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THE EFFECTS OF ETHEPHON ON *CATTELEYA AURANTIACA* (ORCHIDACEAE) SEEDLINGS

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In *Cattleya aurantiaca* seedlings, ethephon (2-chloroethylphosphonic acid, also known as Ethrel), slightly accelerated leaf development at concentrations between 2.5 and 20 ppm but suppressed it at 50 ppm. It inhibited leaf length at concentrations of 2.5, 5, and 50 ppm but enhanced it at 10 and 20 ppm. Root formation was inhibited by concentrations higher than 2.5 ppm. Chlorophyll content of seedlings was highest on a medium containing 5 ppm ethephon.

Introduction

Knowledge regarding the contribution of mycorrhizae to germinating orchid seeds and developing seedlings has increased in recent years, but uncertainties still exist, especially with regard to the functions of plant hormones. The available evidence suggests that germinating seeds and developing seedlings do not require an exogenous supply of auxins but may temporarily benefit from naphthalene-acetic acid (STRAUSS and REISINGER 1976). Effects of cytokinins or gibberellins are unclear (WITHNER 1959, 1974; ARDITTI 1967a, in press; STOUTAMIRE 1974), and there are no reports on the influence of ethylene on orchid seed germination and seedling development.

Ethylene promotes germination of rape, *Brassica napus* (TAKAYANAGI and HARRINGTON 1971), but this by itself is not enough to suggest that it may have a similar effect on orchid seeds and seedlings because they differ from those of other flowering plants. However, a report that mycorrhizal fungi of orchids produce ethylene (HANKE and DOLLWET 1976) suggests that this hormone may affect seed germination and seedling development.

Ethylene, a gas which diffuses easily, is difficult to incorporate in culture media. Therefore, compounds which gradually evolve ethylene are better suited for in vitro experiments. Ethephon or Ethrel (2-chloroethylphosphonic acid) is such a compound (ANONYMOUS 1967; WARNER and LEOPOLD 1969; YANG 1969). It can elicit ethylene-like effects such as flowering of pineapple, abscission, and suppression of asexual embryogenesis in vitro (ANONYMOUS 1967; COOKE and RANDAL 1968; TISSERAT and MURASHIGE 1977). We used ethephon as a nutrient medium component in a study of ethylene effects on seedling development in *Cattleya aurantiaca*.

Material and methods

PLANT MATERIAL.—Six-month-old *Cattleya aurantiaca* seedlings germinated and grown on Knudson

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C (KC) medium (KNUDSON 1946) were transferred onto a solution containing ethephon (100–200 per culture vessel).

CULTURE MEDIUM.—Ethephon (a gift from Amchem Products, Inc.) was diluted in 95% ethanol acidified to pH 3.0–3.3 to prevent premature ethylene evolution, and 2 ml/100 ml medium were added to preautoclaved KC at concentrations of 2.5, 5, 10, 20, and 50 ppm and mixed by swirling. Both KC and KC plus 2 ml of 95% ethanol were used as controls.

CULTURE VESSELS.—Prescription bottles of 100-ml capacity were filled with 20 ml medium. Following inoculation, a cotton plug was inserted in the neck of each bottle and the cap screwed on loosely to prevent the accumulation of ethylene.

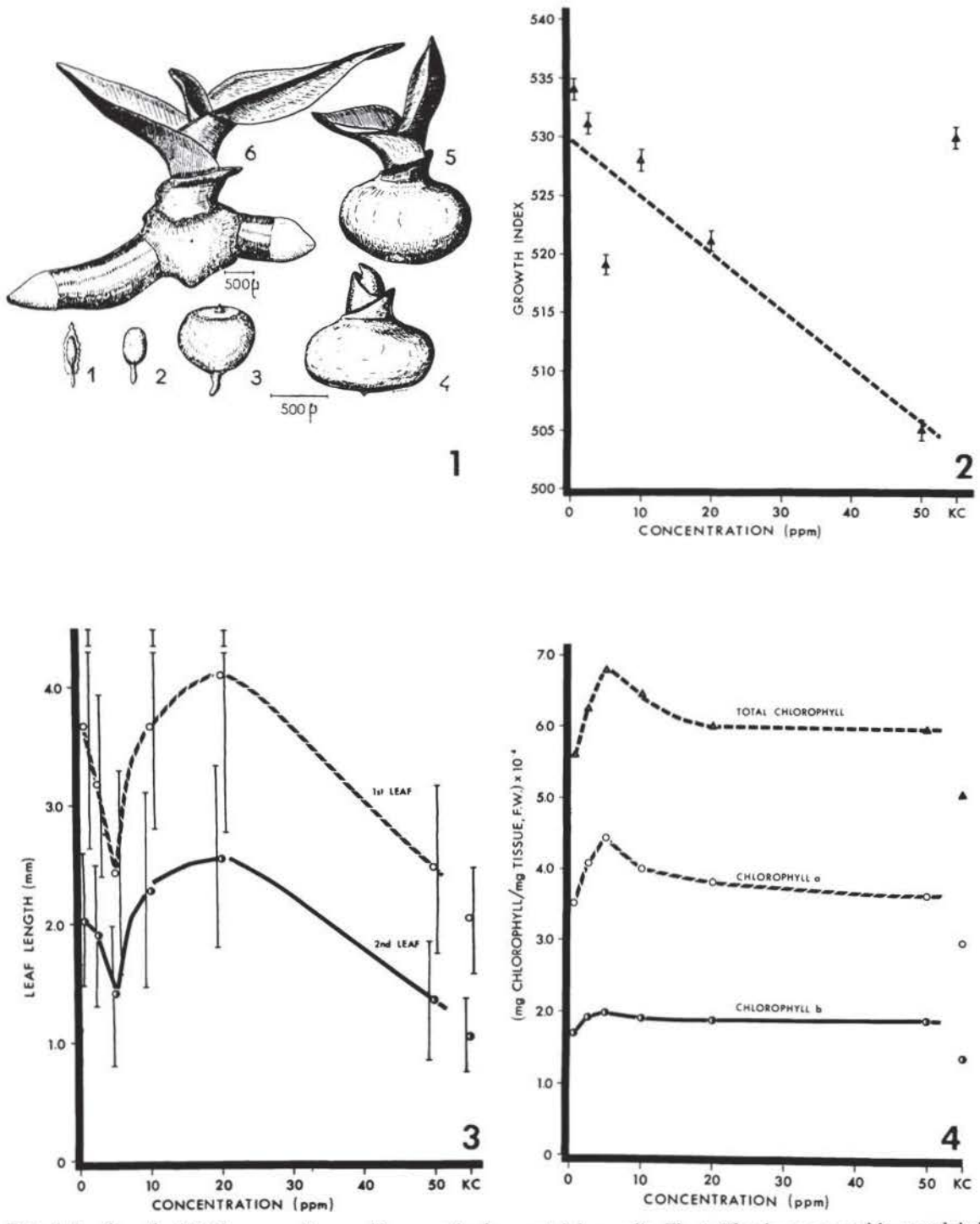
CULTURE CONDITIONS.—Cultures were maintained under banks of Gro-Lux lamps at 1,614 lx and 12-h photoperiods. The temperature was 22–25 C.

EVALUATION OF RESULTS.—Ethephon effects on seedling growth; leaf development, formation, and elongation; and chlorophyll content were evaluated 75 days after the start of the experiment. Development was measured by the growth index method (fig. 1; SPOERL 1948; ARDITTI 1967b). A total of 400 seedlings (100 seedlings per replica) were evaluated for each medium. The lengths of the two youngest leaves (designated as first leaf and second leaf) were measured in 200 seedlings (50 each from four replicas). One replica of each medium was used for chlorophyll determination. Chlorophyll was extracted from all seedlings in a culture vessel and assayed by standard procedures (MACKINNEY 1941; ARNON 1949; BRUINSMAN 1963) adapted for use with orchids (HARRISON 1973).

Results

GROWTH AND DEVELOPMENT.—At low concentrations, ethephon did not affect seedling development, but at 50 ppm it was inhibitory (fig. 2). Seedlings grew better on KC plus 2 ml ethanol than on any other medium. Growth on the KC control and 2.5 ppm ethephon was equal to or better than on the remaining media.

ROOTS.—Ethephon in concentrations exceeding 2.5 ppm inhibited root formation. It suppressed



FIGS. 1-4.—Growth of *Cattleya aurantiaca* seedlings on ethephon-containing media. Fig. 1, The six stages used in growth index determinations (after MARIAT 1952). Fig. 2, Growth and development of seedlings on ethephon-containing media. Fig. 3, Seedling leaf length vs. ethephon concentrations. Fig. 4, Effects of ethephon concentrations on chlorophyll content.

development past stage 5, increasing the number of seedlings with expanded leaves but without roots (fig. 1), and reduced the percentage of those with both (table 1). At concentrations of 5, 10, and 20 ppm, ethephon stimulated the appearance of numerous root hairs on circa 6% of the seedlings.

LEAF DEVELOPMENT AND LENGTH.—Concentrations of 2.5, 5, 10, and 20 ppm slightly enhanced leaf development, as indicated by the decrease in the number of stage 4 seedlings coupled with an increase of stage 5 plantlets (table 1). Leaf development was suppressed by 50 ppm, and as a consequence the number of stage 5 seedlings increased (table 1). Ethephon enhanced first and second leaf elongation at 20 ppm (fig. 3) but, surprisingly, caused a reduction in length at 2.5 and 5 ppm (fig. 3).

CHLOROPHYLL CONTENT.—Chlorophyll a, chlorophyll b, and total chlorophyll content all followed the same trend (fig. 4). At all concentrations of ethephon, chlorophyll content increased relative to the KC and the KC plus 2-ml ethanol controls. The greatest increase occurred at 5 ppm (fig. 4).

ADDITIONAL OBSERVATIONS.—Some excessively swollen seedlings, occurring singly or in aggregates, were observed on the KC plus 2-ml ethanol control and at 20 and 50 ppm ethephon but not at the lower concentrations. Many seedlings died after reaching growth stage 5 (fig. 1) on a medium containing 50 ppm ethephon.

Discussion

Cumulative release of ethylene from ethephon is proportional to its concentration in the medium (TISSERAT and MURASHIGE 1977). During 4 wk a total of 60 $\mu\text{l/liter}$ were released from a medium containing 3 ppm ethephon and "... 225 $\mu\text{l/l}$ for 10 mg/l and 450 $\mu\text{l/l}$ for 30 mg/l" (TISSERAT and MURASHIGE 1977). Comparisons with or extrapolation and interpolation from these data indicate that the amounts of ethylene released in our media were 55 $\mu\text{l/liter}$ (in the presence of 2.5 ppm ethephon),

110 $\mu\text{l/liter}$ (5 ppm), 225 $\mu\text{l/liter}$ (10 ppm), 340 $\mu\text{l/liter}$ (20 ppm), and 600 $\mu\text{l/liter}$ (50 ppm). Therefore, it is reasonable to assume that the effects we observed were brought about by differences in ethylene levels. The large number of excessively swollen seedlings we noted on 20 and 50 ppm ethephon, more than on the ethanol control, also argues for a specific ethylene effect since this enlargement is similar to the typical swelling brought about by the gas (BURG and BURG 1966; CHADWICK and BURG 1967). This effect may be due to disruption of normal polar cell expansion and increases in fresh weight as in pea internodes (EISINGER and BURG 1972). However, it is possible that phosphate produced by the decomposition of Ethrel may have also had an effect, as in asexual embryogenesis in carrot callus cultures (TISSERAT and MURASHIGE 1977). A comparison between our observations and the effects of naphthaleneacetic acid on seedlings of the same species (STRAUSS and REISINGER 1976) indicates that the influence of auxins and ethylene on orchid seedlings is not related.

The reduced or inhibited development of *Cattleya aurantiaca* seedlings brought about by ethephon is reminiscent of its inhibitory effects on Algerian ivy tissue cultures (STOUTEMYER and BRITT 1970) and asexual embryogenesis as well as the development of advanced embryonic stages in carrot tissue cultures (WOCHOK and WETHERELL 1971; TISSERAT and MURASHIGE 1977).

Concentrations of 5 and 50 ppm ethephon severely inhibited foliar elongation; and 2.5 ppm, although inhibitory, had a more moderate effect (fig. 3). The highest concentration, 50 ppm, also inhibited leaf development. This agrees with reports that growth of tomato and marigold leaves was inhibited by low concentrations of ethylene (ABELES 1973). Inhibition by 50 ppm is undoubtedly due to supraoptimal concentrations, as indicated by the death of seedlings past growth stage 5 on this medium. The other

TABLE 1
GROWTH STAGES OF *CATTELEYA AURANTIACA* SEEDLINGS
ON ETHEPHON-CONTAINING MEDIA

MEDIUM	GROWTH STAGES, % OF ALL SEEDLINGS*		
	4	5	6
Controls:			
KC.....	2.8	64.8	32.5
KC + 2 ml 95% ethanol.....	2.8	60.0	37.3
Ethephon:			
2.5 ppm.....	.5	60.3	33.3
5 ppm.....	2.5	76.3	21.3
10 ppm.....	1.8	68.5	29.8
20 ppm.....	1.8	72.3	25.5
50 ppm.....	4.3	88.8	7.0
6-mo-old seedlings at start of experiment.....	20.4	73.5	6.0

* There were no seedlings at stages 1-3.

differences in leaf elongation are those between suboptimal (10 ppm) and optimal (20 ppm) concentrations.

A comparison of the percentages of plantlets at stages 4, 5, and 6 in the ethephon-containing cultures, the controls, and the 6-mo-old seedlings (table 1) indicates that low levels of ethylene can enhance leaf development somewhat, even if the same concentrations may inhibit subsequent expansion.

Root formation distinguishes between stages 5 and 6 (fig. 1). A comparison of the percentages of seedlings at these stages (table 1) is an indication of the effects of ethephon on root production. It shows that the percentage of stage 6 seedlings (i.e., those with roots) is higher on 2.5 ppm than on any of the other ethephon-containing media. This is in line with reports that Ethrel can enhance rooting in apples, blueberries, mung beans, and tomatoes (ABELES 1973). However, in orchids it appears that concentrations above 2.5 ppm are supraoptimal for root formation. The stimulation of root hair formation by 5, 10, and 20 ppm ethephon is similar to the

effects of low ethylene concentrations (ca. 20 ppm) on pea seedlings (SMITH and RUSSELL 1969).

Increases in chlorophyll content (fig. 4) are not an expected response to ethylene. In fact, this hormone induces senescence and, therefore, often brings about reductions in chlorophyll content (ABELES 1973). One possible explanation of our findings is based on the observation that the ethephon concentrations which increase chlorophyll levels (fig. 4) reduce leaf length. It is conceivable, therefore, that these concentrations may reduce the size of cells but not the amount of chlorophyll per cell and, consequently, per leaf and/or seedling. Hence, unchanged chlorophyll content in smaller leaves results in higher pigment levels per unit of weight (fig. 4).

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