Plant pathogens as biological agents for the control of weeds

Background

Weed control is by far the most pervasive and costly need in agriculture, both in underdeveloped as well as in technologically advanced production systems. In 1994, losses due to weeds in U.S. agriculture—including herbicide costs and yield losses—amounted to over $15 billion, and about 96% of the more than 21 million acres of row crops grown in Iowa received at least one chemical herbicide application. Pesticide use statistics reveal that more herbicides are used than any other class of pesticide. Despite the extensive use of herbicides, certain weed species continue to cause problems in agriculture, and current control strategies for some of these are inadequate. Among these weeds are johnsongrass (Sorghum halapense), the morning glories (Ipomoea spp.), nutsedges (Cyperus esculentus), shattercane (Sorghum bicolor), and velvetleaf (Abutilon theophrasti).

Although chemical herbicides are an important component of modern crop production technology, their use can entail significant costs, not only to agriculture, but to society as well in terms of negative effects on nontarget organisms, water quality, and other facets of the environment. Extensive use of herbicides and other pesticides has resulted in documented cases of ground water contamination, and concern is mounting that their continued use may impact human health in both rural and urban areas.

As plants, weeds are subject to attack by the same sorts of biological pests as are crop plants. Biological control, which has been recognized as an essential element of the agriculture of the future, is defined as the management of natural enemies (predators, parasites, and pathogens) of pests and selected beneficial organisms (antagonists, competitors, and allelopaths) and their products to reduce pest populations and their effects to non-harmful levels.

Of three basic approaches to biological weed control, the classical approach introduces exotic agents obtained from the target weed’s native range. Although this approach—the most common—has been successful, it involves a potential risk of unforeseen behavior by the introduced agent in its new habitat due to the absence of its natural enemies.

The second approach, based on conservation, employs manipulation of environmental factors to enhance the effects of existing native or exotic biological control.

The third approach, augmentation, is based on the inundative release and/or distribution of native (endemic) agents to achieve biological control of exotic weeds. This approach has achieved considerable success in the use of plant pathogens as agents for biological control of certain weeds. Plant pathogens typically have a fairly narrow host range, making them relatively safe to use as biological weed control agents in terms of possible nontarget plant effects.

The identification and development of pathogens capable of controlling important agricultural weeds will have significant beneficial impact on crop production systems by reducing the present reliance on petrochemical-based herbicides. While costs will vary, in most instances they will be lower because the pathogen inoculum can be produced on a variety of comparatively low-cost, organic waste
materials. In most instances, the pathogen could be applied in comparatively low numbers and will then increase, utilizing the weed host as its energy base. Because biological agents tend to be rather specific in their host range, off-target and negative environmental effects are expected to be minimal. Depending on the spectrum of weeds present in a field, mixtures of pathogens may be needed.

A number of weeds that cause economic loss in the north central region of the United States could be controlled by proper management of indigenous fungal pathogens already attacking them at low levels. The pathogens occurring on any one of these weed species may vary from location to location and exist as a wide range of biotypes differing in their host and ecological requirements as well as their virulence and cultural characteristics. Because more efficient strains of pathogens may occur or be developed elsewhere, a database on the pathogens that already attack weeds in Iowa will expedite future importation of such strains for use here.

Thus, this project involved the following objectives:

1. To survey for diseases occurring on agricultural weeds in Iowa and contiguous areas.
2. To identify the causal organisms and establish a database on the diseases attacking the major agricultural weeds of Iowa.
3. To select those pathogens with greatest potential for further development as biological weed control agents.

**Approach and methods**

**Disease survey:** Weed observation sites were established in four quadrants of each of Iowa’s seven Extension areas (central, north central, northeast, east central, southeast, southwest, and northwest). These divisions involve large areas of land unique in their predominant soil types and climates. The survey was conducted over a period that included growing seasons from 1991 to 1993. Survey and collection activities were most intensive during 1991 and 1992; however, some sites were also examined in 1993. Surveys were conducted at two-week intervals during different stages of weed development. A plant disease accession form noting the site, location, host, symptoms, and plant parts infected was completed for each sample collected. Materials were collected that expressed all stages of the diseases present in each particular site. Two host samples were taken at each site. One was used for laboratory and greenhouse culture; the other, consisting of the entire plant where practical, was dried for subsequent identification and inclusion in a systematic collection of diseased weeds. When available, seeds of individual weeds were collected for later use in greenhouse studies.

Causal organisms were identified by direct examination and by isolation in pure culture by standard, aseptic culturing procedures on various media. ("Obligate" organisms that can survive only on their host, such as rusts and smuts, were not cultured.) When pure cultures of the various isolates were obtained, they were placed in tube culture under oil for long-term storage. In the course of these processes, the culture, growth, and sporulation characteristics of each microbial accession were noted in detail.

**Identification of causal organisms:** Selection of pathogens for further study was based on these criteria:
- Observed impact on the host plant in the field: including such items as
  1. Nature of impact (defoliation, stunting, and death)—An impact that reduces competitiveness of the host may be all that most biological control agents can achieve. The question to be addressed is whether this is adequate.
  2. Rate of increase of infection in the plant stand—Rapid rates of increase of diseased host tissue is a desirable characteristic of a potential biological control agent.
  3. Portions of plants attacked—
     a. Foliar lesions (the number required to cause early leaf senescence). These pathogens can cause stunting and reduced competitiveness.
     b. Stem lesions (these pathogens have greater potential as biocontrol agents.
because they can cause major damage and even death with less inoculum than foliar pathogens).

(c) foliar and stem lesions: pathogens with this capability would have the best potential because the leaves are an easy inoculation target and the pathogen could then make the transfer to the stems repeatedly on its own.

(d) vascular parasite (systemic) including stems and roots: these pathogens have very high potential for damage to the host, but they usually require wounds to gain entrance into the plant. They would work well if combined with an insect vector that would provide the entrance wound.

• Prolific production of propagules, or spores, is also necessary. Propagules must be persistent and maintain viability for long time periods.

• Pathogens must be capable of germinating and establishing infection under a wide range of environmental conditions occurring during the growing season of their hosts (target plants); they must also be able to germinate and infect rapidly.

Findings

Disease survey: Survey sites tended to be located in abandoned fields, roadsides, and abandoned farmsteads, because these were the areas in which a number of weeds were found in large enough numbers and in dense stands conducive to the development of the various diseases. Cleanly cultivated fields were generally not productive sites in which to hunt for weed diseases. A few pathogens were found early in the growing season; however, none appeared to become severe enough to be debilitating to their host plants until about mid-summer. Only one (Verticillium sp.) was lethal to its host plant under normal field conditions.

Over 2,000 isolations were made. From these, 89 pathogenic organisms were collected from 22 of the major agricultural weed plants found in Iowa:

22 major weeds in Iowa agriculture:
- American Bindweed
- Bromegrass
- Canada Thistle
- Cocklebur
- Common Milkweed
- Curly Dock
- Giant Foxtail
- Giant Ragweed
- Hemp Dogbane
- Lambs Quarters
- Mild Water Pepper
- Pensylvania Smartweed
- Prickly Lettuce
- Reed Canary Grass
- Shattercane
- Smartweed
- Swamp Smartweed
- Velvet Leaf
- Western Ironweed
- White Top
- Yellow Foxtail
- Yellow Nutsedge

Identification of causal organisms: Upon subjecting the organisms to a screening using the criteria listed, investigators identified the following weeds and pathogens as candidates with reasonably good potential for development as biological weed control agents on their specific weed hosts:

Accelerated leaf senescence of X. strumarium (cocklebur) resulting from infection by rust (Puccinia xanthi).
Weed hosts  Pathogens  
Yellow nutsedge  Puccinia canaliculata *  
(Cyperus esculentus)  
Shattercane (Sorghum bicolor)  Puccinia sorghi *  
Bipolaris sorghi  
Fusarium sp.  
Cocklebur (Xanthium strumarium)  Puccinia xanthi*  
Velvetleaf (Abutilon theophrasti)  Colletotrichum coccodes  
*These organisms are obligate parasites, so they cannot be cultured on artificial media. They could, however, be increased in the field and harvested on their host weeds planted for this purpose. Rust spores at the uredial, or repeating stage, are as a rule very persistent and easily stored for comparatively long time periods. The telial (resting) stage of P. xanthi is extremely persistent. The remainder of the organisms listed are prolific producers of spores and can be grown in the laboratory.

The most encouraging results to date have been achieved with Puccinia xanthi on cocklebur and with Colletotrichum coccodes on velvetleaf. Both of these pathogens cause premature senescence of the leaves and defoliation. C. coccodes rarely attacked the stem or petioles of velvetleaf. P. xanthi occasionally infects young stems and petioles of cocklebur; however, the overall impact of these infections does not seem to be especially severe. Neither of these pathogens is lethal to its host, yet they can both cause severe stunting and reduction of branching in the greenhouse. These effects would likely be more severe in a competitive situation in the field. This project did not assess the impact of successive defoliations.

Because P. xanthi is an obligate parasite and cannot be cultured, the inoculum for our greenhouse trials consisted of infected leaves bearing teliospores (present during the resting stage of the fungus) that had been collected in the field, dried, and stored in plastic bags at room temperature. The telia in those leaves germinated when exposed to moisture, producing basidia on which were borne the basidiospores (reproductive spores present during the infective stage of this rust fungus). Because air circulation with a single rust-infected leaf suspended from the center of the dew chamber (0.66 meters above the cocklebur seedlings) resulted in severe infection of all of the leaves of all of the plants, regardless of their position in the dew chamber was rather slow, investigators assumed that the basidiospores had a low sedimentation rate and were therefore easily transported by air currents. This suggests that distribution of these infectious spores from point sources in the field should be relatively efficient.

In greenhouse tests, the Iowa strains of C. coccodes were as virulent as one obtained from Vermont (courtesy of Dr. Alan Gotlieb, plant pathologist, University of Vermont).

Conclusions: A number of endemic pathogens attack weeds in Iowa. Several of these possess attributes that indicate these pathogens have potential for development into biological agents for control of their specific natural hosts. Most of the pathogens found to attack weeds seem to develop rather slowly during the early part of the season, when their hosts, in their early growth stages, would be most vulnerable to control efforts. The key to successful application of these pathogens will result from developing methods of applying them when they will be most effective at limiting the competitive ability of their hosts.

Implications
Some pathogens may function more efficiently in the presence of reduced levels of certain herbicides. In either case, there is potential for reducing chemical herbicide use, which would improve agricultural profitability and reduce any negative impacts of chemical weed control on the environment. Future research should emphasize identifying indigenous pathogens, because their use would entail far less risk than exotic organisms, which require extensive study to make sure they will not cause problems after they are introduced.

Merely identifying pathogens that attack weeds does not insure that these organisms will control these weeds successfully. The pathogen must possess a number of attributes: ease of artificial culture, sporulation in culture, production of long-lived inoculum (good "shelf life"), good formulation and dispersal characteristics, inoculum that is highly infective under field conditions prevailing during criti-
ical weed control periods, quick sporulation, reinfection of remaining host tissue following initial inoculation, and finally, a host range compatible with the cropping systems prevalent in the area of use.

Most of the pathogens attacking the currently important agricultural weeds in Iowa have been identified and recorded in a data base. In time, with relatively modest investments of resources, some of these could be developed for practical use as biological weed controls. Biological weed control agents will not replace chemical herbicides entirely. They do, however, have potential to reduce the present reliance on chemical herbicides by providing viable alternatives for the control of specific weeds. Lastly, as new strains of the various pathogens are found or developed elsewhere, the regulatory process of bringing them to Iowa will be eased because of the data that now exist on the occurrence of pathogens in the state.

The investigators have conducted training sessions for county weed commissioners in Iowa as well as presented results at various gatherings including county Extension meetings.