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HYDRILLA VERTICILLATA ROYLE: I. CONTROL THROUGH LIFE CYCLE INTERRUPTION. II. USE IN AQUATIC PHYTOTOXICITY BIOASSAYS

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HYDRILLA VERTICILLATA ROYLE

I. CONTROL THROUGH LIFE CYCLE INTERRUPTION

II. U.E IN AQUATIC PHYTOTOXICITY BIOASSAYS

By

STEPHEN JAMES KLAINE

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
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DOCTOR OF PHILOSOPHY

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ABSTRACT

HYDRILLA VERTICILLATA ROYLE

I. CONTROL THROUGH LIFE CYCLE INTERRUPTION

II. USE IN AQUATIC PHYTOTOXICITY BIOASSAYS

Hydrilla (Hydrilla verticillata Royle) is an exotic aquatic angiosperm which has become a problematic weed throughout the southern United States. Infestation by aquatic weeds like hydrilla increase loss of valuable water through evapotranspiration, choke waterways used for transportation, depress real estate values, and may present health hazards. Control strategies including herbicide treatment and mechanical harvesting have proven costly and of short term benefit. One reason for this is the vegetative dormant buds which allow the plant to overwinter and reestablish rapidly after attempts at control. This research focuses on elucidation of the bud formation process in hydrilla to provide the basis for development of a control strategy which combines use of herbicides with disruption of bud formation and hence the capacity for regrowth.

The environmental and hormonal control of vegetative dormant bud formation was studied in the laboratory using algal-free cultures of hydrilla. Bud formation is stimulated by a photoperiod of less than 12 hours. This environmental stimulus appears to be sensed by the phytochrome system and hormonally induced by abscisic acid. Field sampling at Lake
Conroe, Texas, confirmed, where possible, laboratory results and indicated that artificially prolonged photoperiods prevent bud formation.

Ethylene, both in laboratory and greenhouse cultures, significantly reduced bud formation at a dose rate of 0.1 mg/l every two days. Coumarin significantly reduced bud formation when applied at $10^{-6}$ M but information in the literature indicates that the mode of action of coumarin may be induced ethylene formation in the plant (Morgan and Cowell, 1970).

Growth characteristics of hydridilla make it suitable for use in a bioassay for the assessment of the relative phytotoxicity of aquatic pollutants. Algae-free cultures of hydrilla were grown in the laboratory in 10% Hoagland's medium. Evaluation of chemical toxicity was obtained starting from two different life stages of the plant: dormant buds and apical fragments. Dormant bud production was also monitored in order to evaluate the potential for life cycle and chronic studies.

Hydrilla appeared more sensitive to ammonium carbamate than were duckweeds and more sensitive to phenol than was a unicellular green alga. Growth rate was not quantitatively related to toxicant concentration, but yield, dormant bud sprouting, and dormant bud production were related. Dormant bud production and sprouting tests indicate that life cycle, or partial life cycle, studies with hydrilla may be both feasible and more sensitive than the acute tests.
ACKNOWLEDGMENTS

As this dissertation represents the culmination of my five plus years at Rice, I find it difficult to summarize my relationships with all the people who allowed me to share this experience with them. My memories include biochemistry, algae and plants that wouldn't grow, the anxiety of preliminary and qualifying examinations, and the exhilaration of finishing my defense, but these memories of people are those I shall cherish:

Ballgames with Joe, backgammon and beer with Hutch, botany at 60 M.P.H. with Shelly, tennis and breakfast with Claire, scrabble and beer with Dick, examining mud at Lake Conroe with Mary and Mike, chess and Mexican food with Carlos, boggle with Nina, arguing with Bill, racquetball and eating with Anita, the pH electrode at 2:00 a.m. with Mason, partying with Phil, last minute papers with Maurine, tennis with Mike, aquatic botany with Virginia and Rebecca, golf with Tracy, Allen, and Tom, purchase orders from Shirley, barbecuing a pig with Herb, and lunch and bartending with Chris.

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I dedicate this work and my life to my love Christine for her unselfishness, patience, and endless love. She is my friend and love and without whom this research would be less meaningful.

Steve
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I. CONTROL OF HYDRILLA THROUGH LIFE-CYCLE INTERRUPTION

1.0 Introduction: Control of *Hydrilla Verticillata*

*Hydrilla (Hydrilla verticillata Royle)* is a rooted aquatic vascular plant, apparently indigenous to Central Africa, which causes serious problems throughout the tropical regions of the world. This plant obstructs water flow, increases evaporation, causes large losses of water through transpiration, and prevents proper drainage of land (Holm et al. 1969).

Attempts at control of this plant, including herbicide treatment and mechanical removal, have been only marginally successful primarily due to rapid reinfestation of the cleared area. Regrowth of hydrilla is accomplished primarily through sprouting of vegetative dormant buds that are not affected by the control methodology. Retreatment with a herbicide is necessary after only six to eight weeks which makes this method of control extremely expensive. If vegetative dormant bud formation could be eliminated or significantly reduced, current herbicide technology would be satisfactory for controlling hydrilla.

The objective of this research is elucidation of the bud formation process in hydrilla to provide the basis for development of a control strategy which combines use of
herbicides with disruption of bud formation and hence the capacity for regrowth.

1.1 Background and Problem

There are four kinds of aquatic plants: algae, emergent and submersed plants, and floating plants. The algae include both planktonic and attached species. Emergent plants are those which, although rooted in the hydrosol, project the majority of their vegetative material above the water surface. Submersed plants may or may not be rooted but all of their vegetative material lies at or beneath the water surface. Floating plants are not rooted in the hydrosol but have roots in the water column through which nutrient uptake occurs. Nutrient uptake can also occur in the submersed vegetative portions of many aquatic plants. Aquatic plants are necessary in waterways to prevent excessive erosion and turbidity and to maintain the delicate nutrient balance between the water, hydrosol, and plants. Shoreline vegetation provides habitat for water fowl, cover for fish, and a significant part of the primary productivity of many ecosystems (Haller, 1977).

Aquatic plants are considered weeds when their growth becomes excessive, and when some type of control or management becomes necessary to insure continued use of a water
body. Rapid colonization of the rivers and lakes of the warm regions of the world by aquatic weeds have forced us to recognize the power of such infestations. Weeds may interfere with navigation, prevent fishing and recreation, depress real estate values, and present health hazards.

Timmons (1960) reported that 17 western states lost 2.42 x 10^9 m^3 of irrigation water annually due to aquatic weeds. This amount is enough water to irrigate 132,000 to 315,000 hectares of cropland. Twenty years ago this water was conservatively valued at $20.00 per 1200 m^3, or $39,230,000 (Holm et al. 1969). Hudson (1973) estimated that more than 10% of the one million surface hectares of inland water in Florida were rendered useless due to infestations of noxious aquatic plants. Species of aquatic weeds that are particularly troublesome include the water hyacinth (Eichhornia crassipes), with its ability to increase the loss of water from a reservoir through evapotranspiration (Holm, 1969). This increase may be as large as 3 or 4 times the normal water loss through evaporation. Water lettuce (Pistia stratiotes) is a preferred host for larvae of several species of mosquitoes. One or more of these species of mosquitoes serve as principal vectors of each of several forms of encephalomyelitis rural filariasis. Both water hyacinth and water lettuce are floating plants. Some of the more troublesome genera of submersed plants,
imported with dire consequences, include *Potamogeton*, *Elodea*, *Myriophyllum*, and *Hydrilla* (Holm, 1969).

The aquarium industry was the apparent vehicle of entry of hydilla into the United States, and this plant was noticed growing wild in a Miami canal and in the Crystal River on Florida's Gulf Coast in 1960. Current distribution of the plant in the U.S. ranges from North Carolina and Florida westward through the southern states into southern California. Migration is accomplished via boats, motors, trainers, bait pails, and waterfowl. Hydilla was first reported in Texas in 1974 (Guerra, 1976). Initially hydilla was found in Lake Livingston, an impoundment located 9.7 km northwest of Livingston, but has spread to Lake Conroe, another impoundment, about 80 km north of Houston, Texas. Infestation jumped from 486 ha in 1976 to nearly 3644 ha in 1978 (Norton, 1979).

1.2 Growth and Physiology of *Hydrilla Verticillata*

Information on the growth and physiology of aquatic vascular plants is sparse. Interest in this area of research is due primarily to the need for control of noxious aquatic weeds. It is important to review the growth and physiology information available for hydilla in order to have sufficient background to examine a particular physiological process such as bud formation.
The nutritional requirements of hydrilla have been investigated during the last decade utilizing algal-free cultures. The majority of the research has used Hoagland's medium (Hoagland & Arnon 1950) or a modification of it (Table 2.1). Steward & Elliston (1973) determined that 10% Hoagland's medium supplemented with NaHCO₃ was optimal for hydrilla growth. At 100% and 50% Hoagland's medium one or more of the microelements appeared to inhibit growth.

Throughout the last decade Martin and his research group at the University of South Florida have looked at selective nutrient removal as a possible means of aquatic plant control (Martin et al. 1970). The presence of a cationic-exchange resin (Amberlite IR-120 HG) in the medium inhibited hydrilla growth while other exchange resins had no effect. They also noted that the addition of sawdust to the medium (particularly cypress) led to plant senescence (Martin et al. 1971).

Iron chelate, Fe(EDTA), was found to stimulate the growth of hydrilla at a concentration of 0.025 to 0.15 ppm. This research was done in well water, however, which may have been deficient in other nutrients interacting with iron utilization, particularly manganese. Basiouny et al. (1977) grew hydrilla in 5% Hoagland's medium with varying concentrations of iron. Growth was proportional to iron
concentration between 0 and 8 ppm. They also varied manganese concentration and found that optimum growth and dry weight of hydrilla was obtained at a Fe/Mn ration of 85. This ration is higher than that reported for terrestrial plants (Fe/Mn=2) (Somers & Shive, 1942; Vlamis and Williams, 1964; Gilfillan and Jones, 1968).

Light requirements and inorganic carbon utilization have been studied to determine hydrilla's apparent competitive edge over other aquatic plants. Steemann-Nielsen (1944) showed through experiments with Elodea canadensis and Ceratophyllum demersum that submersed aquatics could utilize the bicarbonate ion. C. demersum along with Myriophyllum spicatum and H. verticillata showed increased utilization of the bicarbonate ion as pH increased (Van et al. 1976). As pH increases, aqueous CO$_2$ concentration does not change. However, bicarbonate and carbonate concentrations increase. The apparent Km(CO$_2$) value for photosynthesis by hydrilla is 170 µM CO$_2$ at pH 4 while at pH 8, the Km(CO$_2$) was reduced to 90 µM (Van et al. 1978a). The decreased Km (CO$_2$) at high pH may reflect increased HCO$_3^-$ use by hydrilla.

CO$_2$ compensation values have been widely used to differentiate between C$_3$ and C$_4$ plants. Black (1973) reported that the CO$_2$ compensation point, the CO$_2$ concentration necessary to keep net CO$_2$ fixation at zero, was
30 to 70 µl/l for C₃ (Calvin cycle) plants and 1.0 to 10 µl/l for C₄ (phosphoenolpyruvate carboxylase/C₄ dicarboxylic acid cycle) plants. For hydrilla, the CO₂ compensation point appeared to vary both seasonally and with the location from which the plants were collected (Bowes et al., 1977). Values range from 10 to 30 µl CO₂/l in the summer to 70 to 80 µl/l in the winter. Recent results indicate that hydrilla may also be able to carry out substantial dark CO₂ fixation at night (Cam) when free CO₂ is high (Bowes & Bleckenstaff, 1980; Holaday & Bowes, 1980). These results indicate either the existence of a hybrid C₃-Cam pathway or the ability to adapt and develop different pathways under different conditions.

A factor that may influence the distribution and competitive success of aquatic plants is response to light (Brown et al., 1974). Haller and Sutton (1975) indicated that the growth habit of hydrilla in forming a canopy near the water surface reduces light penetration and enables it to outcompete certain native aquatics such as Vallisneria sp. Hydrilla also have a lower chlorophyll A:B ratio than that found in terrestrial plants (Haller, 1974). This low ratio may be an important factor for productivity, enabling the plant to use the shorter wavelengths of light more effectively for photosynthesis (Van et al., 1978). Hydrilla has a lower light compensation point (10 to 12 µ
einstein m\(^{-2}\) sec\(^{-1}\) and a lower Km (light) for net photosynthesis than other submersed freshwater angiosperms (Van et al. 1976). Light quality also affects hydrilla growth. Green light increases stem elongation and decreases branching while red light has the opposite effect (Van et al. 1977). These results correlate well with light penetration in water and the observed pattern of hydrilla growth characteristics. Low light intensity and a predominance of green wavelengths in deeper waters promote internode elongation and a limited amount of branching. This growth pattern causes hydrilla to grow more rapidly toward the surface where more light is available. Red wavelength light is present near the surface and stem elongation decreases while side branching increases. This pattern produces the mat characteristic of hydrilla infestations.

These photosynthetic characteristics may enable hydrilla to outcompete other species under conditions of low irradiance, such as regrowth from the hydrosol or dense vegetation. Most of the photosynthetic activity in a hydrilla canopy occurs during the early morning hours when light is limiting but CO\(_2\) is relatively high (Van et al. 1976). Hydrilla is also capable of adapting its photosynthetic response to changes in irradiance. Low irradiance results in both low light compensation and saturation points enabling the plant to make maximum use
of light received during growth (Bowes et al. 1977). Both nutrient and light utilization by hydrilla allow it to out-compete native species. These growth characteristics are necessary information for hydrilla culturing but do not readily suggest a control strategy.

1.3 Control Techniques

Methods of control differ depending on the type of aquatic vegetation. Chemical and biological control methods are used for all four types of aquatics (algae, emergents, submersed, and floating) while nutrient enrichment (see later in this section) is usually used to control only submersed plants. Mechanical harvesting is used on all vascular plants, but is more efficient for controlling floating plants. Chemical control consists of applying herbicides to an infested water system. Copper sulfate has been used effectively against algae but is not effective against aquatic vascular plants in concentrations safe for the rest of the ecosystem (Klingman & Ashton, 1975). Hormone analog weed killers like 2,4-D (2,4-Dichloro-phenoxyacetic acid) are particularly effective against floating plants (hyacinths) and emergents (alligator weeds). Application of these chemicals to kill submerged vegetation is too costly due to the effects of dilution (Chokder 1965). Submersed plant control, particularly
hydrilla, has been undertaken with a variety of chemicals. Originally, sulfuric acid and nitric acid were used (Phillippy 1967; Zutshi 1964). A number of chemical herbicides have been tested for their effectiveness in controlling hydrilla (Blackburn and Weldon 1969a; Blackburn and Weldon 1969b; Pahuja et al. 1973). Diquat (6,7 dihydrodipyrido-(1,2-a:2', 1-c) pyrazinediiumion) in combination with copper sulfate worked well as did Endothal (7-Oxabicyclo 2.2.1 heptane-2,3-dicarboxylic acid). The uptake of diquat by hydrilla is greatly enhanced by the presence of copper sulfate (Sutton et al. 1970: 1972). Copper also enhances the action of endothal (Sutton et al. 1971b).

Emulsifying techniques, wetting agents, and bottom placement techniques all enhance herbicide effectiveness. Emulsifying the herbicide and adding wetting agents allows for longer contact time (Gates 1972). This increased contact may, however, also increase the toxicity of the herbicide to fish and invertebrates. The bottom placement techniques applies the herbicide via a long hose approximately one foot above the hydrosoil (McClintock et al. 1974). This technique reduces the effect of water dilution by applying the herbicide in that portion of the water column where it is required.

Biological control involves use of any plant-feeding organism or parasite, providing it does not harm plants of
value or create undesirable imbalances in the biotic community. Since hydrilla is an exotic plant, it has no known natural predators in the United States.

The white amur, *Chenopharyngodon idella*, a fish native to the Amur River areas of China and the USSR, has been cultured successfully on aquatic plants. This fish, also known as the grass carp, has been employed for weed control in China, Hungary, and Japan (Andres & Bennett, 1975). Presently, this fish is used for weed control on a limited basis in a number of states including Florida, Arkansas, Louisiana, and is soon to be introduced into Texas (Guerra, 1980). Originally, it was thought that the grass carp spawned only in native waters (Blackburn et al., 1971b). However, young grass carp which escaped from ponds during the flood season of spring 1979 were found recently in the Atchafalaya basin in Louisiana (Donald V. Lee, Louisiana Department of Wildlife & Fisheries, personal communication, 1980). Some natural reproduction may have occurred.

A snail, *Marisa cornuarinetis*, feeds on a number of aquatic plants including *Ceratophyllum, Najas, Potamageton*, and *Elodea*. It also feeds, however, on young paddy seedlings, watercress, and water chestnuts (Gupta, 1973).

A number of pathogens have been identified for possible weed control but none are currently used.
Rhizoctonia solani isolated from a diseased anchovy hyacinth (Eichhornia azurea) in Panama has shown pathogenicity to a number of aquatics (Joyner & Freeman, 1973). Larvae of the insect Parapoyx rugosalis, in preliminary experiments, showed good promise in controlling hydilla (Balcanuas & Center, 1980).

Alligatorweed (Alternanthera phylloxeroides) one of the most problematic aquatic weeds in the United States fifteen years ago, is no longer troublesome due solely to biological control by the flea beetle (Agasicles sp.) (Maddox et al., 1971). This insect feeds solely on alligatorweed and, thus, provides very successful control (Maddox and Resnik, 1969).

Nutrient enrichment results in shading of the submersed plants by encouraging the growth of microscopic unicellular algae with fertilizer. The fertilizer produces an algal bloom which restricts the penetration of sunlight in water and makes photosynthesis by submersed plants impossible.

Harvesting of the aquatic plant biomass has been utilized to free congested waterways. Drainage canals can be excavated by a crane and dredge from the banks. Mechanical harvesters are expensive and bulky and, besides the cost, there is the problem of disposal of the harvested biomass. Since many aquatic plants are greater than 90% water, the material does not have significant value.

1.4 Dormancy and Formation of Dormant Organs

1.4.1 Terrestrial Plants

The potato (Solanum tuberosum) has been studied for over half a century due to its importance as an agricultural crop. This plant, Solanum tuberosum, produces a true tuber; a swelling of a root. How the swelling organ develops is just now beginning to be understood. Peterson and Barker (1979) studied the process starting from axillary buds. After about 2 days in culture, the axes of these buds elongated considerably, and distinct internodes and scale leaves were evident. Radial expansion then took place several internodes basipetal to the stolen apex. Further radial expansion produced an obvious swelling which involved several internodes. The stolen apex was oriented in a vertical plane with respect to the stem axis after this early radial expansion. After approximately 10 days in culture, the tuber apex and lateral organs appeared to stop growing while radial expansion of the stem continued. This verbal explanation is well documented with photomicrographs in Peterson & Barber's paper.

The control process of tuberization involve both environmental and biochemical factors. Undoubtedly, these
are related. The initiation of the entire process is environmental. Temperature and photoperiod are the key parameters for triggering the plant biochemistry necessary for tuberization. Potatoes grown under laboratory conditions can be induced to produce tubers by lowering the temperature and shortening the photoperiod (Forsline & Langille, 1975).

These environmental conditions cause an increase in cytokinin activity (Langille & Forsline, 1974). The highest cytokinin activity was in above ground tissue after 4 days but moved below ground within 6 days after inducing conditions were started. Tuber initiation followed after 8 to 10 days. The cytokinin activity was traced to two compounds (Forsline & Langille, 1975). Utilizing high pressure liquid chromatography, the cucumber cotyledon bioassay, and mass spectrometry, one cytokinin was isolated and identified as cis-zeatin riboside (Mauk & Langille, 1978). Furthermore, addition of kinetin to non-induced plants, via the medium, caused tuberization to occur just as in induced plants (Forsline & Langille, 1976). Addition of gibberellic acid to induced plants prevents tuberization from occurring (Langille, A.R., University of Maine, personal communication, 1980). A continuous supply of nitrate or ammonium inhibited completely tuber initiation (Krauss & Marschner, 1976).
Cassava (*Manihot esculenta*), a tuberous South American shrub used for making tapioca, shows similar properties to the potato. For the tuberization process to occur, a short photoperiod accompanied by reduced temperatures is required (Lowe *et al.*, 1976).

1.4.2 Aquatic Plants besides Hydrilla

Aquatic plants do not form tubers. Some, however, form specialized dormant buds which often function as propagules as well as overwinterizing devices. These turions are released either through abscission or decay of the parent plant.

* Spirodela polyrrhiza*, a floating aquatic, can be induced to produce turions by short days. The critical day-length changes, however, with temperature. Addition of abscisic acid can induce turion development as well as inhibit growth in *S. polyrrhyza* (Perry, 1968), yet no turion formation was seen in two other members of the lemnaceae family, *Lemna minor* and *Wolfiella floridana*. In addition, kinetin has been shown to counteract turion induction by abscisic acid in *S. polyrrhyza* (Steward, 1969).

A rooted aquatic vascular plant, *Myriophyllum verticillatum*, also produces turions. Turion production is initiated by 8-12 hr photoperiods and temperatures of 15°C or lower. Again, abscisic acid enhances turion development.
during 12 hr days at 15°C. It cannot, however, stimulate turion production under long day conditions (Weber & Nooden, 1976). The cytokinin, benzyladenine, blocks turion formation.

During turion development, abscisic acid-like activity increases along with the activity of one or two unidentified inhibitors. At the start of turion formation, cytokinin activity decreases. During turion development, cytokinin activity increases while at abscission it decreases (Weber and Nooden, 1976).

1.4.3 Hydrilla

_Hydrilla verticillata_ produces two types of vegetative reproductive buds; subterranean buds commonly called tubers, and leaf axil buds called turions (Section 1.5). It has been estimated that 1-ha area of hydrilla may produce several million tubers (Haller and Sutton, 1975). Regrowth from turions is insignificant considering the large number of tubers that are formed (Van, unpublished data). Rapid reinfestation by tuber regrowth is the major reason that chemical treatments give only short-term control.

Dewatering or lake drawdown stimulates tuber germination in the exposed areas (Haller et al., 1976). Removal of the above-ground vegetation by contact herbicides or mechanical harvesting also stimulates hydrilla tuber germina-
tion (Haller et al., unpublished data).

In latitudes which experience seasonal change, hydrilla grows much like an annual plant. In fall and winter, tuberization occurs until freezing temperatures. Van et al. (1978) studied hydrilla tuberization under growth chamber conditions. They found tubers could be induced by short photoperiods of less than 13 hours at temperatures ranging from 14°C to 33°C. No tubers are formed after 5 weeks of growth at 9°C and 10-hr photoperiods.

Hormonal control of the tuberization process has been studied (Van et al., 1978). Addition of abscisic acid to non-induced plants caused tuber formation while addition of gibberellic acid to induced plants reduced tuber production by 50%. No endogenous levels of hormonal activity have been measured.

1.5 *Hydrilla verticillata* Royle

*Hydrilla verticillata* Royle is a member of the Hydrocharitaceae including the genera *Vallisneria*, *Egeria*, and *Elodea*. Backer (1968) describes *Hydrilla verticillata* as having leaves along a floating or submerged stem in distinct whorls. These leaves are 3 to 8 - nately whorled, oblong or linear, usually sharply serratedentate, and 0.75 to 4 cm long. Flowers may be white or reddish with sepals
2 to 3 mm long and petals slightly longer and much narrower. The flowers are unisexual. Lakshman (1951) describe the plant as rooted to the bottom of the pools with a linear branch which trails inside the muddy substratum. Branches arise in an axillary position from a condensed shoot which itself is borne in the axil of a leaf of the main trailing axis. In the United States, only the pistillate or female plans has been introduced preventing hydrilla from reproducing sexually by seed production.

As winter approaches, the condensed shoots, which bear the normal vegetative branches, give rise, from the axil of the scale leaves, to positively geotropic shoots which penetrate into the muddy floor of the pool. The apices of these shoots then swell up to form vegetative dormant buds on turions. The turion is boat-shaped and sharply curved at its distal end and is white or whitish in colour (Lakshmann, 1951).

Turions are also formed on the erect stem far above the mud level. These turions can be either sessile or stalked and are invariably green colored. Both types of turions are filled with starchy reserve food material and contain one apical meristem.

The subterranean turion is the primary structure which enables hydrilla to reestablish after adverse conditions such as ice cover, drought, and chemical treatment. Under natural conditions, 10 to 20 times more subterranean turions
are produced per unit area than leaf axil turions (Haller 1977). It is common in the literature to call the subterranean bud a tuber and the leaf axil bud a turion. However, the subterranean bud is not a true tuber but rather a dormant vegetative bud. Both tubers and turions were considered vegetative dormant buds throughout the research.

An apical fragment consisting of a single node, or whorl of leaves, is capable of developing into a mature plant and infesting an entire area. Hydrilla roots and produces stolons above and rhizomes below the surface of the hydrosol. These stolons and rhizomes have nodes which form vegetative buds again capable of infestation. This research deals with the vegetative life cycle of hydrilla involving production of vegetative dormant buds in the fall and sprouting of these buds in the spring (Figure 1.1).

1.6 Research Objectives—Control of *Hydrilla verticillata*

While certain herbicides are effective in killing the hydrilla standing crop, rapid regrowth from dormant vegetative buds allows reinfection to occur within two months. The objective of this research is elucidation of the bud formation process in hydrilla to provide the basis for development of a control strategy which combines use of herbicides with disruption of bud formation and hence the capacity for regrowth.
FIGURE 1.1

VEGETATIVE LIFE CYCLE OF HYDRILLA
PHOTOPERIOD
\(< 12 \text{hr}\)

MATURE PLANT

SPROUTING BUDS

PLANT WITH
BUDS & FLOWERS

TEMPERATURE
\(\geq 15 \text{ C}\)

DORMANT PLANT WITH
MATURE DISCONNECTED BUDS

TEMPERATURE
\(\leq 8 \text{ C}\)
1.7. Research Plan

My first objective in this research was to investigate the environmental and hormonal controls of vegetative dormant bud formation in hydrilla. I proceeded to investigate interruption of this process to prevent bud formation.

In the laboratory, I investigated the influence of photoperiod, temperature, and light quality on bud formation. The role of phytochrome in this process was also examined. Outdoor experiments were conducted to confirm, where possible, the conclusions about the environmental control of vegetative dormant bud formation in hydrilla.

Effects of exogenously supplied plant hormones on bud formation were then investigated. Since abscisic acid was the only hormone to stimulate bud formation, I proceeded to measure the endogenous concentrations of this hormone.

Compounds were then selected on the basis of their reported antagonism to the action of abscisic acid for testing in the laboratory. Finally, the most promising compound, ethephon, was tested in the greenhouse for its effect on vegetative dormant bud formation in hydrilla.

2. Materials and Methods

2.1 Plant Collection

Hydrilla was collected from Lake Conroe, a freshwater impoundment approximately 80 km north of Houston, Texas,
U.S.A. Vegetative dormant buds were collected from the hydrosol by using a sampler constructed of 15.24 cm inner diameter polyvinylchloride (PVC) pipe 45.72 cm in length with a PVC cap on one end. Aluminum pipe, 2.54 cm diameter, was screwed into the PVC cap and an air-tight valve attached to the pipe. Another piece of pipe with a "T" joint formed the handle. The sampler was forced into the hydrosol with the valve open and then withdrawn after the valve was closed. This sampler gave good results in depths up to 3 m.

When a large quantity of vegetative dormant buds were required, they were obtained from Dr. D.L. Sutton, University of Florida, Fort Lauderdale.

2.2 Growth Medium

All plant material was rinsed thoroughly with deionized water and held in well water which had been passed through a carbon column to eliminate any residual chlorine. Growth medium used throughout the laboratory experiments was 10% Hoagland's medium made from reagent grade chemicals, deionized water and supplemented with 200 mg/l NaHCO₃ (Table 2.1). Outdoor experiments were performed using dechlorinated well water.
<table>
<thead>
<tr>
<th>SALT</th>
<th>g/l</th>
<th>ELEMENT</th>
<th>PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>1.1808</td>
<td>Ca</td>
<td>288.5</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>0.5055</td>
<td>N</td>
<td>271.4</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>0.1361</td>
<td>K</td>
<td>234.6</td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O</td>
<td>0.4930</td>
<td>Mg</td>
<td>48.7</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0585</td>
<td>S</td>
<td>67.3</td>
</tr>
<tr>
<td>Fe(EDTA)</td>
<td>0.0504</td>
<td>P</td>
<td>31.0</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>0.0285</td>
<td>B</td>
<td>5.0</td>
</tr>
<tr>
<td>MnSO$_4$·H$_2$O</td>
<td>0.0154</td>
<td>Mn</td>
<td>5.0</td>
</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O</td>
<td>0.0022</td>
<td>Cl</td>
<td>35.5</td>
</tr>
<tr>
<td>CuSO$_4$·5H$_2$O</td>
<td>0.0008</td>
<td>Cu</td>
<td>0.2</td>
</tr>
<tr>
<td>H$_2$MoO$_4$ (85%)</td>
<td>0.0002</td>
<td>Mo</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zn</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe</td>
<td>5.0</td>
</tr>
</tbody>
</table>
2.3 Culture Systems

Three different culture systems were used throughout the experimentation. A miniaturized system consisted of 22 x 175 mm Kimax test tubes suspended in a water bath with temperature controlled at 28 ± 0.5 C by a recirculating Haake thermostatically controlled water circulator. Each tube contained 10 ml agar, 15 ml liquid medium, and one 7 mm apical algal-free fragment of hydrilla. Continuous lighting, 80 μ einsteins m⁻² sec⁻¹ as measured by a Lambda LI 170 quantum radiometer photometer, was provided by four bilateral four-foot cool-white fluorescent tubes. The cultures were aerated with pipettes down the center of the cotton stoppered test tubes. This apparatus will be referred to as the bubble tube apparatus throughout this research.

Another laboratory set up used for experiments with hydrilla consisted of Pyrex storage jars (10 cm diameter x 8 cm high) with petri dish-like lids. Each jar contained 100 ml agar, 150 ml liquid medium, and three algal-free apical growing fragments of hydrilla. The jars were incubated in a Sherer controlled environment growth chamber (temperature, 28 C; light intensity, 80 μ einsteins m⁻² sec⁻¹).

An outdoor culture system was set up in a greenhouse as a final test for the most promising chemical that
interrupted vegetative dormant bud formation. Glass jars (15 l) holding three inches of waterlogged potting soil, ten liters of water, and 12 apical hydrilla fragments were placed in the greenhouse. Tests were conducted from September 15, 1981 to November 15, 1981 and from December 1, 1981 to February 2, 1982 using the natural short photoperiod to induce bud formation. Temperature was monitored but not controlled and ranged from 5 to 30°C.

2.4 Algal Free and Axenic Cultures of *Hydrilla verticillata*

Algal-free cultures worked well for all the laboratory experimentation and were used throughout the research. All transfers and treatments were performed in an ultraviolet light box (Sylvania-Germicidal G 30 + 8). Glassware was washed in laboratory detergent (MCB reagents Extran 300), rinsed with dilute HCL followed by deionized water, and autoclaved for 15 minutes at 15 psi and 121 C.

Algal-free cultures were obtained using vegetative buds treated with 1% NaOCl for 10 min and sprouted in sterile growth medium. This procedure gave algal-free cultures in 90% of the cases. These plants had a resident microbial population that did not present a problem unless a large quantity of organic substrate was introduced into the cultures.
An extended procedure was required to isolate contaminant-free or axenic cultures. The 1% NaOCl treatment was followed by sterile removal of the outer tissue layers of the bud. This bud was then sprouted in 10% Hoagland's medium augmented with 150 mg/l penicillin, 10 mg/l streptomycin, 25 mg/l gentamicin, and 25 mg/l mycostatin. This procedure was used successfully to obtain axenic cultures in 70% of the attempts (Klaine & Ward, 1981).

While algal-free cultures grew as well as cultures contaminated with algae, axenic cultures exhibited a nutrient deficiency after three weeks of growth. Growth was significantly lower in axenic cultures than in contaminated cultures and senescence occurred after four weeks. Since algal-free cultures were used throughout the experimentation, research with axenic cultures of hydrilla has proceeded slowly. Work is still in progress to determine the cause of the poor growth of axenic cultures.

2.5 Chemical Acquisition

The plant hormones, indole acetic acid (IAA), abscisic acid (ABA), and kinetin were purchased from Polysciences, Inc. Gibberellic acid was obtained from Abbott laboratories as Progibb Plus and ethephon (2-Chloroethyl phosphonic acid) was obtained from Union Carbide as Ethrel. The herbicide, EPTAM 7-E (S-ethyl dipropylthiocarbamate) was
obtained from Stauffer Chemical and the phenolic growth regulator, coumarin, was obtained from Monsanto. Other phenolics including trans-cinnamic acid, naringen, and naringenin were purchased from Sigma as was 3-amino 1,2,4-triazole. The ethylene precursors, L-aminocyclopropane-L-carboxylic acid (ACC), was also purchased from Sigma. The toxicants, phenol and ammonium carbamate, were purchased from Aldrich.

2.6 Analysis of Plant Growth

Total length, dry weight (bud, vegetative and root), and number of buds were measured at the conclusion of each experiment. Dry weight was measured after the plant had been in an oven at 80°C for 24 hours (APHA-AWWA-WPCF, 1975). No attempt was made to discern between mature and immature buds or between subterranean and leaf axil buds.

3. Experimental Procedures

3.1 Effects of Temperature and Photoperiod on Bud Formation

These experiments were performed in the storage jar system described in section 2.3.

Temperatures of 15, 25, and 30°C were tested with photoperiods of 8, 10, 12, 14, and 16 hours. A complete factorial experiment with four replicates of each treatment
was performed. Experiments lasted eight weeks with nutrient changes biweekly. Dry weight and the number of buds per container were determined and analysis of variance performed on the data to test for treatment differences.

3.2 Effects of Exogeneous Hormone Addition on Bud Formation

The bubble tube system described in section 2.3 was used in these experiments.

The plant hormones, IAA, ABA, GA and linetin, were added to sterile growth medium at $10^{-6}$M concentrations. Solutions were changed biweekly and the experiments terminated after eight weeks. Dry weight (root, vegetative, and bud) was determined and dormant buds counted. Four replicates of each treatment were tested using a ten hour photoperiod.

3.3 Measurement of Endogenous Abscisic Acid Levels

Freshly harvested plant material (400g) was rinsed well with deionized water and extracted according to the procedure of Sweetser and Vatvars (1976). Homogenized plant material was extracted with 80% methanol in a refrigerator (5°C) overnight. The extract was filtered and the methanol removed from the supernatant using a rotary evaporator. The supernatant was adjusted to pH 8.0 and extracted three
times with ether. The residual aqueous fraction was adjusted to pH 2.8 and again extracted three times with ether. The final three ether extracts were combined, concentrated, and passed through a Sephadex G25 column using 20% methanol (pH 3.0 with H₂SO₄) as the mobile phase. The 40-60 ml elution volume fraction was extracted with ether and the ether phase concentrated to dryness.

This extract was analyzed for ABA using reverse phase High Performance Liquid Chromatography (HPLC) equipped with a Lichrosorb C₁₈ column. A 50% methanol mobile phase adjusted to pH 3 with phosphoric acid was used to elute the ABA at a flow rate of 2 ml min⁻¹ at a temperature of 35 C. Due to the large quantity of plant material required (400g) this analysis was performed only twice (3 replicates each), once in August and once in October, 1981.

3.4 Effects of Light Quality on Bud Formation

Pyrex storage jars, described in section 2.3 contained 100 ml agar, 150 ml liquid medium, and three apical growing fragments of hydrilla. Two jars each were placed in shoe boxes fitted with red, blue, green, yellow, and clear acetate lids. Each filter color treatment was tested under 10 and 16 hr photoperiods at 25 C. Transmission spectra were obtained for all filters using a Beckman DB-G scanning spectrophotometer (figure 3.1). After eight weeks the numbers of buds, branches, flowers, dry weight and length
FIGURE 3.1

THE SPECTRAL TRANSMITTANCE OF LIGHT THROUGH RED, GREEN, YELLOW, AND BLUE ACETATE FILTERS
were determined.

3.5 Night Interruption Experiments

Pyrex storage jars, described in section 2.3 contained 100 ml agar, 150 ml liquid medium changed biweekly and three apical fragments of hydilla. Three different treatment regimes were used: 10 hour photoperiod followed by a 14 hour dark period; 10 hour photoperiod with the dark period interrupted by one hour of red light (λ = 650 nm); and 10 hour photoperiod with the dark period interrupted by one hour of red light immediately followed by one hour of far red light (λ = 750 nm). Each treatment had four replicates and bud production and dry weight were measured after eight weeks. The Newman-Keuls test was used to compare treatment means (Zar, 1974).

3.6 Bud Production in an Outdoor Pond

A metal trough, 3.7 m by 0.3 m by 0.3 m deep, was constructed and set into the ground with a slight slope to allow overflow to spill into a drain at one end. Aluminum pans (29.5 cm by 23.5 cm by 6.3 cm deep) filled with waterlogged potting soil, were placed in the pond. Each pan held 12 apical fragments of hydilla. Rice University chlorinated well water was passed through a
carbon column and into the pond at the elevated end. Residence time in the system was approximately 10 days. The pond was planted on October 15, 1980 and allowed to grow undisturbed until February 23, 1981 when all plants were harvested and bud production measured. The pond was replanted and allowed to grow through the summer of 1981. On August 15, 1981, one pan was harvested and checked for dormant buds. Bud production was followed over the next six months using one pan on the 15th and one pan on the 1st of each month.

3.7 Quantitative Bud Sampling in Lake Conroe

Bud sampling was performed on various portions of Lake Conroe including the marina at Walden, the golf course at Walden, and Fisherman's Reef Marina. The golf course served as a control area that was not treated with a herbicide while the marina at Walden was treated two or three times per summer with the herbicide Endothal (7-Oxabicyclo 2.2.1 heptane-2, 3-dicarboxylic acid). Fisherman's Reef Marina contained an untreated section that was illuminated 24 hours per day. Quantitative sampling was performed using the hydrosoil sampler, described in 3.2, along transects away from the lights at Fisherman's Reef Marina. Flower production was also noted. The golf course site was used as a control.
3.8 Chemical Screening Tests

Chemical compounds were tested for their effects on vegetative dormant bud formation using a three tiered screening system. The bubble tube system described in section 2.3 was used first to provide a large number of individual treatments in a small amount of space. Chemicals which had a marked effect on bud formation were then retested using the pyrex storage jar system described in section 2.3. The storage jar system was larger and allowed for more than one plant per treatment. The chemical showing the greatest promise, ethephon, was then tested in the greenhouse.

Tests in all three systems lasted eight weeks with final dry weight and bud production being measured. All solutions in the first two systems were changed biweekly, while the greenhouse jars were treated with ethephon, and water levels were adjusted on a timetable consistent with the experiment in progress. Plants were treated biweekly in the first experiment with 50, 100, and 250 mg/l ethephon. Plants were treated on a two day, six day, or a ten day schedule in the second experiment with concentrations of 0.1, 1.0, 10.0, and 50.0 mg/l ethephon.
3.9 Monitoring of Ethylene Evolution From Ethephon

Apical fragments of hydrilla were placed in 250 ml erlenmeyer flasks fitted with a ground glass stopper and a side arm covered by a septum. The flasks were filled with 200 ml of test solution, and stoppered immediately. Samples (250 μl) were taken of the head space through the septum using a gas tight syringe. These samples were injected into a tracor 560 Gas Chromatograph equipped with a Porapak R column (Supelco) and a flame ionization detector. Peaks were evaluated using a SP4100 computing integrator (Spectra-Physics).

4. Experimental Results and Discussion

4.1 Environmental Control of Bud Formation

Dormancy is an adaptation to the environmental conditions which prevail where the species originates. As with many plants, hydrilla uses this adaptation to survive the winter. Initiation of dormancy is a result of a non-lethal change in environmental conditions including available moisture, temperature, and photoperiod.

4.1.1 Temperature and Photoperiod

Temperature had no significant effect on bud formation (Table 4.1). A slight increase in bud production was seen
**TABLE 4.1**

EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON BUD FORMATION BY HYDRILLA

<table>
<thead>
<tr>
<th>TEMP. (°C)</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>TOTAL</th>
</tr>
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<tr>
<td>15</td>
<td>35</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>25</td>
<td>40</td>
<td>28</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>71</td>
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<tr>
<td>30</td>
<td>39</td>
<td>31</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>TOTAL</td>
<td>114</td>
<td>83</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

1Numbers represent total number of buds in four replicate flasks.
at higher temperatures, probably due to higher productivity. A photoperiod of less than 12 hr was required for bud formation.

Other aquatic vascular plants form vegetative dormant buds as a primary means of propagation. Spirodella polyrhiza, a floating aquatic, can be induced to produce vegetative dormant axillary buds under short photoperiods (Perry, 1968). The critical day-length changes, however, with temperature. Hydrilla showed no such temperature dependence.

Lack of temperature dependence of this process in hydrilla allows it to gain a competitive advantage by producing buds earlier than other aquatics and thereby increasing its potential population for the next spring.

4.1.2 Light Quality

The quality of light differs with depth in natural waters as blue light penetrates the least and red light penetrates the furthest.

Hydrilla plants grown under different qualities of light showed significant variation in bud formation (Table 4.2). The yellow filter, which transmitted light above 450 nm, did not significantly affect growth or bud formation as compared to the control. Both green and blue filters excluded wavelengths of light in the 600 to 700 nm range which contain photosynthetically active wavelengths
### TABLE 4.2
EFFECTS OF LIGHT QUALITY ON BUD PRODUCTION BY HYDRILLA

<table>
<thead>
<tr>
<th>FILTER COLOR</th>
<th>PHOTOPERIOD</th>
<th>DRY WT. (g)</th>
<th>NO. BUDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>10 hr</td>
<td>0.50</td>
<td>16</td>
</tr>
<tr>
<td>White</td>
<td>16 hr</td>
<td>0.53</td>
<td>0</td>
</tr>
<tr>
<td>Yellow</td>
<td>10 hr</td>
<td>0.46</td>
<td>14</td>
</tr>
<tr>
<td>Yellow</td>
<td>16 hr</td>
<td>0.55</td>
<td>0</td>
</tr>
<tr>
<td>Green</td>
<td>10 hr</td>
<td>0.12</td>
<td>3</td>
</tr>
<tr>
<td>Green</td>
<td>16 hr</td>
<td>0.12</td>
<td>2</td>
</tr>
<tr>
<td>Blue</td>
<td>10 hr</td>
<td>0.23</td>
<td>5</td>
</tr>
<tr>
<td>Blue</td>
<td>16 hr</td>
<td>0.22</td>
<td>2</td>
</tr>
<tr>
<td>Red</td>
<td>10 hr</td>
<td>0.32</td>
<td>2</td>
</tr>
<tr>
<td>Red</td>
<td>16 hr</td>
<td>0.32</td>
<td>0</td>
</tr>
</tbody>
</table>
and may account for the low dry weight of these plants. The red filter did not transmit light between 450 and 550 nm which contains more photosynthetically active wavelengths and again may account for low growth. Bud production occurred in all treatments under 10 hr photoperiods, but, more importantly, bud production also occurred under 16 hr photoperiods with the green and blue filters. This is the first time bud production has occurred under long photoperiods in hydrilla and strongly indicates a molecular mechanism sensitive to light quality. All plants that are photoperiod dependent for a particular physiological process have been shown to utilize the phytochrome system.

4.1.3 Role of Phytochrome

Phytochrome is a photoreversible pigment which can exist in two principal forms: a red absorbing (Pr), \( \lambda \) max 660 nm, and a far-red absorbing (Pfr), \( \lambda \) max 730 nm form (Figure 4.1). Red light causes Pr to be transformed to Pfr and far-red light reverses this process. In the dark Pfr is metabolically converted to Pr (Figure 4.2). The high red/far-red ratio in daylight causes a high Pfr/Pr ratio ringing the day. This ratio can be reversed given a long enough dark period (Salisbury and Ross, 1969). This system forms the basis of a biological clock controlled entirely through photoperiod. In fact, this system is
FIGURE 4.1

ABSORPTION SPECTRA FOR PHYTOCHROME RED AND FAR RED PIGMENTS (RAVEN ET AL. 1976)
FIGURE 4.2

THE PHOTOCHROME PIGMENT SYSTEM
actually controlled by only the red and far-red portion of the spectrum.

Both the blue and the green acetate filters excluded the red light in the 660 nm portion and therefore the ratio of red to far-red light was greatly reduced from the control clear filter. The Pr/Pfr ratio produced under these filters is less than that produced under the clear, yellow, and red filters which contained the red wavelengths. This reduced ratio would be similar to the ratio experienced by plants grown under shorter photoperiods in white light. Plants grown under blue and green filters with long photoperiods had Pr/Pfr ratios similar to those grown under short photoperiods and thus bud formation occurred. Results from these tests were only suggestive due primarily to ambiguity in the spectral extinction of the acetate filters. Light intensity was also not quantified for each filter.

To further test these conclusions, a night interruption experiment was performed. Control plants were grown under 10 hr photoperiods in white light. Another set of plants was grown under 10 hr photoperiods with the 14 hr night interrupted with one hour of red (650 nm) light. A third set of plants was grown under 10 hr photoperiods with the 14 hr night interrupted with one hour of red light followed by one hour of far-red (750 nm) light.

The controls produced buds while plants with the nights interrupted by red light did not produce buds. The red
light interruption presumably increased the Pr/Pfr ratio and the plant experienced a long day or short night effect. Following the red light interruption with an equal amount of far-red light neutralized the effect. Results indicate that phytochrome appears to play a role in bud formation in hydrialla (Table 4.3).

4.1.4 Field Studies

A quantitative sampling study was performed at Lake Conroe to verify photoperiod results obtained in the laboratory. A small outdoor pond was set up at Rice University to augment the field studies. Quantitative sampling was performed, using the hydrosol sampler described in section 2.2, at two different sites on Lake Conroe. The golf course site at Walden served as a control area and the Fishermans Reef Marina site, illuminated throughout the night, was used to test the effect of prolonged photoperiod on bud formation. Neither the portion of the marina site sampled nor the golf course site was treated with a herbicide. No buds were found at the base of the lamps (Figure 4.3), buds were found along transects approximately 30 meters from the light sources. Light intensity at 30 meters was less than 1% of the intensity at the base of the lamps. Bud density at 30 meters from the lights was similar to the golf course site ($4 \times 10^6$ buds/ha) and was
### TABLE 4.3

EFFECT OF LONG NIGHT INTERRUPTION WITH RED AND FAR RED LIGHT ON BUD PRODUCTION BY HYDRILLA

<table>
<thead>
<tr>
<th>Treatment</th>
<th># Buds</th>
<th>Dry WT. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 hr photoperiod</td>
<td>14</td>
<td>0.54</td>
</tr>
<tr>
<td>10 hr photoperiod with red light night interruption</td>
<td>0</td>
<td>0.49</td>
</tr>
<tr>
<td>10 hr photoperiod with red + far red light night interruption</td>
<td>18</td>
<td>0.63</td>
</tr>
</tbody>
</table>
FIGURE 4.3

BUD DISTRIBUTION AT FISHERMAN'S REEF MARINA,
LAKE CONROE, TEXAS. ERROR BARS REPRESENT
95% C.I. ABOUT THE MEAN, n = 3
FISHERMAN'S REEF MARINA

- sample station

* light source

NUMBER OF BUDS

DISTANCE FROM LIGHT SOURCE (meters)

* presented as 95% C.I. about the mean
also similar to that found by Haller and Sutton (1975) in Florida lakes ($3 \times 10^6$ buds/ha) indicating that the area is capable of supporting typical bud production by hydrilla. The prevention of bud formation by artificially prolonged photoperiods in the marina area supports the conclusion that this process is solely photoperiod dependent.

Bud production was measured in the experimental pond at Rice University after four months of growth ending Feb. 23, 1981 ($2.5 \times 10^6$ buds/ha). Results from this small pond are also comparable to those found at the golf course site on Lake Conroe. Bimonthly sampling of bud formation from mid-August to January indicated heavy bud production throughout the fall commencing when day length shortens to less than 12 hr (Figure 4.4).

The laboratory and field studies indicate that vegetative dormant bud formation in hydrilla is environmentally controlled by photoperiod alone. This photoperiod effect appears to be mediated through the phytochrome pigment system. Bud production might be inhibited by artificially prolonged day length as appears to be the case at the marina on Lake Conroe.

4.2 Hormonal Control of Bud Formation

Exogeneous hormone addition was used to examine hormonal control of bud formation (Table 4.4). Using a
FIGURE 4.4

BUD PRODUCTION IN THE EXPERIMENTAL POND AT
RICE UNIVERSITY FROM AUGUST THROUGH
DECEMBER, 1981
# TABLE 4.4

EFFECTS OF HORMONE ADDITION ON
BUD FORMATION BY HYDRILLA

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>BUDS</th>
<th>DRY WT. (g)</th>
<th>% ROOTS</th>
<th>% VEG.</th>
<th>% BUDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0.71</td>
<td>19.3</td>
<td>77.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Gibberellic Acid</td>
<td>3</td>
<td>0.59</td>
<td>15.6</td>
<td>83.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Abscisic Acid</td>
<td>33</td>
<td>0.56</td>
<td>4.3</td>
<td>21.4</td>
<td>76.9</td>
</tr>
<tr>
<td>Kinetin</td>
<td>7</td>
<td>0.69</td>
<td>17.8</td>
<td>80.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Indole Acetic Acid</td>
<td>9</td>
<td>0.77</td>
<td>16.2</td>
<td>79.7</td>
<td>4.1</td>
</tr>
</tbody>
</table>
10 hour photoperiod, gibberellic acid (GA), indole acetic acid (IAA), abscisic acid (ABA), and a cytokinin, kinetin, were added at $10^{-6}$ M to hydrilla cultures growing in bubble tubes. Kinetin and IAA had no significant effect on bud formation while ABA increased it ~400% and Ga decreased it ~50%. Plants treated with ABA had over 75% of their total dry weight in buds while in the other treatments buds were below 5% of total dry weight. The endogenous ABA level in plants harvested in August (long photoperiods) was 0.1 ug/400 g fresh weight as compared with 8.0 ug/400 g fresh weight for plants harvested in October (short days).

Both Spirodea polyrhiza (Perry, 1968) and Myriophyllum verticillatum (Weber and Nooden, 1976) were stimulated to produce vegetative dormant buds by the addition of ABA, but cytokinin reduces bud production in both plants. While ABA increased bud production in hydrilla, cytokinin had no effect on the process. Of the plant hormones tested, only gibberellic acid decreased bud formation, which is in agreement with other work done with hydrilla (Van et al. 1978a).

Wareing (1969) found that dormancy could be induced in birch (Betula pubescens) and sycamore maple (Acer pseudo-platanus) by short days and long nights, and these conditions resulted in increased levels of growth inhibitor in the leaves and buds. They could extract this inhibitor from
leaves of *B. pubescens*, apply it to seedlings of *A. pseudoplatanus*, and induce dormancy. This inhibitor was identified as ABA and the effects of ABA on these species could be reversed by application of GA.

Eagles and Wareing (1963) and El-Antably et al. (1967) investigated the GA/ABA interaction involving bud dormancy. Experiments in which ABA and GA₃ were applied together in various concentrations to tree buds indicated that the growth-inhibitory effects of ABA can largely be overcome by increasing concentrations of GA₃, although it was not possible to determine whether the two hormones were interacting competitively. ABA-induced dormant bud formation in *Lemna polyrhiza* can be reversed by kinetin but not by GA₃ (Perry and Bryne, 1969). Thus, experiments in the literature with externally applied ABA, GA₃, and kinetin give some support for the evidence that bud dormancy may be regulated by an interaction between endogenous hormones of this nature. However, this support is inconclusive and varies greatly between species.

Results of the experiments performed with hydrilla indicate that vegetative dormant bud formation is enhanced by ABA and reduced by GA. IAA and kinetin have no measureable effect on the process at the concentrations tested.
4.3 Discussion of Vegetative Dormant Bud Formation

The physiological effects of ABA on dormancy are not well understood. Aside from the generalities that dormancy can be correlated with high ABA levels in many plants and that exogenous ABA can induce dormancy in other plants, few particulars are known. Progress has been made on the effects of ABA on ion movement but whether or not these are related to vegetative dormant bud formation on dormancy in general is unknown (Walton, 1980).

The environmental stimulus of a short day/long night regime apparently is received by the phytochrome system most probably in the leaves. The connections between the phytochrome system and the increase in endogenous ABA levels are unclear. Tanada (1973) postulated that Pfr controls the endogenous levels of ABA directly, based on experiments performed using Phaseolus vulgaris (mung bean). Erez and Lavee (1969), using peach buds, postulated that naringenin 7-glucoside (Naringin) was synthesized in the leaves as a result of Pfr stimulation and that this highly soluble compound was translocated to the budsite. The glucoside was then enzymatically cleaved by a β-glucosidase to the sparingly soluble aglycone, naringenin which is a natural growth inhibitor and may stimulate ABA production. The results and tentative conclusions in this area are as varied as are the number of investigators, species,
and phytochrome mediated processes examined.

4.4 Interruption of Bud Formation

Information on the environmental and hormonal control of vegetative dormant bud formation suggests that this process consists of several steps. An environmental stimulus consisting of a shortened photoperiod initiates the process. This stimulus is sensed by the phytochrome system in the leaves and is transmitted to the appropriate site in the plant. Asscisic acid biosynthesis is stimulated and increased levels of this hormone result in induction of vegetative dormant bud formation in hydrilla.

Several of these steps seem particularly logical sites for interruption. The first is artificial regulation of photoperiod. Field studies at Fisherman's Reef Marina on Lake Conroe suggest that artificial illumination throughout the night prevents both bud formation and flowering (Figure 4.3). Since this process is phytochrome mediated, night interruption with either white or red light should also inhibit bud formation just as it did in the laboratory (Table 4.3). This approach, if successful, would represent a significant reduction in energy dollars compared to illuminating the area throughout the night.

Phytochrome inactivation might also interrupt dormant bud formation. Chemical interruption of this
process, while possible, requires the use of compounds which are not only phytotoxic but would not be feasible for use in an ecosystem (S. Roux, University of Texas, Personal Communication, 1981).

Interrupting ABA biosynthesis would prevent the increase in hormonal concentration characteristic of plants forming dormant buds. Attempting to interrupt the production of ABA requires that the biosynthetic pathway or at least certain controlling factors of the pathway be understood. Millborrow (1975a;b) has shown that three of the four methyl groups of ABA are derived from the 3' methyl groups of mevalonic acid (MVA) and that the pro-(S)- and the pro-(R)-6' methyl groups of ABA are derived from the 3-methyl and C-2 of MVA, respectively. The result of this work, as well as more recent work (Milborrow, 1978), indicates that the formation of ABA from MVA is consistent with either a direct pathway from farnesyl pyrophosphate or from a C_{40} carotenoid-type precursor. Unfortunately, this work has not progressed enough to distinguish between the two possibilities nor does it allow inferences to be drawn about intermediates in the pathway (Walton, 1980).

The final step for interruption is the action of ABA. There are many reports in the literature of compounds which affect plant processes controlled by ABA. Unfortunately, the results of these studies vary greatly depending upon
investigator, plant species, and physiological process examined. Information in the literature suggested that two classes of compounds, phenolics and hormones, were most appropriate to test for their effects on dormant bud formation in hydrilla.

4.4.1 Plant Phenolic Compounds

Phenolic compounds are a class of plant growth regulators known to inhibit growth and classed as natural growth inhibitors (Kefeli and Kadyrou, 1971). A phenolic compound is one of a large array of chemical compounds possessing an aromatic ring bearing one or more hydroxyl groups together with a number of other substituents. The common phenolic constituents of plants can be divided into two main groups: the first includes phenolic acids and coumarins; the second is the flavonoid compounds, including anthocyanidins (Walker, 1975).

Phenolic compounds and ABA have an apparent similarity in causing inhibition of growth and in antagonizing the action of growth-promoting hormones. Ray et al. (1980) reported just the opposite results when they demonstrated antagonism between ABA and several phenolic compounds. Growth of the hypocotyl in Amaranthus caudatus seedlings was significantly inhibited by ABA. Application of trans-cinnamic acid, gallic acid, ferulic acid, tannic acid,
coumarin, morin, naringenin, quercetin, rutin, and chalcone all reversed the inhibition of hypocotyl growth caused by ABA. Coumarin was most effective: it completely reversed the ABA effect. Because coumarin was such a strong ABA antagonist it was tested for its effects on bud formation.

Coumarin significantly inhibited vegetive dormant bud formation at $10^{-6}$ M when tested in the bubble tubes (Figure 4.5). No decrease in total dry weight yield of the plant was observed. This result indicates no whole-plant stress at concentrations less than $10^{-4}$ M. Coumarin was retested using the storage jars and again bud formation was significantly inhibited even at $10^{-8}$ M (Figure 4.6). Dry weight yield of all treated plants was also significantly reduced indicating stress in the plant at these concentrations apparently contradicting results obtained in the bubble tubes. The storage jar experiments were consistently more sensitive to test chemicals than were the bubble tubes possibly due to the aeration occurring in the latter system.

Naringin and naringenin (Figure 4.7), a glucoside and its sparingly soluble aglycone were chosen as representatives of the flavonoids because naringin was included in the work of Ray et al. (1980). Naringenin had no effect on dormant bud formation at $10^{-6}$ M or $10^{-9}$ M while plant mortality
FIGURE 4.5

EFFECTS OF COUMARIN ON GROWTH AND BUD FORMATION

BY _H. VERTICILLATA_ IN BUBBLE TUBES, n = 4
FIGURE 4.6

EFFECTS OF COUMARIN ON GROWTH AND BUD FORMATION
BY H. VERTICILLATA IN STORAGE JARS. ERROR
BARS REPRESENT 95% C.I. ABOUT THE MEAN,

n=3
FIGURE 4.7

PHENOLIC COMPOUNDS EXAMINED FOR THEIR EFFECTS
ON DORMANT BUD FORMATION BY H. VERTICILLATA
COUMARIN

NARINGENIN
(4',5,7-trihydroxyflavone)

NARINGIN
(4',5,7-trihydroxyflavone 7-rhamnoglucoside)

\[ \text{O} \]
\[ \text{O} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{HO} \]
\[ \text{HO} \]
\[ \text{HO} \]
\[ \text{HO} \]
\[ \text{O}_{5} \text{H}_{11} \text{C}_{6} \text{-O} \]

occurred at $10^{-3}$ M (Figure 4.8). Bacterial growth was extremely heavy at this higher concentration and could have been the cause of plant mortality.

Naringin was also lethal to the plant at $10^{-3}$ M associated with heavy bacterial growth but bud production as well as yield were stimulated at $10^{-9}$ M (Figure 4.9). The different results obtained from naringen and naringenin are probably due to chemical uptake by the plant based on their relative solubilities. Naringin is more soluble and probably more readily taken up by the plant than is naringenin. This apparent enhancement of bud formation supports work by Erez and Lavee (1969) with dormant peach buds which indicates a possible flavonoid link between the phytochrome system in the leaves and bud formation at a particular site.

4.4.2 Plant Hormones

Results of exogenously applied IAA, GA, ABA, and kinetin were reported earlier (Section 4.2). I chose another hormone, ethylene, because it has been associated with breaking of dormancy in potatoes (Rylski et al. 1974) and was antagonistic to ABA in the germination of peanut seeds (Ketring and Morgan, 1972).

Ethylene can be applied as a gas or as a chloroalkylphosphonic acid such as 2-chloroethylphosphonic acid
FIGURE 4.8

EFFECTS OF NARINGENIN ON GROWTH AND BUD FORMATION BY H. VERTICILLATA IN STORAGE JARS. ERROR BARS REPRESENT 95% C.I. ABOUT THE MEAN,

n = 3
FIGURE 4.9

EFFECTS OF NARINGIN ON GROWTH AND BUD FORMATION BY
H. VERTICILLATA IN STORAGE JARS. ERROR BARS
REPRESENT 95% C.I. ABOUT THE MEAN,
n = 3
(ethephon). This compound has been used as a ripening agent in the agriculture industry and is marketed for this purpose by Union Carbide under the trade name Ethrel. Ethylene is evolved from ethephon via attack of the phosphate dianion by a water molecule (Figure 4.10).

Ethephon inhibited bud formation in the bubble tube system at 250 mg/l with no significant loss of dry weight (Figure 4.11). No senescence was apparent even at 500 mg/l which seemed odd at such a high concentration of ethylene. A reexamination of ethephon, using the storage jars, showed increased sensitivity with appreciable loss of dry weight at the higher concentration (Figure 4.12). Bud formation was totally inhibited at 100 mg/l ethephon with a 65% reduction in dry weight indicating senescence. Apparently, aeration in the bubble tubes rapidly stripped the sparingly soluble ethylene from solution requiring a large concentration of ethephon to elicit a significant response. The storage jars had no such aeration and resulted in enhanced sensitivity. The ethylene effect was confirmed using 1-amo cyclopropane carboxylic acid (ACC), the immediate precursor to ethylene (Figure 4.13). Results indicated significant reduction in bud formation at $10^{-4}$ M ACC. Concentrations of 50, 100, and 250 mg/l ethephon were next examined using the greenhouse system (Figure 4.14). Significant bud reduction occurred at 50 and 100 mg/l with little loss in dry weight.
FIGURE 4.10

DEGRADATION OF 2-CHLOROETHYLPHOSPHONIC ACID TO PRODUCE ETHYLENE (BIDDLE et al. 1976)
\[ \text{CH}_2=\text{CH}_2 + \text{Cl}^- + \text{H}_2\text{PO}_4^- \]
FIGURE 4.11

EFFECTS OF ETHOPHON ON GROWTH AND BUD FORMATION

BY H. VERTICILLATA IN BUBBLE TUBES, $n = 4$
FIGURE 4.12

EFFECTS OF ETHYLENETHIOCYANATE ON GROWTH AND BUD FORMATION

BY *H. VERTICILLATA* IN STORAGE JARS. ERROR

BARS REPRESENT 95% C.I. ABOUT THE MEAN,

n = 3
FIGURE 4.13

EFFECTS OF L-AMINO CYCLOPROpane CARBOXYLIC ACID ON GROWTH AND BUD FORMATION BY H. VERTICILLATA IN BUBBLE TUBES, n = 4
FIGURE 4.14

EFFECT OF ETHEPHON ON GROWTH AND BUD FORMATION
BY H. VERTICILLATA IN THE GREENHOUSE. ERROR
BARS REPRESENT 95% C.I. ABOUT THE MEAN,
\[ n = 3 \]
Ethylene evolution from ethephon was next monitored in order to predict ethylene contact time in the culture. Using a 50 mg/l solution of ethephon, at pH 6, ethylene was measured in the head space of a sealed flask. Ethylene evolution ceased after 30 hr. This result indicates that plants treated on a 14 day treatment schedule were receiving ethylene for only about two days during this period.

Ethephon was retested in the greenhouse with treatments made on 2, 6, and 10 day intervals using 0.1, 1, 10, 50 mg/l. Results of treating plants on a 10 day treatment regime show a close correlation between dry weight and bud production indicating that reduction in bud formation was most likely due to whole plant stress (Figure 4.15). A concentration of 1.0 mg/l ethephon, dosed on a six day schedule reduced bud formation 60% while dry weight was not significantly reduced (Figure 4.16). Decreasing the time between treatments to two days resulted in a significant reduction in bud formation with little decrease in dry weight at 0.1 mg/l ethephon (Figure 4.17). These results indicate a significant reduction in bud formation (80%) can be obtained at very low concentration of ethephon (0.1 mg/l) if applied semicontinuously.

4.5 Discussion

Chemicals tested for their effects on bud formation were
FIGURE 4.15

EFFECTS OF A 10 DAY TREATMENT INTERVAL WITH ETHEPHON ON GROWTH AND BUD FORMATION BY \textit{H. VERTICILLATA} IN THE GREENHOUSE. ERROR BARS REPRESENT 95\% C.I. ABOUT THE MEAN, \( n = 3 \)
FIGURE 4.16

EFFECT OF A 6 DAY TREATMENT INTERVAL WITH ETHEPHON ON GROWTH AND BUD FORMATION BY H. VERTICILLATA IN THE GREENHOUSE. ERROR BARS REPRESENT 95% C.I. ABOUT THE MEAN, n = 3
FIGURE 4.17

EFFECT OF A 2 DAY TREATMENT INTERVAL WITH ETHEPHON ON GROWTH AND BUD FORMATION BY H. VERTICILLATA IN THE GREENHOUSE. ERROR BARS REPRESENT 95% C.I. ABOUT THE MEANS, n = 3
selected on the basis of their suggested antagonism to ABA. Interestingly, two such compounds, ethylene and coumarin, reduced bud formation while the flavinoid glucoside, naringin, enhanced bud formation. Two other plant hormones tested, IAA and kinetin, gave no significant response at the level tested while GA also reduced bud formation.

Walton (1980) recently reviewed the biochemistry and physiology of abscisic acid. In discussing dormancy, he presented the inconsistent and contradictory results of investigators attempting to identify the role of ABA. His conclusion was that a role for ABA in the induction or maintenance of bud and seed dormancy has been neither unequivocally demonstrated nor disproven. His review of the subject was confined, however, to terrestrial plants; he did not include work done with aquatics (e.g. Perry, 1968; Weber and Nooden, 1976; Van et al. 1978). The aquatic work as well as my own results demonstrate a stimulatory role for ABA in vegetative dormant bud formation in aquatic vascular plants. A similar conclusion has been reached with Potamogeton pectinatus L. (L.W.J. Anderson, U.S.D.A., Davis, California, personal communication, 1982).

Why are the results more clear for aquatics than for terrestrial plants since temperature, photoperiod, and the photoreceptor for dormancy are the same? Another mechanism, possibly phenolics, may be interacting with ABA to confuse results for terrestrial plants.
Short days have also been shown to increase phenylalanine ammonia lyase (PAL) activity in a number of terrestrial plants (Camm and Towers, 1977). This enzyme is responsible for the deamination of L-phenylalanine to yield trans-cinnamic acid and ammonia. Trans-cinnamic acid is the first compound produced and is the subsequent substrate in a number of reactions involved in the biosynthesis of plant phenolics (Figure 4.18). As stated earlier (section 4.4.1), plant phenolics include coumarins, flavinoids, and lignins and PAL appears to regulate the substrate pool necessary for the biosynthesis of any or all of these phenolics (Camm and Towers, 1977). It should be noted that increased PAL activity might be due to phytochrome changes in membrane permeability providing a flux of soluble metabolites into subcellular sites where enzymes may be sequestered (Smith).

Many plant phenolic compounds have growth inhibitory effects much like ABA. Investigations have indicated that ABA, in general, primarily depresses enzyme synthesis while phenols depress enzyme activity (Kefeli and Kadjiov, 1971). Phenols may play a role in dormancy in terrestrial plants and thus confound results of investigators looking at ABA action only. Ultraviolet light induces PAL activity suggesting a difference between aquatic and terrestrial plants (Craker, 1975). Aquatics are subjected to less
FIGURE 4.18

BIOSYNTHETIC PATHWAY OF PLANT PHENOLICS
ultraviolet light, and thus phenolic activity may be significantly lower which allows the action of ABA to be more easily seen. The question is, do submersed aquatics have significant phenolic activity? Lack of significant lignification in most submersed aquatics eliminates one need for phenolic biosynthesis. Unfortunately there has been little or no work done on phenolic biosynthesis in submersed aquatics.

Since naringin enhances bud formation in hydriilla, the mechanism of action of flavinoids may be available in aquatics even if phenolic biosynthesis is insignificant. Coumarin inhibits bud formation but this compound is not central to the phenolic biosynthetic pathway, rather it occurs on a side branch from cinnamic acid through 0-coumaric acid (Figure 4.18). Little free coumarin occurs in the intact cell (Stoker and Belles, 1962). Furthermore, coumarin does not participate in feedback inhibition of PAL as do the trans-cinnamic acids (Poulton et al., 1980). Thus, in retrospect, coumarin may not have been as good a choice as cinnamic acid for a representative of the plant phenolics and coumarins. The results, however, do indicate a reduction in bud formation using coumarin which suggests a mode of action other than interacting in the phenolic pathway. Coumarin has been shown to inhibit red light-induced opening of etiolated bean hypocotyl hook
An increase in endogenous ethylene was also present possibly mediating the effects of coumarin as well as inducing increased anthocyanin formation (Craker and Wetherbel 1973). These results suggest the interaction of ABA, ethylene, and some plant phenolics (flavonoids?) in control of dormancy. Whether they act independently or in sequence is as yet unknown. Studies involving phenolic biosynthesis and ABA biosynthesis as well as their interactions are necessary, and may be particularly interesting since mevalonate, the precursor to ABA, is also a substrate involved in the biosynthesis of furanocoumarins (Brown et al., 1970; Brown, 1970; Floss and Paikert, 1969).

The involvement of ethylene in the effects of coumarin links the two most effective chemicals tested for inhibiting bud formation. Ethylene, both alone and in combination with GA and kinetin, has been shown to be antagonistic to ABA (Hallion, 1976; Dunlap and Morgan, 1977). Abscisic acid has also been shown to reduce ethylene levels (Gamborg and LaRue, 1971).

Recent results obtained using a new systemic herbicide, DPX-4189 (2-chloro-N-[4-methoxy-6-methyl-1, 3.5-triazin-2-yl-aminocarbonyl] benzenesulforamide) indicated that extremely low concentrations (5ppb) inhibited vegetative dormant bud formation in hydrilla while reducing dry weight by 20% (Van and Steward, 1982). Further studies looking at translocation of these herbicide as well as stress
ethylene formation in vivo may indicate that bud inhibition by this herbicide is controlled through ethylene formation.

5.0 Conclusions

5.1 Vegetative Dormant Bud Formation in *H. Verticillata*

The objectives of this research were to delineate possible points in the process of vegetative dormant bud formation that might be amenable to control. Bud formation in hydrilla is stimulated by a photoperiod of less than 12 hours, possibly mediated through the phytochrome system, and hormonally induced by abscisic acid (Figure 5.1). Areas requiring continued research are how phytochrome controls this process and how abscisic acid exerts its influence.

Ethylene, applied semicontinuously at low concentrations, significantly reduces bud formation. The mode of action appears to be its antagonism to abscisic acid control of dormancy. Abscisic acid biosynthesis, phenolic biosynthesis, and their interactions involving the control of dormancy are important areas for future research.

The results of my research suggest that a potential managerial strategy for hydrilla control would be to incorporate ethephon into a slow release formulation and apply it just prior to the bud production season. If this were followed by herbicide treatment in the spring, the standing crop of hydrilla would be greatly reduced with far less regrowth from vegetative dormant buds.
FIGURE 5.1

EFFECTS OF VARIOUS COMPOUNDS ON VEGETATIVE DORMANT BUD FORMATION IN H. VERTICILLATA, \( n = 4 \)
BUD FORMATION

INCREASE

PHOTOPERIOD ≤ 12hr

↓

ARTIFICIALLY PROLONGED PHOTOPERIOD

↓

PHYTOCHROME SYSTEM

↓

NARINGIN

↓

HORMONE PRODUCTION

↓

INCREASED ENDOGENOUS ABA

↓

ABA

↓

ETHELPHON ACC COUMARIN TOXICANTS

↓

ETHELYLENE

↓

GA

↓

BUD FORMATION
II. USE OF HYDRILLA IN AQUATIC PHYTOTOXICITY BIOASSAYS

6. Literature Survey: Use of Aquatic Macrophytes to Evaluate Phytotoxicity

6.1 Background

Aquatic toxicity is one of the components required to evaluate the relative risk associated with the manufacture, distribution, and utilization of various chemicals and chemical products. Classical assessment of aquatic toxicity has focused on fish and invertebrates primarily due to their economic importance; however, increased awareness of the role of primary producers in aquatic systems has stimulated their use in aquatic hazard evaluations. The evaluation of aquatic phytotoxicity now includes the use of both algae and aquatic macrophytes as bioassay organisms.

Aquatic phytotoxicity evaluations have primarily used algae as the test organism due to the relative simplicity of algal culture systems. Most algal bioassays are based on the United States Environmental Protection Agency Algal Assay Bottle Test which was originally developed to assess nutrient concentrations in eutrophication studies (Miller et al., 1978). More recently, increased recognition of the importance of macrophytes to aquatic productivity has suggested their use as bioassay organisms. Rooted aquatic plants are difficult to work with experimentally, hence,
members of the floating Lemnaceae (duckweed) family have been proposed as "representative" aquatic macrophytes (Federal Register, 1979).

The term "aquatic macrophyte" refers to macroscopic vegetation, including macroalgae, mosses, ferns, and angiosperms that grow in aquatic and wetland habitats. Taxonomic and physiologic characteristics of aquatic macrophytes are presented in detail elsewhere (Sculthorpe, 1967; Correll and Correll, 1972; Hutchinson, 1975). This review concerns the use of aquatic aquatic macrophytes for both inorganic and organic chemical toxicity bioassays.

6.2 Major Problems Associated with Aquatic Macrophyte Toxicity Bioassays

Evaluating the impact of a pollutant on a particular organism requires that typical growth characteristics of the organism be known. Both optimized and reproducible controlled growth of the test species is necessary to minimize variability of test results.

The majority of aquatic macrophytes do not lend themselves to nondestructive quantitative measurements of growth with time, which is necessary if data on growth rate is desired. This may necessitate use of other parameters, such as final yield or photosynthetic productivity, as measures of deleterious effects of pollutants. Rooted
species present an added difficulty if a complex rooting substrate is needed.

6.3 Evaluation of the Toxicity of Inorganic Compounds Using Aquatic Macrophytes

Most inorganic elements are toxic at high concentrations. The majority of studies on the effects of inorganics on aquatic plants have concerned uptake, accumulation, and elimination of these elements (Nakada et al., 1979; Succhcharven, 1980; Harding and Whitton, 1978; Burton and Peterson, 1979; Behan et al., 1979; Bergamini et al., 1979; Kozuchowski and Johnson, 1978); however, few deleterious effects are mentioned.

Members of the Lemnaceae are currently being evaluated as standard bioassay test organisms. These floating aquatics are used for a number of reasons: 1) they can be grown in axenic culture in a defined inorganic medium, 2) they are not rooted in the hydrosoil, and 3) both growth rate and final yield can be determined nondestructively.

The effects of chromium on two species of duckwee were determined using static bioassays (Mangi et al., 1978). Frond numbers and dry weight were used to assess growth response to chromium over a 14-day exposure period. CrO₄ at 10 ppm significantly reduced growth of both *Lemna minor* and *Spirodea polyrhiza*. 
A rooted aquatic, *Elodea canadensis*, has been used to assess copper toxicity (Brown and Rattigan, 1979). A static bioassay was developed in which the growth of *E. canadensis* was optimized with respect to illumination, temperature, and dissolved carbon dioxide. Relative toxicity was judged by reduction of photosynthetic oxygen evolution and visual evaluation of plant damage.

Two other rooted species, *Najas quadulepensis* (Southern Naiad) and *Myriophyllum spicatum* (Eurasian Watermilfoil) have been used in static bioassays of heavy metals to evaluate toxicity. Visual evidence of cadmium toxicity to *N. quadulepensis* was present at 0.007 mg/l (Clearley and Coleman, 1973). Toxicity of a number of heavy metals to *M. spicatum* was determined using reduction of dry weight as the growth response (Stanley, 1974). Fifty percent inhibition of root weight occurred with concentrations of 0.25 ppm Cu$^{+2}$, 1.9 ppm Cr$_2$O$_7^{-2}$, 3.4 ppm Hg$^{+2}$, 2.9 ppm AsO$_2^{-1}$, 7.4 ppm Cd$^{+2}$, 2.5 ppm Al$^{+3}$, 9.9 ppm Cr$^{+3}$, 41.2 ppm Ba$^{+2}$, 21.6 ppm Zn$^{+2}$, 13.3 ppm NH$_4^{+1}$, 22.4 ppm CN$^{-1}$, 143 ppm B$_4$O$_7^{+2}$, 363 ppm Pb$^{+2}$, 10,228 ppm Na$_2$SO$_4$, and 8,183 ppm NaCl. The addition of soil increased the toxicity of Cr$^{+2}$ and Ba$^{+2}$, but decreased the toxicity of Cr$_2$O$_7^{-2}$, Cu$^{+2}$, Cd$^{+2}$, Al$^{+3}$, and Hg$^{+2}$. The author suggested that the soil, besides adsorption of the metals, could influence toxicity by providing Ca$^{+2}$ ions and affecting
membrane transport or by providing chelators that might facilitate metal uptake.

Mercury and methylmercury uptake, release, and transfer in Elodea densa indicated that methylmercury was concentrated in the younger tissues while inorganic mercury was concentrated in the older tissues (Czuba and Mortimer, 1980; Mortimer and Kudo, 1975). Methylmercury was better absorbed and was more toxic than the inorganic form. Inorganic mercury was released more readily from plants containing both forms.

6.4 Evaluation of the Toxicity of Organic Compounds Using Aquatic Macrophytes

Many organic pesticides have been evaluated for toxicity using aquatic macrophytes. The objective of the work was primarily control of troublesome aquatic plants via herbicidal treatment. Literature is available on the use of herbicides to control the Haloragaceae (watermilfoil) (Sutton, et al., 1971) Potamogetonaceae (Fondweeds) (Dutta et al., 1972), Najadaceae (Water nymphs) (Sutton et al., 1970), Pontederiaceae (Hyacinths) (Stewart, 1973), and several others.

A flow-through bioassay using Lemna minor was proposed by Wallbridge (1977) and further developed for toxicity assessment by Bishop and Perry (1980). The
critical parameter used to evaluate toxicity was the EC\textsubscript{50}, defined as the calculated test material concentration expected to reduce growth by 50% relative to the control. The 7-day EC\textsubscript{50} values for front count, root length and dry weight and the time-independent EC\textsubscript{50} for growth rate and doubling time were calculated for the herbicide 6,7-dihydrodipryrido (1,2-a:2', 1'-c) pyrazinidium dibromide (Ortho Diquat, Chevron Chemical Co.) and for the surfactants, sodium dodecyl sulfate (SDS), C\textsubscript{11.8} linear alkylbenzene sulfonate (LAS), alcohol ethoxylate (AE), and cetyl trimethyl ammonium chloride (CTAC). The toxicity of Diquat > CTAC > LAS and AE > SDS for all four parameters measured, with EC\textsubscript{50} values ranging from 0.0015 to 44 mg/l.

Static tests also using duckweeds indicated that 5 \times 10^{-6} M cetyl trimethyl ammonium bromide (CTAB) significantly reduced the growth rate of Spirodella oligorhiza (Walker and Evans, 1978). The toxicity of a homologous series of the herbicide 1,1' alkyl-4,4'-bipyridylium to S. oligorhiza indicated a relationship between herbicidal potency and steric effect with compounds containing the least bulky side chains being most phytotoxic (Ross et al., 1979).

Growth optimized axenic, static cultures of L. minor and S. polyrhiza also have been used successfully for toxicity evaluation of aquatic pollutants (Ward et al., 1980). The EC\textsubscript{50} values generated from static bioassays
showed a difference in sensitivity between the two species for ammonium carbamate and the aquatic herbicide endothal 
(Table 6.1). The authors found that screening of species 
is necessary if the most sensitive species is desired for 
the bioassay.

Rooted species of aquatic macrophytes, despite the 
problems mentioned above, have been used successfully to 
evaluate the toxicity of some chemicals. *Elodea canadensis* 
and *Myriophyllum spicatum* were used to assess the effect 
of the larval lampricide, TFM(3-trifluoromethyl-4-nitro-
phenol) on aquatic flora (Maki and Johnson, 1977). Re-
circulating laboratory stream channels were used with 
reductions of plant length and dry weight as indicators 
of toxicity.

Protoplastic streaming and chloroplast movement were 
used in tests lasting less than 5 hr to determine the 
effects of polyphenols and quinones on *Nitella* sp. and 
*Elodea canadensis* (Stom and Kozhova, 1976; Stom, 1977). 
Polyphenols are oxidized to quinones in aquatic plants 
and these quinones exert a toxic effect by oxidizing 
sulfhydryl groups. The toxicity of polyphenols was 
directly proportional to their ability to be oxidized by 
test plants.

The surfactant sodium desoxycholate caused visible 
damage to *E. canadensis* and *Potamogeton coloratus* in 
batch cultures at 100 to 500 mg/l (Schaefer and Glanzer,
TABLE 6.1
EC$_{50}$ VALUES FOR THE TOXICITY OF
TWO PESTICIDES TO DUCKWEEDS

<table>
<thead>
<tr>
<th></th>
<th>ENDOTHALL (ppm)</th>
<th>AMMONIUM CARBAMATE (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. minor</td>
<td>1.4</td>
<td>205</td>
</tr>
<tr>
<td>S. polyrrhiza</td>
<td>2.1</td>
<td>196</td>
</tr>
</tbody>
</table>
1976). These concentrations are much higher than those showing deleterious effects to duckweeds. The authors did not determine a no effect concentration.

Oil spills have provided in situ tests of the effects of hydrocarbons on aquatic flora. Studies indicate a marked reduction in the standing crop of intertidal algae and surf grasses, but no effect on subtidal species (Strachan, 1972; Foster, 1972). While emulsifiers were the most effective method of dealing with a spill, they were found in some instances to be more toxic than the spill itself. Photosynthetic $^{14}$C-fixation rate in two species of lichens was used to measure the toxicity of the oil emulsifier BP1002 (Brown, 1973). A 1% solution of the emulsifier inhibited photosynthesis in both species. The surfactant fraction of the emulsifier caused loss of lipid-soluble chlorophyll pigments.

Evaluations of the effects of the water soluble fraction of No. 2 fuel oil on S. oligorhiza indicated that the duckweed was more sensitive to this toxicant than the green alga, S. capricornutum (Klaine et al., 1982). The enhanced toxicity may have been due to the floating habit of the duckweed, since sparingly soluble organics collect at the water surface.

The development of a consistent rooted aquatic macrophyte bioassay to assess phytotoxicity would be a
valuable addition to the available tools for the assessment of relative risk of new chemicals. A major barrier to the development of such a bioassay is the lack of in depth physiological data on aquatic species. Research concerning the controls of noxious aquatics, such as hydrilla, can provide this needed physiological information.

6.5 Research Objectives: Development of a Rooted Aquatic Macrophyte Bioassay for Toxicity Evaluation

Few rooted aquatic macrophytes have been used in toxicity bioassays. The large amount of culturing experience and plant physiology data on hydrilla makes it a promising bioassay organism. The objectives of this research are to determine if the response of hydrilla to known toxicants is quantitatively related to the chemical concentration, and to investigate the use of this plant in partial life cycle studies. This information will be valuable for multispecies and microcosm testing in which aquatic macrophytes are included.

7 Materials and Methods

7.1 Toxicity Bioassays Using Hydrilla

Hydrilla was used to assess the relative toxicities of two chemicals. The pyrex storage jar system, described in Section 2.3, was used for these experiments. The
first group of experiments started with dormant buds and measured the effect of either phenol or ammonium carbamate on bud sprouting over a two week period. The second group of experiments started with apical growing fragments and measured growth response by growth rate (length/time), final dry weight, and final length over a two week period. The final group of experiments started with mature plants and measured the effect of these two toxicants on bud production during eight weeks.

8 Results and Discussion

8.1 Use of H. verticillata for Phytotoxicity Evaluation

Results with ammonium carbamate and phenol indicate that measures of bud sprouting, dry weight, and length of hydrilla were quantitatively related to toxicant concentration (Figures 8.1 and 8.2). Phenol significantly inhibited all three parameters at less than 10 mg/l, half of the concentration necessary to produce a significant reduction in the growth rate of Selenastrum capricornutum, a green alga (Ward et al, 1980). The response of H. verticillata to ammonium carbamate indicated it was more sensitive than either L. minor or S. polyrhiza (Grant, 1982).

Bud formation was a more sensitive measure of the toxicity of both phenol and ammonium carbamate indicating that partial life cycle studies might be more sensitive
FIGURE 8.1

TOXICITY OF AMMONIUM CARBAMATE TO

H. VERTICILLATA
FIGURE 8.2

TOXICITY OF PHENOL TO H. VERTICILLATA,

\[ n = 4 \]
than short term tests. A third chemical, 3-amino 1,2,4-triazole, also showed an increased reduction of bud formation as compared to the other test parameters (Figure 8.3). Results obtained from bud production and/or bud sprouting tests might be compared with similar tests currently being performed with animals (e.g., critical life stage, partial fish chronic, etc.).

Multispecies and microcosm testing are currently being investigated for use in hazard assessment of aquatic pollutants. Presently, plants are playing only a minor role in such investigations, probably due to general unfamiliarity with their use in toxic bioassays. Microcosm and the larger mesocosm studies must include both macrophytes and algae if they are to attempt to stimulate a natural ecosystem.

9 Conclusions

9.1 Phytotoxicity Evaluation Using H. verticillata

The objective of this research was to evaluate the use of hydrialla for assessing the relative phototoxicity of aquatic pollutants. Final yield of hydrialla as both dry weight and total length was quantitatively related to toxicant concentration. Hydrialla appeared more sensitive to ammonium carbamate than were duckweeds and more sensitive to phenol than was a unicellular green alga.
FIGURE 8.3

TOXICITY OF 3-AMINO, 1,2,4-TRIAZOLE TO

H. VERTICILLATA, n = 4
Dormant bud production and sprouting tests indicate that life cycle, or partial life cycle, studies with this plant may be both feasible and more sensitive than the acute tests.
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