOCARPIC DEVELOPMENT OF NON-POLLINATED FLOWERS OF TOMATO PLANTS GROWN IN THE GREENHOUSE

Concentration of solution	Average weight of fruit in gm	Average number of normal parthenocarpic fruit per plant	Average number of days between emasculation and ripe fruit	Average number of flowers abscissing per plant	Average number of days between emasculation and abscission of unset flowers
Control (pollinated)	130	0	57	2.3	12
10 ppm	--	0	--	4.8**	12
25 ppm	98	0.3	51	3.4**	12
50 ppm	126	1.3**	57	2.4**	15*
100 ppm	147	2.6**	57	6.0**	11
	No significant difference	**Highly significantly different from any other treatment	No significant difference	**Highly significantly different from any other treatment	*Significantly higher at 5% level

Figure 2. Top -

Normal tomato seeds and undeveloped ovules of parthenocarpic fruit set with 50 ppm of 4-phthalimido-2,6-dimethylpyrimidine

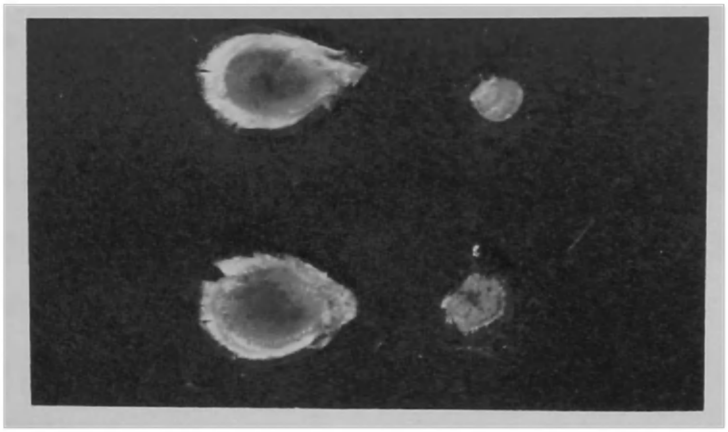
Bottom -

Upper left: Cross section of a pollinated control fruit

Upper right: Cross section of a parthenocarpic fruit set with 50 ppm of 4-phthalimido-2,6-dimethylpyrimidine

Lower left: Side view of a parthenocarpic fruit showing most extreme pointedness of blossom end resulting from setting with 100 ppm of 4-phthalimido-2,6-dimethylpyrimidine

Lower right: Blossom end of a parthenocarpic fruit set with 50 ppm of 4-phthalimido-2,6-dimethylpyrimidine showing the persisting style which frequently occurs



There were many abnormal fruits produced by all of the treatments which were not included in the data above. They were small, weighing less than 65 grams, which is too small to have commercial value. They required more than 85 days to ripen after emasculation and setting, and they were puffy, with large grooves in the carpel walls, and frequently had small, brown masses of tissue the size of aborted ovules. Since there was a break in the frequency distribution curve between these and the normal fruit, with regards to all three classifications, the data of the abnormal fruit were eliminated which was necessary before an analysis of variance could be calculated. The poor nutrition may have delayed the development of some of the fruit until it was corrected as suggested by Leopold (52). Perhaps enough of the growth regulator was applied to the abscission zone of the pedicel which may have been slightly separated during dipping to produce the puffy fruit characteristics described by Zalik (111). The abnormal fruit of this experiment closely resembled those pictured by him.

Field Experiments

Methods and Materials

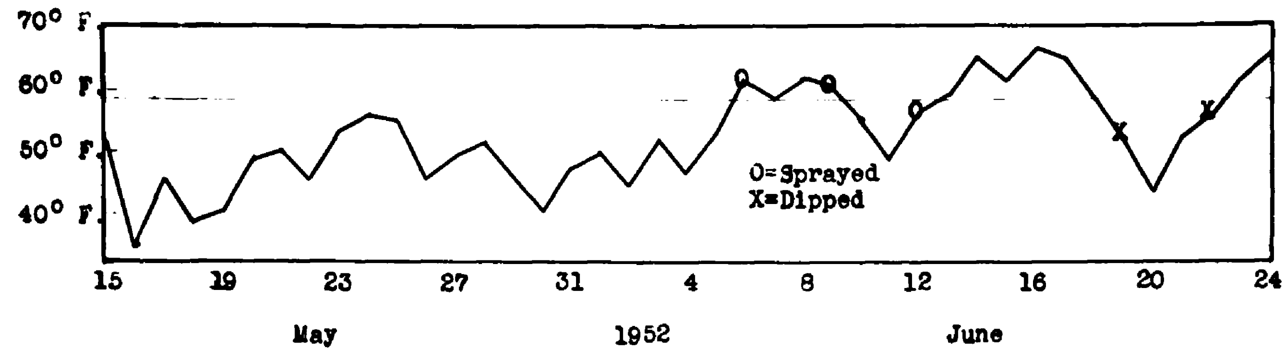
Several flats of John Baer tomato plants were planted April 11, 1952. They were transplanted to four-inch pots during the last week of April and grown in the greenhouse. On May 30 one hundred of the best plants were selected for planting in the field. They all had the flower buds of the first hand well developed and some flower buds were open at this time. The plants were planted in a single row three and a half feet apart. The one hundred plants were divided into five replications. Each replication contained five plots which included four plants each. The soil varied from a Hillsdale sandy loam at the upper end of the row to a silt loam at the lower end. This was the major difference between replications. The plants were watered with a starter solution of "Take Hold" soon after planting.

The hands with open blossoms were sprayed on June 6, 9, and 12. All of the first hand and the second hand of some plants were included in these sprays. The weather was warm for a period so no treatments were applied until June 18 and 22, at which time the flowers were treated by dipping them in the solutions. The minimum daily temperature as recorded at the hydrogeologic station a half mile away is shown in Figure 3. The five treatments used were 100, 250, and 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine, one treatment was sprayed with 30 ppm of para-chlorophenoxyacetic acid, which is the recommendation (107) made to tomato growers, and distilled water was sprayed on the flowers of the control treatment.

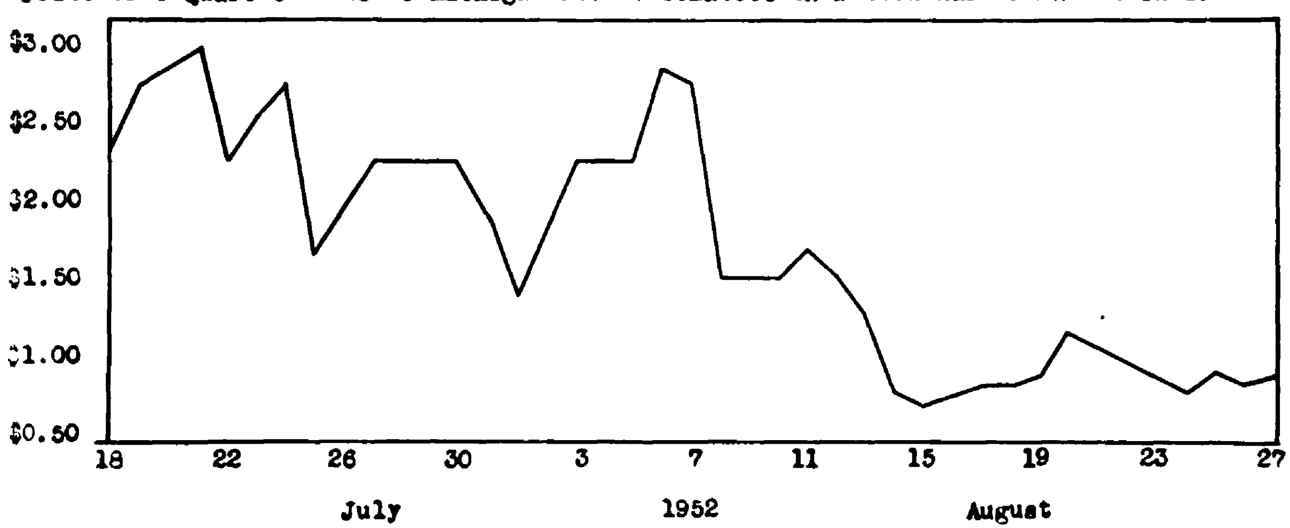
The sprayer used in this and all subsequent experiments consisted of an ordinary knapsack sprayer with an air pump. This was used only as an air reservoir and the spray solution was kept in a 125 ml erlenmeyer flask fitted with a cork and glass tubing like a wash bottle used in a

Figure 3.

Minimum Night Temperature During the Blossoming Period of Tomatoes at East Lansing- 1952.



Price of 8 Quart Cartons of Michigan No. 1. Tomatoes on Benton Harbor Market in 1952.



chemistry laboratory. A fine spray nozzle was attached to the outlet, and a stopcock was attached at the inlet to the air hose so that the sprayer could be turned off. All the parts of the nozzle could be easily cleaned and a clean flask could be used for each different compound.

The first ripe fruits were picked on July 21, and at two-day intervals until August 8. The fruit of the lower hands which had been sprayed were picked by then. They were the only ones which were parthenocarpic. August 8 was the day which the price of tomatoes broke on the Benton Harbor, Michigan, market which is the largest fresh fruit market in the world. The price dropped from about three dollars to less than a dollar and a half as can be seen in Figure 3.

These early fruits were weighed individually, graded according to the grade laws of the United States Department of Agriculture (97) and cut open and inspected for the presence of seeds or abnormalities. The fruits ripening after August 8 were picked at four-day intervals until the middle of September when they were picked about once a week. This was done to determine the effect of setting the fruit of the first hands upon the later yield of the plants.

Results

There was a striking increase in the amount of fruit produced early in the season on the plants set with 4-phthalimido-2,6-dimethylpyrimidine as can be seen in Table II. The

TABLE II

YIELD OF EARLY TOMATOES AS AFFECTED BY FRUIT SETTING SPRAYS APPLIED AT BLOSSOM TIME

(Total yield of 20 plants for four days expressed in kg)

Time interval	Control	p-chlorophenoxy-acetic acid	4-phthalimido-2,6-dimethyl-pyrimidine		
		30 ppm	100 ppm	250 ppm	500 ppm
July 19-23	1.24	0.91	1.77	3.15	5.89
July 23-27	2.77	1.92	2.85	7.43	4.57
July 27-31	2.21	2.27	4.21	5.78	2.95
July 31-Aug. 4	1.69	2.09	3.27	3.03	1.42
Aug. 4-8	1.09	0.83	1.73	0.39	1.00
L.S.D. for testing between any treatment at any date for 5% level is .46; for 1% level .62					
Total yield to July 31	6.22	5.10	8.83	16.36	13.41
L.S.D. for testing between total yields of treatments at 1% level is .78					
Total yield to Aug. 3	9.00	8.02	13.82	19.78	15.83
L.S.D. for testing between total yields of treatments at 1% level is .59					

500 ppm treatment produced a quantity of fruit earliest. The 250 ppm treatment produced the largest amount of early fruit, but the peak of its early production was somewhat later. The 100 ppm treatment also increased the amount of early fruit, and the peak of its early production occurred after that of the 250 ppm treatment. The greatest difference between treatments occurred early in the season in July so the data were divided to show this difference.

The fruit set with this compound were of as high grade as the check fruit, as shown in Table III. Cracks and a little blossom end rot, both of which were partially due to the irrigation practices were the major causes for tomatoes not being U.S. No. 1. The set fruits were also somewhat larger than the check fruit. The higher the concentration of the spray, the larger the percentage of parthenocarpic fruit. Although the 250 and 500 ppm treatments had many more early fruits, there was a highly significant decrease in the actual number of fruits with seeds, which seems to indicate that these sprays decreased the amount of viable pollen, or the amount of fertilization, or hindered the development of fertilized ovules. At no time was the yield of plants treated with 30 ppm of parachlorophenoxyacetic acid significantly different than that of the control treatment. The plants of 250 and 500 ppm treatments were slightly smaller at the middle of the picking season than the plants of the other treatments. This was primarily due to the fact that the plants of the

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TABLE III

CHARACTERISTICS OF THE FRUIT RIPENING EARLY IN THE SEASON WHICH WAS SET WITH GROWTH REGULATORS

Treatment		Average weight of fruit in gm	Percent U.S. No. 1 fruit	Percent parthenocarpic fruit
Control		119	48	0
30 ppm	p-Chlorophenoxyacetic acid	116	24	0
100 ppm	4-Phthalimido-2,6-dimethylpyrimidine	119	37	22**
250 ppm		136	53	70**
500 ppm		144	44	89**
	L.S.D. at 5%	9	All percentages significantly different from each other when tested by chi square	**Highly significantly different from all other treatments
	L.S.D. at 1%	13		

making vegetative growth while the treated plants were producing fruit.

The total yield of fruit was about the same on all the treatments as shown in Table IV. The plants sprayed with 4-phthalimido-2,6-dimethylpyrimidine had more fruit early in the season and late in the season but had less during the peak of the tomato season.

The plants of the replications on the heavier soil matured their fruit somewhat later, but the type of soil upon which the plants were growing did not affect the ability of its flowers to be set by the growth regulators.

Experiments with the Fall Crop of Ten Greenhouse Tomato Varieties

Methods and Materials

The ability of 4-phthalimido-2,6-dimethylpyrimidine and other growth regulators to set fruit of ten different greenhouse tomato varieties was studied in an experiment conducted cooperatively with Dr. S. H. Wittwer. The experiment was laid out in a split plot design so that the production of the ten tomato varieties, the effect of three fruit setting treatments and a fertilizer treatment, and the interaction of the treatments on the different varieties could be studied in the same experiment. The experiment was replicated four times, with only one plant of one variety receiving any one treatment in a replication.

The seed of the different varieties was planted in vermiculite on June 26, 1952. The seedlings were trans-

TABLE IV

EFFECT OF SETTING THE LOWER HANDS OF JOHN BAER TOMATO PLANTS UPON THE YIELD OF FRUIT THROUGHOUT THE SEASON

(Total yield expressed in kg. from the 20 plants in the five replications for the interval between pickings)

	Control	p-chlorophenoxy-acetic acid 30 ppm	4-phthalimido-2,6-dimethylpyrimidine		
			100 ppm	250 ppm	500 ppm
July 23	1.24	0.91	1.78	3.15	5.90
July 27	2.77	1.91	2.85	7.44	4.57
July 31	2.21	2.27	4.22	5.79	2.95
Aug. 4	1.69	2.09	3.27	3.03	1.42
Aug. 8	1.09	0.83	1.73	0.39	1.00
Aug. 12	1.87	1.32	1.23	0.41	0.25
Aug. 16	5.87	5.57	4.29	3.28	2.75
Aug. 20	10.70	11.12	11.42	11.48	7.23
Aug. 24	19.57	25.68	25.22	17.63	14.48
Aug. 28	13.16	12.06	9.74	7.34	4.57
Sept. 1	7.80	9.19	9.09	6.99	4.67
Sept. 5	2.26	2.99	3.25	3.44	2.50
Sept. 12	7.45	4.28	3.73	4.79	7.81
Sept. 17	6.25	4.29	5.43	10.18	12.19
Sept. 23	5.87	3.81	6.60	12.52	14.30
Sept. 29	6.36	9.54	6.85	11.91	8.77
Total	93.16	97.86	100.70	109.77	95.36

L.S.D.	5%	1%
Testing between yields on any date of any treatment	132	1.74
Between total yields of different treatments	No significant diff.	

planted into four-inch pots on July 10. The soil of the ground bed in the horticultural greenhouse was manured, spaded and sterilized. On August 10 the plants were transplanted one and one-half feet apart in rows which were three feet apart in the ground bed. The plants were trained up on strings and when they reached a height of six feet the tops were pruned off. The fruit setting treatments and the foliar fertilizer sprays were applied at weekly intervals from the middle of September to the end of November. The three fruit setting chemicals -- 100 ppm of 4-phthalimido-2,6-dimethylpyrimidine, 100 ppm of benzothiazole-2-oxyacetic acid, and 50 ppm α-o-chlorophenoxypropionic acid -- were dissolved in a one percent solution of ethyl alcohol, and applied by dipping the open flowers into the solution. The plants received ample sunlight all of the time that they were setting fruit.

Results

The growing conditions were so near optimum for normal pollination and fruit setting of tomatoes that there was no great need for supplementary treatments with growth regulators. The plants treated with 4-phthalimido-2,6-dimethylpyrimidine yielded slightly more fruit than did the check treatment as is shown in Table V. This compound also set more fruits per plant than did the other growth regulators as is shown in Table VIII and caused less blotchy ripening as is shown in Table IX. The treatments with this compound were started about two weeks later than the other treatments

TABLE V

THE TOTAL YIELD OF FRUIT PRODUCED BY TEN GREENHOUSE TOMATO VARIETIES AS AFFECTED BY WEEKLY TREATMENT WITH THREE FRUIT SETTING CHEMICALS OR A FOLIAR FERTILIZER SPRAY

(Kilograms of fruit produced per plant)

Greenhouse tomato varieties	Control	4-Phthalimido-2,6-dimethyl-pyrimidine 100 ppm	Benzothiazole-2-oxyacetic acid 100 ppm	α-o-Chlorophenoxy-propionic acid 50 ppm	20-20-20 foliar spray	Variety averages
Spartan Hybrid	4.01	4.97	4.27	5.25	2.73	4.24
Michigan State Forcing	4.07	3.27	2.95	3.89	2.68	3.37
Eureka Hybrid Number 77	3.31	3.32	3.77	3.48	3.40	3.45
Pink Globe	3.05	3.17	3.62	4.20	1.80	3.17
Number 22 Purdue Univ.	2.02	2.06	3.32	2.84	2.24	2.49
WR3 x Mich. State Forcing	5.08	4.97	4.72	4.85	4.04	4.73
Vineland 511	3.61	4.27	2.83	4.32	3.17	3.64
Vineland 512	3.65	3.12	3.22	3.66	3.35	3.40
Vineland 521	3.22	3.61	3.75	3.93	3.49	3.60
WR3 x Waltham	3.56	3.63	3.22	4.20	2.80	3.48
Treatment averages	3.56	3.64	3.56	4.06	2.97	

L.S.D.	5%	1%
Testing between treatment averages	0.33	0.44
Testing between margin variety averages	0.66	0.89
Testing between different treatments of a variety	1.58	2.08

TABLE VI

THE YIELD OF EARLY FRUITS PRODUCED BY TEN GREENHOUSE TOMATO VARIETIES AS AFFECTED BY WEEKLY TREATMENT WITH THREE FRUIT SETTING CHEMICALS OR A FOLIAR FERTILIZER SPRAY

(Kilograms of fruit produced per plant by November 6)

Greenhouse tomato varieties	Control	4-Phthalimido-2,6-dimethyl-pyrimidine 100 ppm	Benzothiazole-2-oxyacetic acid 100 ppm	α-o-Chlorophenoxy-propionic acid 50 ppm	20-20-20 foliar spray	Variety averages
Spartan Hybrid	1.23	1.31	1.46	1.41	0.84	1.25
Michigan State Forcing	0.58	0.53	0.66	0.68	0.27	0.54
Eureka Hybrid Number 77	0.83	1.00	1.23	0.97	0.97	1.00
Pink Globe	0.38	0.21	0.97	0.84	0.50	0.49
Number 22 Purdue Univ.	0.21	0.00	0.79	0.55	0.34	0.38
WR3 x Mich. State Forcing	1.28	1.29	0.97	1.63	0.80	1.19
Vineland 511	0.73	1.14	1.08	1.35	0.69	1.00
Vineland 512	0.99	0.56	0.90	0.89	0.69	0.80
Vineland 521	0.90	1.08	1.35	1.20	0.99	1.10
WR3 x Waltham	1.07	1.06	0.90	1.40	0.96	1.08
Treatment averages	0.82	0.82	1.03	1.09	0.66	

L.S.D.	5%	1%
Testing between treatment averages	0.18	0.23
Testing between margin variety averages	0.26	0.35
Testing between different treatments of a variety	0.71	0.93

TABLE VII

THE AVERAGE NUMBER OF FRUITS SET PER PLANT ON TEN GREENHOUSE VARIETIES AS AFFECTED BY WEEKLY TREATMENT WITH THREE GROWTH REGULATORS OR A FOLIAR FERTILIZER SPRAY

Greenhouse tomato varieties	Control	4-Phthalimido-2,6-dimethyl-pyrimidine 100 ppm	Benzothiazole-2-oxyacetic acid 100 ppm	α-o-Chlorophenoxy-propionic acid 50 ppm	20-20-20 foliar spray	Variety averages
Spartan Hybrid	36	38	33	39	26	34
Michigan State Forcing	36	30	25	33	25	30
Eureka Hybrid Number 77	29	29	31	27	31	30
Pink Globe	28	29	29	28	18	27
Number 22 Purdue Univ.	17	18	23	21	20	20
WR3 x Mich. State Forcing	37	43	35	35	36	37
Vineland 511	45	46	29	45	41	41
Vineland 512	43	37	35	40	33	38
Vineland 521	43	44	38	40	45	42
WR3 x Waltham	37	30	27	29	27	30
Treatment averages	35	34	30	34	30	

L.S.D.	5%	1%
Testing between treatment averages	3	4
Testing between margin variety averages	5	6
Testing between different treatments of a variety	13	17

TABLE VIII

THE AVERAGE WEIGHT PER FRUIT OF TOMATOES FROM TEN GREENHOUSE VARIETIES AS AFFECTED BY WEEKLY TREATMENT WITH THREE GROWTH REGULATORS OR A FOLIAR FERTILIZER SPRAY

(Grams per fruit)

Greenhouse tomato varieties	Control	4-Phthalimido-2,6-dimethyl-pyrimidine 100 ppm	Benzothiazole-2-oxyacetic acid 100 ppm	α-o-Chlorophenoxy-propionic acid 50 ppm	20-20-20 foliar spray	Variety averages
Spartan Hybrid	127	132	127	137	95	124
Michigan State Forcing	112	110	121	120	108	114
Eureka Hybrid Number 77	127	115	120	132	111	121
Pink Globe	113	107	128	150	104	120
Number 22 Purdue Univ.	121	116	148	137	96	123
WR3 x Mich. State Forcing	137	116	134	140	114	128
Vineland 511	78	93	101	97	77	89
Vineland 512	85	83	92	92	86	87
Vineland 521	80	83	98	99	77	87
WR3 x Waltham	109	125	123	145	103	121
Treatment average	109	108	119	125	97	

L.S.D.	5%	1%
Testing between treatment averages	7	9
Testing between margin variety averages	11	15
Testing between different treatments of a variety	30	39

TABLE IX

THE PERCENTAGE OF GRADED FRUIT EXHIBITING BLOTCHY RIPENING FROM TEN GREENHOUSE TOMATO VARIETIES AS AFFECTED BY WEEKLY TREATMENTS WITH THREE GROWTH REGULATORS AND A FOLIAR FERTILIZER SPRAY

Greenhouse tomato varieties	Control	4-Phthalimido-2,6-dimethyl-pyrimidine 100 ppm	Benzothiazole-2-oxyacetic acid 100 ppm	α-o-Chlorophenoxy-propionic acid 50 ppm	20-20-20 foliar spray	Weighted variety averages
Spartan Hybrid	25	28	50	35	25	32
Michigan State Forcing	29	54	48	71	65	53
Eureka Hybrid Number 77	34	37	62	64	34	47
Pink Globe	24	38	32	50	35	36
Number 22 Purdue Univ.	26	50	31	67	48	45
WR3 x Mich. State Forcing	24	28	24	41	34	30
Vineland 511	13	20	20	36	18	22
Vineland 512	30	24	35	53	25	34
Vineland 521	15	22	30	41	23	25
WR3 x Waltham	21	30	34	40	31	31
Weighted treatment averages	23	30	37	48	32	
Actual number of good quality fruits on 40 plants which received same treatment	637	633	520	446	549	

Treatment averages are highly significantly different from each other at the 1% level as tested by chi square.

TABLE X

THE PERCENTAGE OF FRUIT WITH BLOSSOM END ROT FROM TEN GREENHOUSE TOMATO VARIETIES AS AFFECTED BY WEEKLY TREATMENT WITH THREE GROWTH REGULATORS AND A FOLIAR FERTILIZER SPRAY

Greenhouse tomato varieties	Control	4-Phthalimido-2,6-dimethyl-pyrimidine 100 ppm	Benzothiazole-2-oxyacetic acid 100 ppm	α-o-Chlorophenoxy-propionic acid 50 ppm	20-20-20 foliar spray	Weighted variety averages
Spartan Hybrid	0	0	0	0.65	0	0.01
Michigan State Forcing	0	0	0	0.77	0	0.02
Eureka Hybrid Number 77	0	0	0	0	0	0
Pink Globe	0	0	0.85	3.57	0	0.94
Number 22 Purdue Univ.	0	0	0	0	0	0
WR3 x Mich. State Forcing	0	0	0	0.72	0	0.01
Vineland 511	0	0	0	1.11	0	0.03
Vineland 512	0	0	0	1.26	0	0.03
Vineland 521	0	0	0	0	0	0
WR3 x Waltham	0	5.93	0.20	6.84	0.92	2.78
Weighted treatment averages	0	1.07	0.04	1.42	0.01	

Treatment averages are highly significantly different from each other at the .01% level as tested by chi square.

and this fact may explain why the yield of fruit early in the season on the plants treated with 4-phthalimido-2,6-dimethylpyrimidine is not different than the nontreated plants as shown in Table VI.

The benzothiazole-2-oxyacetic acid treatment did increase the amount of fruit produced early in the season. The 100 ppm concentration, which was used then, produced such serious formative effects upon the leaves, even though it was only applied to the flowers, that the concentration was reduced to 50 ppm in October and the total yield of fruit set with this compound was the same as the yield of the nontreated plants. The fruit set with this compound were somewhat larger and a larger percentage of them showed blotchy ripening. Although the treatment with α-o-chlorophenoxypropionic acid increased the total yield of fruit, the amount of early fruit, and the average weight of the individual fruits, it also increased the percentage of fruit with blotchy ripening and the incidence of blossom end rot and resulted in less good quality marketable fruit than any other treatment.

EXPERIMENTS ON THE SETTING OF MUSKMELON AND CUCUMBER FRUITS

Review of Literature

After 4-phthalimido-2,6-dimethylpyrimidine was found to be useful in setting tomatoes, experiments were conducted to determine if it would set cucumbers and muskmelons. Cucumbers and many other cucurbits have been set parthenocarpically with growth regulators, but a satisfactory combination or concentration of growth regulators has not been found for setting muskmelons (35).

Gustafson (31) has reported setting the improved Jersey Pickle cucumber with naphthaleneacetic acid, but he was unable to set the Vaughn variety of cucumber. He also set Hubbard, buttercup, and crookneck summer squash, peppers, eggplant and watermelons, with 10,000 ppm or 20,000 ppm of naphthaleneacetic acid or indolebutyric acid in lanolin. He does not include any data on untreated plants, nor does he describe how pollination was prevented. Wong (109) induced parthenocarpic development of National Pickling variety of cucumbers with 10,000 ppm and higher concentrations of naphthaleneacetic acid in lanolin. He prevented pollination by covering the blossoms with wire cages. He also set watermelons and peppers with naphthaleneacetic acid. Lutokhin (55) set pumpkins with 500 ppm of indolebutyric acid, indolepropionic acid, or 2,4-dichlorophenoxy-α-butyric acid in glycerol. Whitaker (104) reported that 10,000 ppm to 20,000 ppm of para-chlorophenoxyacetic acid applied after hand pollinating muskmelons in a breeding program would give a significant increase in the percentage of fruit set, but of course these were not parthenocarpic. Mann (56) has reviewed the literature relevant to fruit set of the cantaloupe.

Nitsch, Went and others (67) studied the sex expression of cucurbit flowers. They stated that there was a series of flower types progressing from male to female at successively higher nodes. The lower nodes developed normal male flowers, then normal male and normal female flowers were developed, later giant female flowers developed with an inhibited type of male flower, finally only parthenocarpic female flowers were developed at the highest nodes. He stated that cold temperatures, below 76° F and short days, about 12 hours, hastened the development toward parthenocarpy in Boston Pickling cucumbers.

Experiment with Muskmelons

Methods and Materials

Delicious variety muskmelons were planted in plant bands on May 15 and grown in the greenhouse until the second week in June when they were set out on a Fox sandy loam soil on the college farm. They were watered with a starter solution of "Take Hold" soon after transplanting. There were two plants per hill and two hills per replication. Five treatments were replicated three times. The male flowers were pulled off as they formed so that the plants would have more flowers. The muskmelon has a perfect flower, so the flowers were emasculated, or in some cases the pistil as well as the stamens was broken out and the lanolin put in the cup formed by the calyx. The treatments consisted of pure lanolin used as a control, 60 ppm of para-chlorophenoxyacetic acid, 200 ppm, 500 ppm, and 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine mixed into a warmed lanolin paste. From six to fifteen of the first perfect flowers on each plant were treated. The flowers opening the last third of the season were not included to avoid any fruit which would be naturally parthenocarpic, although this is very rare in muskmelons.

Results

No parthenocarpic fruits were obtained from any treatment. This was a large enough population to detect quite small differences between treatments if they had occurred.

Experiment with Cucumbers

Methods and Materials

About 80 Burpee hybrid cucumber plants were grown in 10-inch pots in the greenhouse. They were planted the first week of June and trained up on stakes and strings to a height of about six feet at which height the terminal buds were pinched off. The cucumber is a monecious plant so the female flowers were clipped shut before anthesis with paper clips to prevent pollination. The male flowers were also picked off and there were no other cucurbits within half a mile. Every three days the flowers which had been clipped were dipped. Distilled water was used for the control treatment; 30 ppm of para-chlorophenoxyacetic acid and 100 ppm, 250 ppm, and 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine were the other treatments used to set the flowers. About a hundred flowers per treatment were dipped. The temperatures in the greenhouse during July or August were frequently around 100° F, the days were about 15 hours long, and only the earlier flowers were used to avoid the possibility of natural parthenocarpic fruit.

Results

No conclusions can be reached from this experiment concerning the ability of 4-phthalimido-2,6-dimethylpyrimidine to cause the parthenocarpic development of cucumbers because over one-third of the fruits of each treatment, including the control treatment were parthenocarpic. A parthenocarpic and a normal cucumber are shown in Figure 4. The parentage of the Burpee Hybrid is not published however there are English strains which are naturally parthenocarpic and one of these might be a parent of the seed and explain the large number of naturally parthenocarpic fruit.

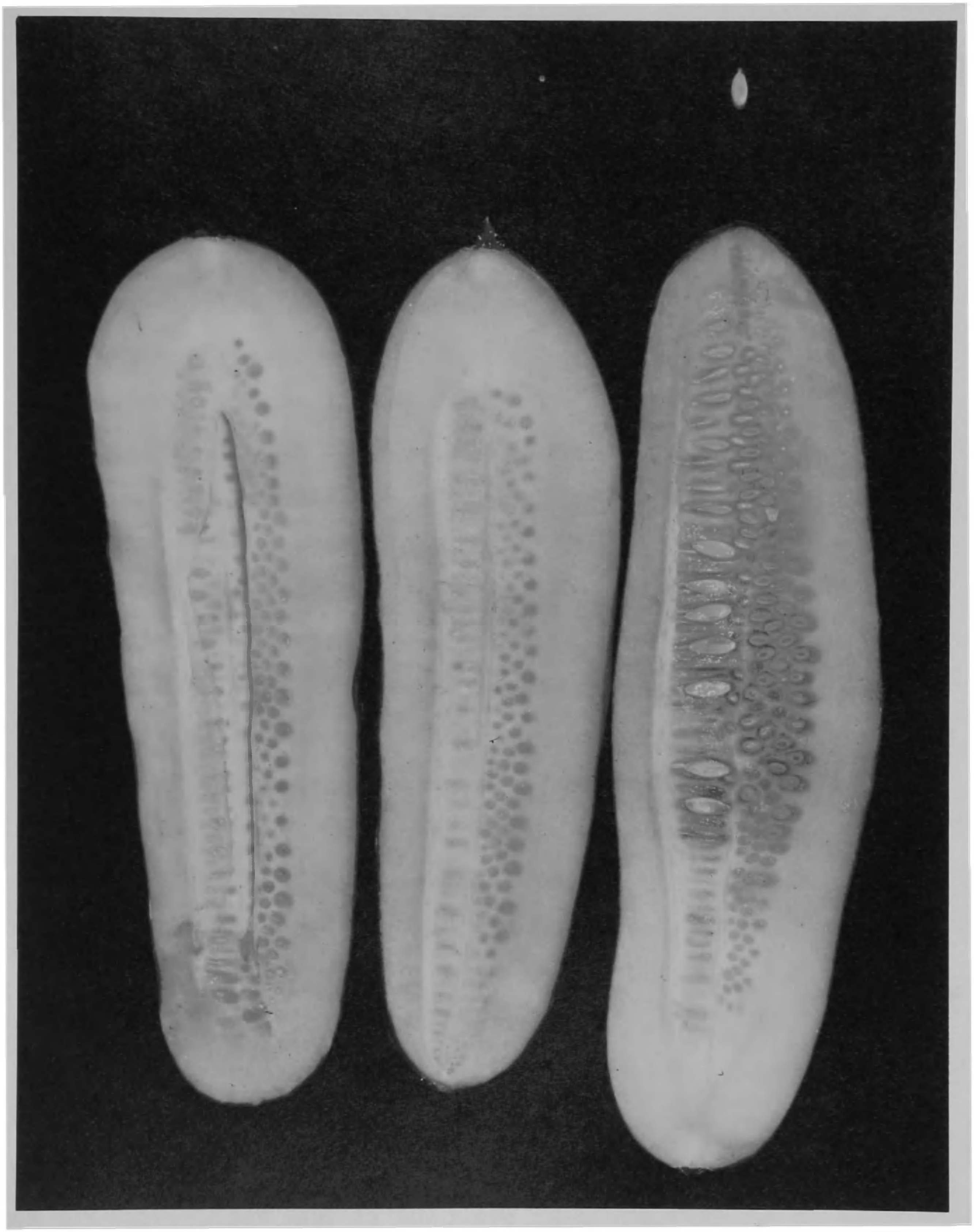


Figure 4. Two parthenocarpic Burpee Hybrid cucumbers on the left and a normal cucumber on the right. Representative ovules from each type are shown directly above the cucumbers.

EXPERIMENTS ON THE ABSCISSION OF COLEUS PETIOLES

Review of Literature

The abscission of various organs is a wide-spread phenomenon of plants. The abscission of leaves, especially of deciduous plants in the fall, the abscission of flowers and immature fruits of plants, the abscission of ripe fruit, and the abscission of branches of some plants are very common occurrences.

Although abscission is frequently accompanied by some cell division and the formation of a primary protective layer, the primary process of abscission usually involves a dissolution of the intercellular substance and a mechanical breaking of the vascular tissue. The biochemists (15) have chosen to call the intercellular pectic substance protopectin, to distinguish it from the other pectic materials. This galacturonic acid polymer which makes up the bulk of the intercellular substance in young cells probably has a definite orientation and the manner of cross-linkage and arrangement of calcium and methyl groups may be entirely changed in any extraction process. The enzyme which attacks protopectin yielding soluble pectin is known as protopectinase and an adequate chemical characterization of it has not been carried out (13). Because of this, the best available method of studying the effect of growth regulators on the process of abscission is by studying the rate of abscission.

La Rue (50) was the first man to study the effect of auxin on the abscission of petioles. He found that by applying a lanolin paste containing 50 ppm of indoleacetic acid to the tip of debladed petioles of coleus plants kept in the dark, the petioles stayed on two and a half times longer than the controls. Beal and Whiting (10) reported that the abscission of stems of the four-o'clock plant, *Mirabilis jalape*, could be inhibited by applying indoleacetic acid in lanolin to the cut surface of a branch. The delaying of the preharvest drop of apples with sprays of growth regulators is another example which will be discussed later.

The physiological processes involved in abscission are not well known. In recent years there has been considerable objection to the theory that abscission is the result of cell divisions and growth of an abscission layer which pushes off the organ (28). The division of cells may or may not accompany abscission. More emphasis has been placed upon the breakdown of the intercellular substance and the biochemical changes responsible for this. Since most chemical changes in living organisms are accomplished by enzymes, many attempts have been made to isolate or identify protopectinase. Kertesz (45) and others have been unable to find an enzyme, and suggest that perhaps just the potassium ion, or sunlight may be the catalyst involved.

Whatever the mechanism may be, indoleacetic acid has been found to inhibit abscission, and ethylene and other compounds have been found to stimulate it. Upon these facts, the hypothesis has been advanced that it is the balance of auxin and ethylene which governs abscission. Ethylene is a normal product of plant metabolism, whose production is increased under anaerobic conditions. In ripening fruits it is known to accelerate its own production, and to accelerate the rate of respiration, which makes the conditions more anaerobic if the fruit is kept in a closed place as fruits are. Hall (34) found that ethyl alcohol, pectin and pentoses were very good substrates for an ethylene producing enzyme which he extracted from yeast. Ethyl alcohol, a two-carbon molecule, is one of the non-volatile end products which pectins and pentoses might be broken down to during anaerobic respiration. Ethylene is a volatile, two-carbon gas which apparently can come from ethyl alcohol. One must remember however that this is an endothermic reaction requiring about 77 kilo-calories per mole.

Hall (33) found that in the presence of indoleacetic acid his enzyme produced little ethylene while in the absence of indoleacetic acid larger amounts of ethylene were produced. Thimann (91) and Bonner (13) and others have suggested that indoleacetic acid is necessary in organic acid metabolism, a process in aerobic respiration. Leaves and flowers are known to produce indoleacetic acid. The hypothesis which has been proposed is that when the supply of auxin to the abscission zone is decreased, and the respiration becomes more anaerobic, ethylene is evolved in the tissue which further stimulates its respiration and the utilization of pectic substances, and pentoses which are responsible for holding the cells together.

Neely et al (66) found the activity of pectin methoxylase was nearly doubled in stems and leaves of red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid. Perhaps this indicates that the auxins affect directly the activity of enzymes responsible for the splitting off of methoxy groups from pectin.

The influence of 4-phthalimido-2,6-dimethylpyrimidine upon the abscission of debladed petioles of coleus plants was studied in a series of experiments on potted plants in the greenhouse. The abscission of debladed petioles of coleus is quite precise, with the lower, older petioles abscissing earlier. The growth regulators were applied in lanolin, in the manner used by La Rue, and as a spray, the common commercial method.

Lanolin Application

Methods and Materials

Cuttings of the Christmas Gem variety of *Coleus blumei* L. were made in January. The lateral shoots which develop in the axils of leaves were cut off to remove that source of auxin. Forty-six plants were graded for size and the blade of one leaf at each of the lower nodes was cut off. The tips of the petioles of the different lots of plants were then covered with a lanolin paste containing 100 ppm, 250 ppm, 500 ppm, 750 ppm, and 2500 ppm of 4-phthalimido-2,6-dimethylpyrimidine. A pure lanolin paste was used as a control and 100 ppm of naphthaleneacetic acid was used for comparison with a standard growth regulator. The growth regulators were dissolved in a small quantity of ethyl alcohol before mixing into warmed lanolin. The concentrations stated above were made up as weight of dry growth regulator per weight of lanolin. The amount of lanolin paste applied per petiole varied somewhat. The potted plants were laid out in a latin square design on the greenhouse bench with each of the seven treatments replicated seven times except the 2500 ppm treatment which contained only three plants. The number of petioles which did not absciss with slight pressure to break the vascular bundles, was recorded once a day.

Results

Only the 100 ppm treatment of naphthaleneacetic acid caused a highly significant decrease in the rate of abscission, while the highest concentration of 4-phthalimido-2,6-dimethylpyrimidine appeared to increase the rate of abscission as can be seen in Table XI. This is not significant and might possibly be due to a higher concentration of alcohol which according to Hall could be converted to ethylene.

TABLE XI

THE ABSCISSION RATE OF DEBLADED COLEUS PETIOLES AS INFLUENCED BY GROWTH REGULATORS APPLIED IN LANOLIN TO THE TIPS OF THE PETIOLES

(Average number of petioles remaining on the plants at periods after treating)

Hours after treating	Control	Naphthalene-acetic acid 100 ppm	4-Phthalimido-2,6-dimethylpyrimidine				
			100 ppm	250 ppm	500 ppm	750 ppm	2500 ppm
0	5.57	5.57	5.57	5.57	5.57	5.57	5.57
12	5.42	5.28	5.28	5.57	5.57	5.57	5.57
34	4.85	4.99	4.71	4.03	4.06	5.27	2.32
66	2.71	4.85	2.28	2.92	1.96	2.78	1.39
96	1.57	4.71	1.71	2.09	1.05	2.05	0.93

L.S.C. at 5% level for testing between different treatments at the same time is 2.35

Spray Application

Methods and Materials

The 4-phthalimido-2,6-dimethylpyrimidine was applied in aqueous solution as sprays in two similar experiments. The entire plant was sprayed with the knapsack sprayer with the attachment allowing a clean flask to be used for each compound. The plants used in these experiments had been used previously, however, the same treatments were applied to the plants in both experiments and considerable time was allowed to elapse between the experiments. None of the treatments caused any formative effects or morphological changes.

Results

Both of these experiments showed the same trend. Tables XII and XIII and Figure 5 show that only the 1000 ppm concentration of 4-phthalimido-2,6-dimethylpyrimidine and the 10 ppm of naphthaleneacetic acid caused a significant decrease in the rates of abscission, and there was no significant difference between these concentrations of these chemicals.

Defoliation

The results of the first experiment with the high concentration of 4-phthalimido-2,6-dimethylpyrimidine applied in lanolin seemed to indicate that this compound might be useful as a defoliant. Salts and acids of 3,6-endoxohexa-

TABLE XII

ABSCISSION RATE OF DEBLADED COLEUS PETIOLES AS INFLUENCED BY SPRAYS OF GROWTH REGULATORS

(Average number of petioles remaining on the plant at periods after spraying)

First Experiment

Hours after treating	Control	Naphthaleneacetic acid 10 ppm	4-Phthalimido-2,6-dimethylpyrimidine				
			10 ppm	25 ppm	50 ppm	75 ppm	1000 ppm
0	4.00	4.00	4.00	4.00	4.00	4.00	4.00
20	3.86	4.00	3.52	3.23	4.00	3.72	4.00
26	3.60	4.00	3.36	3.23	4.00	3.72	4.00
44	3.47	3.87	3.04	2.77	3.45	3.45	4.00
56	2.00	3.48	1.76	2.00	2.07	2.48	3.84

L.S.D. at 5% level for testing between different treatments at the same time is 1.32

TABLE XIII

ABSCISSION RATE OF DEBLADED COLEUS PETIOLES AS INFLUENCED BY SPRAYS OF GROWTH REGULATORS

(Average number of petioles remaining on the plants at periods after spraying)

Second Experiment

Hours after treating	Control	Naphthalene-acetic acid 10 ppm	4-Phthalimido-2,6-dimethylpyrimidine				
			10 ppm	25 ppm	50 ppm	75 ppm	1000 ppm
0	6.00	6.00	6.00	6.00	6.00	6.00	6.00
73	5.32	5.70	5.00	4.54	4.71	5.16	4.59
91	3.71	5.70	4.00	3.66	4.29	4.05	4.00
97	3.54	5.55	3.71	3.51	4.29	3.77	3.88
123	1.77	4.95	2.14	2.34	2.29	1.40	3.29

L.S.D. at 5% level for testing between different treatments at the same time is 1.36

Figure 5. The delay in abscission of debladed petioles of coleus resulting from a spray with 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine is evident on the plant on the left as compared with a non-sprayed plant on the right.

hydrophthalic acid were reported by Tischler, Bates, and Quimba (93) to be defoliants for cotton, beans, hydrangeas and other woody plants and to be herbicides. Pridham (70) found disodium-3,6-endoxohexahydrophthalate effective in defoliating about 80 different species of nursery stock. Coleus plants sprayed with a solution containing 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine and 10,000 ppm of ammonium thiocyanate, a defoliant used on cotton, developed large necrotic areas on the leaves and a drooping of the leaves, but the leaves did not absciss. The N-1 naphthyl phthalamic acid used in thinning pears caused serious defoliation when applied at full bloom. The 4-phthalimido-2,6-dimethylpyrimidine was ineffective as a defoliant on tomatoes, beans, cucumbers, coleus, apples, pears, peaches or cherries.

EXPERIMENTS ON THE THINNING OF TREE FRUITS AT BLOSSOM TIME

Review of Literature

The thinning of the overly abundant number of flowers or fruits which fruit trees frequently have is primarily a process of increasing the abscission of flowers or fruits. Growth regulators have been experimented with and have been used with varying degrees of success for several years for this purpose. Burkholder and McCown (17) were the first to notice that sprays of naphthaleneacetic acid and naphthaletamide would reduce the fruit set of apples. An entirely satisfactory method of thinning peaches has not yet been found (49, 85). The degree of thinning resulting from sprays of growth regulators is frequently greatly influenced by tree vigor, variety, time of application, environmental conditions, and it is very hard to make predictions or recommendations concerning their use. Stebbins (87) and other workers have found that about 20 ppm of naphthaleneacetic acid is the proper concentration for Duchess. The only phthalamic acid derivatives known to have been tested are N-phenylphthalimide, which Detar (20) reported ineffective in setting fruit or thinning Bartlett pears, and N-1 naphthyl phthalamic acid. Chadwick, Miller and Erskine (18) reported that the latter compound successfully eliminated the flowers of Norway Maple, *Acer platanoides*, but caused a moderate twisting of leaves. The flowers of purple crabapple, *Malus purpurea*, and horse chestnut, *Aesculus hippocastanum*, were eliminated with 422 ppm with only a little foliage injury on the crabapple but considerable injury if the horse chestnut was sprayed at full bloom. The use of the common growth regulators in thinning fruit trees is summarized by Batjer and Hoffman (8).

Purpose and General Methods

Experiments were conducted to determine if 4-phthalimido-2,6-dimethylpyrimidine and related phthalamic acid derivatives would be effective in the thinning of apples, pears, or peaches. All of the sprays were applied with the knapsack sprayer with the attachment which allowed a different flask to be used for each treatment. The nozzle was very simple and was rinsed between treatments. The N-1 naphthyl phthalamic acid and the N-2 chlorophenyl phthalamic acid used in these experiments is not soluble in water, but is soluble in water as a diethanol amine salt. The diethanol amine salt was prepared in this and all other experiments by making a paste of the powders of the phthalamic acid derivatives with an equal weight of diethanol amine which is a viscous liquid.

Each treatment was applied to a limb on three different trees which were located next to each other on the horticultural farm at East Lansing, Michigan, in the spring of 1952. The ordinary fungicide and insecticide sprays were applied to control diseases.

Although the thinning and stop-drop sprays were conducted on a very small scale, large differences are necessary for a compound to be useful commercially and experiments of this size should detect such differences.

The number of flowers or fruits on the limbs at the time of spraying was recorded and the number of these which had not abscissed after the June drop, and had developed into normal fruit was counted the last week in June. This data was converted to percentages, the averages of which are recorded in the tables of this section. Since percentages are not normally distributed, the percentages were transformed to the angle equal to the arc sine of the square root of the percentage as suggested by Snedecor (84) and analyzed by analysis of variance.

Duchess of Oldenberg Apples

Methods

Sprays of 100 ppm, 500 ppm, and 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine; 100 ppm, 500 ppm, 1000 ppm, and 5000 ppm of N-1 naphthyl phthalamic acid; and 20 ppm of naphthaleneacetic acid were applied to limbs of Duchess trees during the middle of the day on May 5. The maximum number of flowers were probably in bloom earlier that morning. The average number of flowers per spur (5.05) on the limbs used for the different treatments, was calculated and there was no significant difference at the beginning of the experiment.

Entirely different limbs on the same trees were sprayed on June 4 or 30 days after full bloom to determine if sprays during the normal June drop would increase this drop. Only high concentrations, 1000 ppm and 2000 ppm of the diethanol amine salts of N-1 naphthyl phthalamic acid, which had been effective on pears, and N-2 chlorophenyl phthalamic were used because only a limited supply of 4-phthalimido-2,6-dimethylpyrimidine was left and this type of experiment requires several gallons.

Results

From Table XIV it can be seen that the small number of trees and the large variation between trees make it impossible to draw any definite conclusions from this experiment. The apparent increase in the number of fruits remaining on the limbs sprayed on June 4 with N-1 naphthyl phthalamic acid, and the lower concentration of N-2 chlorophenyl phthalamic as compared with the control limbs is probably an artifact because more vigorous, higher limbs were chosen for the later spray treatments while the control limbs were the same as those used for the earlier spraying and were somewhat lower. The higher concentration of N-2 chlorophenyl phthalamic acid did appear to thin with only a slight amount of leaf injury.

TABLE XIV

THE EFFECTIVENESS OF GROWTH REGULATORS IN THINNING THE FRUITS OF DUCHESS APPLE TREES

Treatment		Average percent of the number of fruits originally on limbs which did not absciss by the end of June
	Sprayed May 5 at Full Bloom	
Control		2.6
20 ppm	Naphthaleneacetic acid	3.5
100 ppm		5.8
500 ppm		2.6
1000 ppm		2.4
100 ppm	N-1 naphthyl phthalamic acid	3.5
500 ppm		1.5
1000 ppm		4.8
5000 ppm		3.4
No significant difference between treatments		
	Sprayed June 4 During June Drop	
Control		21.8
1000 ppm	N-1 naphthyl phthalamic acid	48.4*
2000 ppm		45.3*
1000 ppm	N-2 chlorophenyl phthalamic acid	32.8
2000 ppm		11.9**

*Highly significantly higher at 1% level than control
**Very nearly significantly lower at 5% level than control

Bartlett Pears

Methods

On May 4, the day of full bloom, sprays of 100 ppm, 500 ppm and 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine and 100 ppm and 2000 ppm of N-1 naphthyl phthalamic acid were applied to limbs of bartlett pear trees of medium vigor. There was no significant difference in the average number of flowers (7.20) per spur on the limbs used for different treatments.

Results

The 4-phthalimico-2,6-dimethylpyrimidine was ineffective in thinning. The 2000 ppm concentration of N-1 naphthyl phthalamic acid caused a severe epinasty as shown in Figure 9. It inhibited further increase in the size of leaves which made these leaves appear smaller when compared with the leaves on the control limbs and many of the leaves abscissed. Many of these leaves had black necrotic areas. However, this may not be due entirely to the treatment since there was some pear psylla out then. During the fourth week after spraying at the calyx stage of development, the fruits receiving this treatment, abscissed at a rapid rate. On May 20 about half (average of 48 percent) of the fruits had abscissed from the treated limbs whereas the normal drop had not yet started on the other limbs. The 100 ppm concentration of the same compound did not cause the severe effects on the leaves and resulted in much more nearly the desired amount of thinning as shown in Table XV.

Peaches

Methods

Sprays of 100 ppm, 500 ppm and 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine and 100 ppm and 1000 ppm of N-1 naphthyl phthalamic acid were applied to different limbs on three peach trees on the evening of May 3, 1952, which was about two days after the peak of full bloom. The trees used were in an old block of mixed varieties so that in order to get trees near to each other and of approximately equal vigor, trees of three different varieties, a South Haven, a Salberta, and a Shipper Late Red, were used. On June 4, or 35 days after full bloom, a second group of limbs on the same trees were sprayed with the following solutions: 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine, 1000 ppm and 2000 ppm of N-1 naphthyl phthalamic acid and 1000 ppm and 2000 ppm of N-2 chlorophenyl phthalamic acid.

Results

The N-2 chlorophenyl phthalamic acid appeared to cause a yellowing and curling of leaves about a week after spraying, as shown in Figure 9. Although this compound shows the most promise of the compounds tested for the thinning of peaches, the injury to leaves makes its use unadvisable. The large variation between the trees and the small number of limbs used make it impossible to draw any definite conclusions from this experiment.

TABLE XV

THE EFFECTIVENESS OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE AND N-1 NAPHTHYL PHTHALAMIC ACID SPRAYS APPLIED AT FULL BLOOM IN THINNING THE FRUITS OF BARTLETT PEAR TREES

Treatment	Average percentage of flowers which did not absciss by last week in June
Control	15
100 ppm 4-phthalimido-2,6-dimethylpyrimidine	20
500 ppm	23
1000 ppm	14
100 ppm N-1 naphthyl phthalamic acid	7+
2000 ppm	1*

+Almost significantly less than control at 5% level
*Significantly less than control at 5% level

TABLE XVI

THE EFFECTIVENESS OF SPRAYS OF 4-PHTHALIMIDO-2,6-DIMETHYL-PYRIMIDINE AND PHTHALAMIC ACID DERIVATIVES IN THINNING PEACHES

Treatment		Average percentage of fruits present at time of spraying which did not absciss by July
	Limbs Sprayed May 3, About Full Bloom	
Control		36
100 ppm	4-phthalimido-2,6-dimethylpyrimidine	16
500 ppm		7
1000 ppm		23
100 ppm	N-1 naphthyl phthalamic acid	21
1000 ppm		8
No significant difference between treatments		
	Limbs Sprayed June 4, or 35 Days After Full Bloom	
Control		40
1000 ppm	4-phthalimido-2,6-dimethylpyrimidine	22
1000 ppm	N-1 naphthyl phthalamic acid	26
2000 ppm		40
1000 ppm	N-2 chlorophenyl phthalamic acid	18
2000 ppm		13
No significant difference between treatments		

EXPERIMENTS ON THE DELAYING OF THE PREHARVEST DROP OF APPLES

Review of Literature

One practical application of the effect of growth regulators upon the process of abscission is the use of growth regulators to delay the preharvest drop of fruits. In 1939, Gardner, Marth, and Batjer (26) reported for the first time, using naphthaleneacetic acid to delay the dropping of apples. Since that time it has been found to be quite effective on many varieties of apples, especially the early varieties, on pears, and on some varieties of oranges, grapefruits, and apricots. Batjer and Thompson (9) found that 10 ppm of 2,4-dichlorophenoxyacetic acid was more effective than naphthaleneacetic acid in delaying the drop of Winesap apples and that the best time to apply the 2,4-dichlorophenoxyacetic acid was between 20 and 30 days before the dropping begins. They found 2,4-dichlorophenoxyacetic acid to be entirely ineffective on the Duchess variety. Napthaleneacetic acid sprays used to delay dropping of apples are usually applied within a week before or at the time dropping begins. Recently 2,4,5-trichlorophenoxypropionic acid, sold under the trade name of "Color Set" has been found to be quite effective in delaying the dropping. The use of growth regulators in relation to the production of tree fruits has been reviewed by Gardner (25) and Pearse (71).

Methods and Materials

A small scale experiment was conducted to determine if 4-phthalimido-2,6-dimethylpyrimidine or a few derivatives of 2,4-dichlorophenoxyacetic acid which Hamner (35) had observed to be less toxic in his trials would be effective in the delaying of the preharvest drop. Duchess of Oldenberg is an early maturing variety of apples on which fruit drop is a problem. There were only three trees and only a limited amount of chemicals available so different limbs were used for the different treatments. All of the treatments were applied to each tree, so the three different trees could be considered replications. One of the trees was less vigorous as could be seen from its response to the thinning and stop drop sprays, the circumference of its trunk, and growth of its terminal shoots. This tree also had a heavier crop. All the trees, however, appeared very healthy and, except for a little scab early in the season, diseases were well controlled.

Concentrations of 100 ppm, 500 ppm, and 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine, 10 ppm, 25 ppm and 100 ppm of 2,2,2-trichloroethyl-2',4'-dichlorophenoxyacetate, and 10 ppm, 25 ppm, and 100 ppm of β (2,4-dichlorophenoxy)-α-hydroxypropionic acid were used. The 4-phthalimido-2,6-dimethylpyrimidine is water soluble, the second compound came in a solution which did not form a good solution or emulsion with water even when some ethyl alcohol and some synthetic detergent were added. By shaking vigorously before spraying a fairly good emulsion was obtained, but a perfectly uniform distribution of the compound on the leaves was probably not attained. The third compound was readily soluble in water as a diethanol amine salt. The compounds were applied with a knapsack type sprayer with the attachment which allowed a different flask to be used for each different chemical. The sprays were applied on August 14, 1952, at mid-day when the temperature was about 85° F. Batjer (6) obtained the best response to naphthaleneacetic acid stop-drop sprays when they were applied at temperatures above 80° F. When the limbs were sprayed the seeds of the apples were turning brown, and some apples were falling. A moderate rain occurred 36 hours after spraying.

Results

There was no apparent decrease in the dropping of the fruit and on August 20, 1952, six days after spraying, the records of Table XVII were taken, which also show no significant difference between treatments.

TABLE XVII

THE EFFECTIVENESS IN DELAYING THE PREHARVEST DROP OF DUCHESS APPLES ON LIMBS SPRAYED WITH GROWTH REGULATORS JUST BEFORE DROPPING BEGAN

Treatment		Average percentage of apples on at time of spraying which did not drop in six days
Control		64
100 ppm	4-phthalimido-2,6-dimethylpyrimidine	57
500 ppm		51
1000 ppm		53
10 ppm	2,2,2-trichloroethyl-2,4-dichlorophenoxyacetate	38
25 ppm		60
100 ppm		71
10 ppm	B-(2,4-dichlorophenoxy) α -hydroxy-propionic acid	48
25 ppm		64
100 ppm		71

No significant difference between treatments

EXPERIMENTS ON THE ROOTING OF CUTTINGS

Review of Literature

One of the first uses found for growth regulating chemicals was as an aid in the rooting of cuttings. In 1929, Went (98) found that a non-specific, heat resisting substance could be extracted from leaves or germinating barley which, when applied to cuttings, promoted the development of new roots. One of the most active ingredients of Went's preparations was probably indole-3-acetic acid which Thimann and Went (93) found to aid in the rooting of cuttings in 1934. During the next decade a huge number of different chemicals, primarily different derivatives and formulations of aryloxy-alkyl-carboxylic acids and aryl-alkyl-carboxylic acids, were tried on a large variety of different species under different conditions. This work has been condensed and collected by Thimann and Behnke (92), Avery and Johnson (6) and Pearse (71).

Succulent cuttings of adaptable species are usually quite easy to root naturally, but applications of growth substances frequently increase the number of roots and speed of rooting, and may alter the type of roots formed. Dormant hardwood cuttings, greenwood cuttings and evergreen cuttings are frequently harder to root. Rooting is usually preceeded by the formation of callus tissue around the wound surface. The root primordia are initiated from the pericycle but may arise from surrounding tissues. The roots may emerge through the callus tissue, or through the basal portion of the cutting.

Growth regulators have commonly been applied to cuttings by dipping the cuttings in solutions, by smearing on a lanolin paste, or by dusting with talc containing the regulator.

The physiological mechanism by which growth regulators stimulate the rooting of cuttings is not clearly understood. Stuart (89) analyzed different parts of cuttings of young bean plants and Alexander (5) analyzed detopped bean plants whose cut surfaces were treated with indoleacetic acid. Both men found carbohydrate and nitrogenous food materials were translocated to the treated area. These materials might aid in the growth of roots.

General Methods

Several preliminary experiments were conducted to determine if 4-phthalimido-2,6-dimethylpyrimidine which acted as a growth regulator in other processes was effective in the rooting of cuttings. Two different species each, of succulent, hardwood and evergreen cuttings were included in the experiments. The growth regulators were applied by soaking the cuttings in various concentrations of solutions because the concentration of growth regulator applied could be more accurately known and its entry into the cutting was more certain than when it is applied in a dust or in lanolin. Two control treatments were utilized, one in which the cuttings were dipped in distilled water and another in which they were dipped in a solution of 10 ppm of naphthaleneacetic acid, a standard growth regulator frequently used in the rooting of cuttings.

Succulent Cuttings of Coleus

Methods and Materials

Cuttings were taken from the main shoots of 38 Christmas Gem variety *Coleus blumei* L. plants on March 2, 1952. These plants had been used previously in the experiment on the rate of abscission of leaf petioles as influenced by growth regulators applied in lanolin to the tip of the petiole. Any effects of the previous experiment were minimized by allowing several weeks between experiments, by using only new shoot growth above where the petioles had been, and using the same chemical at the same or a slightly higher concentration in the cutting experiment. The plants appeared normal in every respect at the time of the experiment. Seven cuttings, each were placed in beakers containing the solutions of distilled water used as a control, 10 ppm naphthaleneacetic acid, 10 ppm, 25 ppm, 75 ppm, 250 ppm, and 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine for three-quarters of an hour. The last treatment contained only three cuttings. The cuttings were placed in flats of washed sand, on the greenhouse bench, and watered with tap water. The lower leaves of the cuttings wilted at times, and lost some color as food materials were withdrawn from them.

On March 30, 1952, the cuttings were taken out and the total number of roots and the length of the longest root was recorded.

Results

The optimum concentration of 4-phthalimido-2,6-dimethylpyrimidine in this experiment appears to be between 75 ppm and 250 ppm. The 10 ppm treatment appears to have stimulated the growth of roots. The proper concentration of 4-phthalimido-2,6-dimethylpyrimidine significantly increased the number and the length of the longest root of the coleus cuttings in this experiment, as is shown in Table XVIII.

Succulent Cuttings of Tomato

Methods and Materials

Succulent cuttings were taken from the suckers of the Rutgers variety of tomato plants used in the greenhouse fruit setting experiment. It was over two months since any treatment had been applied to the plants, and it was applied then as a dip of only the flowers. The cuttings were dipped in distilled water used as a control, 10 ppm, 50 ppm, and 100 ppm solution of 4-phthalimido-2,6-dimethylpyrimidine and were rooted in vermiculite, an expanded mica. The roots

TABLE XVIII

THE NUMBER AND MAXIMUM LENGTH OF ROOTS ON COLEUS CUTTINGS 28 DAYS AFTER TREATMENT WITH GROWTH REGULATORS

Concentration of solution	Average number of roots per cutting	Average length of longest root of each cutting in cm	Estimated average length of roots per cutting in cm
Control	48.8	5.5	3.3
10 ppm Naphthaleneacetic acid	75.4	6.8	3.9
10 ppm 4-Phthalimido-2,6-dimethyl-pyrimidine	61.1	8.4	4.3
25 ppm	65.7	6.0	3.0
75 ppm	76.4	4.8	3.3
250 ppm	87.1	5.8	3.3
500 ppm	63.0	5.2	2.8
L.S.D. at 5% level			
Testing between averages of the first six treatments	22.3	1.5	insignificant
Testing between 500 ppm and any other treatment	31.2	2.0	insignificant

were counted and measured after 11 days.

Results

As shown in Table XIX the small number of cuttings (41) used and the large difference between cuttings make it impossible to draw any conclusions from this experiment. However, the low concentrations of 4-phthalimido-2,6-dimethylpyrimidine seemed to stimulate root growth as they did in the coleus experiment.

Hardwood Cuttings of Willow

Methods and Materials

Cuttings of willow *Salix blanca* L. were taken on April 18, just before the buds burst. The cuttings ranged from 3/8 to 1/2 inch in diameter and were graded so that each treatment contained similar sizes of cuttings. The cuttings were placed in beakers containing distilled water used as a check, 50 ppm naphthaleneacetic acid, and 50 ppm and 500 ppm solutions of 4-phthalimido-2,6-dimethylpyrimidine for 13 hours. They were then placed in vermiculite, in flats in a shaded portion of the greenhouse on the floor.

Results

The 50 ppm concentration of naphthaleneacetic acid and the 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine greatly retarded the opening of the leaf buds on cuttings as can be seen in Figure 6. The same treatments caused a significant

TABLE XIX

THE AVERAGE NUMBER AND LENGTH OF ROOTS OF TOMATO CUTTINGS 11 DAYS AFTER TREATMENT WITH DIFFERENT CONCENTRATIONS OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE

Treatment	Average number of roots per cutting	Average length of roots per cutting
Control	31.0	45.7
10 ppm	28.6	53.5
50 ppm	20.1	59.2
100 ppm	21.1	50.6

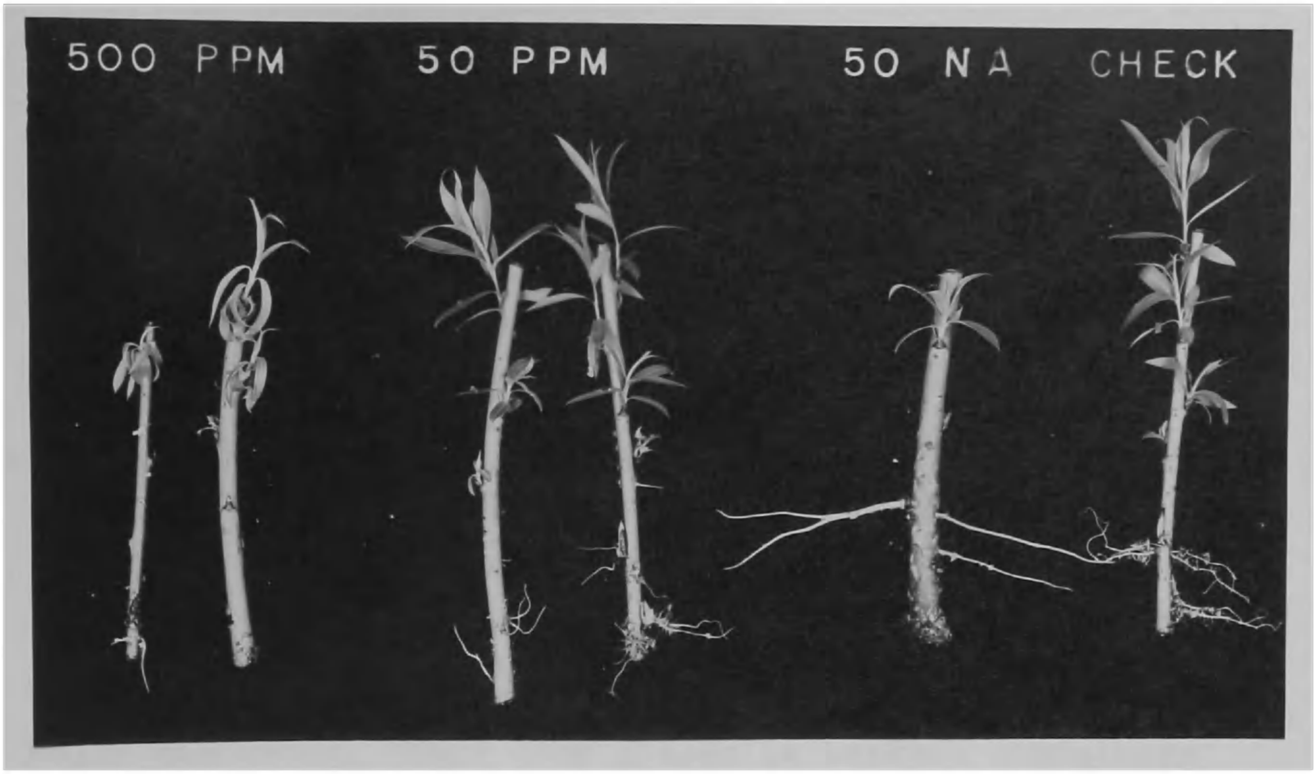
No significant difference between treatments

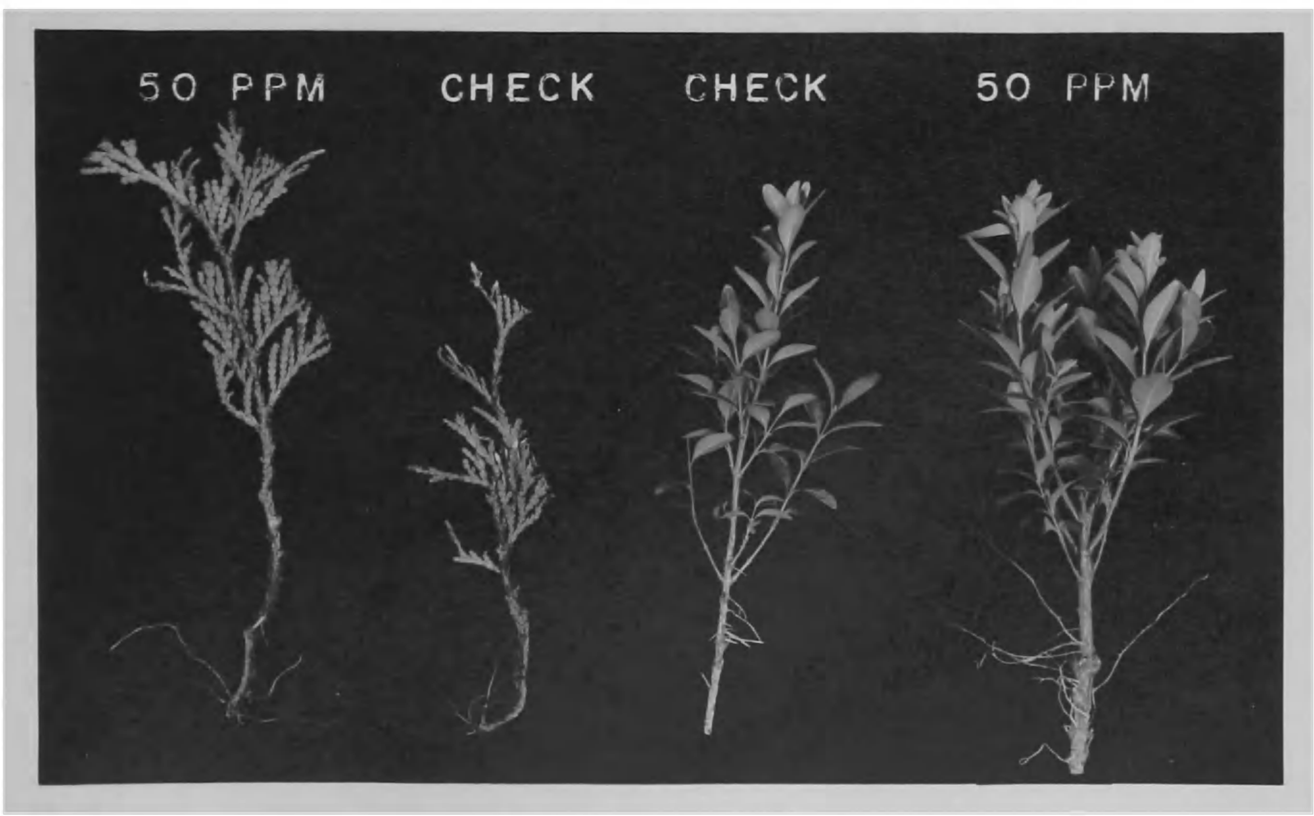
Figure 6. Top -

The effect on rooting and inhibition of bud development after dipping willow cuttings in 50 ppm and 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine, 50 ppm of naphthaleneacetic acid or distilled water.

Bottom -

The effect of dipping arbor vitae cuttings shown on the left, and common boxwood cuttings shown on the right, upon rooting.


500 PPM
50 PPM
50 NA
CHECK


50 PPM
CHECK
CHECK
50 PPM

decrease in the number of cuttings which rooted and a highly significant inhibition of both the number and length of roots as is shown in Table XX and Figure 6. The inhibition of the development of the leaf buds indicates that the 4-phthalimido-2,6-dimethylpyrimidine is translocated in the vascular system, at least for short distances.

Hardwood Cuttings of Apple

Methods and Materials

Cuttings were taken from dormant McIntosh apple shoots. Care was taken to make angular cuts slightly above and below the nodes. They were placed in beakers containing distilled water used as a control, 50 ppm, 250 ppm, and 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine for ten hours. The cuttings were then placed in vermiculite in flats in a shaded area on the floor of the greenhouse. The temperature was probably frequently below optimum for rooting.

Apple cuttings are usually made in the fall and winter and allowed to callus before being set out. This experiment was not anticipated in time so dormant shoots taken for scions were used although they were not callused. This is important because apple is considered to be difficult to root under ideal conditions. The cuttings did not root before the hot days of summer made the greenhouse so hot that the leaves and wood of the cuttings dried out and died. However, some of the cuttings did callus.

TABLE XX

THE EFFECT OF SOAKING WILLOW CUTTINGS IN SOLUTIONS OF GROWTH REGULATORS ON THEIR ROOTING AS MEASURED 14 DAYS AFTER TREATMENT

Treatment	Percentage of cuttings rooted	Average number of roots per cutting	Average length of roots per cutting
Control	94	5.6	43.9
50 ppm Naphthaleneacetic acid	64*	2.9	14.7
50 ppm 4-Phthalimido-2,6-dimethyl-pyrimidine	94	4.4	33.6
500 ppm	67*	1.5	17.1
L.S.D. 1%		2.1	4.6

*Significantly less or probability of getting chi square of 3.513 is less than .05

Results

All of the growth regulators used caused a highly significant reduction in the number of cuttings which callused as compared to the control as can be seen in Table XXI. The 50 ppm concentration of 4-phthalimido-2,6-dimethylpyrimidine caused significantly less inhibition of callusing than the other treatments, except the control.

All the treatments greatly delayed the opening of the leaf buds and the size of the leaves which did develop was reduced. The experiment could not have been continued for the high temperatures of summer were beginning to kill the cuttings.

Magnolia Cuttings

A few mallet type cuttings were made of *Magnolia soulangeana*. Some were soaked 12 hours in distilled water used as a control, and some in 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine. None of these cuttings rooted, but the 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine caused formative effects on the leaves which developed.

Evergreen Cuttings of Boxwood

Methods and Materials

About 70 cuttings of boxwood, *Buxus sempervirens* L., were made while they were still quite dormant before any new growth had started. They were graded so that similar

TABLE XXI

THE EFFECT OF GROWTH REGULATORS ON THE CALLUSING OF McINTOSH APPLE CUTTINGS

Treatment	Percent of cuttings callused three months after treatment
Control	16**
50 ppm Naphthaleneacetic acid	2
25 ppm 4-phthalimido-2,6-dimethylpyrimidine	9*
250 ppm	2
500 ppm	2

**Highly significantly different than other treatments or probability of getting chi square less than 6.64 is .01

*Significantly higher than other treatments except control or probability of getting chi square less than 3.84 is .05

size of cuttings were included in each treatment. The treatments consisted of a 12 hour soak in solutions of distilled water used as a check, 50 ppm of naphthaleneacetic acid, 50 ppm and 500 ppm of 4-phthalimido-2,6-dimethylpyrimidine. The cuttings were then rooted in vermiculite, in flats placed in a shaded place on the floor of the greenhouse. The number of cuttings rooting, the number of roots per cutting, and length of each root was determined. Those cuttings which had not rooted at the first time were placed back in the vermiculite and allowed to root if they would. This was done a third time also. The data on the average number and length of roots per cutting was obtained from the first observation only because the roots were sometimes injured in counting, and a comparison of the number and length of roots of cuttings which had differing amounts of time to grow would not be too valid especially in such a small population.

Results

The 50 ppm of 4-phthalimido-2,6-dimethylpyrimidine was the best concentration tried and that concentration was approximately as effective as the same concentration of naphthaleneacetic acid at the time of the first observation as can be seen in Table XXII and Figure 6. Both of these treatments are highly significantly better than the check when tested by chi square. This treatment was highly significantly better than naphthaleneacetic acid and the

TABLE XXII

THE PERCENTAGE OF BOXWOOD CUTTINGS WHICH ROOTED AND THE AVERAGE NUMBER AND LENGTH OF ROOTS PER CUTTING AS AFFECTED BY TREATMENT WITH TWO GROWTH REGULATORS

Treatment		Percentage of cuttings rooted in			Average number of roots per cutting	Average length of roots per cutting in mm
		two months	three months	four months		
Control		28	28_2	28	24	21
50 ppm	Naphthaleneacetic acid	47_1	47	47	26	19
50 ppm	4-Phthalimido-2,6-dimethylpyrimidine	43_1	63	65	20	19
500 ppm		6	28_2	33		
		Every percentage highly significantly different from all others for that period except those pairs marked with subscripts			No significant differences between number of roots or average length of roots per cutting	

check, if more time was allowed for rooting. There was no significant difference between treatments with respect to the average number of roots per cutting at the first time of observation. The response of the 500 ppm treatment suggests that the concentrations of 4-phthalimido-2,6-dimethylpyrimidine used were too high and only after some of it was inactivated or leached away did the greatest rooting take place.

Evergreen Cuttings of Arbor Vitae

Methods and Materials

Approximately 70 arbor vitae, *Thuja occidentalis* L., cuttings in the same condition as the boxwood were handled in the same way in every respect.

Results

More of the cuttings dipped in 50 ppm of 4-phthalimido-2,6-dimethylpyrimidine had rooted than the cuttings of the check treatment at the end of two months as is shown in Table XXIII and Figure 6. The naphthaleneacetic acid also made the cuttings root faster as would be expected. With more time the cuttings of the check treatment rooted better than those of the other treatments. On arbor vitae, as on boxwood, it appears as though the high concentration of 4-phthalimido-2,6-dimethylpyrimidine was too high and only after some of it had been inactivated or leached away could these cuttings root even as well as the cuttings of the check

TABLE XXIII

THE PERCENTAGE OF ARBOR VITAE CUTTINGS WHICH ROOTED AND THE AVERAGE NUMBER AND LENGTH OF ROOTS PER CUTTING AS AFFECTED BY TREATMENT WITH GROWTH REGULATORS

Treatment	Percent of cuttings rooted in		Average number of roots per cutting	Average length of roots per cutting in cm
	two months	three months		
Control	19	81	4.0	1.0
50 ppm Naphthaleneacetic acid	47	54	16.0*	1.3
50 ppm 4-Phthalimido-2,6-dimethylpyrimidine	32	37	4.3	1.4
500 ppm	0	16	0.0	
	Every percentage highly significantly different from all others as tested by chi square		*Significantly higher than all others at 5% level	No significant difference

treatment. The cuttings treated with naphthaleneacetic acid developed many thin roots while the cuttings of the other treatments and check developed fewer thicker roots.

EXPERIMENTS ON THE RELATION OF CHEMICAL STRUCTURE AND THE BIOLOGICAL ACTIVITY OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE

Introduction

One of the fundamental problems currently receiving a great deal of attention is the structural requirements of compounds which are necessary for them to be active in living organisms. This problem is frequently related to studies on the biochemical mechanism of a reaction because most reactions in living organisms are catalyzed by enzymes, and if a chemical compound has the proper structure and physical characteristics associated with this structure, it may act as an inhibitor, or stimulant for an enzyme system.

If the enzyme system involved is known, it can sometimes be isolated and the effects of the chemicals on it can be studied in vitro. Frequently experiments done in vitro do not correlate well with experiments done with intact living tissue. Because of this, or because the enzyme system is unknown, biological tests have been found to be very useful.

Inhibition of Root Growth by Different Concentrations of 4-Phthalimido-2,6-dimethylpyrimidine

Review of Literature

Ready and Grant (76) found the germination and growth of roots of cucumber seeds to be a fairly accurate bioassay for the amount of 2,4-dichlorophenoxyacetic acid in a water solution. This method has the advantages of requiring no synthetic media since the cotyledons of the seeds contain a fairly uniform supply of food material for growth. It involves a very actively growing meristem in the root tip, and a quantitative measure is readily obtained by measuring the length of the single tap root with an inexpensive metric ruler.

Methods and Materials

The response of the roots of cucumber, a dicotyledonous plant, and wheat, a monocotyledonous plant, to a wide range of concentrations of 4-phthalimido-2,6-dimethylpyrimidine was studied to determine the effective range of this compound.

Fifteen seeds were distributed upon a piece of Whatman No. 1 filter paper in a petri dish. The filter paper was moistened with five milliliters of a solution of a certain concentration which was to be tested. The concentrations used were 0.1 ppm, 1.0 ppm, 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine and another compound which was received from the Phillips Petroleum Company at the same time. The latter substance was a lumpy yellow and brown powder which did not appear homogeneous. It is soluble in water. Its chemical formula was not given so it will be referred to by the manufacturer's number, 475. Four petri dishes of cucumber seeds were used as replications in testing the 4-phthalimido-2,6-dimethylpyrimidine using cucumber seeds, while five petri dishes or replications of each concentration were used in the wheat experiment and the experiment with compound number 475.

The cucumber seed used in all of these experiments was the Burpee Hybrid. Usually about 80 percent of the seeds germinated. Cracked, broken, small or shriveled seeds were not used. Golden Banner wheat, obtained from the Farm Crops Department, was used in the experiment with wheat. There was no mold growth in these experiments.

The seeds germinated and grew in the covered petri dishes on the laboratory bench for exactly five days. Then the length of the primary root of the cucumber seedlings were measured and the length of the five roots of the wheat seedlings were measured and added together. One replication, or the seedlings of one dish of each treatment, was measured before the second replication was measured so as to put the variance due to growth while the measurements were being taken into replications instead of treatments. The records of all the seeds, including the few which did not germinate or grow well were included in the calculation of these experiments.

Results

In general, increasing concentrations of these compounds caused an increased inhibition of root growth although one ppm of 4-phthalimido-2,6-dimethylpyrimidine resulted in an insignificant increase in the length of wheat roots and 25 ppm and 50 ppm of Phillips Petroleum compound 475 also showed an insignificant increase in the length of cucumber roots as shown in Figure 8. These curves were drawn from the average length of the roots of a seedling, which are shown in Table XXIV along with the least difference necessary for significance for each experiment. The scales on the vertical axis for the average length of the cucumber and wheat roots were adjusted so as to be identical at 0.1 ppm which was about the same as the check. The 11 cm mark on Figure 8 for the wheat scale was omitted, so the 12 should be 11 and the 13 should be 12. The concentration was plotted on a logarithmic scale which is frequently done in biological assays to obtain a smoother curve (94).

By reference to such curves the approximate concentration can be estimated, if the average length of roots grown under similar conditions, in the absence of other growth factors, is known. A solution of 4-phthalimido-2,6-dimethylpyrimidine which had been left in the greenhouse in the light for about a month was tested in this way and found to have lost about half of its activity.

Figure 8.

THE INHIBITION OF ROOT GROWTH AS AFFECTED BY VARYING CONCENTRATIONS OF GROWTH REGULATORS.

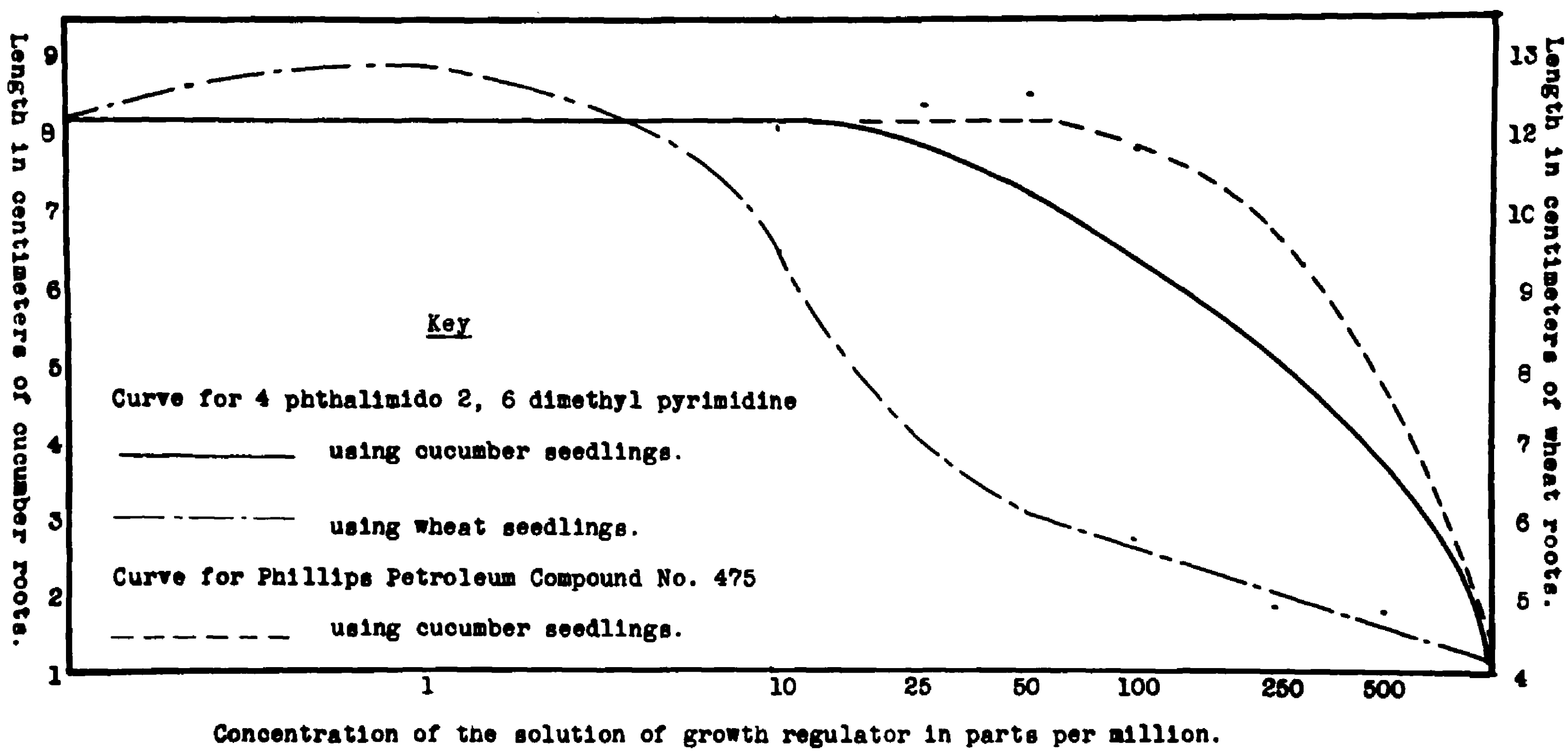


TABLE XXIV

THE EFFECT OF VARYING CONCENTRATIONS OF GROWTH REGULATORS UPON THE GROWTH OF CUCUMBER AND WHEAT ROOTS

(Average length in cm after growing for five days in petri dishes)

Concentration	4-Phthalimido-2,6-dimethylpyrimidine		Phillips compound 475
	Cucumber tap roots	Sum of wheat roots	Cucumber tap roots
Control	8.35	11.28	8.28
0.1 ppm	8.12	11.28	8.53
1 ppm	8.19	11.98	8.09
10 ppm	8.18	9.65	8.42
25 ppm	7.90	6.56	8.67
50 ppm	7.33	6.29	8.84
75 ppm	6.81	--	--
100 ppm	6.44	5.93	8.12
250 ppm	5.19	5.69	7.03
500 ppm	3.76	5.61	5.04
1000 ppm	1.12	4.75	1.75
L.S.D. at 5% level	0.43	1.26	0.81
L.S.D. at 1% level	0.56	1.66	1.07

An Experiment Testing the Interaction of 4-Phthalimido-2,6-dimethylpyrimidine with Thiamin

Review of Literature

Recently it has been found that pyrithiamin, a compound with a chemical structure which differs only slightly from that of thiamin interferes with the normal functioning of this essential vitamin (110). It is thought that pyrithiamin takes the place of thiamin in important enzyme systems, but does not do the work which thiamin normally does, and in this way inhibits the reactions which normally occur. Bonner (12) found that thiamin is required in the culture media of excised roots, and it is generally thought to be essential in the metabolism of all living organisms although most intact higher plants have the ability to synthesize an ample supply of this vitamin.

In a similar manner, Arnow and others (4) have found that triazolo pyrimidine analogs of guanine inhibit the growth of the green algae Stichococcus subtilis.

Schultz (78) reported that an analog of thiamin with methyl groups in the two and six positions on the pyrimidine nucleus, had had about one-three hundredths of the activity of ordinary thiamin which has a methyl group at the number two position and an amino group at the number six position.

Methods and Materials

A small factorial experiment with cucumber seedlings was performed to determine if 4-phthalimido-2,6-dimethylpyrimidine had any major effect upon the influence of thiamin upon the growth of cucumber roots. The four combinations (1) control, (2) 250 ppm of 4-phthalimido-2,6-dimethylpyrimidine, (3) 250 ppm of thiamin, and (4) 250 ppm of 4-phthalimido-2,6-dimethylpyrimidine and 250 ppm of thiamin were added to petri dishes. The four treatments were replicated four times. After the seedlings had grown in the petri dishes on the laboratory bench for five days they were measured.

Results

The 4-phthalimido-2,6-dimethylpyrimidine alone caused the usual inhibition of growth and the thiamin alone caused a slight stimulation of growth. The effect of the combination of the two compounds was approximately additive so there is no reason to suspect that either compound influences the response of cucumber roots to the other compound in this experiment. The actual averages of this experiment are shown in Table XXV.

A Comparison of the Effect of Several Phthalic Acid Derivatives Upon the Growth of Cucumber Roots

Review of Literature

The similarity of the structure of 4-phthalimido-2,6-dimethylpyrimidine with that of many other compounds which have been reported to be growth regulators suggests that the compounds might derive their activity from this similarity. The phthalamic acids are examples which have been

TABLE XXV

THE INTERACTION OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE AND THIAMIN ON THE GROWTH OF CUCUMBER ROOTS

Treatment		Average length of roots in cm
Control		7.2
250 ppm	Thiamin	7.8
250 ppm	4-Phthalimido-2,6-dimethylpyrimidine	4.5
250 ppm 250 ppm	Thiamin plus 4-phthalimido-2,6-dimethylpyrimidine	5.1

No significant interaction

discussed earlier.

Maramorosch (57) found that diethyl phthalate will cause lesions on the leaves of crimson clover, tobacco and asters. Leaves sprayed with this compound died within three to five days. Radar (73) and others reported on tetrahydropyrimidine derivatives as potential foliage fungicides since they would kill without injuring the foliage.

Allen and Skoog (2) conducted a series of experiments with seedlings which were similar to those described in this section, in which they studied the effect of several tetrahydropyrimidine derivatives upon the root growth of seedlings. They found that the most active compounds had a ring nucleus containing nitrogen and carbon atoms linked by one or more double bonds, a lack of polar substituents such as hydroxyl groups, and a side chain of sufficient length to impart surface active properties to the molecule.

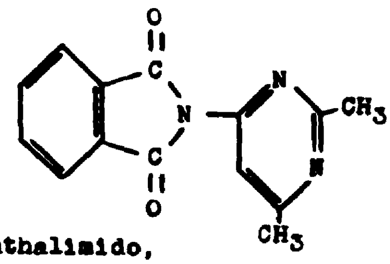
Methods and Materials

A series of experiments were conducted to compare the effects of 4-phthalimido-2,6dimethylpyrimidine with phthalic acid, phthalimide, Phillips Petroleum compound 475, N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid and N-4 chlorophenyl phthalamic acid upon the growth of cucumber roots. The first four compounds are soluble in water and are compared with cucumber seedlings germinated with water. The last three compounds were used as diethanol amine salts, so a second control which contained diethanol amine was added. The structural formulas for these compounds are shown in Figure 7, and the pH of each compound is shown in Table XXVI. In an attempt to obtain greater control of the environment the petri dishes were kept in an insulated room with a temperature between 71° and 75° F at all times. The petri dishes were randomized on a bench about 20 inches below an electric light fixture which contained two fluorescent tubes and six 25 watt incandescent bulbs which were on continuously. In the experiment conducted in the dark the petri dishes were placed in a cardboard box covered with a thick black cloth on a shelf just below the illuminated bench. This experiment in the dark was conducted at the same time as the one in the light with which it was compared.

A single experiment consisted of a comparison between the eight different compounds at a given concentration. There were four replicates or petri dishes per treatment. Each experiment was repeated once. The concentrations of the different experiments were 50 ppm, 100 ppm, and 250 ppm.

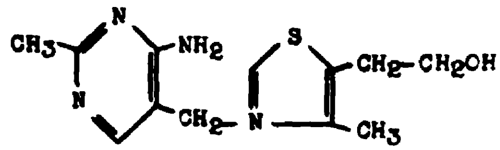
Because all of the 15 seeds in a petri dish did not germinate, only the lengths of the 12 longest roots were used in the statistical analysis, so that the number of measurements per replication would be equal. An examination of the differences necessary for significance (L.S.D.) of Table XXVII and the variation between them shows the necessity of statistical analysis. The actual average root lengths for each experiment were converted to index numbers

Figure 7.

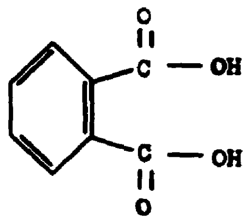


4 Phthalimido,

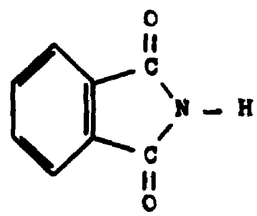
2, 6 Dimethyl Pyrimidine



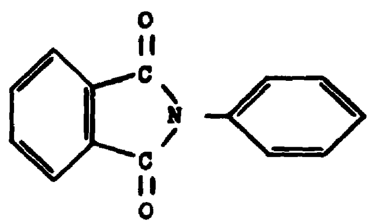
Thiamine



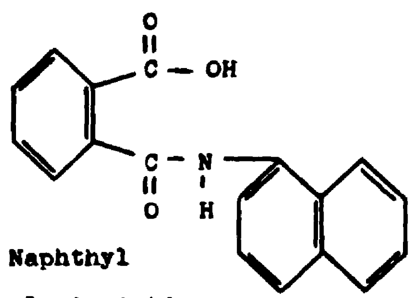
Phthalic Acid



Phthalimide

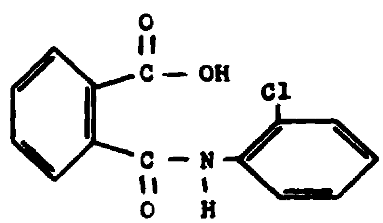


N Phenyl Phthalimide



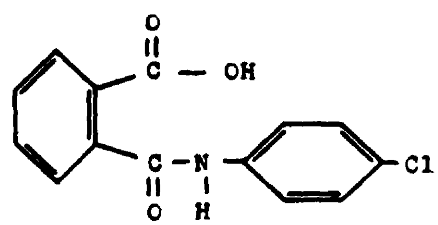
N-1 Naphthyl

Phthalamic Acid



N-2 Chloro phenyl

Phthalamic Acid



N-4 Chloro phenyl

Phthalamic Acid

TABLE XXVI

THE APPROXIMATE pH OF THE GROWTH REGULATOR SOLUTIONS USED IN THESE EXPERIMENTS

Compound	Approximate pH at 1000 ppm
4-Phthalimido-2,6-dimethylpyrimidine	4.1
Phthalic acid	4.0
Phthalimide	4.7
Phillips compound 475	5.2
Diethanol amine	9.3
N-1 naphthyl phthalamic acid	9.2
N-2 chlorophenyl phthalamic acid	9.2
N-4 chlorophenyl phthalamic acid	9.7

The phthalamic acids were used as diethanol amine salts

TABLE XXVII

THE COMPARATIVE EFFECT OF VARYING CONCENTRATIONS OF COMPOUNDS STRUCTURALLY RELATED TO PHTHALIC ACID UPON THE GROWTH IN LENGTH OF ROOTS OF CUCUMBER SEEDLINGS

Compound	In continuous light						In darkness
	50 ppm	50 ppm	100 ppm	100 ppm	250 ppm	250 ppm	250 ppm
Control	100	100	100	100	100	100	100
4-Phthalimido-2,6-dimethylpyrimidine	104	85	98	94	82	90	100
Phthalimide	140	106	125	137	88	137	108
Phthalic acid	140	108	114	98	83	93	106
Phillips Petroleum compound 475	128	137	135	117	105	114	111
Diethanol amine	--	103	92	63	77	85	75
N-1 naphthyl phthalamic acid	110	96	72	62	26	105	103
N-2 chlorophenyl phthalamic acid	86	72	55	61	23	61	68
N-4 chlorophenyl phthalamic acid	76	59	59	41	21	88	112
L.S.D. at 5% level	11	10	28	10	21	17	14

with the check treatments as 100. The differences necessary for significance were kept in proportion.

Results

Some general trends are evident from the data of Table XXVII. Phthalimide, Phillips compound 475, and in some cases, phthalic acid produced a stimulation in the elongation of cucumber roots while the phthalamic acids were inhibitors which were much more toxic than 4-phthalimido-2,6-dimethylpyrimidine. The roots of the seedlings treated with 4-phthalimido-2,6-dimethylpyrimidine had fewer branch roots, and were shorter than those of the control seedlings. This gave them a rat-tailed appearance. The roots frequently exhibited a negative geotropism. The seedlings treated with Phillips Petroleum compound 475 showed similar, although not as extreme, tendencies. The seedlings treated with phthalamic acids had roots whose diameter tapered rapidly from the larger size of the hypocotyl to a thin string-like appearance with a few short branch roots. The roots of the seedling treated with about 250 ppm of phthalamic acids only developed a stubby conical shape with concave sides. The roots of the seedlings treated with phthalic acid and phthalimide appear normal except somewhat longer.

A Comparison of the Formative Effects of Leaves of Cranberry Beans and John Baer Tomatoes Produced by Several Phthalic Acid Derivatives

Review of Literature

The formative effects and curvatures of leaves and stems of tomato and bean plants have been used by Hitchcock and Zimmerman (37) to obtain a quantitative measure of the response of plants to growth regulators.

Methods and Materials

Cranberry bean plants were planted in a soil mixture of Hillsdale loam, sand and peat in four-inch pots in the greenhouse on April 11, 1952. Eight days later all but the two most vigorous plants in each pot were removed. On April 24 the plants were graded for uniformity and different groups of four pots per group were sprayed with 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine, phthalic acid, phthalimide, Phillips Petroleum compound 475, N-phenyl phthalimide, diethanol amine, N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid, and N-4 chlorophenyl phthalamic acid. The plants were removed from the room while being sprayed, and the knapsack sprayer with the attachment which allowed a different flask to be used for each compound was used.

A group of John Baer tomato seedlings were transplanted to a similar soil on May 4 and were sprayed with the same treatments when they reached a height of eight to twelve inches.

Results

A few days after treatment, the young trifoliate leaves of the bean plants sprayed with 4-phthalimido-2,6-dimethylpyrimidine and those sprayed with N-1 naphthyl phthalamic acid developed an epinastic twisting and a mottled dark green color as shown in Figure 9. The formative effects produced by both compounds appeared identical. The trifoliate leaves of the plants sprayed with N-2 chlorophenyl phthalamic acid also developed an epinasty and also necrotic yellow and brown areas along their margins. The other treatments produced no noticeable formative effects. The plants flowered and the beans were allowed to mature. They were then dissected and inspected for abnormalities. The pods, ovules, cotyledons and embryos of the beans of all the treatments appeared normal.

Another group of cranberry beans which were sprayed at an earlier stage of development did not develop many flowers and the apical shoot lost much of its dominance. Apparently the stage of development at the time of spraying influences the response obtained.

The only compound producing formative effects on the tomato leaves was N-1 naphthyl phthalamic acid. It produced an inhibition of leaf growth, and an epinasty of the young leaves as shown in Figure 9. The other compounds did not produce noticeable formative effects when applied at a concentration of 1000 ppm, however, younger plants might have been more sensitive.

Figure 9. Top -

Formative effects of cranberry bean leaves on plants sprayed with 1000 ppm of distilled water, N-1 naphthyl phthalamic acid, 4-phthalimido-2,6-dimethylpyrimidine, and N-4 chlorophenyl phthalamic acid.

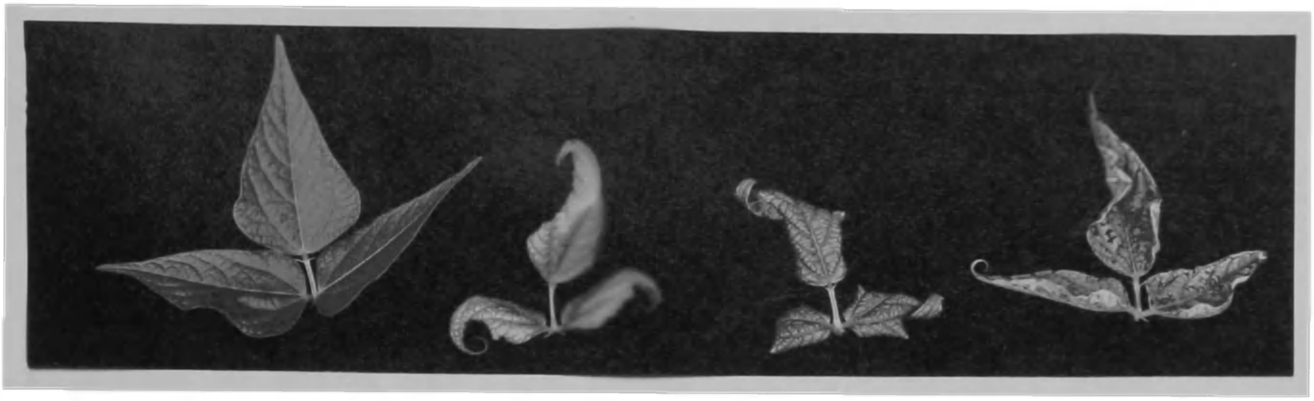
Bottom -

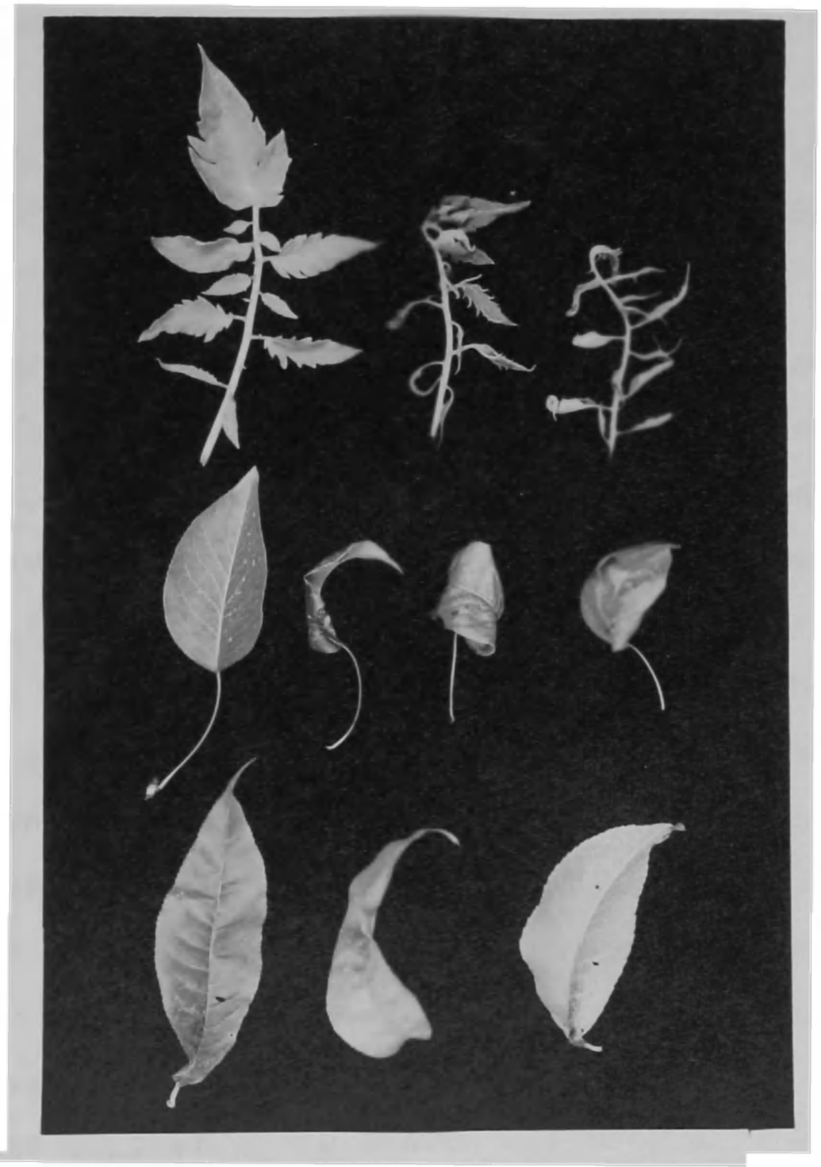
Upper row: Formative effects of John Baer tomato leaves produced by spraying with 1000 ppm of N-1 naphthyl phthalamic acid.

Middle row: Epinasty of Bartless pear leaves sprayed with 2000 ppm of N-1 naphthyl phthalamic acid.

Lower row: Epinasty of peach leaves sprayed with 2000 ppm of N-2 chlorophenyl phthalamic acid.

The leaf on the left of each picture represents the check treatment and other treatments producing no formative effects.





The 4-phthalimido-2,6-dimethylpyrimidine did not cause formative effects in the other experiments which included coleus, cucumber and muskmelon plants, and apple, pear, cherry, and peach trees. Concentrations from 500 ppm to 2000 ppm of N-1 naphthyl phthalamic acid did cause severe epinasty of young pear and apple leaves when they were applied as a spray during full bloom. Peach leaves sprayed with 1000 ppm or 2000 ppm of N-2 chlorophenyl phthalamic acid as shown in Figure 9 also developed an epinasty.

The Effect of Several Phthalic Acid Derivatives Upon the Rate of Respiration of Several Plant Tissues

Review of Literature

The influence of various growth regulating substances upon the respiratory rates of different plant tissues, homogenates, and extracts have been studied by many workers. Kelly and Avery (43) found that in general low concentrations of 2,4-dichlorophenoxyacetic acid stimulated respiration while high concentrations inhibited it. They found that lower concentrations of 2,4-dichlorophenoxyacetic acid were required to cause a stimulation of respiration in the stems of peas which are dicots, than in ceoleopliles of oats, which are monocots. This is in accord with the common observation that dicots are much more sensitive to herbicidal sprays of this compound than monocots. In a later work (44) they found that young tissue was much more responsive than older tissue, which only shows an inhibition of respiration as increasing concentrations of growth regulators are used.

Mitchell, Burris and Riker (60) and Miller and Burris (59) have found that benzoic acid and salicylic acids affect respiration of slices of tomato stems, and of cell-free enzymes of barley in the same way as many of the auxins. All of these chemicals inhibited the respiration of the tissues which were used.

Methods and Materials

The rate of respiration as indicated by the uptake of oxygen by plant tissues was determined with a standard Warburg apparatus as described by Umbreit, Burris, and Stauffer (96). Cucumber seeds were germinated in petri dishes with five milliliters of a 250 ppm solution of the following compounds: 4-phthalimido-2,6-dimethylpyrimidine, phthalimide, phthalic acid, Phillips Petroleum compound 475, diethanol amine, N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid, and N-4 chlorophenyl phthalamic acid. Twenty-four hours after the cucumber seeds were moistened, when the tap root is approximately one-half inch long, the seeds were rinsed in distilled water to remove any fungi, six uniform seedlings were selected and put in the Warburg flasks, and the rate of respiration was determined. The amount of oxygen consumed in consecutive 15 minute intervals was recorded, to observe if the process continued at a fairly uniform rate. The data for the experiments with cucumber seedlings is expressed as microliters of oxygen consumed by the six seedlings per hour. The meristematic regions of a seedling respire so much more rapidly and comprise such a small portion of the total weight of the seedling as compared to the large cotyledons and seed coat that it was felt that this was a less biased way of expressing the data than on the conventional dry weight or fresh weight basis.

The rate of respiration of the top young leaf of the tomato plants was determined. Different plants had been sprayed with a 1000 ppm solution of the following compounds: 4-phthalimido-2,6-dimethylpyrimidine, phthalimide, N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid and control. Since the Warburg apparatus is not designed to accomodate as much tissue as a whole leaf and since a single leaf is not a very reliable sample, duplicate one-tenth gram (fresh weight) samples obtained by slicing several leaves and immersing them immediately in cold buffer solution were used.

In July some cranberry beans were planted in pots as described in the previous experiment on the formative effects. When the primary leaves were from two to three inches in width, before the trifoliates expanded, different pots of plants were sprayed with 1000 ppm of the following solutions: 4-phthalimido-2,6-dimethylpyrimidine, phthalimide, N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid, and N-4 chlorophenyl phthalamic acid. The rates of respiration of duplicate one-tenth gram samples taken by slicing the primary leaves of the plants receiving the different treatment were determined forty hours after the plants were sprayed.

Some Mohawk oats were grown in pots of vermiculite at the same time and sprayed, when the first leaf was about three inches long and the third leaf was not yet unrolled, with the same compounds and concentration used in the respiration studies on the bean leaves. The rate of respiration was determined on duplicate two-tenths gram samples obtained from sections from the middle of the outer, lower leaves of the plants of the different treatments 64 hours after the plants were sprayed.

Results

In all of the respiration studies difficulty was experienced in selecting uniform samples of six cucumber seedlings or small amounts of leaf tissue. The 4-phthalimido-2,6-dimethylpyrimidine and the phthalimide generally caused a slight increase in the rate of respiration, while the N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid, and N-4 chlorophenyl phthalamic acid usually caused very definite increases in the respiratory rates of the different plant materials as shown in Tables XXVIII and XXIX. This is in accordance with the observation that an increase in the toxicity of growth regulators is frequently associated with an increased rate of respiration (74, 81). There certainly is not enough evidence to establish any causal relationship however.

TABLE XXVIII

THE RATES OF RESPIRATION OF CUCUMBER SEEDLINGS 24 HOURS AFTER GERMINATING AND GROWING IN 250 PPM SOLUTIONS OF PHTHALIC ACID DERIVATIVES

Treatment	Microliters of oxygen consumed per hour by six cucumber seeds
Control	108
4-phthalimido-2,6-dimethylpyrimidine	114
Phthalimide	112
Phthalic acid	103
Phillips Petroleum compound 475	116
Diethanol amine	122
N-1 naphthyl phthalamic acid	138
N-2 chlorophenyl phthalamic acid	108
N-4 chlorophenyl phthalamic acid	128
L.S.D. at 5% level	12

TABLE XXIX

THE RATES OF RESPIRATION OF BEAN, TOMATO, AND OAT LEAVES SPRAYED WITH 1000 PPM OF PHTHALIMIDE OR PHTHALAMIC ACID DERIVATIVES

(Microliters of oxygen consumed per hour by 0.2 g tissue slices)

Tissue	Cranberry bean leaves		Tomato leaves	Oat leaves	
Conditions of respiration	Light	Dark	Light	Light	Dark
Control	76	76	72	103	115
4-Phthalimido-2,6-dimethylpyrimidine	84	76	104	101	113
Phthalimide	64	84	80	106	120
N-1 naphthyl phthalamic acid	92	92	183	127	134
N-2 chlorophenyl phthalamic acid	84	94	142	96	105
N-4 chlorophenyl phthalamic acid	244	236	88	105	113
L.S.D. at 5% level	54	10	No significant differences		
L.S.D. at 1% level	84	16	No significant differences		

The stimulation of respiration resulting from treatment with 4-phthalimido-2,6-dimethylpyrimidine would be expected to utilize more of the sugar and fatty acid unless their synthesis was stimulated at a greater rate than their respiration. A decrease in sugars and fatty acid was observed in bean plants analyzed for these constituents. The respiratory quotient of cucumber seedlings treated with 4-phthalimido-2,6-dimethylpyrimidine was 1.29 and of untreated seedlings was 1.24. These differences are well within the limits of experimental error and are not significantly different.

Discussion

The experiments which have been conducted quite clearly demonstrate that 4-phthalimido-2,6-dimethylpyrimidine is a new growth regulator which is capable of inducing parthenocarpic development of tomatoes, of stimulating the rooting of cuttings and causing morphological and chemical changes in plants. This is of special interest because the structure of this compound is quite different than that of the auxins although many of the plant responses are similar.

Koepfli, Thimann and Went (47) have stated that for a compound to have activity as an auxin it should at least have:

1. a ring system as a nucleus
2. a double bond in the ring
3. a side chain

4. a carboxyl group or a structure readily convertible to a carboxyl group on this side chain at least one carbon atom removed from the ring
5. a particular space relationship between the ring and the carboxyl group

It can be seen from Figure 7 that 4-phthalimido-2,6-dimethylpyrimidine would not fulfill any of the last three requirements for activity. Even if the imide linkage were hydrolyzed the compound should have no activity according to this theory.

From its structure it can be seen that 4-phthalimido-2,6-dimethylpyrimidine could be renamed N-4 (2,6-dimethyl pyrimidine) phthalimide. The great similarity of the formative effect of bean leaves produced by 4-phthalimido-2,6-dimethylpyrimidine and N-1 naphthyl phthalamic acid, the fact that both compounds cause a reversal of polarity or a negative geotropism in the roots of bean and cucumber seedlings (35, 58), and the fact that 4-phthalimido-2,6-dimethylpyrimidine is capable of inducing parthenocarpic development of tomatoes as well as the N-aryl phthalamic acids and N-aryl phthalimides all suggest that it may be the phthalimide portion of 4-phthalimido-2,6-dimethylpyrimidine which is primarily responsible for its activity. The difference in activity of N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid in the respiration studies, the experiments on formative effects, the experiments with the cucumber seedlings, and the experiments of Hoffmann and Smith (37) all indicate that the aryl group and the manner in which it is substituted influences the degree of activity of the compound although not the type of activity. These differences are not removed by expressing the concentrations in moles instead of parts per million.

It is the structure of the phthalamic acid or phthalimide portion of all of these compounds, which they have in common, but the different aromatic nuclei (benzene, naphthalene, and pyrimidine) to which the phthalic acid derivatives are attached may be responsible for the differences in toxicity. Veldstra (98) has proposed a theory that the balance between the lipophylic and hydrophilic properties of organic compounds is important in determining their physiological activity. Many modern workers (91) have doubted that action of growth regulators is purely a surface phenomenon, but perhaps these qualities are important in the entry, or translocation of the compound within the plant, or in the movement of the compound into the cells. The phthalamic acids might be considered ortho-substituted benzoic acids, and fit into the schemes of Hansch, Muir and Metzenberg (36). They and Foster, McRai and Bonner (24) have suggested that the auxins are attached at two positions to a protein. One is through a salt linkage with the carboxyl group and the other linkage, perhaps with a sulfhydryl group, occurs at the ortho position of the benzene ring. Phthalamic acid or a hydrolyzed phthalimide might react at its carbonyl group and their electropositive amide group. The substituents or the character of the other aromatic ring might influence the charge at the amide group. A theory such as this affords an explanation of the apparent activity of 4-phthalimido-2,6-dimethylpyrimidine as an antiauxin, since it and the endogenous auxin may be competing for the same points for attachment. Since there is hardly enough consistent evidence to support the two-point attachment theory, and the experiments described herein yield little information concerning this theory, any suggestions such as these are entirely speculative.

The stimulation of the growth of cucumber roots caused by phthalimide and in some cases by Phillips Petroleum compound 475 is interesting. Allen and Skoog (3) have reported that N-phenyl succinimide, especially, and N-para-chlorophenyl succinimide, and N-2,4-dichlorophenyl succinimide stimulated the growth of the roots and shoots of Henry wheat and the root growth of Scarlet Globe radish seedlings, in an experiment similar to the experiments with cucumber seedlings. Bonner and Bandurski (14) have suggested that many antiauxins cause a stimulation of root growth of seedlings.

If phthalimide proves capable of stimulating the growth of seedlings of other species besides cucumber, it might be beneficial to incorporate it in the material used for pelleting seeds.

THE CHANGES IN THE CHEMICAL COMPOSITION OF CRANBERRY BEANS SPRAYED WITH 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE

Review of Literature

The changes in the chemical composition of plants resulting from treatment with growth regulators are important in that they indicate how the normal metabolic processes of the plant have been altered, or the direction and extent of the translocation of materials within the plant. Any increase or decrease in constituents of nutritional value for livestock or human beings is also very important.

In general, the treatment of responsive plants with the auxin-like growth regulators, causes a decrease in the carbohydrate reserves of the plant, and frequently an increase in nitrogenous compounds (61). Stuart (89) found that the total amount of starch, reducing sugar and sucrose in cuttings of kidney bean plants decreased when treated with 100 ppm of indoleacetic acid and that the amount of nitrogen increased. These trends were true for the different organs however there was considerable translocation from leaves and the upper portion of the stem to the base of the hypocotyl. Mitchell (61) found that kidney bean plants treated with naphthaleneacetic acid or naphthalene acetimide had less total sugar, starch, and dextrin in the roots and hypocotyls. The tumorous stems of his treated plants contained more nitrogen, most of which was in water soluble forms. In a later work (64) he reported that the rate of starch hydrolysis in bean leaves was increased after they were sprayed with naphthaleneacetic acid.

Hamner, Sell, and others have extensively studied the physiological responses of the red kidney bean plant to treatments with 2,4-dichlorophenoxyacetic acid and have found that it caused a decrease in the amount of the different carbohydrate fractions in the stems (79). The amount of protein in these stems was approximately doubled and the proportion of the different amino acids making up this protein was altered. The differences in the leaves and roots were not great except that the non-reducing sugar in the treated leaves and roots was completely depleted (99). The amount of thiamin, riboflavin, nicotinic acid, pantothenic acid and carotene in the stems and leaves of red kidney beans were also changed (53).

Similar changes resulting from treatment with 2,4-dichlorophenoxyacetic acid have also been reported for dandelion (75), bindweed (82), tomato (62), wheat (23), annual morning glory (61), and wild garlic (46).

Methods and Materials

Cranberry bean seeds were planted in a uniform soil mixture in five-inch pots. The plants were thinned to two per pot and the pots of plants then graded by eye so as to insure that plants of about the same range of morphological development were included in each treatment and replication. Two replications of plants were sprayed with an aqueous solution of 4-phthalimido-2,6-dimethylpyrimidine when the first trifoliate leaf was well expanded and the second trifoliate was about one-half inch in length. The plants were about nine inches high, with no flowers visible. The sprayed trifoliate leaves developed an epinastic twisting and mottled appearance about three days after spraying, and did not continue to expand appreciably. The plants were harvested by cutting the stems just above the soil, six days after treatment, and promptly taken to the laboratory. There the plants were divided into the primary leaf blades, trifoliate leaf blades, and the remainder which included the stems and petioles, which is here referred to as stems. The fresh weight was obtained and the samples for each analysis were promptly killed.

Total sugar and reducing sugar were determined as described in the Official Methods of Analysis of the Association of Official Agricultural Chemists in Sections 6.48, 22.33, 29.36, 29.39, 41.13 (69). Total nitrogen was determined by the macro Kjeldahl method described in the same book under 2.22. The free fatty acid and unsaponifiable material was determined as suggested by Dill (21).

Results

There was slightly less reducing sugar in the stems and less total sugar in the trifoliate leaves of the treated plants than in the same organs of the control plants as shown in Tables XXX and XXXI. Since the value for sucrose is determined by subtracting reducing sugar from total sugar any inaccuracies in either determination would influence this value, and since there is so little sucrose in young bean plants only quite large differences should be regarded as important.

There was only about half as much fatty acid in the treated plants as in the control plants, and a comparison of the averages indicates that this decrease occurs mostly in the primary leaves and secondly in the stems. There appears to be no great difference in the amount of unsaponifiable material in the treated and control plants. Although this fraction includes many compounds whose metabolism is not well understood, the unsaponifiable material is not commonly regarded as a readily available reserve food material.

The difference in Kjeldahl nitrogen is insignificant statistically, although there appears to be a slight increase in the percent of the nitrogen in the treated trifoliate and primary leaves; however, this difference might be due to a difference in water content of the leaves, and because of the decrease in size of the leaves this is not a real increase in the amount of nitrogen in a leaf.

The increased rate of respiration of bean leaves treated with the same concentration of 4-phthalimido-2,6-dimethylpyrimidine, would be expected to result in a decrease

TABLE XXX

THE COMPOSITION OF CRANBERRY BEAN PLANTS SIX DAYS AFTER SPRAYING WITH 1000 PPM OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE

(Expressed as mg per organ)

	Check				Treated			
	Trifoliate	Primary	Stem	Total	Trifoliate	Primary	Stem	Total
Total sugar	0.18	0.48	2.90	3.56	0.12	0.48	1.89	2.49
Reducing sugar	0.15	0.46	2.79	3.40	0.12	0.42	1.72	2.26
Sucrose (by difference)	0.03	0.02	0.11	0.16	0.00	0.06	0.17	0.23
Fatty acid	1.41	5.59	6.51	13.50	1.31	2.24	4.00	7.55**
Unsaponifiable material	0.51	3.52	3.42	7.45	0.44	3.02	4.29	7.74
Kjeldahl nitrogen	2.85	8.08	9.26	19.92	2.10	7.81	8.73	18.64
Crude protein (N x 6.25)	16.13	50.50	57.87	124.50	13.13	48.81	54.56	116.50
Fresh weight in gm	0.26	1.28	2.86	4.40	0.22	1.31	2.43	3.96

**Highly significantly less at 1% level than check

TABLE XXXI

THE COMPOSITION OF CRANBERRY BEAN PLANTS SIX DAYS AFTER SPRAYING WITH 1000 PPM OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE

(Expressed as percent of the fresh weight)

	Check			Treated		
	Trifoliate	Primary	Stem	Trifoliate	Primary	Stem
Total sugar	.61	.40	.98	.55	.37	.76
Reducing sugar	.51	.38	.94	.55	.32	.68
Sucrose (by difference)	.10	.02	.04	.00	.05	.08
Fatty acid	.59	.42	.24	.57	.17	.17
Unsaponifiable material	.22	.26	.16	.20	.23	.18
Kjeldahl nitrogen	.0106	.0056	.0032	.0111	.0065	.0031

in sugars, fatty acids, and other carbohydrate fractions as these analyses indicate if the capacity to synthesize these materials was not increased so as to overcome the increased rate of breakdown during respiration. This is apparently what happened.

These alterations in the composition of plants are similar to those caused by many other growth regulators such as 2,4-dichlorophenoxyacetic acid, indoleacetic acid and others. The decreases in all of the major constituents which were determined indicate that the food value of these plants was not increased in these respects for animals. The analyses are valuable in that they indicate some of the changes which have occurred in the treated plants.

EXPERIMENTS SUGGESTING THE MODE OF ACTION OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE UPON THE PHYSIOLOGY OF PLANTS

Introduction

The manner in which 4-phthalimido-2,6-dimethylpyrimidine affects the growth of plants was suggested by three experiments. In all of these experiments, 4-phthalimido-2,6-dimethylpyrimidine seemed to lessen or antagonize the main effect of auxin or to interfere with its translocation whether the auxin was of natural or synthetic origin.

According to the theory of Went and Thimann (103) and the large amount of substantiating evidence and experience which is in accord with this theory, auxin is responsible for correlating and controlling many different phases of the morphological development and growth of plants. Auxin is thought to be synthesized in the apical meristematic regions of stems and in leaves, from natural occuring substrates, and to travel in a morphologically downward direction with a naturally occurring polar gradient. The supply of auxin from leaves is thought to be an important factor in preventing their abscission (80). Physiologically high concentrations of auxin are probably responsible for inhibiting the development of the lateral buds of shoots and allowing the apical bud to maintain its dominance. The concentration of auxin which is physiologically active in root growth is much lower than that of the above ground portion, but it is still responsible for root growth, although most all artificially supplied concentrations of auxin severely inhibit growth. Auxin also probably governs the initiation and growth of branch roots.

The Interacting Effects of Indoleacetic Acid and 4-Phthalimido-2,6-dimethylpyrimidine Upon the Growth of Cucumber Roots

Methods and Materials

Artificial or exogenous additions of heteroauxin, which chemically is indole-3-acetic acid, to the normal endogenous supply of auxin within the roots of intact seedlings generally inhibits root growth (1). From the previous experiment with cucumber seedlings it is known that 250 ppm of 4-phthalimido-2,6-dimethylpyrimidine causes a decided but not complete inhibition of the growth of cucumber roots. A small factorial experiment was conducted to determine if 4-phthalimido-2,6-dimethylpyrimidine had any major effect upon the influence of indoleacetic acid upon the growth of the roots of cucumber seedlings. The four combinations of (1) control, (2) 250 ppm of 4-phthalimido-2,6-dimethylpyrimidine, (3) 25 ppm of indoleacetic acid, and (4) 250 ppm of 4-phthalimido-2,6-dimethylpyrimidine plus 25 ppm of indoleacetic acid, were added to the petri dishes. The four treatments were replicated in four dishes each. After the seedlings had grown in the petri dishes on the laboratory bench for five days they were measured.

Results

Either the 4-phthalimido-2,6-dimethylpyrimidine or the indoleacetic acid when applied alone caused an inhibition of growth but when the two were applied at the same time the amount of inhibition was not additive. Statistically this was a significant interaction as shown in Table XXXII. This result might be interpreted in many ways. It could mean that 4-phthalimido-2,6-dimethylpyrimidine inhibits the enzymatic synthesis of the endogenous supply of indoleacetic acid from tryptophane. It might mean that it stimulated the destruction of indoleacetic acid probably by the indoleacetic acid oxidase system. These results also might indicate that the two compounds are involved in two different steps of a series of reactions so that if one step is inhibited the latter step does not occur at its usual rate so its inhibition is not added. Another possibility is that 4-phthalimido-2,6-dimethylpyrimidine interferes with the translocation of the endogenous supply of auxin coming from the root tip to the region of elongation, or the translocation of free auxin arising from the liberation of bound auxin in the cotyledons to the region of elongation.

TABLE XXXII

THE INTERACTION OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE AND INDOLEACETIC ACID ON THE GROWTH OF CUCUMBER ROOTS

Treatment		Average length of roots in cm
Control		7.4
25 ppm	Indoleacetic acid	1.5
250 ppm	4-phthalimido-2,6-dimethylpyrimidine	4.6
25 ppm 250 ppm	Indoleacetic acid plus 4-phthalimido-2,6-dimethylpyrimidine	1.4

Interaction significant at 5% level

The Inhibition of the Translocation of Auxins by 4-Phthalimido-2,6-dimethylpyrimidine in the Petioles of Coleus Plants

Methods and Materials

An experiment was conducted to determine if 4-phthalimido-2,6-dimethylpyrimidine interfered with the translocation of indoleacetic acid or naphthaleneacetic acid. Thirty-two Christmas Gem coleus plants were graded for uniformity and divided into four treatments of eight plants each. The blades of half of the leaves were cut off, as was done in the previous studies of abscission. A lanolin paste containing 1000 ppm of indoleacetic acid was placed on the tips of the petioles of two of the treatments of plants and a paste containing 100 ppm of naphthaleneacetic acid was placed on the tips of the petioles of the other two treatments of plants. The plants of one group treated with indoleacetic acid, and one group treated with naphthaleneacetic acid were treated with a ring of lanolin paste containing 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine applied around the petioles between the abscission zone and the tip. A ring of lanolin containing no growth regulators was applied to the other two groups of plants which served as controls. The rate of abscission of the petioles was used as an index of the amount of inhibition of auxin translocation because auxin usually delays the abscission of coleus petioles (26).

Results

The petioles with a ring of 4-phthalimido-2,6-dimethylpyrimidine between the supply of auxin and the abscission zone did absciss earlier than the petioles with no 4-phthalimido-2,6-dimethylpyrimidine between the supply of auxin and the abscission zone as shown in Table XXXIII. This result might be interpreted as indicating that 4-phthalimido-2,6-dimethylpyrimidine inhibited or interfered with the translocation of indoleacetic acid, or of naphthaleneacetic acid. Both of these auxins responded similarly. These results do not eliminate the possibility that 4-phthalimido-2,6-dimethylpyrimidine and the auxins act independently upon the abscission zone.

A similar experiment using cucumber plants in pots in the greenhouse was conducted. The leaves were not cut off but were left intact to supply a physiologically normal concentration of auxin. A ring of lanolin containing 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine failed to make the leaves absciss. This may be due to the fact that cucumbers do not have a pectolytic enzyme in the abscission zone as reported by Bell (10).

The Inhibition of Apical Dominance by Treating the Apical Shoot of Coleus Plants with 4-Phthalimido-2,6-dimethylpyrimidine

Methods and Materials

An experiment was conducted to determine if the down-

TABLE XXXIII

THE TRANSLOCATION RATE OF AUXINS APPLIED TO THE TIPS OF DEBLADED COLEUS PETIOLES AS AFFECTED BY THE APPLICATION OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE IN LANOLIN TO THE CENTER OF THE PETIOLES AS INDICATED BY THE RATE OF ABSCISSION

Position on petiole	Treatment			
Debladed tip	1000 ppm of indoleacetic acid	1000 ppm of indoleacetic acid	100 ppm of naphthalene-acetic acid	100 ppm of naphthalene-acetic acid
Middle	1000 ppm of 4-phthalimido-2,6-dimethyl-pyrimidine	control of pure lanolin	1000 ppm of 4-phthalimido-2,6-dimethyl-pyrimidine	control of pure lanolin
Hours after treatment	Average number of debladed petioles on plant			
0	4.97	4.97	4.97	4.97
44	4.82	4.97	4.61	4.97
77	4.24	4.97	2.60	4.74
106	3.80	4.97	2.37	4.51
177	3.80	4.97	1.89	4.16
225 approximately	2.05	3.85	0.94	2.66
273 approximately	1.75	3.35	0.82	1.96

L.S.D. for testing between any treatment at the same time is 1.81

ward movement of auxin from the apical region and young leaves of a coleus shoot which is theoretically responsible for the decreased growth rate of the lower axillary shoots, could be decreased by the application of 4-phthalimido-2,6-dimethylpyrimidine between the apical bud and the axillary shoots. On April 18, 1952 twenty-four Christmas Gem coleus plants growing in pots in the greenhouse were paired for uniformity. All of the lateral shoots except the two lowest ones which were of about equal size and the leaves of this lower node and the next few nodes were cut off at their abscission zones. A lanolin paste containing 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine was smeared on about two inches of the apical shoot above the two lower axillary buds of half of the plants. The other half of the plants were treated with a pure lanolin paste and used as controls. The length of the two axillary shoots on each of the treated and control plants was measured at the beginning of the experiment and again 39 days later or on May 27. The final length measurements were adjusted for unequal lengths at the beginning of the experiment by an analysis of covariance.

Results

The axillary shoots of the plants treated with 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine were significantly longer than the axillary shoots of the control plants as shown in Table XXXIV. The 4-phthalimido-2,6-dimethyl-

TABLE XXXIV

THE DECREASE IN THE DOMINANCE OF THE APICAL SHOOT OVER THE GROWTH OF THE LATERAL SHOOTS OF COLEUS PLANTS ACHIEVED BY SMEARING A LANOLIN PASTE CONTAINING 1000 PPM OF 4-PHTHALIMIDO-2,6-DIMETHYLPYRIMIDINE UPON THE APICAL SHOOT

	Average length of lateral shoots in cm	
	Treated	Control
Length at beginning of experiment	1.0	1.2
Length 39 days later	5.1	3.1
Length 39 days later adjusted for differences in original length by analysis of covariance	5.2**	3.0

**Highly significantly longer at 1% level pyrimidine may have inhibited the downward movement of auxin from the apical shoot. This auxin from the apical shoot is responsible for inhibiting the growth of lateral shoots and a decrease in amount of auxin reaching the lateral shoot would theoretically allow them to grow at a greater rate. This is one possible explanation, however, 4-phthalimido-2,6-dimethylpyrimidine may affect growth alone without influencing the translocation or functioning of the natural auxins.

Discussion

These experiments were not extensive enough to serve as a basis for any definite conclusions. However, all of these experiments could be interpreted in the same way. Perhaps 4-phthalimido-2,6-dimethylpyrimidine interferes with the translocation or inhibits the action of auxin. It may be proven to be an antiauxin. The plant physiologists (19) have tentatively defined antiauxins as compounds which inhibit the formation of auxin or competitively inhibit the action of auxin. Only the experiment with cucumber seedlings provides any indication that this might be a competitive inhibition. Since it is difficult to study the effect of auxin in vitro, and an interpretation of the results of in vivo experiments is subject to many of the alternatives which were encountered in these experiments, the absolute classification of a compound as an antiauxin should be done with discretion.

SUMMARY

1. The compound, 4-phthalimido-2,6-dimethylpyrimidine was extensively tested on many plants and was found to be a new plant growth regulator.
2. It is capable of inducing the parthenocarpic development of tomatoes. Spraying or dipping tomato blossoms in aqueous solutions from 100 ppm to 500 ppm of this compound was effective in increasing the yield of early tomatoes which would not normally have set because of the low night temperatures in the field in the spring. Similar treatments increased the yield of fruit of ten different varieties of tomatoes grown in the greenhouse during the fall when light intensity is too low for normal development.
3. An attempt to stimulate parthenocarpic fruit development of a large population of muskmelons by applying a lanolin paste containing from 200 ppm to 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine to the pistils of the flowers was unsuccessful. An attempt to set female flowers of cucumber plants in the greenhouse did not yield conclusive results.
4. This compound was not as effective as naphthaleneacetic acid in controlling the abscission of debladed coleus petioles, in thinning Duchess apples, Bartlett pears, or

peaches, or in delaying the preharvest drop of Duchess apples.

5. Soaking arbor vitae and boxwood cuttings in a 50 ppm solution of 4-phthalimido-2,6-dimethylpyrimidine before rooting, increased the percentage of cuttings which rooted. The length of roots formed by coleus cuttings treated in a similar manner was also increased. The compound does not appear to be superior to naphthalene-acetic acid in the rooting of cuttings of these species.

6. The growth in length of the roots of cucumber seedlings was inhibited by increasing concentrations of 4-phthalimido-2,6-dimethylpyrimidine, N-1 naphthyl phthalamic acid, N-2 chlorophenyl phthalamic acid and N-4 chlorophenyl phthalamic acid in the solution in which the seeds germinated and grew in petri dishes. These phthalamic acids were quite toxic to the seedlings, while phthalic acid was without effect, and phthalimide stimulated the growth of the cucumber roots.

7. Chemical analysis of bean plants six days after they were sprayed with 1000 ppm of 4-phthalimido-2,6-dimethylpyrimidine showed that this treatment decreased the amount of reducing sugar in the stems and trifoliate leaves. The primary leaves and stems of the treated plants contained about half as much free fatty acid as the control plants. These results are in accordance with a higher rate of respiration of treated plant materials as determined with a Warburg apparatus.

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