Pathogens as Biological Control Agents of Weeds

Assoc. Prof. Dr Jugah Kadir
Department Plant Protection
Faculty of Agriculture
Universiti Putra Malaysia, Serdang
Selangor
Weeds

- Cause more than $40 billion in annual global losses through
  - Degraded agricultural and silvicultural productivity
  - Reduced access to land and water
  - Impaired ecstatic
  - Disruption of human activities and well being

- One of chief constrains on agricultural production
- Weeds reduce crop yields by 20-30 % (Labrada 1996)
  - Competition with crop plants for light, water, nutrients, and space.
  - Production of growth-inhibiting compounds (allelopathy).
  - Reducing food, feed and fiber quality.
  - Increase the cost of land and pest management (preparation and harvesting).
Weeds

- Chemical herbicides have dominated weed management strategies in developing countries (Wyse, 1992).
- Nearly 40% of the total amount of all agricultural pesticides applied in Malaysia have accounted as chemical herbicide (FAO, 1998).
Problems with Chemical Herbicide

- Development of resistance to herbicides
  - Build up of resistance within or among species increases with repeated use of herbicides with similar mode of action
  - About 300 resistant biotypes are reported (Heap 2010)

- Appearance of cross-resistance in weeds population
  - Selection pressure exerted by repeated use of herbicide with single mode of action or chemically similar herbicides
  - Herbicide drifts
  - Phytotoxicity and herbicide injury

- Impact on composition and dynamic of microbial, faunal and floral communities
  - Herbicide affected more microorganisms and their activities than insecticides and fungicides
Why Biological Control?

1. Excessive use of agrochemicals has led to environmental problems such as:
   - Residual on crops
   - Persistence in soil
   - Contamination of surface water and ground water

2. Consumer preference for non-chemical alternatives in food production

3. Increasing problems with herbicide-resistance weeds and weed-shifting

4. Phasing out of several older herbicides

5. More stringent and costly registration and regulation.

6. Government-instituted mandates for reducing consumption of herbicide

7. The necessity for non-chemical alternative in environmentally sensitive areas.
Biological Control

- Biological control is the use of parasites, predators, or pathogens to maintain another organism’s population at a lower density than would occur naturally.
Biological Control

• Biological control is the utilization of natural enemies for the control of certain weeds.
• Objective is not eradication but reduction and regulation of the weed population.
Biological Control

• Biological control cannot solve all weed problems.
• Reduces population to where it no longer interferes with human activity
• Mostly used for exotic, invasive perennial weeds on low value land
Biological Control - Agents

- Insects / Mites
- Nematodes
- Pathogens
- Fish
- Mammals
Biological Control – Application methods

- Classical
- Inundative
- Conservative
Biological Control – Classical

• Also called inoculative
  – Importation
  – Natural enemy of plant species
  – Self-perpetuating
  – Single release
  – Build to control level
Biological Control – Inundative

• Also called augmentive
  – Kills in year applied
  – Bioherbicides / mycoherbicides
  – Not self-perpetuating
Biological Control – Inundative

- Plant pathogens better adapted for inundative.
  - Can be applied and stored easily.
  - Can apply millions of spores through spray equipment.
Biological Control – Integration

• Important to integrate into system so the biological control agent is not killed
• May get increased control with a combination of control agents including herbicides plus the biological control agent
Biological Control

• When successful
  – cost effective
  – environmentally safe
  – sustainable
Pathogens can be used as:

- Bioherbicides
- Classical biocontrol agents
- Sources of biochemical herbicides:
  - E.g., Bialaphos and Glufosinate, both derived from *Streptomyces* sp.
- Sources of genes and genetic modes of action:
  - Genes that elicit hypersensitive response (programmed cell death)
Pathogen as A Classical Biocontrol Agent

A pathogen, typically a fungal pathogen, capable of initiating a disease epidemic from a few small, well-planned introductions (= inoculative releases)
Bioherbicide

A plant pathogen (bacterial, fungal, nematode, or viral pathogen) that is mass produced, formulated, standardized, and used in an inundative biocontrol strategy.
Inoculative or Classical method

Apply a small dose of a pathogen’s inoculum over a large weed population and initiate a massive, spreading epidemic

Historically the oldest, preferred and successful method of biocontrol

1. The biocontrol agent is introduced from a foreign location
2. The weed is an exotic (non-native), invasive weed
3. The biocontrol agent is used in the “inoculation” method

weed population
uniformly susceptible
densely occurring
contiguous individuals

pathogen
highly virulent
sustain itself and spread with little or no further human assistance
be able to inflict severe damage on the weed host
native plants in natural communities (i.e., in their native habitat or native range) evolve into a state of equilibrium (homeostasis) with their co-adapted pathogens

- a state of mutual nonextinction
- host/pathogen develop disease constraining strategies
  
  host resistance  
  host discontinuity (i.e., patchy distribution) 
  inadequate inoculum 
  lack phenological synchrony (host/pathogen)
A plant that is separated (in space and/or time) from its natural pathogen (for example, due to emigration and colonization of a new geographic region) tends to lose its resistance to the pathogen over time due to a lack of selection pressure.

The homeostasis wanes and the plant tends to become susceptible to the pathogen.

If the pathogen is introduced into the plant's new home (called the adventive range) and if conditions conducive for disease development occur (i.e., if favorable environmental conditions exist and the constraints listed above are absent), an epidemic is likely to result.

The proof that this can happen is seen in the case of the exotic weed skeletonweed (*Chondrilla juncea*; Asteraceae) in Australia and pamakani weed (*Ageratina riparia*; Asteraceae) in Hawaii.
Host-Pathogen Population Curves

Unmanaged System: Classical Biocontrol

- Blue line: Equilibrium state
- Red line: Host numbers
- Green line: Pathogen/disease units
Inundative method

There are numerous pathogens on native plants (including weeds) that rarely cause major epidemics. Pathogens usually occur in an "endemic" disease state (as opposed to "epidemic" state) due to host/pathogen constraints to disease development.

By applying a large dose of inoculum when the plant is most susceptible and when the environmental conditions are conducive for disease development, a rapid disease development leading to epidemic buildup can be artificially produced. Pathogens that can be grown on artificial media can be used to produce large quantities of standardized inoculum, and the inoculum can be applied at levels that inundate the infection sites on the plants, overcoming inoculum limitation and possible environmental and host-defense limitations. This approach, based on human manipulation of the pathosystem, is called the inundative or bioherbicide strategy.
Historically **indigenous pathogens** belonging to the category of **facultative parasites** have been developed and used as bioherbicides.

Although nonindigenous pathogens can also be used as bioherbicides, due to the current interpretation of the plant quarantine laws, it is more difficult to import and use nonindigenous facultative parasites as bioherbicides.
Host-Pathogen Population Curves

Managed System: Bioherbicides
Augmentation method:

It has been shown in the case of the native weed *Cyperus esculentus* (yellow nutsedge) and the native rust fungus, *Puccinia canaliculata*, that a properly timed release of a small dose of the rust spores over an yellow nutsedge-infested area can accelerate the development of the rust epidemic and the resulting high level of disease can cause the yellow nutsedge population to decline (i.e., less biomass and tuber production and premature death of plants).

Unlike the inoculative method (where a nonindigenous pathogen is used) the biocontrol agent in the augmentation method is indigenous and already present in the area where control is required. But similar to the inoculative method, only a small amount of inoculum is required to initiate the epidemic. This system is also similar to the inundative method in some ways: The level of inoculum used is much higher than the naturally occurring levels in the field. Similar to the inundative method, there is involvement of human manipulation.
Relevance of trophism to biocontrol

**Biotroph**
- Pathogen does not kill the host / host tissue in advance of infection and colonization, but rather grows in intimate association with the host’s cytoplasm.
- Host is essential for the survival and reproduction of the biotrophic pathogen.

**Necrotroph**
- Pathogen kills host tissue in advance of parasitism, derives food (energy) from the dead remains of host cells.
- Pathogen elaborates various types of phytotoxic metabolites and lytic enzymes as their first lines of attack against the host.
<table>
<thead>
<tr>
<th><strong>Inoculative method / agent</strong></th>
<th><strong>Inundative method / agent</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective against exotic weeds, weeds, also naturalized and native weeds</td>
<td>Effective against native also naturalized and exotic weeds</td>
</tr>
<tr>
<td><strong>Host attributes:</strong></td>
<td><strong>Host attributes:</strong></td>
</tr>
<tr>
<td>Should be susceptible</td>
<td>Susceptibility</td>
</tr>
<tr>
<td>Should be in high population densities</td>
<td>Host should be spatially contiguous</td>
</tr>
<tr>
<td><strong>Pathogen attributes:</strong></td>
<td><strong>Pathogen attributes:</strong></td>
</tr>
<tr>
<td>Should be highly virulent</td>
<td>Should be highly virulent</td>
</tr>
<tr>
<td>Should be able to self-disperse, and should be able to develop into self-maintaining population</td>
<td>Should be able to cause rapid and massive host damage or kill</td>
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<td>Should have capacity for density-dependent population buildup</td>
<td>Host specificity not an absolute requirement</td>
</tr>
<tr>
<td>Should have a high degree of host-specificity</td>
<td><strong>Examples:</strong> Biotrophic rust, smut fungi</td>
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<tr>
<td><strong>Examples:</strong></td>
<td>Necrotrophic fungi, bacteria</td>
</tr>
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Examples of Classical Biological Control:
Rust Fungi for Weed Control
Inoculative or Classical method

Historically the oldest, preferred and successful method of biocontrol

In classical biocontrol programs, the introduced pathogen is simply released or inoculated into small weed infestations relative to the total infestation.

If conditions are favorable, the pathogen multiplies on the weed host and spreads, causing a high level of disease that may kill the weed or severely limit its growth. The weed population may then begin to decline.
Apply a small dose of a pathogen’s inoculum over a large weed population and initiate a massive, spreading epidemic.
Since this process depends on a gradual increase in disease, it may take several months or years to obtain significant levels of weed control.

It is impossible to predict the success of an agent or to increase or decrease its efficacy after release and it is also impossible to recall a bioagent once released. Therefore, a careful evaluation of efficacy and safety must precede a classical introduction.
CLASSICAL BIOCONTROL

Exploration  Center of origin / center of diversity

Surveys are made in the native range of the weed
discover and rank pathogens with biocontrol potential

Prospective pathogens, usually rust fungi, are studied in their homelands to determine their host specificity and virulence towards the target weeds

Identity and suitability of individual pathogens

Host-range studies are done to establish the safety of the agents
nontarget plants, including major and minor crops and ecologically important plants

Additional testing: plants of economic and ecological importance to the country

Release / assessment of effectiveness: coordinated with appropriate federal and regional agencies
Rust fungi

Smut fungi

obligate parasites that are generally highly host-specific

have the capacity to multiply on their hosts in a density-dependent manner

These fungi produce wind-disseminated spores that enable the disease to spread from an initial disease focus

Conidial fungi – *Cercopsora* spp.
SUCCESSFUL EXAMPLES
Rust fungus, *Puccinia chondrillina*, to control rush skeletonweed (*Chondrilla juncea*; Compositae) Mediterranean into Australia serious weed in cereal crops and rangelands

Research by the Australian Commonwealth Scientific and Industrial Research Organization

*Puccinia chondrillina* was introduced, along with several insects

The rust was very effective; following inoculative releases, it rapidly spread, created high levels of disease epidemics, and in the process infected, stressed, and killed the most common and susceptible biotype of the weed.
Upon successful establishment of the pathogen, weed density in cereal crops decreased

200 plants per m$^2$

<10 plants per m$^2$

Good control was also obtained in pastures, suggesting an overall projected savings of $25.96$ million per annum (cost to benefit ratio of 1:100 in Australia)

**PROBLEM**

Emergence of resistant weed biotypes

*Puccinia chondrillina* attacks one of three forms of the weed, susceptible type. The other two biotypes became resistant & more widespread.

To counter the emergence of resistant weeds:

introduction of pathogen strains that are effective against the resistant weed biotypes
Release in USA

*Puccinia chondrillina* was also introduced into the United States to control a skeletonweed biotype in the western United States only partially successful.

integrated weed management program

1. Rust pathogen, *Puccinia chondrillina*
2. chemical herbicides
3. Insect biocontrol agents:
   - *Cystiphora schmidti* (a gall-forming *mite*)
   - *Aceria chondrillinae* (a gall-forming *mite*)
SUCCESSFUL EXAMPLES

2
Another successful example of a classical biocontrol program:

Use of a smut fungus, *Entyloma compositarum*, imported from Jamaica to control the weed *Hamakua pamakani* (*Ageratina riparia*; Compositae) in Hawaiian forests and rangelands

**Hamakua pamakani - *Ageratina riparia***

Mexico > Hawaii - 1925 as an ornamental plant

By the 1970s, it had spread to an estimated 62,500 ha in the island of Hawaii and 10,000 ha in Oahu. The weed was also present in the island of Maui.
The fungus, originally misnamed as *Cercosporella* sp., was introduced into Hawaii in 1974

Extensive host range testing established this fungus to be highly host specific and safe biocontrol agent

About **two to three months** after the pathogen was released in the field, **devastating epidemics** occurred in dense stands of *A. riparia* in cool, **high-rainfall sites** in Oahu, Hawaii, and Maui

The weed populations were reduced **80% to less than 5%** in a **9-month period**
At sites with low temperatures and low rainfall there was greater than 50% reduction in the weed population in 8 years after the pathogen’s release. It is estimated that more than 50,000 ha of pasture land have been rehabilitated to their full potential by this pathogen.
SUCCESSFUL EXAMPLES

3
In South Africa

A gall-forming rust fungus, *Uromycladium tepperianum*, (vs)
An invasive tree species, *Acacia saligna* (Leguminosae)

Australia > South Africa

The fungus was introduced from Australia into the Western Cape Province of South Africa

About eight years after introduction and establishment at specific sites, the number of infected trees, rust galls per tree, and severity of rust infections increased at all sites
The rust causes extensive gall formation on branches and twigs, costing a significant energy loss to the tree and disrupting photosynthesis. Heavily infected branches droop from the weight of the galls, and the tree may be eventually killed.

Tree density decreased by at least 80% in rust-established sites. The seed number in soil seed bank stabilized in most sites.

Thus, *U. tepperianum* is proving to be a very effective biocontrol agent, as shown by the greatly reduced population densities of *A. saligna* in South Africa.
An Example: Classical Biocontrol Agent
Biocontrol of Port Jackson Willow (*Acacia saligna*) by the Introduced Rust Fungus, *Uromycladium tepperianum*

Left: Rust galls on a branch of *A. saligna*. Middle: Epidemic spread of the rust has caused *A. saligna* trees to be heavily infected and galled. Right: A “before-and-after” picture illustrating the success of this biocontrol program. Source of pictures: Dr. Mike Morris and Plant Protection Research Institute, South Africa.
AUGMENTATIVE BIOHERBICIDE (INUNDATIVE)
BIOHERBICIDE

• Biological control agents applied in similar ways to chemical herbicides to control weeds

• Active ingredient:
  • Living micro organism, most commonly fungus; referred to as mycoherbicde
  • Applied in inundative doses of propagules: spores or fragments of mycelium
Types of Microbial Pathogens Used in Weed Biocontrol

- Bacteria
- Fungi
- Viruses
Choice of pathogen: Why indigenous: Why Fungi

• Indigenous (Native pathogen):
  – Exempt from quarantine regulations
  – Having co-evolved with native plants and weed hosts, are likely to be selected for host specificity within the flora and to be adapted for the eco-climatic conditions of the region.

• Fungi:
  – Culturable in vitro
  – Readily produce spores
  – Usually aggressive parasites
  – Host specific
  – Fungi are capable of active penetration
If pathogens are such good plant killers:

• Are there examples?
• What about safety to crop and ecologically important plants
• Human and environmental safety?
Mycoherbicide, Bioherbicide, Biopesticide: EPA’s Definition

- According to the U.S. EPA, the term “biopesticide” (incl. bioherbicide, mycoherbicide) includes living (i.e., biocontrol agents) or dead (= killed cells) microorganisms, certain types of biologically derived chemicals, and certain gene-based pest-control systems used to control pests or to mitigate pest problems.
- Mycoherbicide is a bioherbicide composed of a fungal pathogen.
- EPA mandates that any pathogen, whether used as an inoculative agent (released once or a few times) or as an augmentative or inundative agent (used repeatedly and prescriptively) should be registered as a pesticide.
• In the USA, a plant pathogen (i.e., bacterial, fungal, or viral pathogen, but not a nematode) offered for public use (for free or for a price) for pest control purposes falls under the legal authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

• Under this authority, biopesticides must be registered OR properly reviewed and exempted by the EPA

• For commercial purposes, bioherbicide is a pathogen-containing product that is mass produced, formulated, standardized, and used in a prescribed manner

• The active ingredient of the bioherbicide product is the bioherbicide agent (pathogen, usually as spores or vegetative cells)
Two important criteria:

- Efficacy
- Safety
AMOUNT OF CONTROL
How well does the pathogen control the weed?
Completeness of Control

EFFICACY

SPEED OF CONTROL
How quickly is the control achieved?

EASE OF CONTROL
How easy is it to apply and get control?

Solid lines indicate a direct relationship; broken lines suggest that relationship(s) may exist in some weed-pathogen systems.
Safety – Nontarget Plants

- Irrespective of the source of the agent (nonindigenous or indigenous), the agent must be safe to crops and ecologically important plants.

- The nontarget host-range testing is done by screening a large number of plants, including crop-plant species, ecologically important species such as threatened and endangered plants, and others based on specific considerations.
Safety – Nontarget Animals (Incl. Humans) and Environment

- EPA-mandated studies (in addition to nontarget plant trials):
  * Toxicology studies with mammalian, bird, fish, and other appropriate models
  * Potential for mycotoxin production and type(s) of risk
  * Potential to cause allergies in humans
  * Environmental persistence and fate of the bioherbicide agent
- These studies done in a stepwise, “Tiered” system of risk assessment
- Objective is to match the scope of risk assessment to the degree of perceived risk
- Possibility to exempt biocontrol agents from certain risk assessment studies
LIMITATION

• Limited commercial interest
• Complexities in production and assurance of efficacy and self life
• Biological constrains
• Environmental constrains
Limited commercial interest

- Typically market for bioherbicide agents are small, fragmented, highly specialized, and consequently the financial returns from biocontrol agents are too small to be of interest to big industries.
Complexities in Production and Assurance of Efficacy and Shelf-Life

• The inability to mass-produce inoculum needed for large scale use is a serious limitation that has led to the abandonment of several promising agents.
Biological Constrains

- Plant Population: Host variability
  - Within a population of plant species there will be a range of plant resistance types
  - Has not been reported but it can happen
  - Phenological stage of the plants may influence effect of bioherbicide
  - Morphology of the plant species provides them with the ability to produce new growth
Biological Constrains

• Interaction with other microorganisms
  – Especially with microorganisms on the phyllosphere of weed;
    » Nutrients
    » Space
    » Direct antagonism
    » Toxic plant leachates
• Reduce efficacy of foliar bioherbicides
Environmental Constrains

• Aerial Environment
  • Are frequently hostile for biological fungi
  • Most fungi require >12h of dew period for severe infection

• Soil environment
  • Moisture and nutrient status of the soil influence the physiology of the target plants
Important Innovations To overcome the Limitations

• Areas of Inoculum production
• Formulation of agents
• Application methods
Areas of Inoculum Production

- **Liquid fermentations**
  - Most fungi do not produce spores under submerged conditions
  - Suitable for bacterial pathogens
- **Biphasic Production system**
  - Fungus first cultured in liquid shake cultures, followed by slow drying over solid substrates
- **Solid fermentations**
Formulation of agents

• Vital if we are to succeed with the next generation of bioherbicides

• Formulation should predispose the weed to infection by pathogen or it should strongly buffer the pathogen propagules against environmental constraints while promoting disease development.

  • For best result the pathogen should be inherently virulent or possesses a potent weed-killing phytotoxin
Application methods

• Need more intelligent application systems
  • Innovation such
    – as dual nozzle sprayers
    – Sensor controlled sprayers
    – Housed in spray hoods
Research Needs

• Bioherbicides for herbicide-resistant weeds
• Identification and cloning of genes for virulence, host specificity, and host-parasite recognition – all using suitable weed-pathogen models
• Studies on variability in weed biotypes and pathogen populations as a means to improve and predict the suitability of weed-pathogen systems selected for biological control
• More efforts to identify and utilize integrated systems consisting of insect and microbial biocontrol agents (eg. Pathogens and rhizobacteria)
  • Charudattan, 2001.
Worldwide, about eight bioherbicides are registered and used with certain regularity.

Two registered bioherbicides in the USA:
Collego™ - A Bioherbicide for Northern Jointvetch in Rice, *Aeschynomene virginica*

Clockwise from top left: Collego label; Collego-treated (left) and untreated (right) northern jointvetch seedlings; aerial application of Collego. Source: David TeBeest, Univ. Arkansas and David Johnson, Encore Technologies, Minnetonka, MN
Collego™ - A Successful Bioherbicide

- Collego, *Colletotrichum gloeosporioides* f.sp. *aeschynomene*, has been in use for nearly two decades (1982-1992 and 1996-present) to control northern jointvetch (*Aeschynomene virginica*) in rice and soybean crops in Arkansas, USA.

- Collego provides high levels of control of northern jointvetch without adverse side-effects.

- Collego’s efficacy results from the pathogen’s high level of virulence, its capacity to cause rapid secondary disease cycles, and several modes of post-application secondary dispersal of the inoculum.

- Source: David TeBeest, Univ. of Arkansas and David Johnson, Encore Technologies, Minnetonka, Minnesota.
DeVine®: A Bioherbicide for Stranglervine (Morrenia odorata) in Citrus in Florida

Clockwise from top left: product label, effect of DeVine on weed seedlings: control and inoculated plants shown; citrus tree canopies covered by stranglervine; and typical weed control obtained with just one application of DeVine.

Source: R. Charudattan and Abbott Laboratories. DeVine is now produced and sold in the USA by Valent Biosciences, a Sumitomo Subsidiary
DeVine: A Bioherbicide that has Satisfied Growers’ Needs for Over Two Decades

- DeVine is “made-to-order” and shipped and used quickly (“like fresh milk”)
- Unlike chemical herbicides and hand-removal which typically do not remove the regenerative roots that remain close to citrus trees, DeVine can kill these roots
- The pathogen persists and remains active in soil, providing prolonged control of the vine
- Unlike some nonselective chemical herbicides, DeVine can be applied around citrus trunks without harming the tree
- Citrus growers in Florida monitor the vine infestations for a few years and use DeVine when the problem worsens
Dactylaria higginsii, A Leaf-Blighting Fungal Pathogen of Purple Nutsedge

Conidial spores produced on an infected purple nutsedge leaf

Conidiophores, conidia, and an appressorium on a purple nutsedge leaf

Leaf spots and lesions caused by D. higginsii

1 = D. higginsii spores* in 0.05% N-gel
2 = Spores applied in Silwet L-77
3 = Control (water only; no spores)
4 = Spores in water only
5 = Spores + 0.05% Metamucil®

*Spore concentration: $10^6$ spores / ml
In-Field Treatment of Purple Nutsedge with *D. higginsii*

- Plot Layout
- Purple & Yellow Nutsedge

- 3 Applications 2 WK Apart
- $10^6$ Spores / ML w/ 0.05% Metamucil
- 200 Gallons Per Acre
**Dactylaria higginsii: Efficacy**

- **GREENHOUSE:** Nearly 100% control (shoot kill) in purple nutsedge, yellow nutsedge, green kyllinga (*Kyllinga brevifolia* [=*Cyperus brevifolius]*) and other *Cyperus* spp.

- **FIELD:** Greater than 90% control of nutsedge with 3 applications of $10^6$ spores per ml applied at 2 week intervals (note: these tests were conducted in a natural stand consisting purple nutsedge [predominant] and yellow nutsedge)

- **GREENHOUSE:** *D. higginsii* can significantly reduce yield loss in pepper and tomato crops by reducing weed competition from purple nutsedge
Dactylaria higginsii could be of value as an alternative to methyl bromide for control of nutsedges in many high value crops, including:

- Peppers
- Tomatoes
- Citrus
- Carrots
- Spinach
- Cantaloupe
- Corn
- Strawberry
- Squash
- Sugarcane

Other Potential Markets:
- Organically grown crops
- Minor-use crops
- Lawns and golf courses
- Container-grown crops, such as ornamentals
Is the bioherbicide / mycoherbicide technology suitable for small, focused producers?
Consider the following: Bioherbicides are

- Suitable for weed control in intensively managed systems such as crops, water resources, pastures, golf courses, and others
- Ideal for organically grown crops
- This technology is best suited to small biotech companies and low-tech producers than to large, multinational pesticide companies
- Most promising fungal bioherbicide agents require solid-state culturing in order to produce spores
- Whereas plenty of expertise exists worldwide for the liquid-fermentation technology, presently the market is wide open for solid-state production technology
Much of the developmental work is already done in academia

1. Academic research: Discovery, testing, efficacy and safety determination, and validation of results through publication - DONE

2. Patenting and early phase of technology development: production and formulation - DONE

Development of a commercial production system, commercial testing under an EUP, and registration – in cooperation with land-grant institutions and private growers
Pathogens of Aquatic Weeds

Waterhyacinth – *Cercospora piaropi*

Hydrilla – *Fusarium* spp.
Biological Control of Waterhyacinth by Using Weevils and *Cercospora piaropi*: An Example of Integrated Use of Bioagents

**Top left:** Weevils, *Neochetina eichhorniae* (left) and *N. bruchi* (right). Top right: Leaf-spot symptoms caused by *C. piaropi*

**Center left:** a view of plots treated with weevils alone, *C. piaropi* alone, combinations of both agents, or none. Center right: waterhyacinth plants unaffected by the weevils or the pathogen

**Bottom left:** plants attacked by the weevils only. Bottom right: plants attacked by the weevils and *C. piaropi*
Biological Control of Tropical Soda Apple (TSA), *Solanum viarum*, the Plant from Hell!
Tobacco mild green mosaic virus (TMGMV), A Viral Biocontrol Agent of Tropical Soda Apple
Susceptibility of TSA to Three Tobamoviruses

- TMV
- ToMV
- TMGMV: lethal systemic HR (>2 wk)
Plant Age vs. Susceptibility

3-mo old TSA plants 1-2 wk after inoc.

2-mo old plants 2 wk after inoc.

7-mo old plants 1 mo after inoc.

TSA plants prior to inoc.
Fruits from control plants

Fruits from inoculated plants
• Research undertaken in my Laboratory

– Potential of *Exserohilum longirostratum* as bioherbicide for itchgrass
– Potential of *Exserohilum monoceras* as bioherbicide for barnyardgrass
– Potential of *Myrothecium sp*. As bioherbicide for water hyacinth
– Potential of *Exserohilum rostratum* as bioherbicide for *Leptochloa chinensis*
Exserohilum longirostratum

Taxonomy

- Kingdom: Fungi
- Phylum: Ascomycota
- Class: Deuteromycetes
- Order: Pleosporales
- Family: Pleosporaceae
- Genus: Exserohilum (= Drechslera)
- Species: longirostratum (= longirostrata)
Fungal bioherbicides - Production

Incubated, 12 h light / 12 h dark

Liquid shake-cultures

Spores produced, 24-48 h later

Harvested, dry spores
Exserohilum longirostratum
Pathogenicity testing

A = Uninoculated control;  B = Inoculated
Disease progress curve of *E. longirostratum* on 4-leaf growth stage of itch grass
Effect of incubation temperature on diametric growth of *E. longirostratum*
Effect of incubation temperature on sporulation of *E. longirostratum* on different growth media
List of plants tested for the host-range determination of *E. longirostratum* and their disease indices.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Disease incidence (%)</th>
<th>Disease index</th>
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<tbody>
<tr>
<td><strong>Grassy weeds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rottboellia cochichinensis</em></td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td><em>Digitaria sanguinalis</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>Echinochloa colona</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>E. crussgalli</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>E. oryzicola</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>E. oryzicola</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>Eragrostis tenella</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>Ischemium rugosum</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>Paspalum conjagatum</em></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Pennisetum polystachyon</em></td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td><em>Leptochloa chinensis</em></td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>
List of plants tested for the host-range determination of *E. longirostratum* and their disease indices.

<table>
<thead>
<tr>
<th>Turf grass</th>
<th>Disease incidence(%)</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cynodon dactylon</em></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Paspalum notatum</em></td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td><em>Eramochloa ophiuroides</em></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Zoysia zenith</em></td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td><em>Axonopus compressus</em></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Axonopus affinis</em></td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Broad leaves</th>
<th>Disease incidence(%)</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ageratum conyzoides</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Asystasia intrusa</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Euphorbia hirta</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
List of plants tested for the host-range determination of *E. longirostratum* and their disease indices.

<table>
<thead>
<tr>
<th>Crop Plants</th>
<th>Disease incidence(%)</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oryza sativa</em> MR 219</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Oryza sativa</em> MR 158</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td><em>Zea mays</em> var. Putra</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Z. mays</em> var. Thai sweet</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td><em>Z. mays</em> (Sweet corn)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Brassicae chinensis</em> var. dwarf</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Amaranthus</em> sp</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Cucumis sativus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Abelmoschus esculentus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ipomea reptans</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Saccharum officinarum</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Solanum melongena</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Vigna sinensis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Elaeis guinensis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatments</td>
<td>Conidia germination (%)</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------</td>
<td></td>
</tr>
<tr>
<td>Control (Water agar)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Itchgrass</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

Conidia germination 8 h after inoculation
Host Pathogen Interaction

Histological Study
Infection process of *E. longirostratum* on bean leaf
Infection process of *E. longirostratum* on corn leaf
Infection process of *E. longirostratum* on itchgrass leaf
Epidemiological Factors
Effect of *E. longirostratum* on 4-leaf stage itch grass
Effect of *E. longirostratum* on 6-leaf stage itch grass
Effect of *E. longirostratum* on 8 leaf-stage itch grass
Effect of *E. longirostratum* on 8-leaf stage itch grass
Effect of conidia concentration (conidia/ml) on disease development

Control, uninoculated

2.35 \times 10^4

2.35 \times 10^5

2.35 \times 10^6

2.35 \times 10^7
Effect of leaf wetness duration on disease development

Control, uninoculated

0 hr  8 hrs  16 hrs  24 hrs
Herbicide Interaction Study
Gramoxone

The graph shows the diametric growth (cm) over days for different concentrations of Gramoxone. The x-axis represents the number of days, and the y-axis represents diametric growth in centimeters. The graph includes lines for Control, 0.25X, 0.50X, 1.00X, and 1.50X concentrations, each with a distinct marker type and color.
Preliminary field inoculation
24 hr after inoculation
48 h after inoculation

1 month after inoculation
Drechslera cynodontis as Bioherbicide for Controlling Goosegrass
(Eleusine indica (L.) Gaertn.)

Name : Chia Shin Zhi
Matric No. : GS15833
Supervisor : Assoc. Prof. Dr. Jugah Kadir
Committee member: Prof. Dr Sariah Meon
Drechslera cynodontis
Light Microscopy

Germ tube

App formed
Scanning Electron Microscopy

Germ tube

Appressorium
Suitability of *Exserohilum longirostratum* as Bioherbicide for Integrated Management For Barnyard grass (*Echinochloa Spp.*) in Malaysia
In barnyard grass leaves inoculated with conidia:
- the infection hyphae were produced after the appressoria formed over bulliform cells on spore inoculated leaves.
- germ tubes extended on the surface along the junctions of the epidermal cells.

In intracellular penetration:
- the infection hyphae bloated into spherical vessels in colonizing the cells causing necrotic lesions.

The appressoria (ap) formed directly on the epidermal cells from conidia (A) and mycelium (B) based suspensions.
In rice leaves inoculated with mycelium:
~ appressoria formed before the formation of infection hyphae in mycelium inoculation.
~ the appressorium formed at the end of a massive germ tube deposited over the leaf surface.

In Intracellular penetration:
~ The infection hyphae then bloated into spherical vessels and colonized the cells.

Cross-section of leaf showing infection process of *E. Longirostratum* on barnyard grass and rice. In barnyard grass, hyphae colonized the cells by mycelium (A) and conidia (B) -based suspensions.
Mini Plot Trial

Objective:

• To determine field efficacy of *E. longirostratum* in controlling barnyard grass.
• Barnyardgrass seedlings sprayed with mycelium alone and mycelium + pretilachlor were dead, while those sprayed with pretilachlor alone became stunted within a few days and eventually died.
Effects of different treatments on the severity of disease caused by *E. longirostratum* on barnyard grass as represented by the means\(^x\) of Area Under Disease Progress Curve (AUDPC) and disease progress rates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><strong>AUDPC</strong> (unit(^2))</th>
<th>Progress rate (logit/day)</th>
<th>Regression coefficient (R(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-inoculated (Control)</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Pretilachlor alone</td>
<td>357.49 b</td>
<td>0.96 b</td>
<td>0.96</td>
</tr>
<tr>
<td>Conidia alone</td>
<td>468.28 ab</td>
<td>0.51 b</td>
<td>0.87</td>
</tr>
<tr>
<td>Mycelium alone</td>
<td>583.83 ab</td>
<td>0.62 b</td>
<td>0.89</td>
</tr>
<tr>
<td>Conidia + pretilachlor</td>
<td>395.78 ab</td>
<td>0.82 b</td>
<td>0.90</td>
</tr>
<tr>
<td>Mycelium + pretilachlor</td>
<td>610.35 a</td>
<td>1.27 a</td>
<td>0.76</td>
</tr>
</tbody>
</table>

\(^x\)Means with the same letter are not significantly different from each other (P>0.05).
Bioherbicide Potential

- *Exserohilum longirostatum* has shown to have bioherbicide potential for controlling *Rottboellia cochinchinensis*.
- Its host ranges includes most of the grassy weeds.
- Its ability to caused high seedlings mortality makes it a potential biological weed control agents.
- Light did not influence growth and sporulation.
- Temperature has significant effect of growth and sporulation.
Bioherbicide Potential

• Efficacy is influenced by conidia concentration, plant growth stage, and dew period duration
• Dew requirement can be circumvented by using surfactants, oil emulsion, and wetting agents.
• It has the potential for use in integrated approaches to weed control program
• However, its field efficacy needs to be verified