EFFECT OF GIBBERELIC ACID
AND 2,4-DICHLOROPHENOXYACETIC ACID ON
WATERHYACINTH, EICHHORNIA CRASSIPES (MART.) SOLMS

BY
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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1982

I dedicate this work to my mother

MARGARET T. JOYCE

(1928 - 1967)

whose life-long hope and ambition was that I receive a

complete and quality collegiate education.

ACKNOWLEDGEMENTS

This research was partially supported by a cooperative agreement with United States Department of Agriculture, ARS Number 58-7B30-0-177. The grant was administered as a project of the University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic Weeds.

I am extremely grateful to Dr. William T. Haller, my major professor, for his advice and encouragement throughout this study. Appreciation is also extended to the members of my committee: Dr. Daniel E. Canfield, Jr.; Dr. Leon A. Garrard; Dr. Jerome V. Shireman; and Dr. Shirly H. West; and to a former committee member, Dr. Thai K. Van, for their interest and constructive criticisms.

I also wish to express my thanks to Dr. Palakurthi Suresh C. Rao for the use of his carbon oxidizer without which a major portion of this study would not have been possible. Similarly, I would like to thank Dr. George Bowes for the use of his scintillation counter.

My sincere thanks are extended to the U.S. Army Corps of Engineers, Jacksonville District, who afforded me with the opportunity and financial support to pursue this degree. In particular, I would like to thank my supervisors, Mr. Gail G. Gren, Mr. Giralmo DiChiara, and Mr. James D. Hilton and the members of the Natural Resource Management Section, James M. Dupes, James T. McGehee, and Larry T. Taylor for their patience and support. I want to thank Eddie Knight for his assistance in conducting the field portion of this study and to Betty Hyman and Jerrine Hamm for their assistance in preparing this manuscript.

Sincere thanks go to Jim Cobb, Ken Langland, and Daniel Thayer for their assistance and advice throughout this study.

I am most grateful to my wife Pamela T. Joyce and sons, Joseph C. Joyce, Jr. (JJ) and Christopher T. Joyce (Ty) for their support, patience, and sacrifice.

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Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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December 1982

Chairman: William T. Haller

Major Department: School of Forest Resources and Conservation

Effects of combinations of gibberellic acid (GA_3) and 2,4-dichlorophenoxyacetic acid (2,4-D) on waterhyacinths (Eichhornia crassipes (Mart.) Solms) were evaluated to determine if GA_3 increased waterhyacinth sensitivity to 2,4-D under field conditions, and if GA_3 increased translocation of ^{14}C labeled 2,4-D in waterhyacinths grown in growth chambers. Effects of GA_3 on waterhyacinth sensitivity to 2,4-D were evaluated in two phases. In the first phase, small bulbous leafed waterhyacinths were grown outdoors in 70-liter containers and treated during three growing seasons with combinations of GA_3 at 0.0, 23.5, 47.0, 94.0 and 188 g/ha and 2,4-D at 0.00, 0.28, 0.56, 1.12, and 2.24 kg/ha. The second phase was conducted during late summer in a dense population of mature, non-bulbous leafed waterhyacinths in the

St. Johns River, Florida, with combinations of GA₃ at 0.0, 23.5, 47.0, and 94.0 g/ha and 2,4-D at 0.00, 0.56, 1.12, 2.24, and 4.48 kg/ha. Effects of treatment rates were recorded as percent change from initial biomass and initial number of plants. Effects of GA₃ on the translocation of ¹⁴C labeled 2,4-D were monitored through time for levels of ¹⁴C per milligram of plant tissue and percent of total ¹⁴C translocated to separate plant parts.

Regression analysis indicated the lack of significant interaction between GA₃ and 2,4-D in terms of increased efficacy of 2,4-D above routine application rates of 2.24 kg/ha. Additive effects of 2,4-D and GA₃ were suggested, however. Costs analysis of various combinations of GA₃ and 2,4-D indicated that addition of GA₃ in order to lower rates of 2,4-D would increase waterhyacinth control costs by over 300.0 percent.

Translocation of ¹⁴C-labeled 2,4-D to meristematic waterhyacinth tissues was not increased due to pretreatment with 100 mg/l GA₃. Increased translocation to leaves other than 2,4-D treated leaves was suggested. Use of GA₃ to significantly reduce rates of 2,4-D used to control waterhyacinths under field conditions was not justified from either an increased efficacy or economic standpoint.

INTRODUCTION

Man's attraction and search for botanical species which are unique in structure, food potential, floral characteristics, productivity or ability to survive in specific environmental situations have resulted in the introduction of numerous plants outside of their native range. Such introductions may result in an exotic species becoming established in a habitat optimum for growth in the absence of its naturally limiting environmental factors or organisms. Once freed of these naturally limiting conditions, certain exotic species may proliferate to such an extent that the population interferes with man's intended use of its habitat.

The waterhyacinth, Eichhornia crassipes (Mart.) Solms, a perennial, floating aquatic plant, is an example of such an exotic species. The waterhyacinth is generally considered to be a native of Brazil from where it has spread to nearly all sub-tropical and tropical regions of the world where conditions favor its growth (Penfound and Earle, 1948; Bock, 1966). Since its introduction into the United States in the mid 1880's, the waterhyacinth has proliferated to such an extent that it has created serious water resource management problems such as 1) interference with commercial and recreational navigation, 2) reduction in water conveyance capabilities of streams, rivers, and man-made flood control structures and waterways, 3) adverse modification of fish and wildlife habitat, and 4) threatening public health by providing habitat

for disease vectors (Webber, 1897; Penfound and Earle, 1948; Hitchcock et al., 1949; Seabrook, 1962; Anonymous, 1965; Bock, 1966; Buker, 1982.)

Prior to 1946, waterhyacinth control operations consisted of mechanical operations utilizing various conveyor, chopper or shredder machines; containment apparatus such as floating booms, fences or traps; pusher boats to assist the plants to salt water; and the use of various inorganic chemicals such as sodium arsenite (Webber, 1897; Brown et al., 1946; Penfound and Earle, 1948; Hitchcock et al., 1949; Bock, 1966; Wunderlich, 1967; Buker, 1982). Large-scale control of waterhyacinths was revolutionized in the mid-1940's by the discovery of the herbicidal properties of 2,4-dichlorophenoxyacetic acid (2,4-D). Since its development, various formulations of 2,4-D have been routinely utilized for control of waterhyacinths (Anonymous 1946; Brown et al., 1946; Hildebrand, 1946; Hitchcock et al., 1949; Penfound and Earle, 1948). Currently, dimethylamine salt is the only formulation of 2,4-D labeled by the United States Environmental Protection Agency (EPA) for use in public waters which are either flowing and/or potable water supplies. During 1981, governmental agencies in Florida applied approximately 63,700 kg of 2,4-D to public waters for control of nuisance aquatic vegetation, the majority of which was waterhyacinths (Dupes and Mahler, 1982).

Increased public awareness, spurred by the environmental movement and adverse publicity associated with the military's use in Vietnam of the defoliant, "Agent Orange", which was a combination of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), has caused various regulatory agencies to more closely scrutinize labeling of new herbicides and to review existing registrations of herbicide products currently

utilized in agriculture, and more specifically, aquatic environments. In order to reduce public concern over use of chemical control agents and to gain more effective control of pest species, two basic approaches have been pursued; i.e., (a) the use of alternate control methods such as biological agents, mechanical harvesting systems, and/or environmental manipulation and (b) modification of herbicide application techniques, equipment, and/or the use of various chemical adjuvants in order to reduce the amount of herbicide actually introduced into the environment.

This study was designed to determine if the quantity of 2,4-D routinely utilized for large scale control of waterhyacinths could be reduced by simultaneously applying a naturally occurring plant growth substance, gibberellic acid. The study was divided into three major parts. Part one involved small plot evaluations of the efficacy of various rates and combinations of 2,4-D and gibberellic acid for waterhyacinth control. The objective was to determine the presence of an interaction or synergistic effect between 2,4-D and gibberellic acid which would increase the sensitivity of waterhyacinths to 2,4-D. Part two involved growth chamber evaluations utilizing radioactive-labeled 2,4-D and unlabeled gibberellic acid. The objective was to ascertain if the cause of increased sensitivity, if present, to 2,4-D was due to an increase in the quantity of 2,4-D translocated to various locations within the plant when also treated with gibberellic acid. Part three involved field application of a combination of 2,4-D and gibberellic acid under routine operational conditions. The objective of this final phase was to determine if a specified combination of gibberellic acid and a reduced rate of 2,4-D would be efficacious in a large-scale, operational waterhyacinth control program.

LITERATURE REVIEW

Gibberellic Acid

History

Gibberellins are defined by Phinney and West (1961) as substances possessing the same or similar carbon skeleton as gibberellic acid (GA_3) and that are biologically active in stimulating cell division, cell elongation, or both in plants. The earliest known description of the effects of gibberellins was in 1809 by Konishi, a semi-literate Japanese rice farmer, who described rice plants which grew excessively tall (Stowe and Yamaki, 1957). The diseased plants could not support themselves and eventually died due to parasitic action (Yabuta, 1935; Salisbury and Ross, 1978). Salisbury and Ross (1978) indicate that as early as the 1890's the Japanese were referring to these symptoms as the "bakanae" ("foolish seedling") disease. The disease was determined to be caused by a fungus, Gibberella fujikuroi (sexual stage) and Fusarium moniliforme (asexual stage). The active compound was isolated and identified in the 1930's by Yabuta and Hayashi, Japanese pathologists, who named it gibberellin (Yabuta, 1935; Stowe and Yamaki, 1957; and Russell, 1974). Japanese scientists were interested in the pathological aspects of gibberellins rather than physiological impacts (Salisbury and Ross, 1978). Thus, even though the first gibberellins were isolated in the 1930's, Western scientists did not become interested in the physiological effects of gibberellins until the early 1950's due to (1) the

preoccupation with indoleacetic acid (IAA) and synthetic auxins, (2) a lack of contact with the Japanese, and (3) World War II (Salisbury and Ross, 1978). Stuart and Cathey (1961) reported that by 1961 approximately 9 compounds had been isolated which exhibited both gibberellin-like activity and structure. This number increased to 19 by 1965 (Russell, 1974), 29 in 1970 (Lang, 1970), 38 by 1973 (Russell, 1974), 44 by 1974 (Barendse, 1974) and to over 50 by 1978 (Salisbury and Ross, 1978). These gibberellins have been isolated from fungi, algae, ferns, mosses, and many higher plants (Barendse, 1974; Russell, 1974; Salisbury and Ross, 1978). Sircar and Kindu (1960), Bhanja and Sircar (1966), and Sircar et al. (1973) reported the presence of four gibberellin-like substances in the shoot and root extract of waterhyacinths. Russell (1974) attributes this exponential rise in the number of known gibberellins to the initiation of a systematic search for growth promoting compounds which was aided, as observed by Lang (1970), by the simultaneous development of highly effective techniques for the separation and identification of naturally occurring compounds. Both Barendse (1974) and Russell (1974) indicated that due to the limited number of plants examined, the total number of gibberellins and their derivatives is unknown.

Chemical and Physical Characteristics

Gibberellins are isoprenoid compounds and are classified as diterpenes (Lang, 1970; Barendse, 1974; Salisbury and Ross, 1978). All gibberellins have either 19 or 20 carbon atoms which are arranged in either a four or five ring system, and all have at least one carboxyl group (Barendse, 1974; Reeve and Crozier, 1974; Russell, 1974; and

Salisbury and Ross, 1978). Each compound is abbreviated GA, with a subscript such as GA₁, GA₂, etc., to distinguish the different compounds. All could be called gibberellic acid, however, due to the commercial availability of GA₃, it has been studied more extensively than the other forms and is commonly referred to as gibberellic acid (Stuart and Cathey, 1961; Salisbury and Ross, 1978).

Gibberellic acid (GA₃) is a white to pale yellow, crystalline powder, with a molecular weight of 346.37, empirical formula C₁₉H₂₂O₆ and a chemical configuration of

(Abbott Laboratories, 1962; Barendse, 1974; Lang, 1970; Reeve and Crozier, 1974; Salisbury and Ross, 1978).

According to Abbott Laboratories (1962), gibberellic acid solubility in various solvents is as follows:

<u>Solvent</u>	<u>Mg/ml Solvent</u>
Dimethyl Formamide	450
Ethyl Alcohol	200
Methyl Acetone	180
Diacetone Alcohol	120
Isopropyl Alcohol	80
Acetone	40
Tap Water	5

Since gibberellic acid is readily soluble in numerous compounds including water, it is readily formulated for various commercial applications (Abbott Laboratories, 1962). The potassium, sodium, and ammonium salts of the acid are more soluble than the acid alone due to the buffering of the aqueous solutions (Abbott Laboratories, 1962). Stuart and Cathey (1961) indicated that these salts of gibberellic acid were equally as active on the growth of pea and cucumber seedlings as the acid.

Abbott Laboratories (1962) indicated that dried gibberellic acid powder is stable indefinitely; however, its aqueous solution is relatively unstable at room temperatures with a half-life of one month. Stability can be increased by storing the solution at low temperatures or by utilizing sterile water. Gibberellic acid solutions prepared with anhydrous solvents are extremely stable; therefore, commercial concentrates usually utilize non-phytotoxic organic solvents.

Commercially, gibberellic acid is produced by culturing Gibberella fungus in a liquid culture medium from which the acid is

extracted. The process utilized is similar to that used in the production of antibiotics (Borrow et al., 1955; Marth et al., 1956; Russell, 1974).

Relative Potency of Gibberellins

The standard method of assessing the potency of a gibberellin is by various bioassay techniques (Bailiss and Hill, 1971; Reeve and Crozier, 1974). Bailiss and Hill (1971) conducted an extensive review of gibberellin bioassay techniques and listed 33 test systems which were based on such diverse processes as coleoptile, leaf sheath, epicotyl, hypocotyl and radicle growth, bud dormancy, seed germination, induction of α -amylase synthesis, leaf expansion and senescence, and flower and cone induction. Interpretation of bioassay data should be done cautiously because individual bioassays exhibit a great deal of species and even variety specificity (Reeve and Crozier, 1974). As noted by Reeve and Crozier (1974), the barley aleurone and cucumber hypocotyl tests only exhibit response to a limited number of gibberellins, whereas the dwarf rice bioassay responds to almost all gibberellins.

Based on the overall assessment of the relative responses obtained from barley aleurone α -amylase synthesis, dwarf pea growth, lettuce and cucumber hypocotyl growth, and Tan-ginbozu dwarf rice microdrop bioassays, Reeve and Crozier (1974) assessed the relative activities of 38 gibberellins. This ranking indicates that the highest activities are provided by GA_1 , GA_3 , GA_7 , and GA_{32} . Good responses were also induced by GA_5 , GA_6 , GA_{26} , and GA_{37} but not of the same order of magnitude. Other

GA's, such as GA₉, GA₁₀, GA₂₃, and GA₂₄, exhibited species specificity by being highly active in some bioassays yet induced poor responses in others. A ranking of the overall relative activity, indicated that GA₃ was the most active compound (Stuart and Cathey, 1961; Reeve and Crozier, 1974). Consistently low activity was exhibited by GA₈, GA₁₁, GA₁₂, GA₁₃, GA₁₄, GA₁₇, GA₂₁, GA₂₅, GA₂₇, GA₂₈, GA₂₉, GA₃₃, and GA₃₄. In all bioassay systems, GA₂₆ was inactive at all concentrations tested. Barendse (1974) indicated that many of the weaker gibberellins were isolated from immature seeds, and it is not certain if (1) they are also present in the growing plant, or (2) they are merely by-products or intermediates for interconversion during biosynthesis of more active gibberellins.

Toxicology

Gibberellic acid has been evaluated for toxicological effects in rats, mice, guinea pigs, rabbits, dogs, cats, and chickens (Peck et al., 1957; Warden and Schaible, 1958; Kimura et al., 1959). The compound was shown to be asymptomatic and free of pathologic changes in subacute toxicity studies in mice and subchronic toxicity studies in dogs and rats (Kimura et al., 1959). Subacute studies showed gibberellic acid to be tolerated by mice at 2 g/kg, intravenously for 5 days, and at 1 g/kg, subcutaneously for 14 days (Abbott Laboratories, 1962). Studies of acute intravenous toxicity of gibberellic acid in mice yielded an LD₀ of 4.2 g/kg, an LD₅₀ of 6.3 g/kg, and an LD₁₀₀ of 8.7 g/kg (Peck et al., 1957). Peck et al. (1957) indicated that signs of toxicity were nonspecific and no deaths and only minimal signs of toxicity were observed after the oral administration of 25.0 g/kg to mice. Based on

their studies, Peck et al. (1957) stated that gibberellic acid presents no apparent hazard either to the individual who uses the material for agricultural purposes or to the individual who consumes products on which gibberellic acid or its salts have been used.

Biosynthesis and Metabolism

Much of the information available to date on gibberellin biosynthesis has come from studies utilizing cultures of Gibberella fujikuroi (Saw.) W. Barendse (1974) noted that although the pathway of biosynthesis in higher plants is less well known, studies involving higher plants suggest that the pathway follows the same scheme as found in Gibberella fujikuroi. Birch et al. (1958) confirmed the fact that GA_3 has a diterpenoid nature by feeding radioactive-labelled acetate or mevalonate to cultures of Gibberella fujikuroi. By examining relative amounts and positions of the label, they were able to suggest a biosynthesis pathway which was later confirmed (Salisbury and Ross, 1978).

The basic pathway of biosynthesis of gibberellic acid described by Birch et al. (1958), Barendse (1974), and Salisbury and Ross (1978) results in the following sequence of compounds: acetyl coenzyme A; mevalonic acid; isopentenyl pyrophosphate; geranylgeranyl pyrophosphate, a 20-carbon compound which serves as the donor for all gibberellin carbon atoms (Salisbury and Ross, 1978); copalyl pyrophosphate; kaurene; kaurenol; kaurenal; kaurenoic acid; and GA_{12} aldehyde which is the first true gibberellane ring system. This aldehyde of GA_{12} is converted either directly to other gibberellins or to GA_4 , a 19-carbon compound, which is interconverted to other gibberellins (Barendse, 1974; Salisbury and Ross, 1978).

Once formed, gibberellins can be readily converted to bound inactive forms in which they are stored or translocated for release at the proper location and physiological time (Lang, 1970; Barendse, 1974; Salisbury and Ross, 1978). Glucosides are the most prominent known bound form of gibberellin (Barendse, 1974; Salisbury and Ross, 1978). Salisbury and Ross (1978) indicate that other unidentified bound forms are also known to exist, some of which appear to be stable protein-gibberellin conjugates.

Sites of Synthesis

Major sites of gibberellin synthesis are developing seeds, apical buds, young leaves, and root tips (Barendse, 1974; Salisbury and Ross, 1978). Lockhart (1957) implicated the shoot tip as the site of gibberellin synthesis by restoring growth in decapitated pea seedlings by applying GA_3 . The diffusion technique of Jones and Phillips (1964) later confirmed these results and also identified root tips as sites of synthesis. The diffusion technique is based on the following principle: if the amounts of diffusible gibberellins obtained in an agar block over a period of time exceeds the amounts of extractable gibberellins in the same organ, active biosynthesis or conversion occurs in the organ. Barendse (1974) reviewed the role of plant roots in gibberellin synthesis and reported that root tips were the site of conversion of inactive forms of gibberellin synthesized in apical buds to more active forms. Salisbury and Ross (1978) stated that repeated excision of parts of the root system markedly reduced the amount of gibberellin in plant foliage which may partially explain why root pruning inhibits shoot growth. Developing seeds are generally considered to be sites of

synthesis based on observations that they contain large concentrations and many types of gibberellins and the fact that accumulation of gibberellins is inhibited by growth retardants (Baldev et al., 1965; Barendse, 1974; Salisbury and Ross, 1978).

Transport

Gibberellin-like substances have been isolated from both phloem and xylem (Audus, 1972). Hoad and Bowen (1968) isolated gibberellins in sieve tube sap of several species which indicated phloem transport. Exogenously applied GA_3 follows a distribution pattern within the plant and rate of movement typical of substances moving within the phloem (McComb, 1964; Chin and Lockhart, 1965). Barendse (1974) cited various studies which documented xylem transport of gibberellins in a number of species including sunflower, peas, grapes, birch, maple, apple, and pear. According to Salisbury and Ross (1978), the transport pathway from young leaves into the stem below is uncertain, but does not involve vascular transport because young actively growing leaves import but rarely export through either xylem or phloem. Kato (1958a) conducted translocation studies with pea stems and indicated that gibberellic acid does not show a pattern of polar translocation. Clor (1967) confirmed these findings utilizing tritium-labeled GA_3 . However, these experiments utilized concentrations of GA_3 which far exceeded physiological levels and may have affected normal movement of the growth substance (Jacobs and Kaldeway, 1970). Subsequent investigations by Jacobs and Kaldeway (1970) and Jacobs and Pruett (1973) utilizing physiological levels of GA_3 revealed that GA_3 exhibits strong basipetal polar movement in Zea mays roots and Coleus petioles. As with auxins, cortex and pith are

believed to be involved. Thus, the evidence indicates that gibberellins and their conjugates are readily transported in the entire conductive system of plants; however, the physiological significance of this transport has not been determined (Barendse, 1974).

Mode of Action

The literature contains numerous reviews of the physiological, morphological, and biochemical mechanisms of action of gibberellins. Addicott (1970) stated that gibberellic acid has probably been tested more widely for its effects on higher plants than any other naturally occurring substance. However, contradictory results were frequently obtained from seemingly similar experiments. Addicott (1970) and Low (1974) explain the differing results in terms of physiological conditions of the experimental material, i.e., age, size, nutrient and light availability, temperature, species, and type of tissue studied. All of these factors have been shown to alter the level of other endogenous hormones in the experimental tissues and thus change the plant's sensitivity to the exogenous gibberellic acid (Low, 1974). As an example, Goyal and Bajjal (1980a and 1980b) reported different responses between varieties of the same species of rice. The following discussion of the mode of action of gibberellic acid presents the most commonly accepted responses.

Typical morphological effects of sensitive species to gibberellin treatments include germination of dormant seeds (Stuart and Cathey, 1961; Chen, 1974), growth of dormant buds (Shafer and Monson, 1958), stimulation of flowering and inflorescence size (Stowe and Yamaki, 1957; Stuart and Cathey, 1961; Weaver, 1972, Krishnamoorthy,

1974), prevention or delay in flowering (Stowe and Yamaki, 1957; Weaver, 1972), leaf heterophylly (Stowe and Yamaki, 1957; Stuart and Cathey, 1961; Israelstam and Davis, 1979), chlorosis (Stowe and Yamaki, 1957; Ende and Koornneef, 1960; Weaver, 1972; Sarma and Hussain, 1979), inhibition or no effect on root growth (Brian et al., 1955; Kato, 1958b), delay and/or acceleration of senescence (Brian et al., 1955; Stowe and Yamaki, 1957; Stuart and Cathey, 1961; Addicott, 1970; Weaver, 1972; Valdovinos, 1974; Salisbury and Ross, 1978); vernalization (Stowe and Yamaki, 1957; Stuart and Cathey, 1961; Weaver, 1972), stem elongation (Brian et al., 1955; Marth et al., 1956; Stowe and Yamaki, 1957; Phinney and West, 1961; Stuart and Cathey, 1961; Audus, 1972; Weaver, 1972; Low, 1974; Watson, 1982) and increases in shoot to root weight ratio (Stowe and Yamaki, 1957; Stuart and Cathey, 1961; Weaver, 1972; Low, 1974).

Effects on fresh and dry weights reported for gibberellic acid treatments are varied. Morgan and Mees (1956) reported increased vegetative growth of a majority of the species tested but no increases in net yield. Ende and Koornneef (1960) reported a 25 percent increase in stem length in tomato plants treated with gibberellic acid; however, there was no significant difference in plant weights when compared to untreated plants. Stuart and Cathey (1961) noted increases in yield of dry matter in winter pasture grasses but no increase in total weight of Eucalyptus. Stowe and Yamaki (1957) reviewed the effects of gibberellins on fresh and dry weights of plants and concluded that gibberellin-induced elongation does not always cause a parallel increase in dry weight and, in fact, may result in a decrease depending upon the species. Marth et al. (1956) investigated the effects on the weight of soybean

plants after foliar application of gibberellic acid. After one week both the fresh and dry weights were significantly higher; however, after two weeks there was no difference between treated and control plants.

Physiological effects of the gibberellins which account for the change in growth, metabolism, and morphology of plants are as varied and as contradictory as those reported above for the morphological effects. These effects, however, can be grouped into the following categories: cell division; cell elongation; osmotic potential; respiration through enzyme metabolism; carbohydrate and lipid metabolism; and changes in membrane permeability.

The exogenous application of gibberellins has been shown to produce a pronounced increase in cell division in the subapical meristem of Hyoscyamus niger and Samolus parviflorus (both of which are rosette species), Phaseolus vulgaris (a non-rosette), and various other species (Sachs and Lang, 1957; Weaver, 1972; Shininger, 1974). In a review by Shininger (1974), increased cell division was noted in 13 of 21 species treated with gibberellins. Salisbury and Ross (1978) hypothesized that increased cell division by gibberellins may be caused by an increase in the number of sites on the chromosome where DNA and RNA synthesis can occur by unmasking initiation sites for DNA and RNA synthesis. Nitsan and Lang (1966) demonstrated that elongation of lentil epicotyls in response to gibberellic acid required DNA synthesis. Nakamura and Takahashi (1973) also reported gibberellic acid enhanced DNA synthesis. The response of a given cell to divide or elongate appeared to depend upon its age or stage of development. Younger cells respond by dividing while older cells respond by elongation only (Marth et al., 1956; Mann, 1974; Shininger, 1974).

Shininger (1974) reported gibberellin caused cell elongation in 9 of 21 species tested and both cell elongation and increased cell division in 4 of the 21 species. The physiological mode of action for cellular elongation results from numerous inter-related processes; however, the sequence in which these processes occur remains uncertain. Gibberellins have been shown to increase hydrolysis of starches, fructosans, and sucrose molecules (Paleg, 1965; Audus, 1972; Kaufman, 1974; Salisbury and Ross, 1978). Paleg (1960) demonstrated an increase in α -amylase content of barley endosperm treated with gibberellic acid. Chen and Park (1973) also demonstrated gibberellic acid enhanced amylase synthesis and reported enhanced synthesis of proteins and RNA in Avena fatua seeds. Weaver (1972) cited studies which attributed increased enzyme activity to increased synthesis; however, Kaufman (1974) attributed increased enzyme activity in barley aleurone to increased release of enzymes rather than increased synthesis. Cleland et al. (1968) reported increased levels of activity of cell wall hydrolases in Avena, i.e., cellulase, hemicellulase, and pectinase, which has been shown to result in increased plastic extensibility of the cell wall within one hour after treatment with gibberellic acid (Adams et al., 1973; Montague et al., 1973). The above conditions have been shown to result in changes in osmotic potential at the cellular as well as whole plant level. Ende and Koornneef (1960) reported a higher osmotic potential in tomato plants; however, Castro (1976, cited in Castro and Rossetto, 1979) reported lower osmotic potential in tomatoes treated with gibberellic acid. Other plants in which lower osmotic potentials have resulted from gibberellin treatment include lettuce (Stuart and Jones, 1977), sunflower (de la Guardia and Benllock, 1980), cucumber (Katsumi,

et al., 1980), and cotton (Castro and Rossetto, 1979). Castro and Rossetto (1979) reported that gibberellic acid treatment lowered the osmotic potential of cotton plants such that it interfered with aphid feeding. Thus, gibberellic acid increased activity of hydrolytic enzymes results in conditions leading to increased cellular growth by (1) increased cellular osmotic concentration which permits water to enter the cell more rapidly thus diluting the sugars and causing cell expansion (Kato, 1956; Audus, 1972; Cleland et al., 1968; Salisbury and Ross, 1978; de la Guardia and Benllock, 1980); (2) increased cellular plasticity which allows the cell walls to stretch in response to the change in osmotic potentials (Addicott, 1970; Stuart and Jones, 1977; Molz and Boyer, 1978); and/or (3) increased availability of hexose molecules which provide energy for respiration and the formation of pectin and hemicellulose, the cell wall matrix polysaccharides and cellulose, the microfibril fraction of the cell wall (Cleland et al., 1968; Kaufman, 1974; Salisbury and Ross, 1978).

Kaufman (1974) and more recently Rappaport (1980) reviewed control points for the physiological activity of gibberellins. Figure 1, as taken from Kaufman (1974), provides a schematic representation of mechanism of gibberellic acid in growth metabolism at the cellular level as discussed above. This scheme has the following basic features:

(a) Under natural conditions photosynthetically produced sugars function as the substrate for tissue growth.

(b) Sugars enter the tissue at rates determined by transport mechanisms and membrane permeability and become incorporated into a pool of simple or phosphorylated sugars.

(c) Some of the saccharides are converted to storage carbohydrates (starches and fructans); others are utilized in respiratory metabolism or enter metabolic pathways leading to synthesis of cellular components.

(d) Respiratory cellular energy (ATP) is used by endergonic processes such as active transport and synthetic pathways.

(e) Cell wall components and properties are adjusted in order to allow for cellular growth.

(f) Protein synthesis is stimulated in order to allow for growth. Many of these proteins are specific enzymes needed for catalyzation of growth metabolism.

(g) The asterisks in Figure 1 represent possible control points at which gibberellins are thought to regulate key metabolic pathways.

The above discussion indicates that most workers have concentrated on metabolic pathways to explain gibberellin actions. An increasingly popular explanation is in terms of alterations of membrane permeability (Rappaport, 1980). Wood and Paleg (1972) were the first to clearly demonstrate that gibberellic acid can influence the permeability of model membranes. Wood and Paleg (1972) proposed that effects on sub-cellular membrane permeability were due a biophysical alteration of one or more of the membrane components through some sort of bond formation. Involvement of endoplasmic reticulum and increased enzyme synthesis was summarized by Mann (1974) and further confirmed with electron microscopy by Pyliotis et al. (1979). However, as noted by Kaufman (1974), no one mechanism has been elucidated for gibberellins and regulatory sites of this hormone appear to occur at several points in plant growth metabolism.

Figure 1. Schematic representation of GA in growth metabolism (see text for explanatory details). (Modified from Kaufman, 1974)

Interaction with Other Plant Growth Substances

Interaction or synergism between gibberellins and other plant growth substances, auxins in particular, has been evaluated in a variety of plant species and experimental conditions. Nitsch and Nitsch (1956) evaluated effects of various combinations of IAA and GA₃ on oat coleoptiles and the first internode and demonstrated less than additive growth responses in all but one combination of the two substances. Kato (1958b) noted increased growth response of GA and IAA in cucumber over IAA alone; however, since the response was less than additive, no synergism was implied. Marth et al. (1956) commented that wounding of several species prior to application of GA₃ increased the GA₃ response, suggesting synergism with wound hormones. Stowe and Yamaki (1957) cited increased responses to combinations of IAA and gibberellin which were more than additive in pea epicotyls. Ng and Audus (1964 and 1965) demonstrated the requirement for an unidentified endogenous substance from Avena internodes in order to induce a synergistic interaction between GA₃ and applied IAA or 2,4-D on Avena stem segments. Audus (1972) reported a three-fold increase in sensitivity of rice leaves to IAA when also treated with GA₃. Pieterse et al. (1980) and Pieterse and Roorda (1982) reported a tenfold increase in the activity of 2,4-D on Eichhornia crassipes when applied in combination with GA₃.

The observed interactions between gibberellins and applied natural and synthetic auxins have revolved around two possible mechanisms (1) an alteration of the endogenous auxin levels, or (2) an alteration of the rate of translocation of auxins. Numerous studies have reported enhanced biosynthesis of IAA gibberellin (Galston and Purves, 1960;

Paleg, 1965; Varga and Humphries, 1974; Maheshwari et al., 1980).

However, others have reported that observed synergistic interactions were due to an auxin-sparing reaction wherein applied gibberellins inhibited or reduced the concentration of auxin degrading enzymes such as IAA oxidase (Brian and Hemming, 1958; Kogl and Elema, 1960, cited in Weaver, 1972; Galston and Purves, 1960; Sarma, 1979).

Ashton (1959) postulated that the reason 2,4-D was more effective as a herbicide in plants that were actively growing was due to an increase in translocation; therefore, the growth promoting properties of GA might increase the effectiveness of 2,4-D. To test this hypothesis red kidney bean plants were pretreated with 100 mg/l of the potassium salt of GA₃ prior to the treatment of a single primary leaf with radioactively labeled 2,4-D. Twenty-four hours after 2,4-D treatment the amount of 2,4-D in the whole plant was higher than plants not receiving GA₃ treatment. This effect disappeared after 72 hours and was shown not to be due to a reduction in the breakdown of 2,4-D by GA₃. Similar results were reported with 2,4-D by Basler (1959) and 2,4,5-T by Basler (1974) utilizing Phaseolus vulgaris. Pilet (1965) reported pretreatment of Lens culinaris epicotyl segments with GA₃ increased the uptake and velocity of movement of applied IAA out of apical growing regions. Basler (1974) demonstrated that increased translocation of 2,4,5-T by GA₃ appeared to be specific to 2,4,5-T, since the translocation of labeled sucrose-³H and glycine-¹⁴C were relatively unaffected by GA₃ after 4 to 24 hours post treatment. Pieterse and Roorda (1982) also hypothesized that increased sensitivity-of waterhyacinths to 2,4-D when combined with GA₃ was due to increased translocation. The only

study discussed above which either suggested or investigated a possible mechanism for GA₃-enhanced translocation of auxin compounds was that of Basler (1974). Utilizing cycloheximide, a protein synthesis inhibitor, it was shown that continuous protein synthesis was needed to maintain high rates of 2,4,5-T translocation in bean seedlings. The simultaneous treatment of 2,4,5-T, GA₃ and cyclohexamide negated the enhancement of translocation of 2,4,5-T by GA₃. In an unrelated study utilizing corn (Zea mays) seedlings, which does however demonstrate the interrelationship of various plant growth substances, synergism was demonstrated between GA₃ and fluridone, a herbicide which blocks carotenoid synthesis (Devlin et al., 1980). The exact mechanism of synergism was unknown; however, Devlin et al. (1980) suggested that since carotenoids are precursors of abscisic acid (ABA) and since ABA and GA had been shown to be mutually antagonistic (Corcoran, 1974), reduced ABA levels caused by the absence of carotenoids might allow greater expression of GA₃ activity.

Audus (1972) summarized the interaction of plant hormones by stating that there may be no underlying interdependence of gibberellins and auxin-like compounds because (1) gibberellins will produce different metabolic responses in different tissues, and (2) growth or developmental process may be subject to the regulatory action of a balance of several hormones whose points of action may be quite independent but appear to interact due to the mutual association of each component to the total growth system of a given species.

Commercial Applications

The numerous physiological and morphological effects of gibberellins have led to investigations into potential commercial uses. Initial

applications of gibberellins to seeds, soil, or growing plants for the purpose of increasing crop yields generally have not produced encouraging results. In comprehensive field trials by Morgan and Mees (1956), treatment with gibberellic acid failed to increase yields in wheat, potatoes, turnips, carrots, peas, runner beans, lettuce, celery, black currants, kale, and corn. During these trials, vegetative growth of most of the treated plants was stimulated but no increase in crop yield occurred. Marth et al. (1956) conducted a similar series of trials utilizing 42 different species of plants and also reported increases mainly in vegetative growth. Stuart and Cathey (1961) reviewed the applied aspects of gibberellins and reported that gibberellins are applied to plants cultivated for their flowers in order to (1) replace the requirement for cold temperatures for flowering; (2) accelerate flowering; and (3) enlarge and extend the lasting quality of inflorescences. Current routine commercial uses of gibberellins, as reported by Salisbury and Ross (1978), include increasing the size and distance between Thompson seedless grapes, increasing the rate of the malting processes in breweries, increasing stalk length and crispness of celery, delaying senescence in various fruits, and as an aid in fruit set. Abbott Laboratories, Inc., markets gibberellic acid for the treatment of turf grasses in order to initiate growth and prevent color change during cold stress. Abbott Laboratories has also registered gibberellic acid under the brand name Pro-Gibb® (EPA registration numbers 275-20, 275-15, and 275-12) for use on various crops. Rates of application under these registrations vary from 1.1 g/ha to obtain uniform bolting and increase lettuce seed production

to 208 g/ha to increase sucrose yield in sugar cane. No routine commercial use of gibberellins in aquatic environments could be found.

Stuart and Cathey (1961) observed that gibberellins often induce maximum responses when growth conditions are adversely affected by temperature, nutrition, light or other environmental factors, and postulated that under these conditions the synthesis of endogenous gibberellins and gibberellin inhibitors may be retarded permitting the greater response from applied gibberellins. The major limiting factors to the use of gibberellins have been the additional cost, the failure to increase yields of major crops, and the fact that the only method of producing them is through the culture of the Gibberella fungus (Salisbury and Ross, 1978). Stuart and Cathey (1961) stated that future commercial applications will depend upon additional data concerning methods and timing of application and the interaction or synergism with other endogenous and applied plant growth regulators. Based on this review, this statement still appears to apply to gibberellin technology.

Effects on Waterhyacinths

Pieterse et al. (1976) reported that the formation of bulbous or "float" type waterhyacinth leaves could be inhibited by growing the plants in low concentrations (0.03 mg/l) of GA_3 in water under greenhouse conditions. The ratio of petiole length and the greatest circumference was approximately 1.0 at 0.00 mg/l GA_3 , 4.20 at 0.03 mg/l GA_3 and 12.4 at 1.00 mg/l GA_3 . The general growth pattern of leaves following treatment with low concentrations of GA_3 was characterized as similar to morphological responses of plants growing in dense stands or rooted in the shallow hydrosol as described by Penfound and Earle

(1948) and Center and Spencer (1981). GA_3 also markedly inhibited vegetative reproduction and induced profuse flowering. Watson et al. (1982) reported similar effects on leaf morphogenesis, vegetative reproduction, and flower production when GA_3 (0.00 to 0.05 mg/l) was applied to waterhyacinth roots. However, at concentrations greater than 0.05 mg/l GA_3 , higher rates of production of inflorescences and daughter rosettes were observed. Watson et al. (1982) also reported an increase in stolon elongation at GA_3 concentrations up to 0.03 mg/l. However, above 0.05 mg/l, GA_3 caused a decrease in stolon length and suppressed stolon production at 1.0 mg/l. Foliar application of GA_3 at a rate of 6.0 g/ha was also shown to inhibit the formation of float-type leaves although apparently not as dramatically as when GA_3 was applied to the roots (Pieterse et al., 1980; Pieterse and Roorda, 1982).

2,4-Dichlorophenoxyacetic Acid

History

The chemical, 2,4-dichlorophenoxyacetic acid (2,4-D), is a systemic herbicide which is routinely used for the control of broadleaf weeds (Weed Science Society of America, 1979). The synthesis of 2,4-D was a result of research initiated in the 1880's by Julius Sachs, a German botanist, who suggested the presence of plant organ-forming substances (Salisbury and Ross, 1978). The existence of naturally occurring compounds which stimulated plant growth was first demonstrated by Seubert in 1925 (Weaver, 1972). In 1926, Went developed a procedure for quantitative isolation of growth-promoting compounds which stimulated their isolation and identification. Went was also the first to call these compounds auxins (Weaver, 1972; Salisbury and Ross, 1978).

Auxin has become a general term for a group of compounds which typically induce elongation of shoot cells (Brian et al., 1955; Weaver, 1972) and they typically produce physiological responses similar to indoleacetic acid (IAA), a naturally occurring growth substance (Brian et al., 1955; Weaver, 1972; Black and Buchanan, 1980). The auxin discovered by Went was indoleacetic acid, IAA (Salisbury and Ross, 1978) and is considered to be the most common auxin occurring in higher plants.

IAA was found to be relatively unstable and a search was begun for synthetic compounds of similar chemical constitution and growth-promoting activity. In 1942, work was begun with a series of substituted phenoxyacetic acids of which 2,4-D is a member (Zimmerman and Hitchcock, 1942). 2,4-D is considered to be a synthetic auxin because it causes growth reactions similar to the naturally occurring indole auxins; however, it is more active and persists in the plant for a longer period of time than IAA (Van Overbeek, 1964). With the advent of World War II the idea developed that auxins might be used in high concentrations to kill enemy crops or limit crop yields (Norman, 1946), and the initiation of tests to study the potency of these new compounds including 2,4-D was begun. Many of these studies were conducted in 1944 and 1945 as a part of the activities of the Special Projects Division, Chemical Warfare Service at Camp Detrick in Fredrick, Maryland (Norman, 1946). Publication of this early work was delayed due to wartime security policies (Norman, 1946); however, the June, 1946 issue of the Botanical Gazette contained 18 papers describing the results of the Camp Detrick studies. Commercial application of 2,4-D began soon after early trials such as those conducted by Blackman (1945) which demonstrated that broad-leaved weeds growing in grain

fields could be selectively killed without injury to adjacent cereal crops. Herbicidal effects of 2,4-D were described by F. D. Jones in U. S. Patent No. 2,390,941 and this patent was assigned to the American Chemical Paint Company (Weed Science Society of America, 1979). Black and Buchanan (1980) credit recognition of the herbicidal properties of 2,4-D as the catalytic discovery which led to development of many chemically related herbicides and to development of weed control as a scientific discipline.

Physical and Chemical Characteristics

Commercial concentrates of 2,4-D are generally formulated as salts or esters. The pure acid is a white, odorless crystal with a molecular weight of 221 and a molecular formula of $C_8H_6Cl_2O_3$ (Weed Science Society of America, 1979). According to the Weed Science Society of America (1979), the solubility of 2,4-D acid formulation in various solvents is as follows:

<u>Solvent</u>	<u>g/100g solvent</u>
Acetone	85.0
Diesel oil and kerosene	0.10 to 0.35
Ethanol, 50 percent	10.3
Ethyl alcohol, 95 percent	130.0
Isopropanol	31.6
Water	0.09

The dimethylamine salt formulation is extremely soluble, 300 g/100g of water, soluble in alcohols and acetone, but insoluble in kerosene and diesel oil. The butoxyethanol ester of 2,4-D is insoluble in water, but soluble in most organic solvents.

The pure acid formulation of 2,4-D is prepared by combining 2,4-dichlorophenol and monochloroacetic acid. The salts are formulated by addition of amines or inorganic hydroxide to the pure acid. Esters are produced by reaction of 2,4-D with the appropriate alcohols.

Toxicology

Effects of various formulations of 2,4-D have been evaluated for a wide range of organisms in both the aquatic and terrestrial food chains. Studies have shown that toxicity of 2,4-D varies with the formulation utilized in the evaluations (Davis, 1970; Duke, 1971; Weed Science Society of America, 1979; Halter, 1980). Acute oral toxicity (LD_{50}) of the various formulations of 2,4-D has been reported to be 300 to 1,000 mg/kg for rats, guinea pigs, and rabbits (Weed Science Society of America, 1979). Hansen et al. (1971) reported that rats fed from 0 to 1250 mg/kg 2,4-D acid for two years exhibited no significant effect on growth rate, survival rate, organ weights, or hematologic values. Ninety-three percent of dogs fed from 0 to 500 mg/kg 2,4-D acid in their diet for two years were clinically normal with no 2,4-D related effects noted (Hansen et al., 1971). In a critical review of the effects of 2,4-D on the aquatic environment, Halter (1980) reported no effects of 2,4-D acid on phytoplankton at rates as high as 300 mg/l. Following a large-scale application of the BEE formulation at a rate of 49.7 kg/ha (a.e.), Whitney et al. (1973) reported no difference in plankton populations following herbicide treatment. A review of the effects of both the DMA and BEE formulations on benthic invertebrates following routine aquatic weed control operations indicates no adverse effects due to the herbicide, other than those caused due to habitat

changes (Halter, 1980). Davis and Hardcastle (1959) reported a 48 hour LC_{50} of 375 and 350 mg/l of the DMA formulation for bluegills (Lepomis macrochirus) and largemouth bass (Micropterus salmoides) respectively. However, LC_{50} values for bluegills as low as 2.1 mg/l have been reported for the liquid BEE formulation and 34.5 mg/l for granular BEE (Hughes and Davis, 1965). Halter (1980) indicates that 2,4-D is essentially non-toxic to waterfowl. Duke (1971) reported avoidance by mosquitofish (Gambusia affinis) of water treated with the BEE formulation of 2,4-D. Mosquitofish sought water free of 1.0 and 10.0 mg/l 2,4-D, but did not seek water free from 0.1 mg/l (Duke, 1971). Some formulations may cause skin irritation in humans, but no characteristic symptoms of poisoning are documented for humans (Weed Science Society of America, 1979). Moore (1974) reported that electron microscope assay procedures for herbicide and membrane interactions indicated that animals and algae lack biochemical mechanisms (specific binding sites) to respond to 2,4-D. Tolerances for residues resulting from the aquatic application of 2,4-D in food and raw agricultural commodities have been established at 0.10 mg/l for potable water (21 Code of Federal Regulations 123.100, dated December 16, 1975) and 1.0 mg/l for fish and shellfish (40 Code of Federal Regulations 180.142, dated December 9, 1975).

Persistence in the Environment

In terrestrial situations, 2,4-D undergoes microbial breakdown in warm, moist soils in one to four weeks. The actual rate of decomposition depends upon the temperature, moisture, organic matter, and other soil characteristics (Hemmett and Faust, 1969; Weed Science Society of America, 1979; Halter, 1980). Halter (1980) reviewed

thirty-four papers concerning the persistence of 2,4-D in water under both laboratory and field conditions and concluded (1) under laboratory conditions, 2,4-D repeatedly decomposed in water in periods of hours to days; (2) under some warm water field conditions, 2,4-D has repeatedly been shown to be reduced to non-detectable levels (low ppb range) in closed water bodies in approximately one month; and (3) persistence of 2,4-D at extremely low levels may be encouraged by water movements in lakes, reservoirs, and streams. Joyce and Sikka (1977) randomly monitored 2,4-D levels in a large riverine system for seven months in conjunction with routine waterhyacinth control operations utilizing 2.24 to 4.48 kg(a.e.)/ha 2,4-D DMA and reported 2,4-D levels from non-detectable to 1.3 $\mu\text{g/l}$. Smith and Isom (1967) reported similar residue levels in large freshwater reservoirs in the Tennessee Valley in conjunction with Eurasian watermilfoil (Myriophyllum spicatum L.) treatments at 44.8 to 112.0 kg(a.e.)/ha 2,4-D BEE. Schultz (1973) reported that 2,4-D DMA persists in the hydrosol at the mg/l level for about one month, whereas Smith and Isom (1967) documented the persistence of 2,4-D BEE in hydrosol at 58.8 mg/kg 10 months after treatment. Hemmett and Faust (1969) demonstrated that biodegradation of 2,4-D follows zero-order kinetics, with the oxidation rate independent of substrate (2,4-D) concentration. The rate was dependent upon (1) period of time in which the system has acclimatized to 2,4-D; and (2) the natural condition of the aquatic environment. The various formulations of 2,4-D also do not persist or bioaccumulate in fish (Schultz, 1973; Whitney et al., 1973; Sikka et al., 1977; Halter, 1980), blue crabs, Callinectes sapidus (Joyce and Sikka, 1977) and benthic invertebrates (Whitney et al., 1973; Halter, 1980). Hildebrand (1946) during the first documented

application of 2,4-D to waterhyacinths noted "no adverse effects to water fauna" during and after the application of 2,4-D. After over 30 years of continuous use in aquatic environments, monitoring continues to indicate no adverse effects to water fauna due directly to 2,4-D (Smith and Isom, 1967; Whitney et al., 1973; Moore, 1974).

Mode of Action

The mode of action of 2,4-D has been studied more than any other herbicide (Ashton and Crafts, 1973; Mullison, 1982). Most reviews of the mode of action of 2,4-D indicate that it affects almost every biological activity of a plant (Brian, 1964; Carns and Addicott, 1964; Kiermayer, 1964; Wort, 1964a; Ashton and Crafts, 1973; and Mullison, 1982). However, the primary mechanism and site of action has not been clearly established (Ashton and Crafts, 1973, Weed Science Society of America, 1979; Black and Buchanan, 1980; Mullison, 1982). Van Overbeek (1964) and Black and Buchanan (1980) suggested that the growth of a plant is regulated by rhythmic fluctuations in levels and locations of plant growth substances. This fluctuation is interrupted by 2,4-D and orderly plant development is altered. Immature cytoplasm is prevented from maturing and mature cytoplasm reverts back to an immature physiological state (Van Overbeek, 1964). Therefore, 2,4-D can be effective throughout the life of susceptible species, but is especially effective during immature stages when endogenous levels of growth hormones are highest and the plant is actively growing (Black and Buchanan, 1980).

Morphological and physiological responses by plants to 2,4-D depend upon the sensitivity and physiological condition of the treated species

(Ashton and Crafts, 1973; Mullison, 1982) and the rate and type of formulation applied (Weaver, 1972). At low application rates, responses are typical of those caused by plant growth substances and may induce rooting, blossom set, ripening of fruit, and delaying preharvest drop (Wort, 1964b; Weed Science Society of America, 1979). At higher application rates, 2,4-D, typically causes epinasty or downward twisting and bending of stems and petioles and curling of leaves (Cardenas et al., 1968; Weaver, 1972; Black and Buchanan, 1980; Mullison, 1982). Young leaves cease expanding due to cell elongation, photosynthesis is reduced, and chlorosis may occur (Turkey et al., 1945; Van Overbeek, 1964; Aston and Crafts, 1973). As the herbicide is translocated through the plant, mature parenchyma cells tend to first swell and then divide radially more rapidly producing callus tissue and root primordia which results in the blockage of phloem tissue and the cessation of assimilate transport in the phloem (Turkey et al., 1945; Van Overbeek, 1964; Ashton and Crafts, 1973). Meristem activity is inhibited and new organ or lateral bud growth may occur (Weaver, 1972). Growth of mature or primary roots is inhibited and roots may lose the ability to take up water and salts (Van Overbeek, 1964; Wort, 1964a; Cardenas et al., 1968; Mullison, 1982). The progression of these responses ultimately leads to the withering, collapse, and death of 2,4-D sensitive species due to a combination of new and irregular leaf and root growth and inadequate nutrition because of phloem blockage (Ashton and Crafts, 1973). At high application rates, 2,4-D functions as a contact herbicide, does not translocate throughout the plant and thus may not completely kill meristematic tissue (Ashton and Crafts, 1973).

The physiological response of plants to 2,4-D has been attributed to the inhibition and/or stimulation of respiration, blockage of protoplasmic streaming, alteration of DNA transcription and/or RNA translocation, and interference with enzyme regulatory systems (Brian, 1964; Wort, 1964a; Ashton and Crafts, 1973; Yamada et al., 1974; Mullison, 1982). During the mid-1950's numerous papers appeared which linked auxin action with nucleic acids (Ashton and Crafts, 1973). West et al. (1960) reported an increase in the protein and RNA content of cucumber stem tissue when treated with herbicidal concentrations of 2,4-D. This led to the assumption that the cytochemical basis of 2,4-D action was a stimulation of nuclear activity and a reversion to meristematic metabolism (Chrispeels and Hanson, 1962). It was later shown that this increase in RNA was primarily in the ribosomal fraction which was also accompanied by an increase in a messenger type RNA (Key and Shannon, 1964; Key, 1964). Shannon et al. (1964) suggested that 2,4-D induced protein synthesis and excess nucleic acids would preclude normal cell function and that this was the biochemical basis for the herbicidal action of 2,4-D. Chen et al. (1972) experimented with a 2,4-D tolerant wheat species and a 2,4-D sensitive cucumber species and demonstrated that as 2,4-D concentrations increased, the RNA content of wheat showed a net decrease, whereas the cucumber RNA content increased by over 200 percent. Chen et al. (1972) postulated that the ability to resist alteration of RNA species by a plant was the basis for the selectivity of 2,4-D. Ashton and Crafts (1973) summarized the effects of 2,4-D on nucleic acid and protein content of susceptible species and indicated that this was the main biochemical reaction to 2,4-D. This increase in RNA was attributed to an inhibition of the synthesis of ribosomal RNase

which prevented RNA catabolism. The presence of high levels of ribonucleases in other resistant grasses has furthered this hypothesis (Ashton and Crafts, 1973). Wort (1964a) reviewed the effects of 2,4-D on cellular enzymes and documented effects on the activity of 16 enzymes including amylase, ascorbic acid oxidase, IAA oxidase, invertase, phosphorylase, and proteolytic enzymes. These changes were postulated to be caused by changing (1) the cellular conditions such as pH or hydration under which enzymatic progress occurs; (2) the supply of material for enzyme formation; and/or (3) the supply of energy necessary for endergonic reactions through alteration of ATP production (Wort, 1964a). In support of these latter two mechanisms, Mostafa and Fang (1971) hypothesized seven sites where 2,4-D either inhibits or regulates respiratory breakdown of glucose thus increasing or decreasing the concentration of various metabolites. Various studies have documented inhibition of the Hill reaction and oxidative phosphorylation; however, Ashton and Crafts (1973) consider these effects to be of secondary importance.

Much of the discussion surrounding 2,4-D mode of action deals with which response is the cause and which is the effect of auxins and auxin-like substances (Audus, 1972). Morgan and Hall (1962) documented that 2,4-D treated cotton plants exhibited an increased release of ethylene, a gaseous plant hormone which can cause epinasty. Ashton and Crafts (1973) attributed the initial epinastic effects to cell elongation and not to an ethylene effect; however, subsequent cell divisions and cell proliferation are perhaps caused by ethylene, resulting in phloem blockage and eventually death.

Ashton and Crafts (1973) summarized this issue by stating, "the mode of action of the chlorophenoxy compounds must consist of a great number of structural and biochemical reactions revolving around the central theme of prolonged abnormal growth with failure of those changes characteristic of maturity and senescence. In no other way may the great number and diversity of structural and metabolic changes be reconciled." (Ashton and Crafts, 1973, page 284).

Translocation

Polar salts of 2,4-D are absorbed more readily by the roots of most species, whereas ester formulations are more readily absorbed by leaves (Weaver, 1972; Weeds Science Society of America, 1979). Foliar-absorbed 2,4-D is transported polarly within the phloem with assimilated sugars, and root-absorbed 2,4-D moves upward in the xylem during transpiration (Audus, 1972; Weaver, 1972). Movement is towards rapidly growing tissues, such as developing flowers and meristematic shoots and roots (Audus, 1972; Weaver, 1972; Ashton and Crafts, 1973). Thus, thorough distribution of a herbicide, such as 2,4-D, is dependent upon the active movement of foods in the plant. However, not all species absorb and translocate 2,4-D at the same rate. Fang (1958) demonstrated with radioactive labeled 2,4-D that the rate of translocation of 2,4-D was slower in peas and tomatoes than in bean plants. Morphological characteristics that prevent 2,4-D absorption and translocation and the plant's ability to conjugate or metabolize 2,4-D have also been suggested as mechanisms of selectivity by tolerant species (Fang, 1958; Mullison, 1982).

Metabolism by Plants

A herbicide has been defined as a compound which deranges the physiology of a plant over a period long enough to kill it (Van Overbeek, 1964). Thus, those plants which can more effectively metabolize or inactivate the herbicide generally exhibit less sensitivity to the herbicide. Numerous studies indicate that plants exhibit varying abilities to either metabolize or inactivate 2,4-D once it has entered the plant (Weintraub et al. 1952; Fang, 1958; Crafts, 1964; Van Overbeek, 1964; Ashton and Crafts, 1973; Feung et al., 1978). The most common mode of metabolism appears to be decarboxylation and hydrolysis to free phenol (Crafts, 1964; Ashton and Crafts, 1973). One of the first investigations into the metabolism of 2,4-D by plants was conducted by Weintraub et al. (1952) utilizing radioactive 2,4-D with ^{14}C in either the carboxyl, ethylene, or ring positions. These studies indicated that $^{14}\text{CO}_2$ was readily released from the carboxyl position and was not released from phenol ring positions. Weintraub et al. (1952) also documented the presence of a wide variety of metabolites. Ashton and Crafts (1973) suggested that a plant's ability to oxidize carboxyl and ethylene carbon atoms of 2,4-D correlated with its tolerance to 2,4-D. Other mechanisms of 2,4-D metabolism or inactivation by plants include (1) ring hydroxylation followed by oxidation of hydroxyls to carboxyls with ring splitting (Crafts, 1964; Ashton and Crafts, 1973; Feung et al., 1978); (2) complexing with proteins (Fang, 1958; Crafts, 1964; Van Overbeek, 1964); (3) complexing with amino acids followed by ring hydroxylation (Feung et al., 1978); (4) sugar conjugation (Feung et al., 1978); and (5) ring hydroxylation followed by sugar conjugation (Feung et al., 1978; Weed Science Society of America, 1979).

Efficacy of 2,4-D on Waterhyacinths

The first recorded use of 2,4-D for the control of waterhyacinths appeared to be in April 1945 near Tampa, Florida (Hildebrand, 1946). Hildebrand (1946) applied 0.059 to 0.125 percent (by volume) 2,4-D solutions to 0.006 ha plots and reported nearly complete control. Seale and Allison (1946) evaluated five 2,4-D esters, four 2,4-D amine salts, and five inorganic salts of 2,4-D and reported that the ester formulations were the most effective; however, at higher rates (> 2.24 kg/ha in 934 l/ha aqueous solution) the amine and inorganic salt formulations were equally effective. Seale and Allison (1946) reported the first effective aerial application of 2,4-D to waterhyacinths. Hitchcock et al. (1949) conducted extensive evaluations at various rates (2.24 to 17.92 kg/ha) and spray volumes (56.0 to 168 l/ha) and concluded that the principal limiting factors affecting 2,4-D efficacy for waterhyacinth control were (a) concentration and rate of application, (b) rate of delivery of spray solution, (c) stage of growth and development, and (d) atmospheric conditions. Application of 2,4-D to mature waterhyacinths with daughter plants attached by stolons resulted in death of the parent plants; however, daughter plants survived (Hitchcock et al., 1949). Singh and Muller (1979b) used radioactively labeled 2,4-D to confirm these observations by demonstrating maximum transport of labeled 2,4-D to daughter plants from the parent up to the two-leaf stage of the daughter plants and no translocation after the daughter plants reached the four-leaf stage. Singh and Muller (1979a) also demonstrated that radioactively labeled 2,4-D when applied to a single waterhyacinth leaf was transported (a) in small amounts (23.5 percent of total applied); (b) at

a slow rate (maximum amount in six days); and (c) almost entirely towards the newly developing leaves (20.8 percent of total applied). Singh and Muller (1979a) used radioactively labeled 2,4-D to demonstrate that three hours after spraying single waterhyacinths at a rate of 0.75 kg/ha in a spray volume of 800 l/ha, 53.3 percent of the total sprayed solution was in the water culture medium. It was also shown that waterhyacinths grown in culture medium containing labeled 2,4-D can absorb and translocate sufficient 2,4-D from treated water to result in their death if the 2,4-D concentration is above 1.0 mg/l. Thus, Singh and Muller (1979a) suggested that immediately after spraying 2,4-D in the field, the upper surface layer of water, if exposed, might contain concentrations of 2,4-D which would be readily available for uptake by waterhyacinth roots. Others have suggested that 2,4-D which is added to the water surface from spray drift or runoff has an added effect and may be a factor in explaining why waterhyacinths grown in small containers in greenhouse experiments appear to be more sensitive to lower rates of 2,4-D as compared to field studies (Hildebrand, 1946; Hitchcock et al., 1949; Koch et al., 1978).

Koch et al. (1978) reported an aerial application rate of 4.5 kg/ha in spray volumes as low as 15 l/ha in the White Nile River, Sudan. This rate was deemed effective due to the low relative humidity and high temperatures in southern Sudan. The reliability of applications was also deemed to be reduced at spray volumes <100 l/ha. Public agencies responsible for aquatic plant control in Florida routinely apply 2,4-D to waterhyacinths at rates of 2.24 kg/ha in aqueous spray volumes of 467 to 934 l/ha for ground applications and up to 4.48 kg/ha in spray volumes of 56.7 l/ha for aerial applications. The U.S. Army Corps of

Engineers, Jacksonville District, applies these latter rates of 2,4-D under a control program which maintains the waterhyacinth population at a minimum non-problematic level (Joyce, 1977). This approach has reduced by 50 percent the quantity of herbicide utilized annually on the St. Johns River, Florida, for waterhyacinth control (McGehee, 1982).

PART 1 - SMALL PLOT EVALUATIONS

Introduction

Pieterse et al. (1980) reported a ten-fold increase in the sensitivity of waterhyacinths to 2,4-D due to a synergistic effect of 2,4-D and gibberellic acid. These investigations were conducted under greenhouse conditions in 200 x 100 x 50 cm concrete reservoirs. Plants were first treated with an atomizing spray system with concentrations of 2,4-D (amine salt) ranging from 0 to 1000 g/ha at a volume rate of 200 l/ha. GA was then applied to control plants and plants which received 0, 50, 100, and 200 g/ha of 2,4-D. Concentrations of GA₃ utilized were 0, 2, 4, 6, and 8 g/ha at a volume rate of 200 l/ha. Results of these experiments indicated that combinations of GA and 2,4-D at 6 to 8 g/ha and 100 g/ha, respectively, caused death of the plants within one week. The same response was noted in plants which received only 1000 g/ha 2,4-D. Pieterse and Roorda (1982) reported similar results when the 2,4-D and GA were applied simultaneously in the same solution at an extremely low volume rate of 40 l/ha.

Based on results of these two studies, it was hypothesized that (1) such a large reduction in the quantity of 2,4-D might lower the costs of control programs even though costs of GA is relatively high (Pieterse et al., 1980) and (2) a decrease in the 2,4-D concentration would lower the risk to nearby native vegetation or crops (Pieterse and Roorda, 1982). The objective of this part of the overall study was to evaluate this

reported synergism of GA and 2,4-D for the control of waterhyacinths in an outdoor environment in order to eliminate the "greenhouse" effect reported by Hitchcock et al. (1949) and Koch et al. (1978).

Materials and Methods

Waterhyacinths used in this study were collected from Lake Ocklawaha near Palatka, Florida, and a small tributary stream of the St. Johns River in Jacksonville, Florida. Plants were maintained in outdoor pools prior to experimental use or placed directly into the experimental containers after field collection. Regardless of initial source, all plants were allowed to remain in the experimental containers for a period of three to four days prior to initial weighing and treatment. Experiments were conducted during the 1980, 1981, and 1982 growing seasons.

Initial experiments were conducted outdoors in full sunlight in approximately 70-liter metal barrels lined with polyethylene bags. All barrels were filled with tap water to within 2.5 cm of the top. Eight grams of commercially prepared 20-20-20 soluble plant nutrients and micronutrients were dissolved in the barrels to yield a calculated nitrogen concentration of 20 to 22 mg/l (approximately equivalent to 10.0 percent Hoagland's solution). Solution levels were maintained as necessary by addition of tap water. Experiments were set up in three replications of five to six plants each, depending upon size and weight of the plants. Waterhyacinth plants used in the experiments were selected on the basis of appearance, freedom of disease, and uniformity in size. Prior to placement into barrels, all dead plant material, flower spikes, and daughter plants were removed. Plants were allowed to drain and were lightly shaken by hand in order to remove excess water from the

roots prior to weighing. Initial fresh weights were obtained on a Mettler balance and recorded to the nearest 0.0 g. During the first five series of treatment replications, 45 individual plants were sampled for determination of the percent dry weight which was used for calculation of initial dry weights. The percent dry weight was consistent (4.72 percent) and always within reported values (Penfound and Earle, 1948; Bock, 1966; Westlake, 1963; Knipling et al., 1970).

Treatments were conducted in a completely randomized block design. Treatments consisted of the simultaneous application of various combinations of gibberellic acid (GA_3 , Eastman Co.) and 2,4-D (Union Carbide Corporation) at the following rates 0.0, 23.5, 47.0, 94.0, and 188 g/ha GA_3 and 0.0, 0.28, 0.56, and 1.12 kg/ha 2,4-D. During each series of treatments, an additional treatment was made at a rate of 2.24 kg/ha 2,4-D to simulate routine operational field application rates. Each treatment rate was replicated three times per treatment series and all treatment series were replicated at least twice as separate trials (blocks). One additional treatment series in which the 2,4-D concentration was held constant and GA_3 concentration was varied between 0.0 to 188 g/ha was also included in the analysis. Treatment volumes were approximately 934 l/ha. All applications were made with a hand-held sprayer from a height of 30 cm.

Each treatment series was observed at least twice weekly for evidence of treatment effects and to adjust the water level in the barrels. Plants were harvested after 14 to 17 days. Efficacy evaluations consisted of counting the number of viable plants as determined by the presence of a living meristem (Seale and Allison, 1946), removing necrotic

tissue, and weighing the plants to the nearest 0.0 g in order to determine post-treatment biomass. Periodically, plants were placed in a drying oven at 65 C for 72 hours to determine dry to fresh weight ratios. Dry to fresh weight ratios obtained from subsamples were used to calculate final dry weights of all treated plants.

Results were analyzed by general linear regression procedures. The resulting analysis of variance was used to conduct a trend analysis for regression of the response of the mean percent change in biomass and number of plants caused by the main effects of GA₃ and 2,4-D and their interaction (Chew, 1977). Means of the percent change in biomass and number of plants due to discrete treatment levels were also compared using the Waller-Duncan procedure. The Waller-Duncan procedure was employed in lieu of other multiple comparison methods because it has the advantage of using the observed overall F-value in the calculation of the least significant difference (LSD). This characteristic provides a mechanism for accounting for both the comparisonwise and experimentwise Type I error rates, the lack of which has been a major criticism of the standard Duncan Multiple Range Test (Chew, 1977).

Results and Discussion

Tables 1-1 and 1-2 present the analysis of variance of mean percent changes in dry weight and number of waterhyacinths, respectively, due to treatment with combinations of GA₃ and 2,4-D excluding the 2.24 kg/ha 2,4-D treatment. Analysis of Tables 1-1 and 1-2 indicates that the variability between trials was highly significant ($\alpha=0.01$) and accounted for more of the variability than the differences between replications within trials. The overall response of waterhyacinths to GA₃ and 2,4-D

Table 1-1. Analysis of variance of the effects of GA₃ and 2,4-D on waterhyacinths utilizing general linear models procedures (percent change in weight as the dependent variable).

Sources of variation	d.f	s.s	m.s.	f	
TREATMENT	19	348888.1	183625.3	22.64	**
GA ₃	4	85621.6	21405.3	2.64	*
Linear	1	59705.7	59705.7	7.36	**
Quadratic	1	257.5	257.5	0.03	N.S.
Other	2	25658.4	12829.3	1.58	N.S.
2,4-D	3	3319331.0	1106443.7	136.43	**
Linear	1	2513039.7	2513039.7	309.86	**
Quadratic	1	744276.0	744276.0	91.77	**
Residual	1	62015.3	62015.3	7.65	**
Interaction (GA x 2,4-D)	12	83929.7	6994.14	0.86	N.S.
Linear x Linear	1	2672.8	2672.8	0.33	N.S.
ERROR	196	515763.2	2631.4		
Trial (Treatments)	52	421723.1	8110.1	12.42	**
Reps. (Trials x Treatments)	144	94040.1	653.1		
TOTAL (Corrected)	215	4004644.5			

** Significant at 0.01

* Significant at 0.05

N.S. Not significant

Table 1-2. Analysis of variance of the effects of GA₃ and 2,4-D on waterhyacinths utilizing general linear models procedures (percent change in number as the dependent variable).

Sources of variation	d.f	s.s.	m.s.	F	
TREATMENT	19	8125895.2	427678.7	32.59	**
GA ₃	4	317602.8	79400.7	6.05	**
Linear	1	222983.1	222983.1	16.99	**
Quadratic	1	17977.1	17977.1	1.37	N.S.
Other	2	76642.6	38321.3	2.92	N.S.
2,4-D	3	7680433.9	2560144.6	195.10	**
Linear	1	6210423.2	6210423.2	473.27	**
Quadratic	1	1390888.5	1390888.5	106.00	**
Residual	1	79122.2	79122.2	6.03	*
Interaction (GA x 2,4-D)	12	12785.5	10654.9	0.81	N.S.
Linear x Linear	1	13380.1	13380.1	1.02	N.S.
ERROR	196	1108430.3	5655.3		
Trial (Treatments)	52	682368.0	13122.5	4.44	**
Reps. (Trials x Treatments)	144	426062.3	2958.8		
TOTAL (Corrected)	215	9234325.5			

** Significant at 0.01

* Significant at 0.05

N.S. Not significant

treatments was linear with respect to GA_3 and both linear and quadratic in response to 2,4-D. There also existed a significant undescribed relationship above the quadratic function for the effects of 2,4-D. There was no significant interaction between GA_3 and 2,4-D ($\alpha=0.05$). Thus, 2,4-D treatment levels had a much greater influence over the response observed for waterhyacinths in the barrel studies than did combinations of GA_3 and 2,4-D.

Tables 1-3 and 1-4 present summaries of regression analyses and regression coefficients for the treatment levels of GA_3 and 2,4-D, excluding the 2.24 kg/ha 2,4-D treatment, on the percent change biomass and number of plants, respectively. These analyses demonstrated that (1) the response to GA_3 was linear, (2) the negative response to 2,4-D was both linear and quadratic, and (3) the interaction coefficient for GA_3 and 2,4-D was insignificant, suggesting that at best the effect of GA_3 was only additive. The resulting regression models for the response of waterhyacinths to GA_3 and 2,4-D accounted for 82.9 percent of the variability of mean change in biomass (Table 1-3) and 85.1 percent of mean change in number of plants (Table 1-4). In order to account for an additional portion of the variability of the response to treatments, pretreatment weights of the waterhyacinths was included in the model but found to be insignificant.

Tables 1-5 and 1-6 provide another representation of the results in terms of minimum and maximum values observed, treatment means, standard error of the mean, and Waller-Duncan groupings for percent change in biomass and number of plants. Figures 1-1 and 1-2 provide a graphical representation of the percent change in biomass and number of plants, respectively. The Waller-Duncan comparisons detected significant

Table 1-3. Results of regression analysis to determine the response of waterhyacinths grown in barrels to treatment with GA₃ and 2,4-D. The general form of the equation is $Y = b_0 + b_1x_1 + b_2x_2 + b_3(x_1)^2 + b_4(x_2)^2 + b_5(x_1)(x_2)$ where Y = the percent change in biomass due to treatment and b = the regression coefficients for each independent variable (X). The coefficient of determination for the regression model (R^2) is 0.829 and the regression analysis of variance F-value is 203.65 (5, 210 DF, $P = 0.0001$).

Parameter	Coefficient (b)	T*	Prob. > T
Intercept (b_0)	230.91	26.16	0.0001
GA (b_1)	-0.39	-1.84	0.0675
2,4-D (b_2)	-739.96	-22.12	0.0001
(GA) ² (b_3)	0.0002	0.24	0.8135
(2,4-D) ² (b_4)	414.54	15.13	0.0001
(GA)x(2,4-D) (b_5)	0.13	0.91	0.3663

*T-statistics for the null hypothesis that the coefficient = 0.

Table 1-4. Results of regression analysis to determine the response of waterhyacinths grown in barrels to treatment with GA₃ and 2,4-D. The general form of the equation is $Y = b_0 + b_1x_1 + b_2x_2 + b_3(x_1)^2 + b_4(x_2)^2 + b_5(x_1)(x_2)$ where Y = the percent change in number of plants due to treatment and b = the regression coefficients for each independent variable (X). The coefficient of determination for the regression model (R^2) is 0.851 and the regression analysis of variance F-value is 239.32 (5, 210 DF, P = 0.0001).

Parameter	Coefficient (b)	T*	Prob. > T
Intercept (b_0)	440.26	35.14	0.0001
GA (b_1)	-1.11	-3.70	0.0003
2,4-D (b_2)	-1072.92	-22.60	0.0001
(GA) ² (b_3)	0.0023	1.62	0.1070
(2,4-D) ² (b_4)	567.72	14.61	0.0001
(GA)x(2,4-D) (b_5)	0.28	1.43	0.1549

*T-statistics for the null hypothesis that the coefficient = 0.

Table 1-5. Mean percent change from initial dry weight of water-hyacinths grown in barrels and treated with combinations of GA₃ and 2,4-D.

Treatment Rate 2,4-D (kg/ha)	GA ₃ (g/ha)	Waller Duncan Grouping ¹	Mean	N	Min.	Max.	Std Error
0.00	23.5	A	287.70	6	170.74	353.41	25.52
0.00	94.0	AB	248.41	9	117.56	392.78	31.36
0.00	0.0	BC	220.80	27	88.40	374.05	16.04
0.00	47.0	C	180.82	9	107.59	241.53	18.10
0.00	188.0	C	180.00	9	95.17	274.32	17.39
0.28	0.0	D	45.13	24	-38.13	220.00	13.08
0.28	23.5	D	39.81	6	-24.37	91.60	15.46
0.28	94.0	D	17.55	6	-1.95	44.44	6.70
0.56	0.0	E	-25.53	27	-85.02	85.84	9.60
0.28	47.0	EF	-41.88	6	-93.63	16.49	18.51
0.56	23.5	EF	-52.02	6	-86.96	3.67	16.08
0.56	47.0	EFG	-65.94	9	-98.28	-10.96	9.15
0.28	188.0	FG	-77.34	6	-99.29	-54.95	6.22
0.56	94.0	FG	-81.76	9	-99.71	-61.84	4.08
1.12	0.0	FG	-82.90	24	-100.00	-52.30	2.84
0.56	188.0	G	-94.25	9	-100.00	-69.97	3.34
1.12	47.0	G	-97.82	6	-100.00	-89.70	1.69
1.12	94.0	G	-98.96	6	-100.00	-97.38	0.52
1.12	23.5	G	-99.72	6	-100.00	-98.99	0.18
2.24	0.0	G	-99.89	27	-100.00	-97.80	0.08
1.12	188.0	G	-100.00	6	-100.00	-100.00	0.00

¹ Means with the same letter are not significantly different ($\alpha=0.05$, K ratio =100).

Table 1-6. Mean percent change from initial number of waterhyacinths grown in barrels and treated with combinations of GA₃ and 2,4-D.

Treatment Rate 2,4-D (kg/ha)	GA (g/ha)	Waller Duncan Grouping	Mean	N	Min.	Max.	Std Error
0.00	0.0	A	444.78	27	240.00	750.00	23.54
0.00	94.0	A	432.59	9	340.00	583.33	24.11
0.00	23.5	A	429.44	6	360.00	516.67	25.83
0.00	188.0	B	340.00	9	220.00	460.00	27.49
0.00	47.0	B	318.52	9	240.00	400.00	19.01
0.28	0.0	C	177.64	24	40.00	433.33	20.07
0.28	23.5	CD	146.67	6	116.67	183.33	12.85
0.28	94.0	D	116.11	6	60.00	220.00	22.15
0.56	0.0	E	45.06	27	-80.00	216.67	16.12
0.28	47.0	EF	33.33	6	-60.00	160.00	35.65
0.56	23.5	EFG	-6.11	6	-83.33	120.00	32.16
0.56	47.0	EFGH	-13.33	9	-80.00	60.00	18.86
0.28	188.0	FGH	-23.33	6	-80.00	60.00	22.16
0.56	94.0	GHI	-39.47	9	-80.00	0.00	10.26
1.12	0.0	GHIJ	-54.86	24	-100.00	0.00	5.41
0.56	188.0	HIJ	-68.89	9	-100.00	40.00	15.32
1.12	94.0	IJ	-87.22	6	-100.00	-60.00	6.58
1.12	47.0	IJ	-93.33	6	-100.00	-80.00	4.22
1.12	23.5	IJ	-93.33	6	-100.00	-80.00	4.22
2.24	0.0	IJ	-97.78	27	-100.00	-60.00	1.63
1.12	188.0	J	-100.00	6	-100.00	-100.00	0.00

¹ Means with the same letter are not significantly different ($\alpha=0.05$, K ratio =100).

Figure 1-1. Mean percent change from initial dry weight of waterhyacinths grown in barrels and treated with combinations of GA₃ and 2,4-D.

Figure 1-2. Mean percent change from initial number of waterhyacinths grown in barrels and treated with combinations of GA₃ and 2,4-D.

differences ($\alpha=0.05$) between the GA_3 treatment levels at 2,4-D rate of 0.00 kg/ha; however, the differences were not in proportion to the rate of GA_3 . It was also observed that when 2,4-D rates were held constant, the addition of GA_3 increased the mean response of waterhyacinths and this response was frequently significant particularly at 2,4-D rates of 0.28 and 0.56 kg/ha. This observation supports the observation that the effect of GA_3 on waterhyacinth sensitivity to 2,4-D recorded for this series of observations was additive rather than synergistic.

Summary

Based on regression analysis, the response of waterhyacinths grown outdoors in 70-liter containers to treatments with combinations of 2,4-D and GA_3 does not indicate a synergistic effect or interaction between GA_3 and 2,4-D either in terms of the percent change from initial biomass or a percent change from the initial number of plants. However, at lower rates of 2,4-D, the response does appear to be additive. These findings are not in agreement with results reported for the effects of GA_3 and 2,4-D on waterhyacinths by Pieterse et al. (1980) and Pieterse and Roorda (1982). However, differences in experimental design and environmental controls (greenhouse versus non-greenhouse, open air situations) may account for the lack of agreement.

PART 2 - TRANSLOCATION EVALUATIONS

Introduction

Various studies have shown increases in rates and amounts of translocation of radioactive labeled auxins and 2,4-D when plants were pretreated with GA (Ashton, 1959; Basler, 1959; Pilet, 1965; Basler, 1974). The studies utilized either whole, immature plants or stem segments of bean plants. Singh and Muller (1979b) reported that asulam and amitrole have higher rates of translocation in waterhyacinths than 2,4-D, and this may be the reason they are more efficacious against waterhyacinths. Pieterse et al. (1980) and Pieterse and Roorda (1982) suggested that increased translocation was the mechanism by which waterhyacinths exhibited a ten-fold increase in sensitivity to 2,4-D when also treated with GA. The objective of this portion of the overall investigation was to determine if increased translocation of 2,4-D in waterhyacinths was the mode of action of increased sensitivity of waterhyacinths to 2,4-D when also treated with gibberellic acid.

Materials and Methods

Waterhyacinths for this study were collected from a small tributary stream of the St. Johns River in Jacksonville, Florida. Plants were selected for uniformity of size (approximately seven leaf stage) and apparent freedom from disease and insect damage. All plants were allowed to remain in the growth chamber for 3 days prior to treatment.

A total of 32 waterhyacinths were placed in individual one-liter beakers which were filled with 5 percent Hoagland's solution to within 2 cm of the top. Nutrient solution volumes were replenished approximately every 48 hours. Temperature in the growth chamber was maintained between 16 (night) and 32 (day) C. Light was supplied by eight fluorescent and eight incandescent bulbs which provided 330 micro-Einsteins/ M^2 /sec at plant height. Based on the results of Patterson and Duke (1979), the plants could be expected to photosynthesize at approximately 78 percent of the rate expected at full sunlight. The photoperiod simulated a 14-hour day and a 10-hour night. Prior to treatment with the radioactive labeled 2,4-D, one-half or 16 of the plants were individually removed from their beakers, the roots were shielded with plastic, and the foliage was sprayed to the point of "runoff" with 100 mg/l aqueous solution of the potassium salt of gibberellic acid (Eastman Company). The plants were replaced in the growth chamber, and the GA was allowed to dry on the plants prior to the application of 2,4-D.

Fifty milligrams of ^{14}C ring-labeled 2,4-D, specific activity 940 $\mu Ci/mM$, was dissolved in 5 ml of ethanol. One ml (10 mg of 2,4-D) of this solution was then added to 9 ml deionized water to yield a 1,000 mg/l solution with an activity of 4.25 $\mu Ci/ml$. A total of 0.216 μCi was applied by placing 51 μl of the 1000 mg/l labeled 2,4-D solution in three separate 17 μl droplets on the lamina of a single leaf of each of the plants, according to methods described by Singh and Muller (1979a and 1979b). Treated leaves were either the fourth or fifth leaf from the outside of the rosette of leaves, depending upon which was

most horizontal in orientation to minimize droplet movement. Treated leaves were marked with small gummed labels for ease of identification. Plants were placed at random in the growth chamber.

Four plants treated with GA_3 and 2,4-D and four treated with 2,4-D only were harvested at 1, 3, and 6-day intervals. The above procedure was replicated for the six-day treatment series only. The presence of necrotic tissue, daughter plants, and flower spikes was noted. Plants were then separated into the following fractions: treated lamina, petiole of treated leaf, individual leaves, daughter plants, meristem and youngest leaf, and roots. Plant parts were placed in paper bags and placed in a forced-air drying oven for 72 h at 65 C. Dried plant parts were weighed separately to the nearest 0.01 mg and wrapped in a low-ash, unscented, ungummed cigarette papers. Weighed samples were combusted in a Packard TRICARB sample oxidizer to collect $^{14}CO_2$ for assaying radioactivity. Oxidizer sample burn time was set at 1.5 minutes; however, all samples were completely combusted within 0.5 minute. Six milliliters of OXISORB 2 and 10 ml of OXIPREP (New England Nuclear Ltd., Boston (USA)) were used to absorb the $^{14}CO_2$ and serve as a scintillation fluor, respectively. Radioactivity was assayed by a PACKARD TRICARB scintillation spectrometer. Samples were counted for 10 min and counts per minute (cpm) were converted to disintegrations per minute (dpm) based on a linear regression obtained from a quench curve. The quench curve was obtained by oxidizing blank samples of varying weights. After combustion, known levels of radioactivity were added to the scintillation fluor and the counting efficiency was obtained. All results were reported as dpm/mg of plant material and

as percent of total translocated material present in a given plant part.

Mean dpm/mg of plant material and mean percent of total translocated ^{14}C -labeled material per plant part per harvest day were calculated. Mean dpm/mg and mean percent of translocated 2,4-D of plants receiving pretreatment with gibberellic acid were compared with means from untreated plants. A simple t-test for determining the presence of significant differences between two means was used (Walpole and Myers, 1978). Prior to comparison of the mean percentages of translocated radioactivity, an arcsine transformation was performed in order to normalize the distribution (Sokal and Rohlf, 1969).

Results and Discussion

In general, results presented below indicate that, without regard to gibberellic acid treatment, movement of ^{14}C labeled 2,4-D follows a pattern of polar movement towards rapidly growing tissues such as meristematic shoots and newly emerging daughter plants as previously documented for waterhyacinths and other species (Audus, 1972; Ashton and Crafts, 1973; Singh and Muller, 1979b).

Tables 2-1 and 2-2 summarize the results of the movement through time of the ^{14}C -labeled 2,4-D within treated waterhyacinths expressed as dpm/mg dry plant material and percent of total amount of translocated radioactivity, respectively. Tables 2-3, 2-4, 2-5, and 2-6 present a detail analysis of the results on a dpm/mg basis for the plants harvested on days 1, 3, 6 (trial 1), and day 6 (trial 2), respectively. Tables 2-7, 2-8, 2-9, and 2-10 present a similar analysis of the results on a percent of total amount of translocated ^{14}C -labeled 2,4-D.

Table 2-1. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths; (data expressed as mean dpm/mg dry weight).

	Day 1 (Trial 1)	Day 3 (Trial 1)	Day 6 (Trial 1)	Day 6 (Trial 2)
Treated Petiole (minus lamina)				
With GA	94.58	131.41	71.19	94.92
Without GA	88.50	121.45	146.80	100.16
Other Leaves				
With GA	2.51	5.89	6.00	5.08
Without GA	1.86	3.86	9.93	7.42
Meristem and Youngest Leaf				
With GA	15.05	19.34	14.39	14.96
Without GA	12.50	27.68	35.87	15.35
Daughter Plants				
With GA	15.50	17.29	9.74	15.50
Without GA	17.74	21.66	32.71	55.51
Roots				
With GA	1.85	4.24	5.06	3.94
Without GA	2.03	3.25	10.44*	3.71
Total Translocated				
With GA	13.59	18.23	14.71	14.45
Without GA	12.60	15.08	20.77	18.52

** Significant at $\alpha=0.01$

* Significant at $\alpha=0.05$

Table 2-2. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths; (data expressed as mean percent of total ^{14}C translocated).

	Day 1 (Trial 1)	Day 3 (Trial 1)	Day 6 (Trial 1)	Day 6 (Trial 2)
Treated Petiole (minus lamina)				
With GA	60.74	51.21	43.11	44.06
Without GA	60.64	52.64	33.50	36.83
Other Leaves				
With GA	11.27	15.39	24.29	17.25
Without GA	8.05**	13.16	17.42*	21.49
Meristem and Youngest Leaf				
With GA	19.47	15.37	10.59	6.57
Without GA	19.29	18.00	18.15*	6.08
Daughter Plants				
With GA	3.73	11.91	12.29	19.70
Without GA	5.70	11.13	19.07	29.43
Roots				
With GA	4.77	6.16	9.71	12.42
Without GA	6.33	5.06	11.85	8.16

** Significant at $\alpha=0.01$

* Significant at $\alpha=0.05$

Table 2-3. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the first day post treatment; (means expressed as dpm/mg dry weight).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole (minus lamina)						
With GA	3	94.58	78.69	116.68	11.40	
Without GA	3	88.50	81.63	93.10	3.50	0.637
Other Leaves						
With GA	3	2.51	2.17	3.18	0.33	
Without GA	3	1.86	1.43	2.16	0.22	0.180
Meristem and Youngest Leaf						
With GA	3	15.05	8.66	19.38	3.26	
Without GA	3	12.49	10.60	14.27	1.06	0.497
Daughter Plants						
With GA	3	15.50	7.43	24.60	4.99	
Without GA	3	17.74	16.29	20.19	1.24	0.685
Roots						
With GA	3	1.85	1.78	1.98	0.06	0.165
Without GA	3	2.02	1.90	2.18	0.08	0.165
Total Translocated						
With GA	3	13.59	10.13	17.20	2.04	
Without GA	3	12.60	10.01	14.00	1.29	0.70

Table 2-4. Effects of gibberellic acid (100 mg/l) on the translocation of ¹⁴C-labeled 2,4-D in waterhyacinths on the third day post treatment; (means expressed as dpm/mg dry weight).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole (minus lamina)						
With GA	3	131.41	59.26	167.99	36.08	
Without GA	4	121.45	81.24	175.91	19.82	0.805
Other Leaves						
With GA	3	5.89	2.49	11.84	2.98	
Without GA	4	3.86	2.69	5.21	0.68	0.473
Meristem and Youngest Leaf						
With GA	3	19.34	10.17	24.50	4.59	
Without GA	4	27.68	21.77	39.94	4.17	0.240
Daughter Plants						
With GA	3	17.29	11.18	28.08	5.41	
Without GA	4	21.66	14.85	33.60	4.10	0.539
Roots						
With GA	3	4.24	3.43	4.87	0.46	0.165
Without GA	4	3.25	2.47	4.91	0.56	0.253
Total Translocated						
With GA	3	18.24	6.94	26.76	5.88	
Without GA	4	15.08	12.08	19.31	1.55	0.574

Table 2-5. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the sixth day post treatment in trial 1; (means expressed as dpm/mg dry weight).

	N	mean	min.	max.	Std. Error	Prob. > T
<hr/>						
Treated Petiole (minus lamina)						
With GA	3	71.19	54.85	102.89	15.85	
Without GA	3	146.82	92.26	219.54	37.85	0.139
Other Leaves						
With GA	3	6.00	4.16	9.65	1.82	
Without GA	3	9.30	7.40	11.47	1.05	0.135
Meristem and Youngest Leaf						
With GA	3	14.39	5.67	26.89	6.41	
Without GA	3	35.87	28.92	47.24	5.73	0.067
Daughter Plants						
With GA	3	9.74	5.25	17.43	3.86	
Without GA	3	32.71	16.69	56.82	12.27	0.149
Roots						
With GA	3	5.06	4.26	6.36	0.65	
Without GA	3	10.44	8.09	13.85	1.75	0.045
Total Translocated						
With GA	3	13.59	10.13	17.20	2.04	
Without GA	3	12.60	10.01	14.00	1.29	0.702

Table 2-6. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the sixth day post treatment in trial 2; (means expressed as dpm/mg dry weight).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole (minus lamina)						
With GA	4	94.92	66.22	149.93	18.78	
Without GA	4	100.16	67.56	136.29	17.73	0.846
Other Leaves						
With GA	4	5.08	3.11	7.98	1.04	
Without GA	4	7.42	4.63	10.98	1.32	0.215
Meristem and Youngest Leaf						
With GA	4	14.96	6.58	22.24	3.93	
Without GA	4	15.35	9.95	19.54	2.30	0.935
Daughter Plants						
With GA	4	15.50	10.13	29.17	4.56	
Without GA	4	55.51	10.88	170.80	38.50	0.342
Roots						
With GA	4	3.94	2.82	4.86	0.442	
Without GA	4	3.71	1.56	7.36	1.267	0.870
Total Translocated						
With GA	4	14.45	11.10	23.04	2.87	
Without GA	4	18.52	9.79	26.78	3.47	0.402

Table 2-7. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the first day post treatment in trial 1; (means expressed as percent of total amount of translocated ^{14}C).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole (minus lamina)						
With GA	3	60.74	58.78	63.72	1.51	
Without GA	3	60.64	58.89	61.99	0.92	0.950
Other Leaves						
With GA	3	11.28	10.95	11.77	0.43	
Without GA	3	8.05	7.10	8.64	0.83	0.004
Meristem and Youngest Leaf						
With GA	3	19.47	15.53	22.83	2.13	
Without GA	3	19.29	17.76	20.91	0.91	0.938
Daughter Plants						
With GA	3	3.73	1.88	6.80	1.55	
Without GA	3	5.70	4.25	7.07	0.82	0.325
Roots						
With GA	3	4.77	3.25	7.10	1.18	
Without GA	3	6.33	4.42	9.18	1.45	0.454

Table 2-8. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the third day post treatment in trial 1; (means expressed as percent of total amount of translocated ^{14}C).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole (minus lamina)						
With GA	3	51.21	42.59	56.88	4.38	
Without GA	4	52.64	44.88	62.66	3.96	0.814
Other Leaves						
With GA	3	15.39	7.14	19.88	4.13	
Without GA	4	13.16	9.97	17.80	1.68	0.598
Meristem and Youngest Leaf						
With GA	3	15.37	14.37	16.20	0.53	
Without GA	4	18.00	14.70	23.89	2.04	0.332
Daughter Plants						
With GA	3	11.91	4.95	17.92	3.77	
Without GA	4	11.13	4.75	21.37	3.74	0.896
Roots						
With GA	3	6.16	3.51	10.39	2.14	
Without GA	4	5.06	4.47	5.87	0.29	0.573

Table 2-9. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the sixth day post treatment in trial 1; (means expressed as percent of total amount of translocated ^{14}C).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole (minus lamina)						
With GA	3	43.11	35.94	46.98	3.59	
Without GA	3	33.50	30.57	35.14	1.47	0.069
Other Leaves						
With GA	3	24.28	23.59	25.22	0.49	
Without GA	3	17.42	14.53	20.66	1.78	0.020
Meristem and Youngest Leaf						
With GA	3	10.59	8.39	13.02	1.34	
Without GA	3	18.16	16.35	20.54	1.24	0.014
Daughter Plants						
With GA	3	12.29	9.35	17.40	2.56	
Without GA	3	19.07	13.93	25.85	3.54	0.197
Roots						
With GA	3	9.71	8.42	11.24	0.82	
Without GA	3	11.85	8.47	11.30	1.74	0.330

Table 2-10. Effects of gibberellic acid (100 mg/l) on the translocation of ^{14}C -labeled 2,4-D in waterhyacinths on the sixth day post treatment in trial 2; (means expressed as percent of total amount of translocated ^{14}C).

	N	mean	min.	max.	Std. Error	Prob. > T
Treated Petiole						
(minus lamina)						
With GA	4	44.06	35.31	49.67	3.27	
Without GA	4	36.83	27.26	44.04	3.52	0.180
Other Leaves						
With GA	4	17.25	11.24	21.82	4.72	
Without GA	4	21.49	15.95	27.85	6.37	0.324
Meristem and						
Youngest Leaf						
With GA	4	6.57	3.05	13.17	2.25	
Without GA	4	6.08	2.78	9.56	1.39	0.857
Daughter Plants						
With GA	4	19.70	10.36	28.22	9.00	
Without GA	4	27.43	13.70	37.36	11.37	0.324
Roots						
With GA	4	12.42	9.13	16.04	1.85	
Without GA	4	8.16	4.72	16.23	2.72	0.254

Tables 2-3 to 2-10 contain the actual alpha levels at which the difference in the treatment means of plant parts harvested on the same day may be considered significant; however, in the discussion which follows, alpha levels greater than 0.05 are not considered to be "statistically significant" (Sokal and Rohlf, 1969).

For ease of discussion, results are discussed in terms of treatment means of individual plant parts through the three harvest periods.

The petioles of treated leaves consistently contained the highest ^{14}C -labeled 2,4-D activity on both a dpm/mg and percentage of total translocated radioactivity, as would be expected due to the close proximity and connection to treated lamina. No clear pattern of distribution due to pretreatment with GA_3 was evident on either dpm/mg or percentage basis. Results obtained for day 6 (trial 1) highlight the lack of correlation of GA_3 treatment with levels of ^{14}C -labeled 2,4-D translocation. On day 6, trial 1, plants which did not receive GA_3 pretreatment contained over twice as much radioactivity as plants receiving GA_3 ; however, on a percentage basis GA_3 pretreatment plants contained more ^{14}C activity. Due to the variability of the data these differences are not considered significant but are noted merely to demonstrate the lack of consistency in the results.

Mean dpm/mg and mean percentage of translocated ^{14}C in the meristems and youngest leaves did not reflect any significant differences due to GA_3 treatment in plants harvested on days 1 and 3. However, on day 6, trial 1, meristems of plants which did not receive GA_3 pretreatment had a significantly higher percentage (18.15)

of the total amount of translocated material at the $\alpha=0.014$ level than meristems of plants which did receive GA_3 pretreatment (10.59 percent). On a dpm/mg basis, the same pattern of distribution occurred; however, it was not significant at the $\alpha=0.05$ level.

Daughter plants which were produced after initiation of the treatment period consistently contained higher amounts of ^{14}C activity on both a dpm/mg and percentage basis when the parent plant did not receive GA_3 pretreatment. However, due to the variability in the data as reflected by the standard error term, none of the differences in the means were significant at the $\alpha=0.05$ level. Six-day, trial 2, plants yielded similar results but were also non-significant at the $\alpha=0.05$ level.

A significant difference in mean dpm/mg was noted for roots of six-day, trial 1, plants, i.e., 5.06 dpm/mg for the plants with GA_3 and 10.44 dpm/mg for the plants without GA_3 ($\alpha=0.04$). A similar trend was also observed on a percentage of total translocated radioactivity for roots of six-day, trial 1, plants, however, the difference between treatment means was not significant at the $\alpha=0.05$ level. Significant differences were not observed for roots of six-day, trial 2, plants on either a dpm/mg or percentage basis.

Results obtained for leaves other than the leaves which received the direct application of ^{14}C -labeled 2,4-D provided the only indication of increased translocation of 2,4-D due to GA_3 pretreatment. Leaves of both the one-day and six-day (trial 1) plants which were treated with GA_3 contained significantly higher ($\alpha=0.004$ and $\alpha=0.021$, respectively)

mean percentages of the total translocated ^{14}C -labeled 2,4-D than plants which did not receive GA_3 pretreatment. No significant differences were noted on a dpm/mg basis.

An analysis of the cumulative amount of ^{14}C -labeled 2,4-D translocated from treated lamina to plants taken as a whole revealed no apparent significant differences ($\alpha=0.05$) due to the treatment of waterhyacinths pretreated with gibberellic acid.

Summary

Results of the ^{14}C -labeled 2,4-D translocation experiments, while highly variable, suggest that under growth chamber conditions, pretreatment of waterhyacinths with 100 mg/l gibberellic acid (1) does not increase the translocation of 2,4-D to meristematic tissues, and (2) may increase the movement of 2,4-D to previously emerged leaves on a percentage of total radioactivity basis. This suggestion may appear to be somewhat in conflict with other studies which have reported significant increases in translocation of auxins and/or 2,4-D due to treatment with GA_3 (Ashton, 1959; Basler, 1959; Pilet, 1965; Basler, 1974); however, none of these studies were conducted with rosette species such as waterhyacinths. It is possible, as suggested by Audus (1972), that there may be no interdependence of gibberellins and auxin-like compounds because gibberellins have been shown to produce different responses in different plant species and tissues within the same species.

PART 3 - FIELD EVALUATIONS

Introduction

Various public agencies routinely utilize 2,4-D for the control of waterhyacinth in the United States. One such agency, the U.S. Army Corps of Engineers, Jacksonville District, annually utilizes approximately 8,200 kg of 2,4-D at rate of 2.24 kg/ha in a 934 l/ha aqueous solution to control waterhyacinths on the St. Johns River, Florida (McGehee, 1982). Pieterse and Roorda (1982) reported a ten-fold enhancement of 2,4-D sensitivity of waterhyacinths when the plants were simultaneously treated with GA₃ at 6 to 8 g/ha under greenhouse conditions. It was also suggested by Pieterse and Roorda (1982) that such a large reduction in the amount of 2,4-D would lower the risk of damage to nearby crops or vegetation and may be attractive from an economical point of view due to the large reduction in the amount of 2,4-D required to control waterhyacinths. In order to test this possibility, the following investigations were conducted (1) the treatment rates tested in the small plot evaluations (Part 1) were repeated in a natural stand of dense waterhyacinths; (2) a selected rate of 2,4-D and GA₃ was evaluated under actual waterhyacinth control operational conditions; and (3) an economic analysis of the use of GA₃ in conjunction with 2,4-D was performed utilizing the assumption that GA₃ would reduce the amount of 2,4-D required for waterhyacinth control by a factor of 10.

Materials and Methods

Field applications were conducted on Lake Dexter, one of a chain of lakes located on the St. Johns River, 6.7 km southeast of Astor, Florida. The waterhyacinths appeared free of any disease, but did exhibit evidence of moderate feeding by waterhyacinth weevils, Neochetina spp. The plants were growing in a large stand of Nuphar luteum which prevented movement of the waterhyacinth mat during the treatment period.

Field evaluations were conducted in two phases. In phase one, the experimental plots were 9.2 m x 3.0 m. Three experimental plots were established in each of 17 separate 46.0 m x 3.0 m transects such that each of the 51 9.2 m x 3.0 m plots were separated by an untreated plot. The untreated plots served as buffer areas between treated plots. Prior to treatment, three random 0.33 sq m samples were taken from the plots to be treated. Plants within the 0.33 sq m samples were counted, allowed to drain of excess water, and weighed to the nearest 0.05 kg in order to determine pretreatment biomass and number per sq m. In phase two, experimental plots were laid out as three separate 0.40 ha-plots each separated by an untreated strip of waterhyacinths. Pretreatment biomass determinations were not made because the efficacy of treatments was based on visual evaluations of individual plants to obtain a proportion of dead plants per plot.

In phase one, the 51 individual plots were treated by use of an airboat equipped with a tank-mix spray system calibrated to deliver a spray volume of 467 l/ha. Each plot was treated twice in order to obtain a spray volume of 934 l/ha. Applications were made with a fixed boom equipped with a single Delavan Type-D20 flooding nozzle. The boom

was adjusted to approximately 45 cm above the plant canopy such that the swath width was equal to 3.0 m. Treatments consisted of simultaneous applications of combinations of gibberellic acid (Asgrow Florida Company, EPA Accession No.08728) and 2,4-D (Union Carbide Corporation EPA Registration No. 264-2AA) at the following rates: 0.0, 23.5, 47.0, and 94.0 g/ha gibberellic acid and 0.56, 1.12, and 2.24 kg/ha 2,4-D. An additional 2,4-D treatment at a rate 4.48 kg/ha was made due to the higher biomass present in the field plots compared to the small plot evaluations discussed in Part 1 of this study.

In phase two, three 0.40 ha-plots were treated by an airboat which contained a tank mix spray system calibrated to deliver 934 l/ha through a hand-held spray gun equipped with a Delavan Type DFA Dela-foam nozzle. The application was made by a spray crew employed by the U.S. Army Corps of Engineers, Jacksonville District. The crew was instructed to treat each plot in the same manner in which they conduct routine control operations. Treatments consisted of 0.84 kg/ha 2,4-D; 0.84 kg/ha 2,4-D plus 94.0 g/ha gibberellic acid; and 2.24 kg/ha 2,4-D. These rates of 2,4-D and gibberellic acid were chosen based on results of the small plot evaluations (Part 1) and results of phase one, above.

All treatments were examined weekly for evidence of treatment effects. At the conclusion of 24 days of phase one, the plants in the plots treated with 2.24 kg/ha and 4.48 kg/ha 2,4-D appeared to exhibit 100 percent control, and the decision was made to complete the efficacy evaluation on day 25. Phase one efficacy evaluations consisted of harvesting the plants in three random 0.33 sq m samples from each treatment plot, counting the number of viable plants, removing obvious necrotic

tissue, and weighing the remaining plant material to the nearest 0.05 kg in order to determine post treatment biomass. Results of phase one were analyzed for the mean percent change in fresh weight and mean percent change in number of plants from initial pretreatment levels. The means of the percent change by treatment were analyzed for the presence of significant differences utilizing Waller-Duncan procedure for the comparison of multiple means. The Waller-Duncan procedure was employed because it has the advantage over other such methods in that the observed F value is used in the calculation of the LSD (least significant difference). The use of the observed F value provides a method of accounting for both the comparisonwise and experimentwise Type I error rates (Chew, 1977).

Phase two efficacy evaluations consisted of visual assessment of the presence of viable meristematic tissue in the treated plots (Seale and Allison, 1946). At the conclusion of 22 days post-treatment, plants treated with 2.24 kg/ha 2,4-D appeared to exhibit near 100 percent control and the decision was made to complete the efficacy evaluation on day 24 post-treatment. Thirty random plants on three transects through the treated plots were examined and the proportion of dead plants per plot calculated. Proportions of dead plants per treatment plot were analyzed for significant differences utilizing a method described by Walpole and Myers (1978).

An economic evaluation of the use of 2,4-D was performed by calculating the costs of converting the waterhyacinth control operation conducted by the U.S. Army Corps of Engineers on the St. Johns River, Florida, to a control program utilizing various combination of GA_3 and

lower than normal rates of 2,4-D. No costs were included for labor, conversion of the spray equipment to allow the use of GA_3 , or for increased storage and transportation of GA_3 .

Results and Discussion

The mean pretreatment biomass (fresh weight) of the Lake Dexter waterhyacinth population was 21.98 kg/sq m (standard error 0.67) and the mean pretreatment number of plants per sq m was 70.76 (standard error 2.92). These means are within the ranges reported by Center and Spencer (1981) for mature stands of waterhyacinths in a North-Central Florida lake during August. At the conclusion of phase one, mean fresh weight and mean number of plants per sq m of the control plots were not significantly different from the pretreatment levels ($\alpha=0.05$) which indicates that the plants were physiologically mature and had become space-limited as reported by Richards (1980) and Center and Spencer (1981).

Tables 3-1 and 3-2 summarize the results of the phase one evaluations of the 17 treatment combinations of GA_3 and 2,4-D in terms of mean percent change in fresh weight and mean percent change in number due to treatment, respectively.

Comparisons of Table 3-1 and 3-2 reveals that within 2,4-D rates of 0.56 and 1.12 kg/ha and any rate of GA_3 , the mean percent change in fresh weight per sq m was always greater than the mean percent change in number of plants per sq m. This was a reflection of the morphological response of the waterhyacinths to the treatments. At the lower rate of 2,4-D, the older leaves became necrotic at the base and readily separated from the plant, such that the only remaining viable tissue was the meristem, youngest leaves, and roots. However, the plants remained

Table 3-1. Mean percent change in fresh weight of waterhyacinths/sq m in Lake Dexter, Florida, treated with combinations of gibberellic acid and 2,4-dichlorophenoxyacetic acid.

Treatment 2,4-D (kg/ha)	Rate GA (g/ha)	Waller- Duncan Grouping ¹	Mean	n	Min.	Max.	Std Error
0.00	23.5	A	32.11	3	15.87	44.45	8.48
0.00	94.0	A	25.86	3	9.59	51.68	13.05
0.00	47.0	A	21.76	3	10.75	37.01	7.87
0.00	0.0	B	1.15	3	-7.12	7.43	4.32
0.56	94.0	C	-81.33	3	-83.98	-78.99	1.45
0.56	47.0	C D	-82.33	3	-88.20	-74.80	3.96
0.56	0.0	C D E	-86.44	3	-89.13	-82.74	1.91
0.56	23.5	C D E	-88.15	3	-88.85	-87.77	0.35
1.12	0.0	C D E	-94.25	3	-95.48	-93.16	0.67
1.12	47.0	D E	-96.61	3	-97.43	-95.49	1.60
1.12	94.0	E	-97.44	3	-97.99	-96.93	0.31
1.12	23.5	E	-99.05	3	-99.77	-98.60	0.36
2.24	23.5	E	-99.23	3	-99.79	-98.46	0.40
2.24	0.0	E	-99.37	3	-99.66	-98.91	0.23
2.24	94.0	E	-99.50	3	-100.00	-98.59	0.45
2.24	47.0	E	-99.68	3	-100.00	-99.47	0.16
4.48	0.0	E	-100.00	3	-100.00	-100.00	0.00

¹Means with the same letter are not significantly different ($\alpha=0.05$).

Table 3-2. Mean percent change in the number of waterhyacinths/sq m in Lake Dexter, Florida, treated with combinations of gibberellic acid and 2,4-dichlorophenoxyacetic acid.

Treatment 2,4-D (kg/ha)	Rate GA (g/ha)	Waller- Duncan Grouping ¹	Mean	n	Min.	Max.	Std Error
0.00	0.0	A	3.47	3	-15.66	15.48	9.67
0.00	94.0	A	1.63	3	-16.13	12.50	8.95
0.00	47.0	A	1.28	3	-9.26	20.00	9.41
0.00	23.5	A	-12.23	3	-39.60	1.59	13.69
0.56	47.0	B	-51.53	3	-67.69	-36.00	9.15
0.56	0.0	B	-54.43	3	-57.89	-51.11	1.96
0.56	23.5	B	-55.66	3	-68.37	-45.28	6.77
0.56	94.0	B D	-64.78	3	-80.00	-51.85	8.21
1.12	0.0	B D	-67.00	3	-76.56	-59.62	5.01
1.12	47.0	B D	-70.58	3	-73.77	-68.75	1.60
1.12	94.0	C D	-82.65	3	-82.82	-82.35	0.14
1.12	23.5	C D	-85.12	3	-93.51	-72.97	6.22
2.24	0.0	C	-94.90	3	-95.45	-94.19	0.37
2.24	23.5	C	-96.12	3	-98.86	-94.12	1.42
2.24	94.0	C	-96.57	3	-100.00	-90.91	2.85
2.24	47.0	C	-99.16	3	-100.00	-98.59	0.43
4.48	0.0	C	-100.00	3	-100.00	-100.00	0.00

¹Means with the same letter are not significantly different ($\alpha=0.05$).

viable and retained the ability to reinfest the plot. For this reason, the percent change in number would appear to be a more realistic estimator of efficacy.

According to the Waller-Duncan multiple range test, there was no significant difference in the mean change in weight or number between the three treatment rates of GA_3 (2,4-D rate = 0.00 kg/ha). There was a significant increase in the mean weight change of the three levels of GA_3 (2,4-D rate = 0.00 kg/ha) when compared to the control plot; however, the GA_3 treatments did not affect the mean number of plants per sq m when compared to the control plot. Tables 3-1 and 3-2 indicate that there were no significant ($\alpha=0.05$) increases in the efficacy of any fixed rate of 2,4-D when combined with any of the four levels of GA_3 .

Table 3-3 presents the results of the large scale application of 2,4-D and gibberellic acid to a dense stand of waterhyacinths. Twenty-four days post-treatment, plot 3, which received 2.24 kg/ha 2,4-D, and 0.0 g/ha GA, had the highest percentage (80.40) of dead waterhyacinth plants per transect. The next highest percent dead plants was plot 2 which received 0.84 kg/ha 2,4-D, and 94.1 g/ha GA, with 34.70 percent, followed by plot 1 which received 0.84 kg/ha 2,4-D, and 0.0 g/ha GA, with 24.20 percent dead plants per transect. The proportions of dead plants in plots 1 and 2 were not significantly different from each other at $\alpha=0.05$; however, the proportion of dead plants in plot 3 was significantly different from plots 1 and 2 at $\alpha=0.05$. A visual inspection of the phase two plots was conducted 63 days post-treatment. Quantitative evaluations were not possible due to disturbance of the plots by wind, currents, and the 24-day post-treatment sampling procedure. However, the visual evaluation did indicate that the waterhyacinth populations in plot 3 had been

Table 3-3. Effects of large-scale operational application of 2,4-D and gibberellic acid (GA₃) to waterhyacinths; 24 days post-treatment.

Plot No.	Treatment Rate		Proportion of Dead Plants per Plot
	2,4-D (kg/ha)	GA ₃ (g/ha)	
1	0.84	0.0	23/95 = 0.242 b
2	0.84	94.1	33/95 = 0.347 b
3	2.24	0.0	74/92 = 0.804 a

Proportions followed by the same letter are not significantly different at $\alpha=0.05$.

(1) reduced to a non-problematic level and replaced by a monoculture of water lettuce, (Pistia stratiotes L.) and (2) plots 2 and 3 still contained problematic levels of waterhyacinth and were in need of retreatment.

Table 3-4 presents a cost comparison for use of a combination of GA_3 and 2,4-D for control of waterhyacinths on the St. Johns River, Florida, as conducted by the U.S. Army Corps of Engineer's Jacksonville District. Based on an average of 3642 ha of waterhyacinths controlled per year, the normal application rate of 2,4-D, 2.24 kg/ha, results in an annual herbicide cost of \$27,096. With the use of GA_3 at 8.0 g/ha and a ten-fold reduction in the amount of 2,4-D required for an equivalent level of control (0.224 kg/ha), as suggested by Pieterse and Roorda (1982), the annual herbicide costs would be \$35,947 or 32.7 percent higher than the current rate. Based on the results of the field application at Lake Dexter, Florida (Table 3-2), the lowest combination of rates of GA_3 and 2,4-D which would provide a level of control (number per sq m) not significantly different from 2.24 kg/ha, 2,4-D was 23.5 g/ha and 1.12 kg/ha, respectively. This option would result in an annual herbicide cost of \$111,117 or 310.1 percent higher than the current rate.

Summary

The results of the field test of various treatment rates of 2,4-D and GA_3 indicated that on an operational basis, GA_3 does not enhance the effect of 2,4-D on water hyacinths to a significant degree. The additional costs of using GA_3 and 2,4-D at the relative levels suggested by Pieterse and Roorda (1982) are not justified from an economic standpoint at the current market prices of GA_3 and 2,4-D.

Table 3-4. Costs comparison for the use of a combination of GA₃ and 2,4-D compared to normal application rates of 2,4-D for the control of waterhyacinths on the St. Johns River, Florida.

Option A: Normal rate of 2,4-D (2.24 kg/ha)

$$1982 \text{ 2,4-D costs}^1 = \$3.32/\text{kg} \times 2.24 \text{ kg/ha} = \$7.44/\text{ha}$$

$$\text{Annual costs} = \$7.44/\text{ha} \times 3642 \text{ h/yr}^1 = \$27,096/\text{yr}$$

Option B: 8.0 g/ha GA₃ and 0.10 of normal application rate of 2,4-D (0.224 kg/ha)²

$$1982 \text{ 2,4-D costs} = \$3.32/\text{kg} \times 0.224 \text{ kg/ha} = \$0.75$$

$$1982 \text{ GA}_3 \text{ costs}^3 = \$1.14/\text{g} \times 8.0 \text{ g/ha} = \$9.12$$

$$\text{Total } \$9.87/\text{ha}$$

$$\text{Annual costs} = \$9.87/\text{ha} \times 3642 \text{ ha/yr} = \$35,947/\text{yr}$$

Option C: 23.5 g/ha GA₃ and 1.12 kg (a.e.)/ha⁴

$$1982 \text{ 2,4-D costs} = \$3.32/\text{kg} \times 1.12 \text{ kg/ha} = \$3.72/\text{ha}$$

$$1982 \text{ GA}_3 \text{ costs} = \$1.14/\text{g} \times 23.5 \text{ g/ha} = \$26.79/\text{ha}$$

$$\text{Total } \$30.51/\text{ha}$$

$$\text{Annual costs} = \$30.51/\text{ha} \times 3642 \text{ ha/yr} = \$111,117/\text{yr}$$

1. Current bid price (McGehee, 1982).
2. Pieterse and Roorda (1982).
3. Current market price (Asgrow Florida, Inc.).
4. Lowest rates which produced a level of control which was not significantly different from 2,4-D at 2.24 kg/ha (see Table 3-2).

DISCUSSION

Results of this investigation are in conflict with findings of Pieterse (1979) and Pieterse and Roorda (1982) who reported a ten-fold enhancement of the effects of 2,4-D when simultaneously treated with gibberellic acid. However, differences in experimental design and environmental controls probably account for the lack of agreement. Pieterse and Roorda (1982) conducted their studies in concrete reservoirs in a heated greenhouse, utilized an atomizing-type spray apparatus, and applied spray volumes of 40 l/ha or 4 ml/sq m. This study was conducted outdoors in plastic-lined metal containers using a hand-held non-atomizing sprayer and in an undisturbed infestation of waterhyacinths in the St. Johns River using an airboat spray system. Spray volumes were 934 l/ha or 93.4 ml/sq m.

Hitchcock et al. (1949) reported quicker killing of individual waterhyacinths when sprayed with an atomizer as compared to similar rates of 2,4-D sprayed on undisturbed plants in the field. Hitchcock et al. (1949) and Koch et al. (1978) reported increased 2,4-D efficacy under greenhouse conditions due to more effective wetting and the fact that spray solution which does not fall directly on the target plants may be trapped on the water surface of the experimental containers, allowing greater absorption by plant roots than would be the case under natural conditions. These observations also provide a partial explanation for the field trials in Lake Dexter (Part 3) not producing the same level of efficacy observed in the small plot evaluations (Part 1).

The physiological state of plants in each separate evaluation also contributed to the lower level of activity in the field plots. The plants grown in plastic-lined metal containers (Part 1) were young, rapidly growing specimens, whereas, the plants treated in Lake Dexter, Florida (Part 3) were mature, spaced-limited specimens. This condition was evident since the controls in Part 3 exhibited insignificant growth in terms of fresh weight and number of plants produced per sq m during the study. Addicott (1970) and Low (1974) supported these observations by explaining the differing results from seemingly similar experiments with gibberellins in terms of physiological conditions of the experimental material, i.e., age, size, nutrient and light availability, temperature, species, and type of tissue examined.

The apparent lack of interaction or synergism observed in this study is also partially explained by the above referenced comments of Addicott (1970) and Low (1974). However, numerous other researchers reported that synergistic interactions between gibberellins and auxin-like compounds were due to an auxin-sparing reactions wherein the applied gibberellins inhibited or reduced endogenous concentrations of auxin degrading enzymes (Brian and Hemming, 1958; Kogl and Elema 1960, cited in Weaver, 1972; Galston and Purves, 1960; Sarma 1978). Such mechanisms in which levels of endogenous auxins are increased by GA_3 could possibly account for supposedly increased sensitivity of plant tissues to reduced concentrations of auxins or 2,4-D applied with gibberellins. The physiological state of the plant and the environmental conditions of the experiment could then determine the level of production of other endogenous plant growth substances, as suggested by Low (1974). The magnitude of this production could result in either additive or synergistic effects.

The nature of response observed in the ^{14}C -labeled 2,4-D translocation studies can be partially explained by a recent study by Mulligan and Patrick (1979). GA_3 was shown to promote the transfer of ^{14}C and ^{32}P -labeled photosynthates to the site of GA_3 application rather than to competing "sinks" such as roots and meristematic tissues. This increase in transfer away from normal "sinks" to the site of GA_3 application was shown not to be caused by increased photosynthesis rates, increased assimilate export rate from "sources", nor by altering the mobilizing ability of other competing sinks. Mulligan and Patrick (1979) provided evidence that GA_3 was not acting on any transfer process remote from its point of application but was acting locally. A similar relationship in waterhyacinths could account for the suggestion of increased translocation to GA_3 treated petioles in this study. The data also suggested reduced translocation to meristematic sinks in GA_3 treated plants; however, due to variability of the data, significance was not demonstrated consistently.

The costs analysis of the use of GA_3 in conjunction with lower than normal rates of 2,4-D indicates that its use is not justified economically. However, a substantial increase in 2,4-D prices, a reduction in GA_3 costs, or a documented environmental concern over the quantity of 2,4-D applied to public waters could alter this analysis. Pending the development of more environmentally compatible and efficacious herbicides, the most prudent way to reduce the quantity of 2,4-D used in waterhyacinth control and thus herbicide expenditures is to begin control operations before the plants reach problematic levels (Hitchcock et al., 1949) and maintain a low level of plants through a regular patrol system to prevent reestablishment (Seale and Allison, 1946).

This concept is the essence of current maintenance control concepts for waterhyacinth control on large riverine systems (Joyce, 1977).

As a final summary to this discussion, the following comments are offered. Despite years of intensive research, the role of growth substances in the life of the intact growing plant appears far from clear. In fact, a new concept appears to be emerging in plant growth substance theory. Trewavas (1981) stated, "Those who work in the area will be only too familiar with the often-confusing contradictions, the apparently endless and puzzling interactions and the plain uncertainties of supposedly established facts. Even the outline of a simple physiological mechanism of control for any growth substance in the intact plant cannot be deduced with any certainty" (Trewavas, 1981, page 203). The entire concept of plant "hormones" as substances which have localized biosynthesis, control physiological and biochemical events by changing their concentration, and cause actions at a distance from their synthesis stems from mammalian hormone theory and was challenged by Trewavas (1981). Much of the confusion surrounding plant growth substances was blamed on the prevailing trend of explaining the actions of these substances in terms of mammalian hormonal theory. Trewavas (1981) suggested that growth substances might represent, instead, a form of cell-to-cell interaction or communication and that the varying responses to substances such as gibberellins by different species and tissues can be explained by differing sensitivity of plants to gibberellins. This concept may explain the variation in responses of waterhyacinths to GA_3 and 2,4-D under environmental and physiological conditions referenced and investigated in this study.

CONCLUSIONS

Evaluation of effects of combinations of GA₃ and 2,4-D on waterhyacinths in this study indicate that

1. Under field conditions existing during this study, there was no significant synergism between 2,4-D and GA₃ in terms of increased efficacy of 2,4-D. At best, the response of waterhyacinths to combinations of GA₃ and 2,4-D was additive. However, the use of these compounds under other conditions may yield differing results.

2. Pretreatment of waterhyacinths with 100 mg/l GA₃ does not significantly increase the translocation of ¹⁴C labeled 2,4-D to meristematic waterhyacinth tissues on either a dpm/mg or percent of total ¹⁴C translocated basis. The data suggest a possible increase in translocation to leaves other than the leaf receiving direct 2,4-D treatment.

3. Costs analysis of utilizing GA₃ in conjunction with 2,4-D in order to lower rates of 2,4-D used to control waterhyacinths on an operational basis indicated that the addition of GA₃ was not economically justified. However, the use of these compounds under differing field conditions and/or a significant change in the cost of 2,4-D or GA₃ may alter this situation.

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