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Influence of Thidiazuron on Propagule Formation in *Hydrilla verticillata*

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ABSTRACT

The plant growth regulator, thidiazuron, at concentrations of 10^{-8}, 10^{-6}, and 10^{-4}M was evaluated for its effects on growth and propagule formation of dioecious *Hydrilla verticillata* Royle. Greenhouse cultures of hydrilla growing either in pyrex storage jars (algal-free) or in glass aquaria were monitored for tuber and turion production from 1 September 1984 to 15 April 1985. A single treatment of 10^{-6} and 10^{-4}M completely inhibited both tuber and turion production throughout the 227 d test period. Ethylene evolution from treated plants was proportional to thidiazuron concentration. Visual observations of the growth of the treated plants indicate a cytokinin-like effect on apical dominance resulting in extensive branching.

Key words: growth regulators, ethylene, ABA, growth, tubers, turions, reproduction, retardation.

INTRODUCTION

Hydrilla control is usually expensive and short term via usual control methods. Regrowth of hydrilla often occurs from the vegetative propagules (turions or tubers) produced by this plant. If tuber and turion production could be eliminated or significantly reduced, treatment with currently available herbicides may provide more cost effective, longer term control of hydrilla. Exogenous ethylene, applied as ethephon, reduced propagule production in greenhouse cultures by 80% when applied at a level of 0.1 mgL^{-1} ethylene at 2 d intervals (Klaine and Ward, 1984).

Recent work by a number of investigators indicates that many agricultural chemicals stimulate endogenous ethylene formation. Treatment of etiolated mung bean hypocotyl segments with 30 nanomolar thidiazuron (N-phenyl-N'1, 2, 3-thidiazol-5-ylurea) stimulated an increase in ethylene evolution over controls (Suttle, 1984). Treatment of sunflower seedlings with 10 μg chlorsulfuron (2-chloro-N-[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) aminocarbonly] benzenesulfonamide) stimulated ethylene evolution over control levels 1 d after herbicide application and reached a maximum 2 to 3 d after treatment (Suttle, 1983). Applications of cycloheximide induced ethylene production in intact citrus (*Citrus sinensis*) fruits (Riov and Yang, 1982).

Thidiazuron (N-phenyl-N'1, 2, 3-thiadizol-5-ylurea) is currently registered for use as a cotton defoliant. Arndt *et al.*, (1976), first reported the defoliating activity of thidiazuron and the physiological basis of this activity was hypothesized to be the ability of thidiazuron to stimulate endogenous ethylene production (Suttle, 1984). This paper describes the effects of thidiazuron on growth, ethylene formation, and propagule formation by hydrilla.

MATERIALS AND METHODS

Dioecious female hydrilla plants for this study came from stock cultures maintained in the laboratory. These algal-free cultures were originally grown from tubers collected from Lake Conroe, a freshwater impoundment approximately 80 km north of Houston, Texas. Algal-free cultures were originally obtained by treating tubers with 3% NaOCl for 10 min and sprouting them in the dark (Klaine and Ward, 1981). Hoagland's medium (10%) was used throughout the study (Hoagland and Arnon, 1950). The culture medium was made from reagent grade chemicals, deionized water, and supplemented with 200 mgL^{-1} $NaHCO_3$. Semi-solid medium for rooting and tuber development was made with 10% Hoagland's medium and 1.5% Bacto-Agar (Difco Laboratories). Stock cultures of hydrilla were held at 25°C under continuous light (80 μE/m^2/sec; cool white fluorescent tubes). All transfers and treatments were performed aseptically in an ultraviolet light box (Sylvania-Germicidal G30+8). Glassware was washed in laboratory detergent (MCB reagents Extran 300), rinsed with 10% HCl followed by deionized water, and autoclaved for 15 min at 121°C and 20 psi.

Initial experiments were conducted in Pyrex storage jars (10 cm in diameter by 8 cm in height) with Petri dish-like lids. Each jar contained 100 ml semi-solid medium, 150 ml liquid medium, and three apical fragments (7 cm) of hydrilla. Plants were allowed to grow under long photoperiods for 2 wk before treatment with thidiazuron. The liquid medium in each jar was then supplemented with 0, 10^{-8}, 10^{-6}, or 10^{-4}M thidiazuron. Each treatment contained three replicates. The storage jars were kept in the greenhouse for the duration of the experiment. Natural photoperiod was less than 12 h. Hoagland's medium was added to each jar biweekly to maintain nutrient and liquid levels. Numbers of both tubers and turions were monitored from 1 September 1984 to 15 April 1985 (227 d). At the end of the period, plant dry weight, total length, number of branches, and number of nodes were determined.

Aquaria (76 L) holding 7.5 cm waterlogged commercial potting soil, 50 L well water, and 12 apical hydrilla fragments were also placed in the greenhouse from 1 September 1984 to 15 April 1985. On 15 September 1984, each aquarium was treated with thidiazuron at 0, 10^{-8}, 10^{-6}, or 10^{-4}M. Total numbers of tubers and turions were counted on April 14, 1985 (227 d). Tests in aquaria were not replicated.

Ethylene evolution from treated hydrilla was followed in 125 ml glass containers that contained 50 ml of 10% Hoagland's medium and four apical fragments 5 cm in

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length. Care was taken to select uniform fragments of about the same fresh weight (approximately 4 g/flask). Flasks contained the same concentrations of thidiazuron used in the greenhouse studies and each treatment was performed in triplicate. Aminoethoxyvinylglycine (100 μM) was used to inhibit ethylene formation as a negative control. All flasks were sealed with septum stoppers and incubated for 96 h in the dark. At that time the headspace was sampled and the ethylene content determined by gas chromatography using a 60/80 Carbosieve G, 150 cm × 0.31 cm stainless steel column at 70 C and a flame ionization detector.

RESULTS AND DISCUSSION

Production of hydrilla propagules in the storage jars was inversely proportional to thidiazuron concentration (Table 1). No tubers or turions were produced in treatments of 10^{-6} M thidiazuron or higher concentrations. Similar results were obtained in the aquaria (Figure 1). Propagule production in the control aquarium was approximately 1×10^6 buds/ha, similar to other reported densities. A single treatment of 10^{-6} M thidiazuron on 15 September 1984, completely inhibited both tuber and turion production during the entire period through 15 April 1985. Storage jar systems produced predominantly more turions while aquarium systems produced predominantly more tubers (Figure 1). The overall effect on propagule formation, however, was similar in both systems.

Ethylene production was proportional to thidiazuron concentration (Table 1). Aminoethoxyvinylglycine (AVG) is an inhibitor of 1-aminocyclopropane-1-carboxylic acid synthase. The ability of AVG to inhibit thidiazuron-stimulated ethylene production suggests that de novo synthesis of 1-aminocyclopropane-1-carboxylic acid, the immediate precursor to ethylene, is required in hydrilla. These results support earlier work that indicates ethylene can inhibit tuber and turion formation (Klaine and Ward, 1984).

Previous research has indicated that exogenously applied abscisic acid can induce tuber and turion formation (Van *et al.*, 1978; Klaine and Ward, 1984). Endogenous concentrations of abscisic acid in hydrilla were measured using methods described by Klaine and Ward, 1984. Data not reported in that paper indicate that the endogenous level of abscisic acid in plants harvested in August (long photoperiods) was 0.1 ± 0.02 μg/400g fresh weight as compared with 8.0 ± 0.11 μg/400g (mean of 3 replicates ± 95% C.I.) fresh weight for plants harvested in October

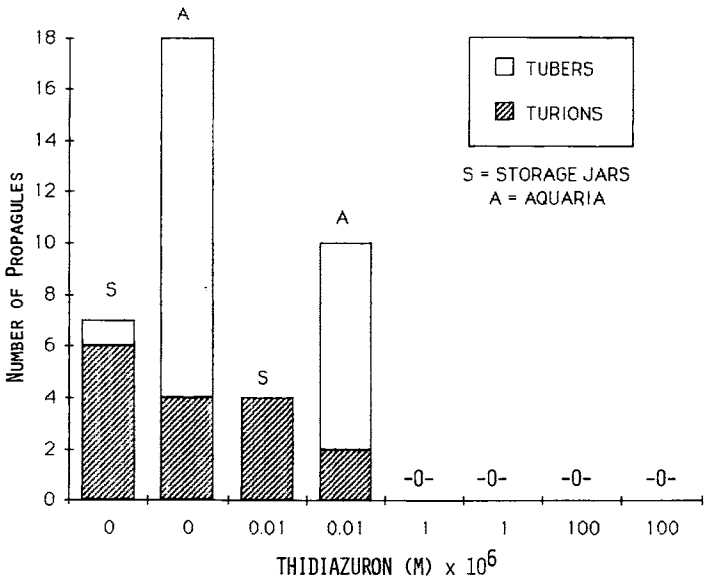

Figure 1. Tuber and turion production in storage jars and aquaria after treatment with thidiazuron. In both systems, the values for the two highest concentrations are zero.

(short days). These results suggest that abscisic acid is the tuber inducing plant hormone, and that ethylene may be antagonistic to abscisic acid by preventing tuber formation.

Other investigators have reported the antagonism between ethylene and abscisic acid. Ethylene, both alone and in combination with gibberellic acid and kinetin, was shown to be antagonistic to abscisic acid (Hallion, 1976; Dunlap and Morgan, 1977). Hallion (1976) showed the antagonism between ethylene and abscisic acid in breaking dormancy of cottonseed. Thidiazuron may also be useful in the breaking of dormancy of the tubers and turions already present in an infested area. Additional data are needed to confirm this hypothesis.

Thidiazuron has been shown to have cytokinin-like activity in a number of bioassay systems (Baskakov *et al.*, 1981; Kulaeva *et al.*, 1982; Thomas and Katterman, 1983). Hydrilla growth in both the 10^{-6} M and 10^{-4} M thidiazuron treatments showed obvious cytokinin activity. This was quantitatively assessed by counting the number of branches present in the storage jar systems (Table 1). The typical loss of apical dominance was readily apparent from the development of multiple shoots ("witches-broom") from a single shoot; and this was present in both the storage jar cultures and the aquaria. Cytokinin-like com-

TABLE 1. EFFECTS OF THIDIAZURON ON GROWTH AND PROPAGULE PRODUCTION OF *HYDRILLA VERTICILLATA* GROWN IN THE STORAGE JAR SYSTEM.

TDZ conc.	Nanoliters ethylene[2]	Number of propagules[3]	Final plant dry weight[3] (g)	Number of branches[3]	Total length[4]	Number of Nodes[3]
0	5.4 ± 0.5	7 ± 3.0	0.21 ± 0.04	9.0 ± 2.0	111.7 ± 22	285 ± 69
10^{-8} M	6.6 ± 0.3	4 ± 1.3	0.20 ± 0.05	12.5 ± 6.5	86.2 ± 36	237 ± 95
10^{-6} M	20.3 ± 0.8*	0*	0.17 ± 0.06	70.0 ± 53.1*	220.8 ± 193	256 ± 110
10^{-4} M	35.4 ± 1.3*	0*	0.18 ± 0.04	31.1 ± 19.1*	95.3 ± 71	312 ± 189
10^{-6} + 100 μM AVG[1]	6.1 ± 0.4					

[1]aminoethoxyvinylglycine.
[2]Nanoliters/4 fragments/96h, mean ± 95% C.I., n= 3.
[3]Mean ± 95% C.I., n = 3.
[4]Length in cm, mean ± 95% C.I.
*Indicates treatment results significantly different from the control, $p \leq 0.05$ (Snedecor and Cochran, 1976).

pounds have been shown to stimulate ethylene production (Lau and Yang, 1974; Yu *et al.*, 1981).

These results indicate that tuber and turion formation can be inhibited by the plant growth regulator, thidiazuron. Furthermore, one treatment of 10^{-6} M was effective for the entire tuber producing season.

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