PLANT PATHOGENS AS BIOCONTROL AGENTS IN NATIVE HAWAIIAN ECOSYSTEMS

Donald E. Gardner

Abstract

Several examples, some spectacular, are available of successful applications of weed biocontrol approaches with plant pathogenic agents in agricultural and rangeland situations. The overall percentage rate of success of all such programs attempted cannot yet be documented, however. Both the "mycoherbicide" method, in which large quantities of inoculum are directly applied to target plants, and the "conventional" method, in which the pathogen is allowed to distribute itself from an introduction point, are used. Two successful biocontrol programs in Hawai'i using plant pathogens are the fungus *Fusarium oxysporum* control of prickly pear cactus (*Opuntia ficus-indica*), and Hāmākua pāmakani (*Ageratina riparia*) control by the fungus *Entyloma ageratinae* or *E. compositarum*. A third project, in which the fungus *Colletotrichum gloeosporioides* has been released on Koster's curse (*Clidemia hirta*), is currently under evaluation. Little precedent exists for management use of biocontrol with plant pathogens in native habitats with the goal of preservation of intact ecosystems, as distinct from agricultural applications, however.

Certain limitations, both of a biological and an administrative or political nature, must be considered in National Park Service biocontrol programs. Perhaps the most important potential biological limitation may be the unavailability of suitable control agents. Suitable agents must be both host specific and sufficiently virulent to effectively control the target species.

Conflicts between National Park Service and agricultural (including horticultural and rangeland) management objectives may also occur. Possibilities for success must be evaluated for each target species individually, taking into account all biological factors as well as other factors involved. Biocontrol offers certain potential advantages over herbicidal or mechanical methods, including longevity and self-dispersal of the agent in the environment; disadvantages include slower, often less perceptible, and less complete control as compared with herbicidal or

mechanical removal. Biocontrol, with insects or diseases, may be the only feasible approach for certain widely distributed alien species, however.

The following pathogenic fungi are currently being evaluated under controlled conditions as potential biocontrol agents for target alien plants in Hawaiian National Park Service areas: *Fusarium oxysporum* f. sp. *passiflorae* for banana poka (*Passiflora mollissima*); *Gymnoconia nitens* and *Kuehneola uredinis* for brambles (*Rubus* spp.); and *Ramularia destructiva*, *Nectria galligena*, and *Cryphonectria* sp. for firetree (*Myrica faya*). None of these agents has yet been released in the field.

INTRODUCTION

Biocontrol research on weeds has most frequently been conducted by entomologists and has focused on the use of phytophagous (plant-feeding) insects as control agents. However, the potential of plant pathogens for this purpose, although as yet relatively unexplored, is becoming increasingly apparent (Shrum 1982). Biocontrol research with plant pathogens (fungi, viruses, and nematodes) is currently under way in at least 13 countries in temperate to tropical zones and in 18 states of the U.S. (Templeton 1982).

Two approaches to weed biocontrol with plant pathogens have been pursued: the use of mycological herbicides (termed "myco-" or "bioherbicides"), and the use of organisms, usually from a foreign country, in conventional, or "classical," programs (Templeton 1982). The mycological herbicide technique was developed largely by plant pathologists in Arkansas and Florida and is applicable mainly in annual monoculture crop production (Templeton 1982). It involves the selection of diseases that already occur on target weeds, and artificially culturing the pathogen in large quantities. Masses of fungal spores are then collected, sometimes combined with an inert carrier, and sprayed or dusted on the field in much the same manner that a chemical herbicide is applied. Mass-produced inoculum of the endemic fungus *Colletotrichum gloeosporioides* f. sp. *aeschynomene* was sprayed in Arkansas rice fields infested with northern jointvetch, *Aeschynomene virginica* (Daniel *et al.* 1973; Smith *et al.* 1973). Success rates in the control of this weed averaged as high as 99% by this method (Daniel *et al.* 1973). For large acreages, the mycoherbicide may be applied with crop duster aircraft. Weed control by this approach, as with conventional herbicide application, usually requires the reapplication of the mycoherbicide on a regular schedule each growing season. Success depends on the amplification of otherwise insignificant effects of pathogens through concentration to abnormally high levels. Some mycoherbicides are sufficiently effective to be patented, commercially produced, and marketed under trade names (Bowers 1982).

The conventional approach to biocontrol with plant pathogens usually involves the introduction of a new pathogenic agent into the environment, followed by natural dispersion from the introduction point(s) (Templeton 1982). Little or no attempt is made to artificially increase inoculum amounts or to apply infective material widely to target plant populations. The success of this approach is enhanced by the ability of large quantities of inoculum to survive unfavorable environmental conditions and to efficiently disperse (Shrum 1982).

As might be expected, conventional biological control would be usually most applicable in natural areas or other non-agricultural situations in which weeds are widely scattered and difficult to locate or to approach for treatment on an individual basis. Even in natural areas, however, alien grasses and woody species may become established to the exclusion of other vegetation types in monoculture-like stands. The alien grasses *Andropogon virginicus* and *Schizachyrium condensatum* form such stands in Hawaii Volcanoes National Park.

SUCCESSFUL APPLICATIONS OF BIOCONTROL WITH PLANT DISEASES IN HAWAI'I

Two significant weed biocontrol programs have been undertaken in Hawai'i in the past. Carpenter (1944) reported a notable example of the successful application of a fungal disease agent already present in the environment for biocontrol of an unwanted plant. A variety of the pathogenic fungus *Fusarium oxysporum*, which causes a soft rot, was used to control prickly pear cactus (*Opuntia ficus-indica*). Researchers manually inoculated the plants by creating wounds in the cladodes (pads) into which small portions of fungal cultures were introduced. This approach, which was particularly well-suited to the growth habit and distribution of prickly pear in Hawai'i, may perhaps be considered a hybrid biocontrol technique since it involved elements of both the mycoherbicidal and the conventional concepts. A naturally-occurring pathogen was cultured and its successes in distribution and infection were greatly enhanced through artificial manipulation. However, the pathogen was not produced in large quantities for widespread, general application. This is among the most successful of any biocontrol work thus far attempted in Hawai'i (Gardner and Davis 1982; Davis *et al.*, this volume).

A second outstanding success in Hawai'i has been achieved with *Entyloma ageratinae* or *E. compositarum* (Barreto and Evans 1988; Trujillo *et al.* 1988), a pathogenic leaf-spotting fungus in the control of Hāmākua pāmakani, *Ageratina riparia* (= *Eupatorium riparium*) (Trujillo 1976; Templeton 1982; Davis *et al.*, this volume). The weed is from Mexico, and the fungus was brought to Hawai'i from Jamaica specifically for the purpose of biocontrol. Upon establishment in the field, this pathogen has caused dramatic reductions to the point of complete elimination of host populations in many areas between 1,500 and 6,500 ft (455-1,980 m) elevation. Resprouting has been observed in wetter areas, but other plants shade out young seedlings. The pathogen was capable of distributing itself by natural means, particularly at lower elevations, and thus required little subsequent manipulation (Trujillo 1976; C.J. Davis, pers. comm.). The self-distribution of the Hāmākua pāmakani fungus to infestations of this weed in natural areas is a good example of the effectiveness of biocontrol with plant pathogens in native communities.

A third project, directed at the control of the forest weed commonly known as Koster's curse or clidemia, *Clidemia hirta*, which was introduced to Hawai'i from tropical America, is under way (Trujillo *et al.* 1986). An isolate of the fungus *Colletotrichum gloeosporioides*, which causes anthracnose of *Clidemia hirta*, was collected on this weed in Panama by E.E. Trujillo of the University of Hawaii. A special form of *Colletotrichum gloeosporioides* was mentioned above as a mycoherbicide for control of northern jointvetch in Arkansas. Pathogenicity tests with the Panama isolate were conducted on *Clidemia hirta* at the U.S. Department of Agriculture Foreign Disease-Weed Science Research Unit at Fort Detrick, Maryland. The fungus was subsequently released at a number of clidemia-infested sites in Hawai'i, where it shows initial promise, but its effectiveness is still under evaluation.

SUCCESSES ELSEWHERE WITH PLANT PATHOGENS AS BIOCONTROL AGENTS

Plant disease agents have proven successful or are showing promise in biocontrol programs elsewhere. Approximately 83 pathogens under current study for the control of 54 target weed species throughout the U.S. and in other countries were listed by Templeton (1982). The attractiveness of fungi as biocontrol agents, in particular, focuses on their general ubiquitousness, high degree of host specificity, destructiveness to the host, persistence, dispersal efficiency, and ease of culture and maintenance in the laboratory (U.S. Department of Agriculture 1978).

The plant rust fungi are typically wind dispersed, may infect directly through the host epidermis or through stomata and do not require wounds, are virulent, and are highly host specific. Members of this group of fungi are therefore particularly well suited as biocontrol agents (Quimby 1982; Gardner, pers. observ.). On the other hand, rust fungi are obligate parasites (*i.e.*, they cannot be cultured on artificial media), a possible disadvantage (Quimby 1982).

Successful and well-documented examples of fungi, particularly rusts, in biocontrol programs include the use of *Puccinia chondrillina* to control rush skeleton weed (*Chondrilla juncea*) in the U.S. and Australia (Hasan 1972; Cullen *et al.* 1973; Hasan and Wapshere 1973; Wapshere 1975; U.S. Department of Agriculture 1978; Emge *et al.* 1981). Rush skeleton weed, a perennial weed of Eurasian origin, has become a serious pest in farm and range land in the western U.S., as well as in the wheatlands of Australia, where it is a strong competitor for moisture and nitrogen. Infection with *P. chondrillina* has been shown to drastically reduce seed production, seed germination, and plant vegetative growth. A gradual reduction in the weed population results from the disease (Emge *et al.* 1981).

The rust fungus *Phragmidium violaceum* in Germany was recognized as a potential biocontrol agent for two bramble (*Rubus*) species, *R.*

constrictus and *R. ulmifolius*, in Chile (Oehrens 1977; Oehrens and Gonzalez 1977) and was introduced to Chile for evaluation. The subsequent success of this approach has led to the further investigation of *P. violaceum* for control of brambles in other countries as well.

Successful control of yellow nutsedge, *Cyperus esculentus*, under experimental conditions by another rust fungus, *Puccinia canaliculata*, has been reported in the southeastern U.S. (Phatak *et al.* 1983). Yellow nutsedge occurs on a worldwide basis and is considered one of the most troublesome weeds. In recent years it has spread rapidly throughout the U.S. and in 1981 was considered the most important perennial weed in most of the Midwest (Stoller 1981). The fungus inhibited nutsedge flowering and new tuber formation, and dehydrated and killed plants.

Uromyces rumicis is credited with the suppression of curly dock (*Rumex crispus*) in Europe (Inman 1971; U.S. Department of Agriculture 1978). Curly dock is a common pasture weed throughout the U.S. and is a significant problem in certain southern states. Based upon the severe attack of *U. rumicis* on curly dock in Europe, and the apparent host specificity of this pathogen, research efforts to evaluate its introduction into the U.S. showed promise (Inman 1971). Current reports of the study could not be located, however.

Wilson (1969), Zettler and Freeman (1972), Goeden *et al.* (1974), Hasan (1974, 1980), and Charudattan and Walker (1982) have provided comprehensive discussions and literature reviews of the subject of weed biocontrol with plant pathogens.

CONSIDERATIONS IN USING BIOCONTROL IN NATIONAL PARK SERVICE AREAS

From the above examples of successful applications, it is evident that little precedent exists for the concept of biocontrol in natural environments. The weed control objective of the National Park Service in preserving native ecosystems differs significantly from that of agricultural, including horticultural or range management, objectives. Whereas the goal of other biocontrol research programs discussed has been the preservation of particular crop or range species in a severely manipulated agricultural environment, a purpose of the National Park Service is to maintain, as nearly as possible, the overall integrity of native environments. Thus, cultural practices recommended for agricultural applications, such as frequent cultivation or favorable timing of crop plantings, are not applicable to weed control in natural systems.

Alien plants in Hawai'i's national parks for which biocontrol is considered are those with little or no value as crops, ornamentals, or forage species elsewhere in the Islands, and those which are not so closely related to economically valuable plants that introduced pathogens or insects would pose a threat (Gardner and Davis 1982; Markin and Yoshioka, this volume). Once released into the environment, biocontrol agents may become naturally distributed throughout their biological ranges and are not limited by political boundaries. The movement of soil-borne pathogens, or of those transmitted by insects or rain, is presumably more easily controlled than is that of wind-dispersed fungi (Shrum 1982). Potential biocontrol agents would most likely, but not necessarily, be found in the country of origin of the problem alien plant (Charudattan 1982; Shrum 1982). Locally occurring pathogens also may be evaluated as biocontrol agents in situations where artificial manipulation and application appear feasible.

Experience indicates that insect agents may exhibit a significant difference in activity between the old and new habitats due to predation and parasitism in the former. Thus, subdued activity observed in the native habitat does not necessarily indicate a potential ineffectiveness of the insect in the new environment where it may be free of its enemies. However, while hyperparasitism is known among some plant pathogens, this is not usually an important factor in limiting the activity of these agents. Therefore, given the consistency of other environmental factors, the observed effectiveness of a disease in the native habitat is more nearly an indication of its projected effectiveness in the new habitat.

Procedures and Limitations

Biocontrol programs must focus on knowledge of suitable agents that attack the target plant, and perhaps equally important, closely related species. Although preliminary review of the scientific literature is necessary, little information is available on alien plants in Hawai'i (Gardner and Davis 1982). Problem alien plants in Hawai'i are often of little significance in their native habitats, and few entomologists or phytopathologists are available in developing countries who can devote attention to research projects of less than high priority in those regions.

Firsthand exploration in the native regions of the target plant to discover its insects and pathogens may be essential to the success of a biocontrol program (Charudattan 1982). Strawberry guava (*Psidium cattleianum*) is an aggressive, widely distributed alien species invading National Park Service areas in Hawai'i and is targeted for biocontrol (Hawaii Volcanoes National Park 1985). Literature searches have revealed no reports of insects or diseases on this species in its native South American habitats. Neither diseases nor insects of apparent significance have been observed on this plant in Hawai'i. However, during recent exploration in Brazil, sponsored by the National Park Service, C.S. Hodges found several apparently unreported insects and diseases on the yellow-fruited variety of strawberry guava. Some of the insects, in particular, showed promise as possible biocontrol agents and should be further investigated (Hodges 1988).

Scientists undertaking exploratory trips should be accompanied by local scientists, if possible, who are familiar with the region and can facilitate research efforts. Characteristics and activities of the potential agent(s) under natural conditions can thereby be observed and information on host ranges, life cycles, fruiting habits, distribution, and abundance gathered. Although these data are useful, caution should be exercised since the agent, like the target plant itself, may behave differently in the new environment than in the native ecosystem (Wilson 1969; R. Burkhart, pers. comm.; D. Gardner, unpub. data).

Ideally, exploration should be conducted during different seasons to provide the greatest opportunity for observation of potential biocontrol agents and their effects. Initial evaluation and screening of a potential agent is best conducted in the native country before importation into the U.S. (Charudattan 1982). When this is not possible, agents not already present in the U.S. have to be tested under quarantine. They should be hand carried by the researcher into the U.S., to the quarantine facility, to insure proper care and the greatest chance of survival (G. Markin, pers. comm.).

The researcher may find it necessary to extend his or her stay in a foreign country; six-month visits are normal. Use of laboratory facilities, supplies, equipment, and other needs at a local research establishment may be required, or contract funding for local scientists, if available, to conduct the work may be necessary.

Foreign exploration presupposes the cooperation of the foreign government in permitting access by U.S. researchers. Likewise, conditions of political unrest, dangers associated with drug trafficking activities, and similar situations that cause travel in particular countries to be unsafe for Americans may lead the U.S. Government to declare such localities off limits to foreign travel. The U.S. Forest Service imposed such a prohibition on travel by its personnel to Colombia in 1985, where exploration is currently under way for biocontrol agents for banana poka (*Passiflora mollissima*) in Hawai'i (G. Markin, pers. comm.).

U.S. Department of Agriculture Animal and Plant Health Inspection Service and Hawaii State Department of Agriculture regulations require that a permit be obtained prior to the importation of foreign insects or pathogens not already present into the U.S. and into Hawai'i (Charudattan 1982; State of Hawaii Department of Agriculture Chapter 71 Administrative Rules). Requirements specify that the potential biocontrol agent must be maintained in a certified quarantine facility to prevent premature escape (Klingman and Coulson 1983). Since pathogenic fungi and other plant disease agents are microscopic or produce microscopic spores that are often air-borne, specifications for quarantine facilities in which pathogens are to be contained are more stringent than are those for facilities in which insects are maintained (Melching *et al.* 1983; Gardner and Smith 1985).

Relatively few certified facilities for entomological research are in operation throughout the U.S., presumably due to the high cost of construction. The new quarantine facility at Hawaii Volcanoes National Park is among these. Even fewer containment facilities certified for work with plant pathogens are in operation (Charudattan 1982; Melching *et al.* 1983). The Hawaii Department of Agriculture has recently constructed a small biocontrol facility for plant pathogens and added a plant pathologist to its staff (P.-Y. Lai, pers. comm.). A full description of the U.S. Department of Agriculture quarantine facility for plant pathogens, located at Frederick, Maryland, has been published (Melching *et al.*

1983). Kahn (1983) published a comprehensive discussion of the proper design and maintenance of quarantine facilities for all types of biocontrol programs.

For Hawai'i, as is presumably true for other states that impose restrictions on importation of foreign organisms, the enforcement authority is with the local agriculture department. Until recent years, all biocontrol work in Hawai'i was focused on the protection of agricultural or horticultural products. Current involvement of other State and federal agencies, including the National Park Service, in biocontrol research has complicated the objectives. The Interagency Steering Committee for Biological Control of Forest Pests, chaired by the Hawaii Department of Land and Natural Resources, was created to alleviate this problem. The University of Hawaii, Hawaii Department of Agriculture, U.S. Forest Service, and National Park Service representatives also serve on the committee.

The purpose of the Steering Committee is to coordinate biocontrol interests among agencies so that conflicts and duplications of effort are minimized, and to provide a forum where priorities are set and cooperative efforts facilitated. The Steering Committee serves only to advise the Hawaii Department of Agriculture, however, since that agency retains final authority in biocontrol research matters. Following review by the Hawaii Department of Agriculture, permit requests for importation or release of foreign organisms are further reviewed by representatives of major agricultural industries, such as those of the Hawaiian Sugar Planters' Association. Upon issuance of a State permit, application for a similar U.S. Department of Agriculture Animal and Plant Health Inspection Service permit must be initiated. All restrictions and conditions imposed by these agencies must be complied with. Importation requirements can often be more readily addressed if information is available on the characteristics of the organism to be imported prior to introduction.

Whereas a fully certified insect containment facility for biocontrol research is currently in operation at Hawaii Volcanoes, a comparable National Park Service facility for plant pathogens is not yet available. Biocontrol research with plant pathogens has been conducted at the University of Hawaii under confined laboratory conditions by both University and National Park Service researchers working independently of one another. The Park Service work is presently conducted in Plexiglas plant growth chambers designed and constructed for this purpose. Future research accommodations remain to be determined, although it is possible that development of specific cooperative agreements will enable use of the new Hawaii Department of Agriculture facility within constraints imposed by that agency on a space-available basis (P.-Y. Lai and G. Funasaki, pers. comm.).

Expectations from Successful Biocontrol Programs

Unlike other methods of weed control, such as the use of herbicides, the effects of a successful biocontrol program may be subtle and not immediately or readily perceptible, particularly to persons not closely involved with the program. Gradual reduction in stands of lantana

(*Lantana camara*) has become apparent over a period of several years, as has been the decline in prickly pear cactus populations on ranchland on the island of Hawai'i (Davis *et al.*, this volume). A program may be regarded successful if reduction in vigor, growth rate, or ability of the alien plant to reproduce occurs and may, over time, allow native species to successfully compete where they could not previously (C.W. Smith, pers. comm.). Attack by disease agents may only reduce to manageable levels, rather than eliminate, target weed populations. Alien plant populations, while usually maintained at low and controllable levels by biocontrol agents operating successfully in the environment, may be expected, on occasion, to irrupt. These periodic rapid increases may correspond to environmental conditions which unusually favor the host and/or disfavor the pathogen (Holcomb 1982; Shrum 1982).

Although, as indicated above, some successful biocontrol programs have depended on a single control agent, the possibility of integrating biocontrol approaches with other methods of plant control should not be overlooked (Smith 1982). Thus, any complementary effects of insects, pathogens, mechanical, cultural, and chemical methods that can be applied to particular alien species are utilized.

Potential Advantages and Disadvantages of Biocontrol

Although the potential for success of biocontrol in natural ecosystems is not known, National Park Service resource managers consider this approach an important long-term solution to widespread, aggressive aliens (Gardner 1982; Hawaii Volcanoes National Park 1985). The National Park Service discourages use of chemical pesticides where other methods will suffice, but considerable use occurs for many reasons. A 1981 memorandum from Park Service Director Russell Dickenson, updating the 1978 National Park Service Management Policies Handbook, stated:

> Chemical pesticides of any type will be used only where feasible alternatives are not available or acceptable. The Service's use of all pesticides shall be approved by the Director. Application shall be in accordance with applicable laws, Departmental and Service guidelines, and Environmental Protection Agency and Occupational Health and Safety Administration regulations.
>
> Service policy does not prohibit, as such, the use of chemical pesticides. However, chemical controls are to be allowed only if (a) there is a clear and present danger to the health and safety of man; and/or (b) there is danger of destruction of significant property or resources and a determination has been made that the control methods of no action, mechanical, cultural and/or biological control are non-existent, unavailable, or unacceptable.

Since, as stated, mechanical, cultural, and biocontrol approaches are considered preferable to use of chemical pesticides, these methods should of necessity be thoroughly evaluated in any Park Service alien plant control program. As with other insular systems, the problem of invasion by alien species in Hawai'i is particularly severe as compared with that of continental systems (Gardner 1982). For this reason, although official pesticide usage regulations still apply, these guidelines may be relaxed in actual practice in favor of expeditious alien plant control in Hawaiian National Park Service areas. Manual removal of alien plants, sometimes in combination with herbicidal application, has proven effective in local, relatively restricted populations of alien plants (Tunison and Zimmer, this volume).

Notwithstanding this, labor-intensive approaches have not proven successful for widespread, well-established alien plant species over large areas. Cultural techniques, which involve manipulation of the environment to favor the desired and/or disfavor the undesired entity, appear more applicable under conditions of crop or ornamental cultivation than to natural habitats.

On the other hand, the field of biocontrol, especially as it is applied to native ecosystems, is controversial. Introduction of foreign organisms, including biocontrol agents, into native habitats is considered undesirable environmental tampering by some individuals concerned with environmental preservation (Howarth 1983). The long-term effects of introduced organisms in fragile ecosystems are unpredictable, possibly causing more harm than they were introduced to prevent. Howarth (1983) stated "experience has shown that there are serious risks to the natural environment involved when foreign organisms are purposefully established in a new land." Introduction of foreign organisms, especially indiscriminately, causes "biological pollution" (Howarth 1983) and results in a native environment becoming nonnative by definition. Some of the objections to biocontrol approaches are presumably less applicable to agricultural situations, in which there is no intention of maintaining environmental integrity, than to natural systems, but agricultural agents may spread to native systems.

A major potential problem in relying on biocontrol approaches for particular alien species prior to an investigation is the lack of assurance that suitable control agents exist at all. Since such agents must be both sufficiently host-specific and virulent to be effective, it is quite possible that pathogens or insects found on a given alien species meet neither, or only one, of the requirements but fail to meet both. Problems associated with locating potential agents may also pose a significant drawback.

A related difficulty is the loss of research time if, after considerable time has been invested in host testing, the candidate agent is shown to attack desirable plants and must be discarded. As an example, in 1955 F.A. Bianchi, a Hawaiian Sugar Planters' Association entomologist, traveled under the sponsorship of the Hawaii Territorial Board of Agriculture to the Azores, Madeira, and the Canary Islands to search for biocontrol agents of firetree (*Myrica faya*) (Hodges and Gardner 1985; F. Bianchi, unpub. corres.). The most promising discovery was that of a fungus, *Dothiorella* sp., which caused significant shoot dieback of firetree. However, host range testing by a plant pathologist in Portugal eventually indicated that the fungus would also attack several trees of value in Hawai'i, including mango (*Mangifera indica*) and avocado (*Persea* *americana*). It was subsequently necessary to discontinue the research (Azevedo 1960; Hodges and Gardner 1985; F. Bianchi, unpub. corres.).

Costs associated with a biocontrol program are initially high, and results are usually not quickly evident. Although the testing process in quarantine is designed to be comprehensive, it is not possible to evaluate the behavior of a potential biocontrol agent under every combination of environmental condition and host plant that it may encounter in the field. Due to space limitations, host range testing in quarantine is often restricted to seedlings or small plants, whereas certain plants are known to be more susceptible to particular pathogens at certain developmental stages than at others (Shrum 1982). Misleading results arising from these variables may presumably lead to unwarranted release of the agent. Mutability among microorganisms following release and innate genetic heterogeneity among plant populations are also unpredictable dangers (Leonard 1982). Thus, although the problems are minimized, even extensive testing under quarantine cannot fully assure continued reliability of the released organism.

On the other hand, although initial research expenses may appear prohibitively high, the long-term cost of a successful biocontrol program may be much lower and less labor intensive, requiring a small effort relative to conventional programs. An efficiently self-dispersed agent does not cause the detrimental environmental effects incurred by the activities of laborers with their equipment in conventional control efforts in sensitive ecosystems. Tactical problems, such as transportation of large numbers of laborers to remote areas, with their supplies and equipment, are also lessened in biocontrol programs.

The long-term environmental impacts of neither biocontrol, for the above stated reasons, nor herbicidal use can be fully assessed with current information. Although manufacturers are required to specify permissible uses on the labels of each commercially available pesticide, these are usually agriculture-oriented applications; little information is currently available on persistence and activities of these materials, or their degradation products, in native Hawaiian ecosystems. Proven parkwide control methods for the particular, widespread, well-established alien species in Hawaiian National Park Service areas, whether chemical, mechanical, or biological, therefore do not appear to exist at present.

CURRENT STATUS OF NATIONAL PARK SERVICE RESEARCH IN BIOCONTROL WITH PLANT PATHOGENS

Banana Poka

Banana poka, generally known as *Passiflora mollissima* in Hawai'i (LaRosa 1985), although some researchers consider *P. tripartita* the correct name (E. Trujillo, pers. comm.), is among the highest priority forest weeds designated by Hawaii Volcanoes National Park natural resource managers for biocontrol research (Hawaii Volcanoes National Park 1985). The ability of this aggressive vine to invade and destroy native forests, where it covers the canopy with dense mats of foliage, is also recognized by the Hawaii Department of Land and Natural Resources, which is participating in biocontrol efforts against this plant (Tanimoto and Char, this volume).

Literature reports were found of a destructive vascular wilt disease affecting *Passiflora edulis* forma *edulis*, the purple-fruited passion fruit variety favored for human consumption, in Queensland, Australia (Purss 1954, 1958). The causal fungus was *Fusarium oxysporum* f. sp. *passiflorae*, a form considered specific to this host. A later report indicated that effective control of this soil-borne disease could be obtained by grafting *P. edulis* f. *edulis* scions to *P. edulis* f. *flavicarpa* rootstocks, which were disease resistant (Purss 1958; Inch 1978). *Passiflora edulis* f. *flavicarpa* is the yellow-fruited variety more common in Hawai'i, known locally as liliko'i. It is of some commercial value in Hawai'i. Varieties of passion fruit resistant to the *Fusarium* wilt disease (*e.g.*, Redlands Triangular) have been developed and are currently available to the passion fruit industry (Inch 1978). No genera of Passifloraceae occur in Hawai'i other than *Passiflora*, and no members of this genus are native. With the exception of liliko'i, none of the approximately 30 species of *Passiflora* in Hawai'i, and their varieties, are of any commercial value. Banana poka is presumed to have been introduced as an ornamental that has escaped cultivation. Whereas banana poka fruit may be consumed as human food in the native countries of this species (E. Trujillo, pers. comm.), it is not used for this purpose in Hawai'i.

The special forms of *F. oxysporum* are characterized by their host specificity, often attacking only one or a few closely allied host species (Gordon 1965). The likelihood was not considered great that *F. oxysporum* f. sp. *passiflorae* would attack banana poka. Even though both are members of the genus *Passiflora*, *P. edulis*, classified in the subgenus *Passiflora*, and *P. mollissima*, subgenus *Tacsonia*, are less closely allied. It was nevertheless desirable to confirm this assumption by inoculation tests.

Upon obtaining Hawaii Department of Agriculture and U.S. Department of Agriculture Animal and Plant Health Inspection Service permits, cultures of *F. oxysporum* f. sp. *passiflorae* were obtained from Queensland, Australia. Seedlings of banana poka, purple liliko'i, yellow liliko'i, *P. suberosa*, *P. ligularis*, and *P. foetida* were inoculated by a root-dipping method, whereby the seedling was removed from the growth medium, the roots were immersed in a spore/water suspension, and the seedling was replanted. Although the root-dip method is an artificial method not applicable in the field, it is useful as a direct, uniform screening approach to determine basic resistance or susceptibility. Similarly, seedlings of many crop and ornamental species, unrelated to Passifloraceae and representing a number of plant families, were tested by the root-dip method to establish host specificity.

Mortality rates among representatives of the Passifloraceae tested (Table 1) have shown banana poka to be significantly susceptible, with more

Table 1. Mortality of *Passiflora* spp. seedlings inoculated by dipping roots in a spore suspension of *Fusarium oxysporum* f. sp. *passiflorae*.

Species	% Mortality rate	Sample size
P. mollissima	69	459
P. mollissima (older seedlings)	46	307
P. edulis f. *edulis*	37	250
P. edulis f. *edulis* (older seedlings)	30	63
P. edulis f. *flavicarpa*	0.29	345
P. edulis f. *flavicarpa* (older seedlings)	0	101
P. foetida	32	233
P. ligularis	26	70
P. suberosa	0	224

than 90% of seedlings killed in some tests. None of the inoculated seedlings of species not in the family Passifloraceae became diseased, nor did ratoons of sugar cane (*Saccharum officinarum*) and pineapple (*Ananas comosus*), Hawai'i's most important agriculture crops.

Banana poka was further tested by a more natural method, in which seeds were sown in soil amended with inoculum (Gardner 1989). Resulting mortality reached 66% (of 79 emerged seedlings). The fungus was re-isolated from stem tissue of diseased plants and recultured on artificial medium. No disease-related mortality was obtained in the non-inoculated control treatment (Gardner 1989). As is common among vascular wilt diseases associated with *F. oxysporum* (Gardner and Burge 1980), the disease in infected *Passiflora* spp. plants was severe, and no infected plants recovered.

Although *Fusarium* wilt disease occurred in larger, fruit-bearing plants in Australia (Purss 1954, 1958), space limitations in the growth chambers did not allow testing of large *P. mollissima* plants in the National Park Service study. Current results with seedlings suggest, however, that *F. oxysporum* f. sp. *passiflorae* has a promising degree of virulence on banana poka. Furthermore, these tests indicate that this fungus may not pose a serious threat to *P. edulis* f. *flavicarpa* in Hawai'i, as demonstrated by the use of *Passiflora edulis* f. *flavicarpa* for resistant rootstocks in Queensland. Field testing under the actual environmental conditions at which banana poka occurs in Hawaiian forests, with particular reference to soil and air temperatures, is necessary before further conclusions concerning its effectiveness can be drawn.

A high-pressure stem injection method of inoculation, developed for biocontrol experimentation with a related vascular wilt disease (Gardner and Burge 1980), should also be tested on large banana poka plants in the field.

Firetree

Among the most intensive of any mechanical and chemical control efforts have been directed against firetree during the past two decades by Hawaii Volcanoes National Park natural resource management (Gardner and Davis 1982). Through constant surveillance, the plant is now controlled in limited areas of the Park, designated as Special Ecological Areas, but is spreading out of control elsewhere. There are no native or otherwise valued members of the Myricaceae family in Hawai'i, making this plant a good candidate for biocontrol. In previous investigations of biocontrol of firetree, Krauss (1964), a Hawaii Department of Agriculture entomologist, explored the Azores, Madeira, and the Canary Islands in 1960 and 1962 for potential control agents. He also searched for insects on other species of *Myrica* in the southeastern and northeastern U.S., Mexico, Costa Rica, and various parts of Africa. A list of insects and fungi he found was published (Krauss 1964). Attempts to establish several of the insect species on firetree in Hawai'i were not successful, however (Krauss 1964; Hodges and Gardner 1985).

In 1984, C.S. Hodges of the U.S. Forest Service and the author, of the National Park Service, traveled to the Azores, Madeira, and Canary Islands in a further attempt to discover potential biocontrol agents for this plant in Hawai'i (Gardner 1984; Hodges and Gardner 1985). A second exploratory trip to the Azores and Madeira was undertaken in 1987 (Gardner *et al.* 1988). Three fungus diseases were found, which appeared to be sufficiently important to warrant further study. However, the diseases did not appear to significantly limit populations of firetree in the native environment (Gardner 1984; Hodges and Gardner 1985). A stem canker disease associated with an unidentified species of *Cryphonectria*, however, was not widespread, and its potential should be further investigated (Gardner *et al.* 1988). Insects found, including the fruit-boring species *Carposina atlanticella*, are reported by Krauss (1964) and discussed elsewhere.

Florida Blackberry

Florida blackberry (*Rubus argutus* = *R. penetrans*) is a high-priority designated species for biocontrol (Hawaii Volcanoes National Park 1985; L.L. Loope, pers. comm.). This thorny, aggressive bramble forms dense, impenetrable thickets in native forests. Other alien species of *Rubus* pose similar threats but are more limited in distribution at present. Although blackberry fruit is sometimes consumed by individuals, none of the alien species of *Rubus* in Hawai'i are of commercial value. Two endemic species, *R. hawaiiensis* and *R. macraei*, occur in native forests on the islands of Kaua'i, Maui, and Hawai'i, however. The protection of these species is of primary concern in any biocontrol program against the alien species of *Rubus*.

Successful biocontrol with the rust fungi *Kuehneola uredinis* and *Phragmidium violaceum* of other species of *Rubus* in South Africa and

South America was described in the literature (Oehrens 1977; Oehrens and Gonzalez 1977; Hasan 1980). The probability of *P. violaceum* attacking any of the alien species of *Rubus* in Hawai'i was not considered great, since *Rubus* is an extremely genetically diverse genus throughout the world. A small amount of *P. violaceum* inoculum was provided the National Park Service, under permit, by E. Oehrens in Chile, but upon its arrival the fungus was apparently nonviable. No further work with *P. violaceum* has been attempted.

Kuehneola uredinis was discovered to be already present in Hawai'i, where it attacks leaves of *R. argutus* (Gardner and Hodges 1985). Subsequent field observation and inoculation experiments showed the pathogen to be capable of producing small, isolated sori on leaves of both *R. hawaiiensis* and *R. macraei*. The extent of infection appeared insignificant on both native species, however, and caused no damage.

In further work, root cuttings of the two endemic species and four prominent alien species (*R. argutus, R. ellipticus, R. rosifolius, R. glaucus*) occurring in Hawaiian National Park Service areas were established at North Carolina State University. A small number (usually two to four) of plants of each species was inoculated with *Gymnoconia nitens*, a rust fungus capable of causing severe systemic infection (as distinct from leaf infection only) on species of *Rubus* in the Southeast (Gardner 1984). Inoculation was accomplished by spraying a spore/water suspension on the leaves and maintaining plants under conditions of high humidity for two days. The native plants did not thrive in the new environment and eventually died, but without evidence that they had become infected. Among the alien species of *Rubus*, infection occurred only on *R. argutus*, some plants of which died from the disease (J. Ballington, pers. comm.). Further work to establish conclusively the pathogenicity of *G. nitens* on *R. argutus* from Hawai'i and to evaluate its effects on the native species is under way in North Carolina. Also, *K. uredinis*, which is reported to cause systemic infection that significantly impacts the development of species of *Rubus* in the Southeast (C. Hodges, pers. comm.), should be tested on the native and alien species from Hawai'i. Even though this fungus in Hawai'i is considered to be the same species as that in the Southeast, the possibility exists that a more virulent strain occurs in the Southeast that may be useful in Hawai'i.

CONCLUSIONS

The overall percentage rate of success among all biocontrol efforts undertaken with plant pathogenic agents cannot yet be documented (Templeton 1982). Several examples of successful, and even spectacular, results of biocontrol approaches with plant pathogenic agents in agricultural situations are known, and success with Hāmākua pāmakani in natural areas of Hawai'i has been acknowledged (Davis *et al.*, this volume). These successes indicate more possibilities for application of biocontrol in natural environments. A prerequisite of most biocontrol programs is that target species be of little or no value outside designated control areas, since biocontrol agents usually can neither be contained within political boundaries nor recovered once released. Furthermore, suitable agents may not be available for all species for which biocontrol would be desirable. For these reasons, opportunities for application of biocontrol techniques may be limited and controversial, and other alien plant control approaches should also be used. The future possible use of genetic engineering techniques to manipulate the virulence or host specificity of pathogens and thus create suitable agents for biocontrol purposes may have merit.

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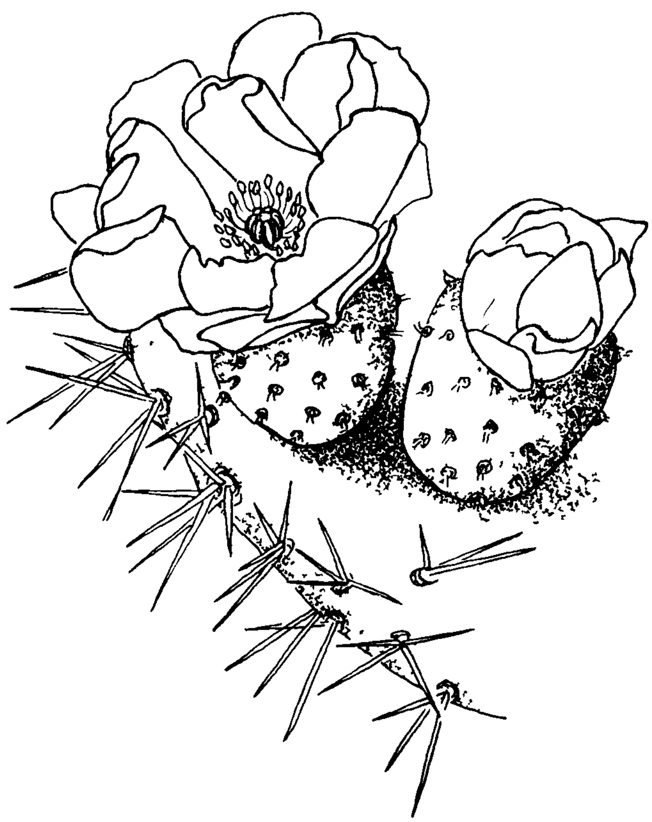

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