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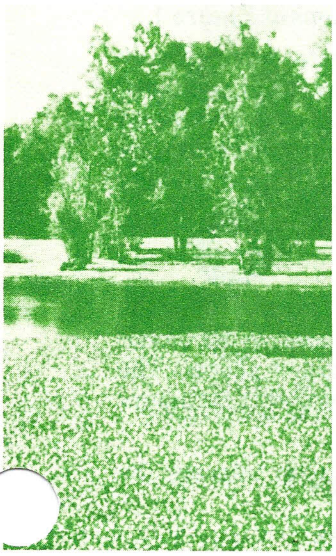

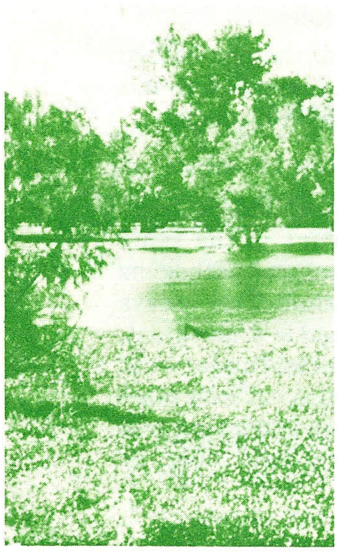

ENVIRONMENTAL LABORATORY

AQUATIC PLANT CONTROL RESEARCH PROGRAM

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FEASIBILITY OF APPLYING GENETIC ENGINEERING TECHNOLOGY TO AQUATIC PLANT CONTROL

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ABSTRACT—Application of genetic engineering technology to the development of a biocontrol agent for Eurasian watermilfoil and hydrilla is feasible. The research tasks required to develop such an agent are discussed.

INTRODUCTION

Problem

Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle) are two noxious submersed aquatic plants that interfere with drainage, irrigation, boat traffic, and recreational use of waterways. Eurasian watermilfoil is distributed worldwide and is present in every state in the continental United States. It reaches most severe proportions in the Pacific northwest, Gulf and southern Atlantic Coast states, and some mid-Atlantic states. Hydrilla occurs in 100 to 150 thousand acres of the nation's waterways. Most extensive growth occurs in the Gulf and southern Atlantic Coast states, but hydrilla has been found in isolated sites as far north as Delaware and Iowa. Therefore, hydrilla has tremendous potential for expanding its present range.

Conventional Approach

The conventional approach for finding biocontrol agents for such nuisance species has been to conduct searches for natural enemies in the country of origin. The U.S. Department of Agriculture, working under a funding agreement with the U.S. Army Corps of Engineers' Aquatic Plant Control Research Program (APCRP), initiated a systematic world survey in 1978 for natural insect enemies of hydrilla. Several insects that feed on the plant have been identified and will be studied in their countries of origin for efficacy as biocontrol agents. A domestic survey for pathogens of Eurasian watermilfoil was initiated by the Waterways Experiment Station (WES) in 1984. The search could yield pathogenic biocontrol agents, but no results are yet available.

Such overseas and domestic searches will continue, but many years will be required to develop any agents found. If no successful biocontrol agents are found, conventional approaches will have been exhausted and management of these noxious aquatic plants will become increasingly difficult.

Other Approaches

Since biological control affords long-term, environmentally compatible plant management and because conventional approaches have not yet produced a successful biocontrol agent for submersed aquatic plants, exploration of less conventional approaches has become necessary. Dr. Haim Gunner at the University of Massachusetts is attempting to develop microbial control agents by inducing pathogenicity in lytic enzyme-producing microorganisms that are normally associated with Eurasian watermilfoil. Similar work on lytic enzyme-producing microorganisms associated with hydrilla is in progress at WES.

Application of genetic engineering technology is another possible approach. Genetic engineering has produced microorganisms, some of which are now commercially available, to address problems in such diverse areas as production of insulin and other pharmaceuticals, treatment of oil spills, and control of agricultural pests. A meeting with three experts involved in different aspects of genetic engineering was held at WES in 1983 to outline the research tasks required for applying genetic engineering technology to biocontrol of aquatic plants. The rest of this article represents a synthesis of that meeting.

RESEARCH TASKS

Host Specificity

The first, and perhaps most crucial, requirement for developing a biocontrol agent by applying genetic engineering technology is finding a host-specific microorganism. The microorganism need not be pathogenic because genes for pathogenicity can be introduced, but candidates must be intimately and exclusively associated with the target plant. Since host specificity usually involves several interactions between the microorganism and host plant, several genes are probably involved. If these genes are widely separated on the chromosomes, extensive manipulations that are impractical at this time would be necessary to make use of the genes.

Identification of Desired Trait

The second task would involve analysis of the host plant to find substances or mechanisms that injure the plant. All plants, including Eurasian watermilfoil and hydrilla, are susceptible to a wide variety of toxins, growth inhibitors, bacteria, and fungi. Knowledge of target plant susceptibilities would suggest traits needed by a biocontrol agent.

Important characteristics of the candidate microorganism and the target plant must be described, and their interactions examined to discover mechanisms potentially useful for bioengineering. All of the foregoing efforts could proceed simultaneously before the actual engineering process is initiated.

Application of Bioengineering Technology

Once the desired trait for the candidate microorganism has been chosen, a search for genes capable of conferring that trait must be made. Genes that perform very specific functions are available from both university and industrial laboratories involved in molecular genetics studies. Once a gene(s) for the desired trait is obtained, it can be transferred to the candidate microorganism using genetic engineering technology.

One technique that can be used for this purpose is introduction of genes for the desired trait into plasmids, which are readily taken up by bacteria (Figure 1). Plasmids are circles of DNA that impart traits to a cell that are not characteristic of the cell's original genetic material. The stepwise process of splicing the desired genes into a plasmid followed by uptake of the plasmid by a bacterial cell is represented in the red portions of Figure 1.

Steps necessary for such a transfer have already been performed successfully many times on microorganisms for other purposes. All of the required technology is currently available; however, unique adaptations of the technology are required for each new system to which it is applied.

Bioassays

When the new gene(s) has been transferred to the candidate microorganism, assays must be developed to determine the effects of the new gene(s). The following are some of the questions that these assays would be designed to answer:

Does the recipient microorganism tolerate the new gene(s) and its products?

Is the desired trait expressed?

Does the new gene(s) impair any of the normal desirable functions of the recipient?

As these questions are answered, the engineered microorganism will be fine-tuned to ensure that it is capable of accomplishing its intended purpose.

Efficacy Studies

The newly engineered candidate microorganism must be tested on the target plant in the same manner that newly discovered natural pathogens are tested. Test tube and aquarium-level assays must be conducted to prove that, and to what extent, the candidate can damage the plant and under what conditions it is most effective. Host specificity must be reconfirmed. Methods for mass-producing and applying the candidate microorganism must be developed.

Formulation Development

A successfully engineered microorganism must be formulated for large-scale field application by commercial interests. A field-ready formulation must have effectiveness as guaranteed by good quality control methods, extended shelf life, ease of

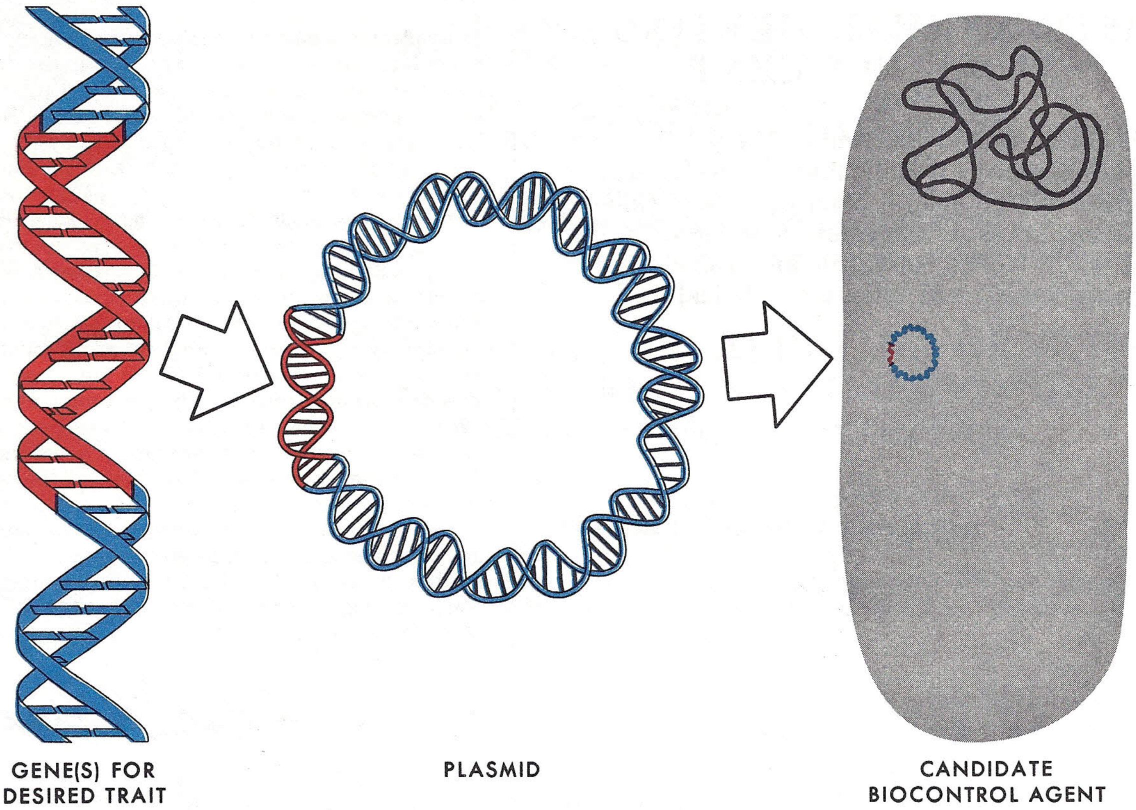

Figure 1. Diagram of one mechanism for genetic engineering of a candidate bacterial biocontrol agent

application, and environmental compatibility as evidenced by registration with appropriate agencies.

TIME REQUIRED

The estimated time from initiation of the effort through development of a commercial product for field use is twelve years. Figure 2 shows estimated time for each task.

CONCLUSIONS

Application of genetic engineering technology to the development of a biocontrol agent for Eurasian watermilfoil and hydrilla is feasible. The technology is sufficiently advanced to initiate such an effort as soon as host-specific microorganisms can be found. The research plan described will begin as a part of the Fiscal Year 1986 APCRP, contingent upon availability of funding.

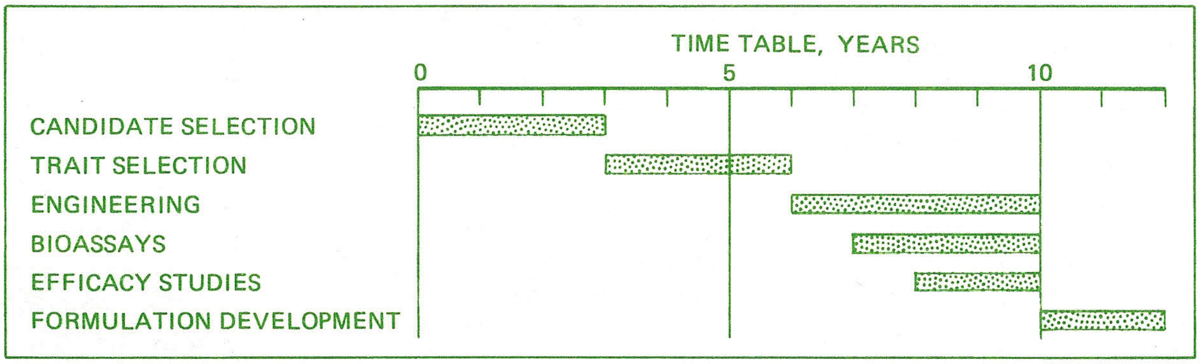

Figure 2. Timetable for developing genetic engineering application for biological control of aquatic plants

19TH ANNUAL MEETING ANNOUNCEMENT

The 19th Annual Meeting of the U. S. Army Corps of Engineers Aquatic Plant Control Research Program will be held at Marriott's Hotel Galvez, Galveston, Texas, on 26-29 November 1984. The FY 1986 Civil Works Research and Development Program Review will follow the technical sessions.

This bulletin is published in accordance with Army Regulation 310-2. It has been prepared and distributed as one of the information dissemination functions of the Environmental Laboratory of the Waterways Experiment Station. It is principally intended to be a forum whereby information pertaining to and resulting from the Corps of Engineers' nationwide Aquatic Plant Control Research Program (APCRP) can be rapidly and widely disseminated to Corps District and Division offices as well as other Federal agencies, State agencies, universities, research institutes, corporations, and individuals. Contributions are solicited and will be considered for publication so long as they are relevant to the management of aquatic plants as set forth in the objectives of the APCRP, which are, in general, to provide tools and techniques for the control of problem aquatic plant infestations in the Nation's waterways. These management methods must be effective, economical, and environmentally compatible. This bulletin will be issued on an irregular basis as dictated by the quantity and importance of information to be disseminated. Communications are welcomed and should be addressed to the Environmental Laboratory, ATTN: J. L. Decell, U. S. Army Engineer Waterways Experiment Station, P. O. Box 631, Vicksburg, Miss. 39180, or call 601-634-3494.

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