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Many grasses, because of their beneficial uses as forage, landscape, or groundcover plants, have been spread around the world for economic development and use. However, during the past century several grasses have turned out to be quite invasive and weedy, for example, tropical signalgrass (*Urochloa subquadripara*).

Tropical signalgrass has been identified as one of the most troublesome weeds in sod farms, golf courses and lawns in Florida (Busey, 2001). In the tions for postemergence control of tropical signalgrass (Busey, 2001). Two applications of MSMA at 1.5 Kg a.i./ha at 13- to 14-day intervals have been found to reduce tropical signalgrass canopy to less than 10 percent, compared with up to 100 percent in untreated plots.

Tank mixing with Sencor does not enhance signalgrass control. Teuton et al. (2002) have identified a few preemergent herbicides including some that control tropical signalgrass when applied early postemergent. Although MSMA is effective in bermudagrass, there are no postemergent herbicide treatments available for St. Augustinegrass, because

A Multiple-Pathogen Bioherbicide System With Potential To Manage Signalgrass In Turf And Sod In Florida

BIOHERBICIDE



Figure 1. A germinating spore of Exserohilum rostratum, one of three fungi used in a bioherbicidal mixture tested on tropical signalgrass.

Figure 2. Tropical signal grass uninoculated (left) and inoculated with a mixture of three fungal pathogens. In greenhouse tests, up to 90 percent of the shoots were blighted on inoculated plants.

northern part of the state, tropical signalgrass is sensitive to frost, but in southern Florida it continues to spread vegetatively in successive years.

Tropical signalgrass is particularly troublesome in sod farms. It is difficult to control because of its tolerance to several chemical herbicides or its ability to outgrow control measures. It is essentially resistant to atrazine and asulox, two commonly used turf herbicides. The lack of selectivity of many chemical herbicides precludes their use to control tropical signalgrass in bermudagrass and St. Augustinegrass sod farms (Busey, 2001).

Chemical Control of Signalgrass in Florida Turf. Currently, MSMA, Illoxan (diclofop-

methyl), Drive 75DF (quinclorac), and Sencor (metribuzin) are being evaluated in various combinathe herbicides are either ineffective against the tropical signalgrass or cause damage to the St. Augustinegrass (Brecke, pers. comm.)

Need for An Alternative Technology for Control of Tropical Signalgrass.

Sod growers, golf course managers, and lawn care managers in Florida depend on chemical herbicides because of their effectiveness and ease of use. Concerns about groundwater contamination by agricultural chemicals and build up of resistance to chemical herbicides in use necessitate environmentally-safe, alternative technology to complement existing weed management options.

Conventional weed management strategies, relying largely on chemical herbicides, are in need of refinements to make them more sustainable (Mersie and Singh, 1989). Refinements can be in the form of improved application efficiency of chemical herbicides, use of adjuvants to enhance efficacy, and selective spraying of only weed-infested areas in a crop.

Nonchemical weed control methods, such as biological control (bioherbicides), if they can be deployed in an integrated approach, can help enhance the effectiveness and sustainability of weed-management practices.

Bioherbicide Strategy.

The bioherbicide strategy, a form of biological control, consists of using certain highly virulent native pathogens of weeds that are mass-produced, formulated, and applied like a pesticide to obtain rapid development of disease and a high level of weed kill. Typically, these pathogens are registered as bioherbicides by the EPA and are used in accordance with their labels. They are applied when environmental conditions and weed-growth stages are conducive for disease development.

The use of host-specific plant pathogens as bioherbicides could be a practical weed management method for signalgrass control. Bioherbicides can be used as a supplement to conventional herbicides or as a component of integrated control. Bioherbicides can be highly effective in terms of efficacy, environmental benefit, and economics (Charudattan, 2001). Currently, six bioherbicides are registered in Canada, Japan, South Africa, and the United States (Charudattan, 2001). Among these is a bacterial bioherbicide, Camperico, registered in Japan for the control of annual bluegrass (*Poa annua*) in turf.

An example of a registered bioherbicide in the United States is DeVine", the first bioherbicide registered by the EPA. It is used for the control of milkweed vine, *Morrenia odorata*, a major weed in Florida citrus groves. DeVine consists of a pathotype of the fungus *Phytophthora palmivora*, which is capable of killing both seedlings and fully grown vines. On the basis of extensive host range and efficacy studies, this pathogen was found to be a safe biocontrol agent for use in citrus. Abbott Laboratories, Chicago, registered the bioherbicide in 1980 and it is now produced and sold by Encore Technologies, Minnetonka, Minn., on an as-needed basis.

Multiple-Pathogen Bioherbicide System for Broad-Spectrum Weed Control

Among the major challenges facing bioherbicide technology is economics. Since bioherbicidal pathogens developed as bioherbicides are highly host specific, a bioherbicide typically can control only one out of many weeds affecting the crop. This limits the commercial potential of the bioherbicide and consequently there is little economic incentive to develop and register bioherbicides.

Inadequate or incomplete level of weed control is another problem.

However, these problems may be overcome by using mixtures of pathogens that are effective against several weeds. All susceptible weeds can be controlled simultaneously without loss of efficacy and host-specificity of the pathogens. Chandramohan and Charudattan (2001) have shown that several weedy grasses, including those that affect agricultural crops as well as natural areas, could be controlled by using a mixture of three fungal pathogens applied with suitable adjuvants.

The use of a mixture of pathogens is advantageous in that if one of the pathogens in the mixture fails the others may compensate. Also, using a pathogen mixture may reduce the chances of development of resistance in weeds that is possible if a single pathogen is used repeatedly. In addition, it may be possible to take advantage of possible synergistic interactions among pathogens in the mixture, which will enhance the efficacy of the bioherbicidal mixture. The level of weed control can be further improved with repeated applications.

Discovery and Development of a Bioherbicide System for Control of Several Grasses.

In 1994, we isolated three fungal plant pathogens, *Drechslera gigantea, Exserohilum longirostratum*, and *Exserohilum rostratum* (Figure 1), which were isolated from naturally infected large crabgrass (*Digitaria sanguinalis*), crowfootgrass (*Dactyloctenium aegyptium*), and johnsongrass (*Sorghum halepense*), respectively (Chandramohan, 1999; Chandramohan, and Charudattan, 2001).

These fungi occur in several Florida counties and are therefore indigenous to this state. These fungi were tested for pathogenicity to various grasses and determined to cause severe disease on many weedy grasses. Some grasses were killed, while some were moderately susceptible, and others immune.

The range of grasses that were infected and killed was also determined in greenhouse trials. Thirty-six economically important crop plants were tested to ascertain the potential risks to nontarget plants; however, none of these plants was harmed by the pathogens, whether they were used individually or in a mixture.

The crop plants tested were bean, beet, blackeye cowpea, broccoli, brussels sprouts, cabbage, cantaloupe, carrot, cauliflower, cliantro, collards, corn, cucumber, eggplant, endive, green pepper, head lettuce, Indian mustard, oat, okra, onion, parsley, pea, peanut, radish, romaine lettuce, rye, sorghum, spinach, squash, sweet corn, tomato, turnip, watermelon, wheat, and zucchini. Also, the pathogens did not damage orange and grapefruit, crops in which the bioherbicides are intended to be used.

The pathogens were then field-tested at two locations in Florida - Lake Alfred and Ft. Pierce. (Chandramohan, et al., 2002).

At Lake Alfred, it was possible to control four-week-old plants of large crabgrass (*Digitaria sanguinalis*), crowfootgrass (*Dactyloctenium aegyptium*), johnsongrass (*Sorghum halepense*), guineagrass (*Panicum maximum*), southern sandbur (*Cenchrus echinatus*), Texas panicum (*Panicum texanum*), and yellow foxtail (*Setaria glauca*), which were transplanted randomly into replicated field plots.

The grass seedlings were sprayed with spore suspensions of each pathogen alone or a mixture of the three pathogens in equal proportion of their spores. The fungi were applied as foliar sprays at the rate of 500,000 spores per ml in one of three carriers: water, 0.5 percent aqueous Metamucil", or an emulsion (Sunspray" 6E 80 ml, paraffin oil 20 ml, and spores in water 100 ml). Appropriate controls were included, and the fungi were applied two or three times at two-week intervals.

The emulsion-based inoculum preparation of each pathogen as well as the pathogen mixture yielded the best level of weed control (nearly 100 percent) of all the grasses tested, and the control lasted for more than 12 weeks.

At Fort Pierce, the pathogens were tested on a natural population of guineagrass. The emulsionbased inoculum of individual pathogens as well as the mixture of three pathogens gave nearly 100 percent control, and the control lasted for a period of 10 weeks.

We have also field-tested the bioherbicidal system to manage guineagrass in Florida sugarcane in two field trials. The pathogen mixture caused a high level of disease on guineagrass and an 82-99 percent reduction in panicle numbers per square meter. Currently, the pathogen mixture is being tested to control torpedograss (*Panicum repens*), an invasive weed species threatening native plants in Lake Okeechobee.

Bioherbicidal Control of Signalgrass in Florida

A mixture of the above-mentioned fungal pathogens applied twice to tropical signalgrass in greenhouse tests blighted up to 90 percent of the shoots (Figure 2) (Chandramohan, et al., 2002a, b). In a separate study, these pathogens were tested in a greenhouse for nontarget effects on various species of cultivated turfgrasses.

However, the cultivated grasses tested under the same experimental conditions as for tropical signalgrass were immune or resistant to each of the pathogens as well as the pathogen mixture. Bermudagrass (*Cynodon dactylon*) (cvs. FloraTex, Floradwarf, Tifway, and Sahara) sustained some injury from the pathogen/emulsion mixture, but recovered.

Bahiagrass (*Paspalum notatum*) (cv. Pensacola), creeping bentgrass (*Agrostis stolonifera*) (cvs. Crenshaw, Pencross), centipedegrass (*Eremochloa ophiuroides*), and seashore paspalum (*Paspalum vaginatum*) (cv. Sea Isle 1) were immune.

St. Augustinegrass (*Stenotaphrum secundatum* (cvs. Floratam, 1996-7, Palmetto, Seville, and TXF) and zoysiagrass (*Zoysia japonica*) (cv. Empire) were resistant.

The susceptible weedy grasses included in this study (large crabgrass, guineagrass, tropical signalgrass) continued to remain diseased, while the cultivated grasses continued to grow and remain healthy if they were immune. If resistant, they recovered from the initial hypersensitive response and remained healthy. These results indicate that the pathogen mixture could be further developed as a biocontrol for tropical signalgrass in turf and sod in Florida.

In the case of cultivated grasses that are immune or resistant, for example St. Augustinegrass sod or lawn, the pathogens could be used as an overthe-top application. In bermudagrass, spot treatments of the tropical signalgrass with the bioherbicidal mixture may be necessary because of the potential for slight injury from the pathogens in the emulsion mixture. From our earlier field tests, we have observed that the pathogens are confined to the treated area (Chandramohan et al., 2002).

Novelty and Future Potential

In summary, we have developed a practi-

cal biocontrol system with potential to manage several grasses including tropical signalgrass, a problematic weed in Florida turf and sod. This multiple-pathogen approach offers the possibility to custom-blend various pathogens to suit particular groups of weeds. This is a novel approach to weed control, and the University of Florida has been issued a U.S. patent (U.S. Patent No. 6,265,347. Issued, July 27, 2001. Chandramohan, S., and Charudattan, R. "Enhanced Bioherbicidal Control of Weeds using Multiple Pathogens"). We are exploring the possibility of commercial development of this bioherbicide system.

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For information about the authors, see page 32.

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