with Cycocel required less water than control plants. Curtis (7) states that reduced transpiration could result in increased salt accumulation in plant leaves.

The pH of the media at each fertility level was satisfactory for plant growth. The pH's of the media ranged from 5.5 to 6.2 with no significant differences due to treatments. The pH range is within the range claimed (13, 14) to be best for growth in a high organic medium.

Varying the fertility levels to treated and control transplants prior to field setting had very little influence on yields. However, experiments with post-transplanting applications of fertilizers may show that plants treated with growth retardants may require a different soil fertility program than that of control plants for greatest influence on yields.

**Abstract.** Chrysanthemum, ‘#3 Indianapolis White’, subjected continuously to atmospheres containing 1–4 ppm ethylene failed to initiate and develop flower buds under short day conditions. The plants showed typical epinastic symptoms, shortening of internodes, thickening of stems and loss of apical dominance. The plants developed many short axillary shoots, each with a few small leaves. The top leaves on the plant became smaller and smaller and were less dissected than the controls. Subjecting plants alternatively to ethylene-containing and normal atmospheres generally prevented flowering also, but occasionally crown budding occurred. Bioassay of endogenous auxins showed that these growth promoting substances were maintained at high levels in the ethylene-treated plants, which may account for their failure to flower. In addition, ethylene also seemed to affect the polar auxin transport system of the plant.

**Effects of Ethylene on Morphology and Flowering of Chrysanthemum morifolium Ramat**

Benny O. S. Tjia, 2 Marlin N. Rogers and David E. Hartley, University of Missouri, Columbia

The source of their problem appears to come mainly from the exhaust gases of gas or oil heaters and open-flame burners used for heating purposes during cold weather when ventilation in their tightly constructed plastic houses is restricted. Chrysanthemums grown under these conditions showed abnormalities in growth and failed to enter the reproductive stage, even though subjected to photoinductive conditions favorable for flowering. Gas analyses of air samples collected from affected greenhouse ranges showed the presence of ethylene in minute quantities.

Crocker and Knight (8) were among the first to recognize plant injury symptoms caused by ethylene. They found that ethylene in the air at concentrations as low as 0.1 ppm caused injury to carnations. Recent work by Heck and Pires (11) has shown marked ethylene injury in more than 100 species of plants fumigated with 2–10 ppm ethylene for 10 days. Other unsaturated hydrocarbons such as acetylene, propylene and butylene were also found to be injurious to plants, but it required much greater quantities of the latter gases to produce injurious symptoms. It takes 500, 5000 and 500,000 times as much acetylene, propylene and butylene respectively to produce injury equal to that caused by ethylene (10). Thus, it seems that ethylene is the only unsaturated, low-numbered hydrocarbon that is active enough to cause physiological responses at very low concentrations.

The effects of ethylene have been investigated intensively by many workers (5, 6, 9). It is now known that ethylene induces physiological changes

**LITERATURE CITED**


9. Lindstrom, R. S., and N. E. Talbert. 1960. (2-chloro-ethyl)-trimethyl am-
such as fruit ripening, abscission of parts, proliferation of tissue, breaking of dormancy, inhibition of growth and variations in cellular metabolism. In 1954 Van der Laan (12) considered the possibility that ethylene, directly or indirectly, acts on the auxin system in the plant. He postulated that the auxin content was decreased since ethylene caused a decrease in elongation of stems. Though his observations conflict with those of more recent workers (1, 3), he was the first to demonstrate the possibility of such a relationship. Morgan and Gausman (15), working with cotton and cowpea, found that incubation of either pieces of tissue or intact plants in ethylene-containing atmospheres reduced the degree of polar auxin transport. Further work by Burg (4) on pea tissue also showed a reduction of polar as well as lateral transport.

The objective of the present work was to describe the morphological abnormalities of chrysanthemum caused by ethylene and to develop a hypothesis which could explain its inhibition of flowering by determining and analyzing the changes in activity of the endogenous growth hormones.

**Materials and Methods**

The work was conducted during the winter months of 1965–1967 in the University of Missouri growth chambers and greenhouses. The study was carried out using the chrysanthemum cultivar, '#3 Indianapolis White'. Rooted cuttings were received from Yoder Brothers, Inc., Barberton, Ohio. After 3 weeks growth the plants were again dipped in liquid air and normal atmospheres above, (c) Plants of '#3 Indianapolis White' after 9 weeks and successive leaves (from left to right) of similar plants; 3-4 ppm ethylene below and normal atmospheres above. (c) Plants of '#3 Indianapolis White' after 9 weeks exposure to ethylene-containing atmospheres. From left to right; continuous (24 hours daily) ethylene and long photoperiods; continuous ethylene and short photoperiods; ethylene during 9-hour dark period only and long photoperiods. Note the absence of lateral vegetative shoots on plants not receiving continuous ethylene treatment.

**Experiment 1.** This experiment was designed to determine the morphological characteristics of plants subjected continuously or intermittently to ethylene-containing atmospheres. Plants were grown in controlled environment chambers at 65°F night and 75°F day temperatures. Long day conditions were provided by the use of incandescent lights over the plants from 10 PM to 2 AM at a minimum intensity of 10 ft-c at plant height. After 3 weeks growth the plants were well established and ready for use in experimental treatments.

**Long Day Treatment (15 hours light daily).**

1. Ethylene continuously.
2. Ethylene during light period only.
3. Ethylene during dark period only.
4. Ethylene neither during dark or light period.

**Short Day Treatment (9 hours light daily).**

5. Ethylene continuously.
6. Ethylene during light period only.
7. Ethylene during dark period only.
8. Ethylene neither during dark or light period.

Ethylene gas (99.5% purity) was released in the tightly sealed, ethylene-containing room twice daily and/or each time the room was entered. A quantity of gas sufficient to provide 1–2 ppm ethylene in the chamber was displaced with water from a glass storage container. Periodic air samples were collected and analyzed by gas chromatography to verify ethylene levels being maintained.

**Experiment 2.** This experiment was carried out to collect apical tissue for bioassay. The plants received continuous ethylene treatment in a plastic greenhouse, especially designed and built as nearly gastight as possible. The air in the plastic house was continuously recirculated through a pipe duct system to an oil-fired, hot-air furnace located outside the growing structure and was heated as necessary to maintain a 65°F minimum temperature under the control of a thermostat located inside the plastic house. The ethylene was introduced continuously into the atmosphere by releasing a calculated amount of pure gas from a Mariotte bottle through a glass, water-filled bulb inside the plastic house. A concentration of 3–4 ppm was maintained throughout the treatment period.

At the beginning of the experiment, 300 established potted chrysanthemum plants which had been under long day treatment were placed in the plastic house, under the natural short day conditions of January (effective day-length 10½ to 11½ hours). Samples of apices were then collected after 1, 2, 3, 4, 5 and 6 days and after 2, 3, 4, 5, 6 and 9 weeks of growth in the ethylene-containing atmosphere. Apices of 25 plants were removed between 10 and 11 AM on each sampling date. These were labelled, immediately frozen in liquid air and then stored under sub-zero temperatures until all samples had been collected. When harvesting was complete, the samples were again dipped in liquid air and lyophilized. The freeze dried tissue was then ground into a fine powder in a Spex Mixer-Mill and stored in a desiccator at −3°C in the dark until used for auxin determination.

Four hundred mg of ground, lyophilized apical tissue was extracted 3 times for 20 min with 20 ml absolute methanol. All extractions were carried...
out in dim diffused light at \(-3^\circ\). The 3 filtrates were combined and evaporated under reduced pressure under a black cloth cover in a water bath (40\(^{\circ}\)) using a Rinco evaporator. Although replicated extractions and bioassays were not executed for each treatment in this particular experiment, the results obtained were in general agreement with those obtained from other similar experiments conducted earlier.

The growth substances were separated using paper chromatography. Strips of Whatman #1 filter paper, 15 cm wide were used. The dried extract was taken up in 0.25 ml of methanol and streaked on a straight line 13 cm long about 2.5 cm from the base of the chromatographic paper strip with a blunt hypodermic syringe. The paper was equilibrated overnight (12 hr) with isopropanol : water = 80 : 20 v/v. After equilibration, the paper was dipped in the same solvent and it was allowed to rise 20 cm. The chromatograms, after being dried, were cut into 1 cm sections; thus each section represented an Rf of 0.05. Strips 1 cm below the streak were used as controls. The strips were eluted in small glass vials in which a sucrose-citric acid-phosphate buffer of pH 5.0 was added.

The activity of the growth substances was determined by the coleoptile straight growth test using 'Monon' wheat variety. The wheat seeds were soaked in tap water for 8 hr in the dark room. Then the water was discarded and the seed rinsed with fresh water and then broadcast on moist, sterilized quartz sand in large pyrex or plastic dishes. To avoid excessive thickening of stems, shortening of internodes and epinasty of leaves (Fig. 1a). Short day treated plants remained vegetative. In addition, both groups of plants developed lateral vegetative shoots about 1 inch long (Fig. 1c). The upper leaves became smaller and smaller and these were less dissected than leaves from untreated control plants (Fig. 1b). The lower leaves yellowed and died on plants exposed to ethylene.

Plants subjected to ethylene either during the dark or light period only, under both long day and short day treatment displayed less marked symptoms. The plants exposed to ethylene for only 9 hr daily (during the dark period for plants receiving long day treatment, or during the light period for plants receiving short day treatment) did not show any appreciable signs of injury or epinasty. The lower leaves did not become yellow and senescent, and lateral shoots did not break (Fig. 1c). However, the younger leaves became smaller in size and some plants developed crown buds.

Plants exposed to ethylene for 15 hr daily (during the light period for plants receiving long day treatment, and during the dark period for plants receiving short day treatment) showed more marked injury symptoms. Internodes were somewhat shortened, the leaves became smaller and lower leaves senesced and died. The plants developed small lateral vegetative shoots. Some of the plants developed crown buds. A summary of the visible characteristics is presented in Table 1.

**Table 1. Morphological characteristics of Chrysanthemum morifolium (variety 'Indianapolis White') subjected to 1–2 ppm ethylene.**

<table>
<thead>
<tr>
<th>Plant responses</th>
<th>Ethylene treatment</th>
<th>Short day</th>
<th>Long day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day and night</td>
<td>Day only</td>
<td>Night only</td>
</tr>
<tr>
<td>Terminal flower buds</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Crown buds</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Multiple crown buds</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shortening of nodes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thickening of stem</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Retardation of flowering</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Small lateral vegetative shoots</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Yellowing of lower leaves</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Curling and drying of leaves</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Abscession of lower leaves</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Vegetative growth</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Indicates that not all of the 12 plants subjected to the same treatment possessed the mentioned characteristics. (- = absence, + = presence, ++ = higher degree and +++ = extreme degree of mentioned characteristics).
Active vegetative growth in chrysanthemum, a short day plant, is also associated with high auxin content. When these plants are subjected to the naturally shortening days of fall, the auxin levels decrease steadily and eventually the flowering mechanism is set in motion. Shaw (16), working with a thermonegative variety of chrysanthemum subjected to short days and grown at elevated night temperatures, showed that the growth factors remained at relatively high levels and flowering was inhibited.

Thus it seems that flower initiation in short day plants is preceded by a decrease in auxin activity. This fact was further confirmed by Lindstrom and Asen (13). They sprayed chrysanthemums with indoleacetic acid (IAA) at different intervals after the beginning of short days and a delay or inhibition of flower initiation took place.

It has also been shown that both intact plants and excised tissue respond to the application of ethylene by increasing their internal auxin levels. If this is reversed, that is, if IAA is applied rather than ethylene, the tissue responded by producing more ethylene. Thus a very close relationship exists between auxin and ethylene and many ethylene responses can be duplicated with auxin treatment (3).

Our results showed that when the test plants were grown in ethylene-containing atmospheres they completely failed to initiate flower buds, even though the plants were given favorable photoinductive periods. Thus it seemed that the low levels of auxin necessary to set the flowering mechanism in motion were somehow prevented from developing. Instead, auxin activity was maintained at high levels as can be seen from the histograms presented here.

A steady decrease of auxin activity is generally observed when short day plants are subjected to the gradually diminishing daylengths of fall. However, if an abrupt change of daylength from 15 to 9 hr occurs, a different pattern of auxin activity is observed. Instead of having a gradual decrease in auxin levels, the sudden change in daylength temporarily results in a higher net accumulation of the growth hormones, followed after 5 or 6 days by a marked decrease. Although a plausible hypothesis to explain the reason for this pattern of behavior is difficult to develop, the fact of its occurrence is quite clear from our bioassay results. The rapid surge in stem elongation of chrysanthemum plants for the first 2 or 3 days following the initial application of black cloth is a commonly observed phenomenon and would also tend to substantiate the fact that this temporary surge of auxin activity in the apical meristem actually occurs.

The absence of flowering in our test plants is associated with high auxin activity, but this alone does not provide an adequate explanation for the thickening of stems and other responses of the chrysanthemum caused by ethylene. Therefore, other physiological reactions or changes due to the presence of high auxin activity must have taken place.

The literature reveals that in plants exposed to low concentrations of ethylene, simple soluble substances tend to increase at the expense of the more complex soluble and insoluble forms. Osmotic potentials are thereby affected and the permeability of cell membranes changes. Auxin application has been shown to increase the flexibility and extensibility of cell walls. Thus a combination of all these factors may have taken place, which resulted in the stem thickening observed.

Definite balances between the levels of auxins and kinins mediate cell division within tissues and growth activity within cells. Leaf expansion depends also on the balances of auxins and kinins. Thus if this balance is disrupted, leaf expansion should also be affected (Fig. 1b).

Burg (4) suggested that polar and lateral transport of auxins are inhibited by ethylene. The transport system of plants in our experiments appeared to be affected also, since the cells located in the lateral axillary buds were activated, resulting in the development of axillary side shoots.

The phenomenon of apical dominance regulates axillary growth. This is inhibited as long as the apical meristem is present, synthesizing substantial quantities of auxin. However, as soon as the terminal growing point of the plant is removed, eliminating the primary site of auxin synthesis, the auxin-kinin ratio in the plant system...
is changed and the cells within the axillary buds respond by dividing and beginning growth. In our experiment we noted prolific growth of the axillary shoots present on plants with intact apical meristems, which upon bioassay proved to be still high in auxin activity. This would seem to support Burg’s findings that auxin transport is inhibited in plants in ethylene-containing atmospheres and might further explain the senescence and dying of lower leaves on our ethylene-treated plants.

Literature Cited


Interactions of Applied Growth Regulators and Temperature on Root Initiation in Salix Cuttings

R. Domanski, T. T. Kozlowski, and S. Sasaki

University of Wisconsin, Madison

Abstract. The effects of various combinations of naphthaleneacetic acid (NAA), gibberellic acid (GA), and benzyladenine (BA) at 5 or 50 ppm of each and temperature (22 and 25°C) on root initiation in Salix viminalis, L. cuttings were studied. Highly significant influences of temperature, exogenous growth regulators, and temperature-growth regulator interactions on root initiation were demonstrated. Root formation generally was stimulated by increasing temperature from 22 to 25°C. NAA stimulated root formation markedly and its effects were further enhanced for at least 10 days by increasing temperature from 22 to 25°C. In contrast to NAA, both GA and BA inhibited root initiation. The inhibitory effect of GA was greater at 25°C than at 22°C; BA had the greater depressive effect at 22°C. Addition of GA or BA, or both, to NAA generally reduced the stimulation of root formation by NAA. As temperature increased toward an optimum for root initiation the balance of endogenous growth regulators and possible cofactors may have changed to bring about the stimulatory effects of auxins over the inhibitory influences of cytokinins and gibberellins.

Introduction

MANY factors have been shown to affect rooting of cuttings from trees. As Kramer and Kozlowski (8) and Bacher and Stowe (5) emphasized, these factors include age of the tree from which the cutting is taken, the position of the cutting on the tree, the type of cutting (c.g., softwood or hardwood), the time of year when cuttings are taken, sex of the parent tree, nutrient status of the cutting, and environmental conditions under which cuttings are rooted. Although all these factors have a physiological basis, the internal control of root initiation on cuttings is not fully understood. Domanski (5) observed that some exogenously regulated growth regulators, such as auxin, stimulate rooting and others, such as exogenous gibberellin or cytokinins, suppress it. Although synthetic auxins stimulate rooting of cuttings in many species they do not overcome the limitations of the factors listed above and they generally do not stimulate rooting on cuttings which are not known to root without them (2). Experiments on rooting of cuttings in juvenile and mature phases of Pyrus prunifolia Willd., var. robusta Bailey (Malus robusta, Rehd.) and Hedera helix, L. indicate that cofactors acting with endogenous auxins are involved in root initiation (6, 9, 11). A contributory role of endogenous inhibitors to rooting also has been suggested by Tizio et al. (13).

During plant development the amounts of endogenous growth regulators including auxins, gibberellins, and cytokinins vary in plants and it is possible that the balance among them may be influenced by temperature. The present experiments were conducted to observe the effects of a few combinations of applied synthetic growth regulators at 2 temperatures on root initiation in Basket Willow cuttings.

Materials and Methods

Experiments were conducted on cuttings of Salix viminalis, L. obtained from the Salicarium in Poproc, Poland. Experimental cuttings were 20 cm long. These had been cut from median portions (20–80 cm) of 1-year-old shoots which were approximately 1.2 m long. The bases of experimental cuttings were soaked for 12 hours in various combinations and concentrations of gibberellic acid (GA), benzyladenine (BA), and naphthaleneacetic acid (NAA) (Table 1). Treated and control cuttings with their bases immersed in water in test tubes were