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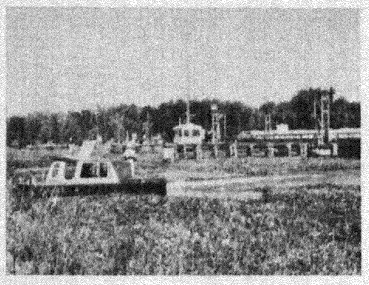

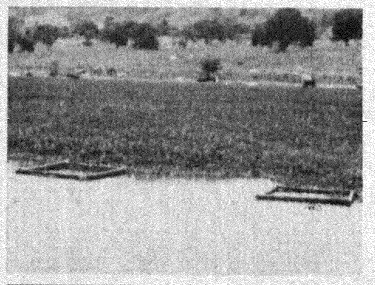

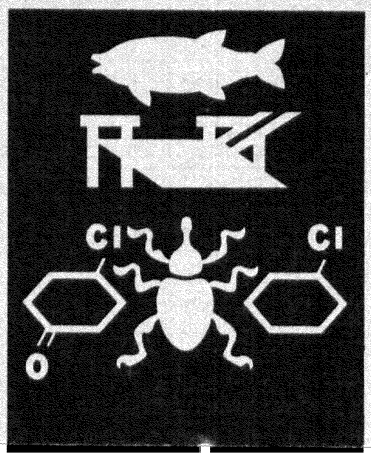

MISCELLANEOUS PAPER A-79-8

STUDY OF WATERHYACINTHS SHOWING POSSIBLE RESISTANCE TO 2, 4-D CHEMICAL CONTROL PROGRAMS

By William T. Haller and Mirghani Tag El Seed

Department of Agronomy
University of Florida
Gainesville, Fla. 32611

November 1979

Final Report

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The fact that heterostyly exists in waterhyacinths has been known for years, but a recent observation that long-style plants might be somewhat resistant to 2,4-D control resulted in the need for this study. To further evaluate the situation, the work involved field observations of midstyle and long-style populations, self- and cross-pollination studies, and herbicide treatments. Although long-style waterhyacinth plants do appear to be somewhat

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more resistant to 2,4-D than midstyle ones, this probably will not be a serious consideration in the Corps' aquatic plant control operations. However, the situation is very curious from a botanical standpoint and should be studied further.

Preface

This report presents results of a brief study conducted for the Aquatic Plant Control Research Program (APCRP) by the Department of Agronomy, University of Florida, Gainesville, Fla. The purpose of this study was to evaluate the phenomenon of heterostyly in waterhyacinths and the possible resistance of long-style waterhyacinth plants to 2,4-D control measures. Funds for this effort were provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES).

The principal investigators for the work were Drs. William T. Haller and Mirghani Tag El Seed of the University of Florida, who prepared this report.

The work was conducted at WES under the general supervision of Mr. W. G. Shockley, Chief of the Mobility and Environmental Systems Laboratory, and Mr. B. O. Benn, Chief of the Environmental Systems Division, and under the direct supervision of Mr. J. L. Decell, Chief of the Aquatic Plant Research Branch. As a result of reorganization at WES, Mr. Decell is now manager of the APCRP, which is a part of the Environmental Laboratory of which Dr. John Harrison is Chief.

The Commanders and Directors of WES during this period were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

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STUDY OF WATERHYACINTHS SHOWING POSSIBLE RESISTANCE TO 2,4-D CHEMICAL CONTROL PROGRAMS

Introduction

1. Waterhyacinth plants have three distinct floral types that are determined by the length of the female flower in relation to the anther. The most common flower type is the mesostyle (or midstyle) (Figure 1). In this type, the style is slightly shorter than the anthers, resulting in the stigma being in close proximity to the pollen sacks. Penfound and Earle (1948)* reported this floral type in essentially all waterhyacinths they examined in Florida and Louisiana in the 1940's. In addition, studies of waterhyacinths on the Nile River have also shown essentially 100 percent of the mesostyle-type plants. There have been (as far as the authors know) no studies conducted on other than mesostyle waterhyacinths.

2. The rare types of hyacinth flowers are the long style (Figure 2) in which the style is elongated, elevating the stigma significantly above the anthers, and the short style in which the style is almost nonexistent and the stigma is located near the ovary. Short-style plants are extremely rare. The authors have examined tens of thousands of waterhyacinths and have found only one short-style flower. In contrast, long-style plants were found in some areas in greater proportions than mesostyle plants. This finding is contrary to what would be expected when the literature is reviewed. The history of each area where long-style plants were found indicated that long-style plants are found in areas where 2,4-D (dichlorophenoxyacetic acid) has been used in a control program. The authors proposed that 2,4-D may be selecting for the long-style population by possibly killing the more susceptible mesostyle

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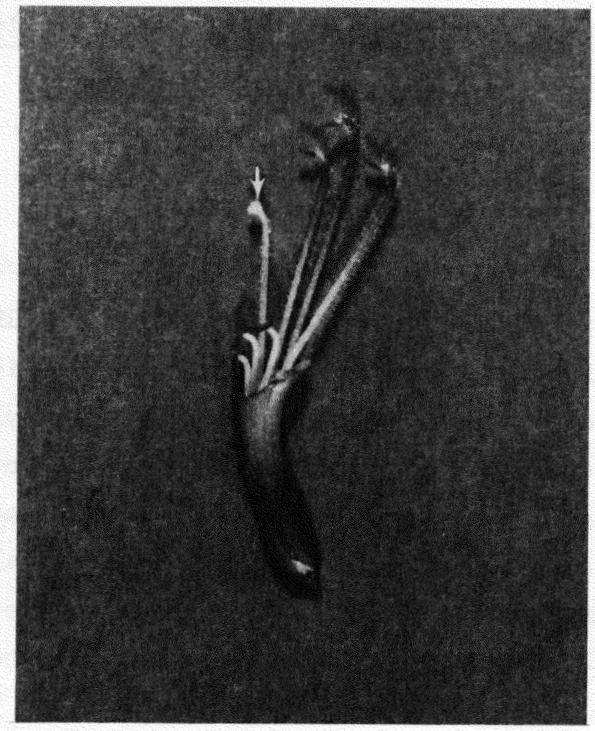
* Penfound, W. T., and Earle, T. T. 1948. "The Biology of the Waterhyacinth," Ecol. Monog., Vol 18, pp 447-472.



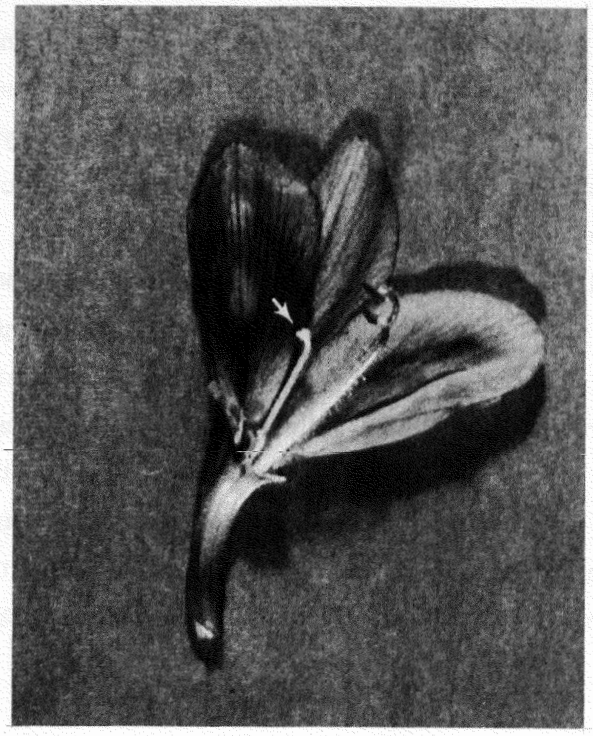
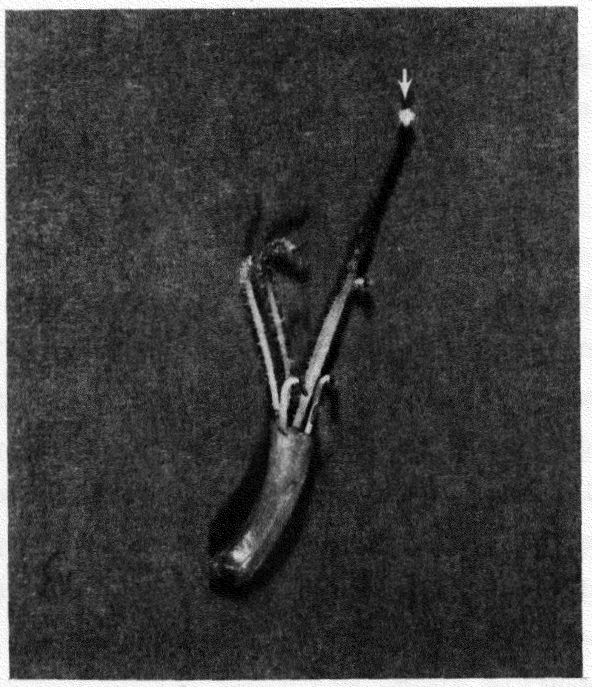


Figure 1. Florets of waterhyacinth flowers dissected to illustrate the mesostyle type. Note the position of the stigma (arrow) with respect to the upper three anthers



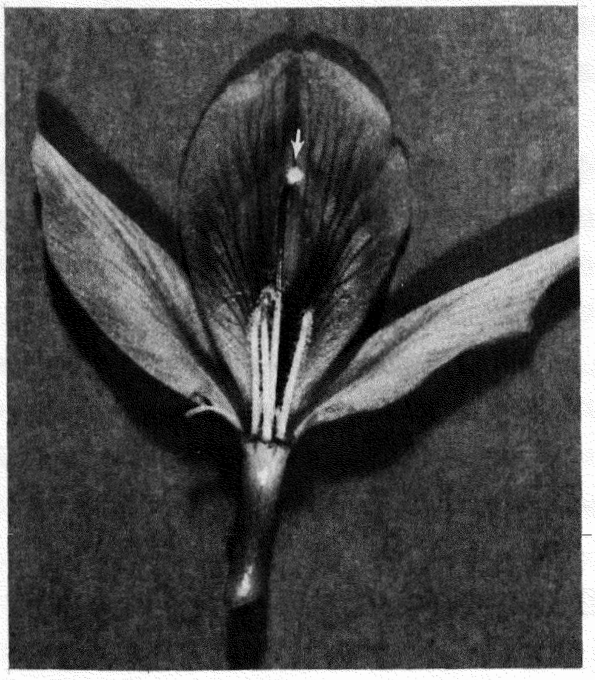


Figure 2. Florets of waterhyacinth flowers with elongated or long styles. Note the relative position of the stigma (arrow) with respect to the anthers. The length of the style is nearly as long as the petal. These plants are apparently very rare under natural conditions but are common in areas treated with 2,4-D

plants. Field and laboratory studies were conducted to study heterostyly in waterhyacinths.

Field Studies

3. Floral characteristics of waterhyacinths were studied in several Florida ecosystems beginning in the fall of 1977 (Table 1). When the large number of long-style plants became apparent, several other areas were studied. The only places where long-style plants were found were in water bodies that had a previous history of chemical weed control with 2,4-D. The existence of long-style plants could possibly be due to environmental conditions in late fall; however, this theory was disproved when Orange Lake was again studied in the early summer, and again a high proportion of long-style plants was found.

4. Lakes were sampled by steering an airboat into waterhyacinths in several littoral areas around each lake and examining several dozen flowers. Sampling of Rodman Reservoir again indicated some relationship between 2,4-D and long-style plants. Chemical weed control has not been widely used in Rodman Reservoir as plant pathogens, winter dieback, and other factors maintain the reservoir essentially free of hyacinths. Most 2,4-D spraying (what little there is) is concentrated in the inflowing creeks, particularly Deep Creek and Orange Creek. After studying the entire reservoir, the only long-style plants found were located in the mouth of Orange Creek. In summary, there appears to be a definite correlation between long-style plants and 2,4-D control programs. Additional lakes will be studied to continue testing this correlation.

Self- and Cross-Pollination Studies

5. It is possible that, as maintenance control programs are conducted over the years, vegetative reproduction is essentially stopped, and new infestations of waterhyacinth are derived from germinating seeds. If only seeds from long-style plants are viable, which is not the case, then as the plant populations are 100 percent controlled this

year, next year's population would come from long-style seeds and result in a high proportion of long-style flowers. In order to test this hypothesis, several pollination crosses were made (Table 2).

6. Waterhyacinths are easily fertilized by brushing the pollen-laden anther on the stigma of any particular plant. The fertilized flower is then covered by a small polyethylene bag, and in about 3 weeks seeds can be removed from the seed capsule. Hyacinths naturally produce very few seeds, but, by artificial pollination, millions of seeds have been produced. If long-style plants were genetically pure, either dominant or recessive, one would expect certain ratios of long-style:mesostyle plants in the F_1 progeny. It is apparent that waterhyacinths have already hybridized, as all crosses resulted in production of mesostyle plants (Table 2).

7. Germination of waterhyacinth seeds has been extensively studied, but it is still neither well understood nor predictable. Researchers have varied temperature and light; alternated wetting and drying; and studied after-ripening, cold treatments, soils, and seed scarification. The results have been very contradictory.

8. Surprisingly, the authors had little trouble germinating hyacinth seeds. Nearly 100 percent germination was obtained on laboratory window sills, in the greenhouse, and in growth chambers. Effects of water depth on seed germination (Table 3), effects of light on germination (Table 4), and effects of various gases on germination (Table 5) were studied. One factor that apparently affected the seed germination studies was an atmosphere of carbon dioxide (Table 5). This is not too surprising as carbon dioxide-oxygen ratios have been shown to be extremely important in the germination of other aquatic plants (hydrilla propagules). Also, seeds held at low light intensity (10 percent, Table 4) showed slightly lower germination after 8 weeks than seeds subjected to higher light intensities.

9. Once the seed has germinated, growing the seedling to a mature stage becomes a major problem. The small seed has a very low starch reserve, and the seedling cannot survive for over a few days. The authors were able to grow waterhyacinth seedlings best by placing the germinated

seed in a flat pan filled with organic soil at field capacity and wetted until almost flooded. Water had to be added almost daily. Even with special care, growing seedlings to flowering plants required nearly 20 weeks.

10. These basic studies on seed germination and fertilization were required to obtain basic information on hererostyly in the plants. The authors are now able to predict germination rates and plant behavior, abilities which are essential to further studies.

Herbicide Treatments

11. In order to test the resistance of waterhyacinths to 2,4-D, long-style and mesostyle waterhyacinths were placed in barrels and acclimated for 7 days. At this time, the plants were upright and appeared healthy and normal in every respect. Ten barrels each of long-style and mesostyle plants were randomly placed and sprayed with a compressed air sprayer and boom (flat fan). Weedar 64 (2,4-D dimethyl amine) was applied to all barrels at 1.75 lb active ingredient per acre (0.794 kg/4046.8 sq m) formula. A 1 percent solution of Big Sur (S-235) was added to the spray formula as a surfactant.

12. As a result of hot, dry weather, this normally sublethal rate was toxic to all plants. The petiole on the long-style plants never became completely chlorotic. Ordinarily an evaluation would indicate 100 percent control of both long-style and mesostyle plants. However, it appears at this time (3 weeks post-treatment) that the regrowth of the long-style plants is going to be more complete than that of the mesostyle plants. Retreatment at 1.0 lb of 2,4-D per acre (0.453 kg/4046.8 sq m) was conducted on another 10 replicates during the second week of September. Results and regrowth cannot be recorded for another 4 to 6 weeks; however, it appears that knockdown of mesostyle and long-style plants is equal. The difference in survival rates seems to be in the potential for regrowth after treatment. A more descriptive term for this phenomenon, rather than herbicide resistant, is probably herbicide tolerant. Basically, the long-style plants at sublethal doses apparently do not

translocate the herbicide to the crown meristems as well as the mesostyle plants. Thus, in a mixed population, regrowth is more apt to occur from long-style plants.

Future Considerations

13. This is the first report of differences in herbicide tolerance in waterhyacinths, as the authors are sure that this reporting is the first artificial crossing of long-style and mesostyle plants. The existence of tolerant plants is probably not extremely important to ongoing maintenance programs since a little extra 2,4-D will kill the tolerant long-style plants. The authors think that long-style populations arise out of the use of hand-held sprayers where an even coverage of 2 to 3 lb/acre (0.907 to 1.360 kg/4046.8 sq m) is essentially impossible. However, the discovery of tolerant hyacinths and the basic work this study has initiated is extremely interesting from a botanical standpoint. The authors' laboratory will continue work on this discovery, and the authors are currently preparing to publish more of their work. Copies of future publications will be provided to the U. S. Army Engineer Waterways Experiment Station, and all concerned will be kept aware of their future research activities.

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Table 1

Types of Heterostylic Plants Found in Several Areas of Florida

Bodies of Water	Date	No. of Mesostyle Plants	No. of Long-Style Plants	Spray History
Orange	10/77	1239	970	2,4-D
Alice	10/77	2945	0	Diquat
Baldwin	10/77	1120	0	Diquat
Cypress	11/77	815	0	Never sprayed
Bivens	11/77	365	219	2,4-D
Orange	6/78	187	507	2,4-D
Paynes	5/78	1029	0	Never sprayed
Alachua	5/78	528	0	Never sprayed
Rodman	7/78	1060	8	2,4-D

Table 2

Artificial Fertilization of Various Flowers to Determine Genetic Makeup of Heterostyly

Male	Female	Flowers Produced
Long	Long	Mesostyle
Long	Meso	Mesostyle
Meso	Long	Mesostyle
Meso	Meso	Mesostyle

Table 3

Effects of Water Depth on Percent Waterhyacinth Seed Germination in Large Flasks Without Soil

Weeks from Beginning of Germination	Percent Germination for Cited Water Depth, cm					
	3	10	20	30	40	50
2	1	0	1	4	0	1
4	40	28	38	25	25	24
6	84	77	84	81	69	88
8	96	99	100	96	100	99

Table 4

Effects of Sunlight Intensity on Percent Germination of Waterhyacinth Seeds

Weeks from Beginning of Germination	Percent Germination for Cited Sunlight Percentages			
	100	70	30	10
2	33	30	33	1
4	76	61	65	9
6	93	97	81	37
8	99	100	96	88

Table 5

Effects of Atmospheric Gases on Percent Germination of Waterhyacinth Seeds in Erlenmeyer Flasks

Days after Beginning of Germination	Percent Germination for Cited Conditions								
	Comp. Air	N_2	O_2	CO_2	H_2	CH_4	C_2H_6	Sealed	Open
3	100	98	100	12	91	62	96	100	100
7	100	98	100	45	98	100	100	100	100
14	100	98	100	67	98	100	100	100	100