



Landcare Research  
Manaaki Whenua



# *Microgels* to replace synthetic herbicides to control willow in water-catchment wetlands

Landcare Research & EnForm Tech  
Stan Bellgard, Von Johnson, Dan Than, Nitish Anand, Chris Winks, Gabriela Ezeta\* and Sarah Dodd



Auckland  
Council  
Te Kōwhiri o Tāmaki Makaurau



# Types of Biocontrol

- **Classical** – agents (usually insects or fungi) are introduced from their exotic range, reared here, and released with little or no further intervention.
  - E.g. classical use of a plant pathogen is the white smut fungus *Entyloma ageratinae* on mist flower *Ageratina riparia*.
- **Inundative** – a naturally occurring pathogen is mass produced and applied in a manner of ways - does not persist and needs to be reapplied.
  - Cut-stump paste formulation
  - Targeted to the pest species (not broad spectrum)

# Drivers for change

- Increased public awareness about chemical residues
- Removal / de-registration of herbicides
- New Zealand Forest Certification scheme – push for residue free timber products



# Issues with glyphosate

Journal of Applied Ecology



British Ecological Society

*Journal of Applied Ecology* 2010, **47**, 498–504

doi: 10.1111/j.1365-2664.2010.01791.x

## Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival

David W. Kelly<sup>1,2\*</sup>, Robert Poulin<sup>2</sup>, Daniel M. Tompkins<sup>1</sup> and Colin R. Townsend<sup>2</sup>



ELSEVIER

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: [www.elsevier.com/locate/toxinvit](http://www.elsevier.com/locate/toxinvit)



A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels

Émilie Clair<sup>a,b</sup>, Robin Mesnage<sup>a,b</sup>, Carine Travert<sup>a</sup>, Gilles-Éric Séralini<sup>a,b,\*</sup>

<sup>a</sup> Université de Caen Basse-Normandie, EA2608, Institute of Biology, Esplanade de la Paix, 14032 Caen Cedex, France

<sup>b</sup> Université de Caen Basse-Normandie, Risk Pole MRSH-CNRS, and CRIIGEN, 40 rue de Monceau, 75008 Paris, France

# Residues and public health

- A portion of all herbicides applied to forests, croplands, roadsides and gardens are inevitably lost to water bodies
- Four glyphosate formulations have been demonstrated to induce apoptosis (i.e. cell death) and necrosis in human umbilical, embryonic and placental cells.
- Adjuvants amplified the toxicity induced by glyphosate through changes to human cell permeability
- Conventional willow control in wetland areas is undertaken in New Zealand using an application of glyphosate to cut stumps – including water catchments.

# Microbial formulations

- Formulation of either micro-organisms or their metabolites, that is mass-produced to have a pathological effect on a target weed are part of a new micro-technology platform.
- Can include; fungi, bacteria, viruses, protozoans
- Specifically, for a fungal-microbial formulation, the active ingredient is a wood-rot / parasitic fungus, e.g.  
*Chondrostereum purpureum* /  
*Sclerotium sclerotiorum*

# Micro-technologies

- Micro-technologies: involves rearing the fungus / bacteria, in bulk, whilst preserving its infectivity, and ability to function e.g. degrade lignin
- This requires knowledge of ;
  - fungal biology
  - reproductive physiology and
  - Optimum medium for inoculum production
  - Optimum conditions for inoculum production



# Current commercial products

## Chontrol™ Paste

A biological herbicide for the inhibition of resprouting and regrowth from cut stumps of alders (red and sitka) in rights-of way and forest vegetation management

COMMERCIAL

READ THE LABEL BEFORE USING  
POTENTIAL SENSITIZER  
KEEP OUT OF REACH OF CHILDREN

REGISTRATION NO: 27823 PEST CONTROL PRODUCTS ACT

GUARANTEE: *Chondrostereum purpureum* Strain PFC2139  $10^5$  to  $10^7$  Colony  
Forming Units per Kg.  
Net contents: 1.0 kg

Store the product under refrigeration temperatures (4°C)

Lot #:                      Date of Manufacture:

Mycologic Inc., c/o IDC, University of Victoria, Victoria, BC Canada V8W 2Y2  
(250) 721-7104



- **Chontrol Paste™** cut-stump paste for woody tree weeds.
- **Sarritor** for dandelion (*Taraxacum officinale*)
- **Camperico** for turf grass (*Poa annua*)
- **Smoulder** for dandelion (*Taraxacum officinale*)



# Mode of action

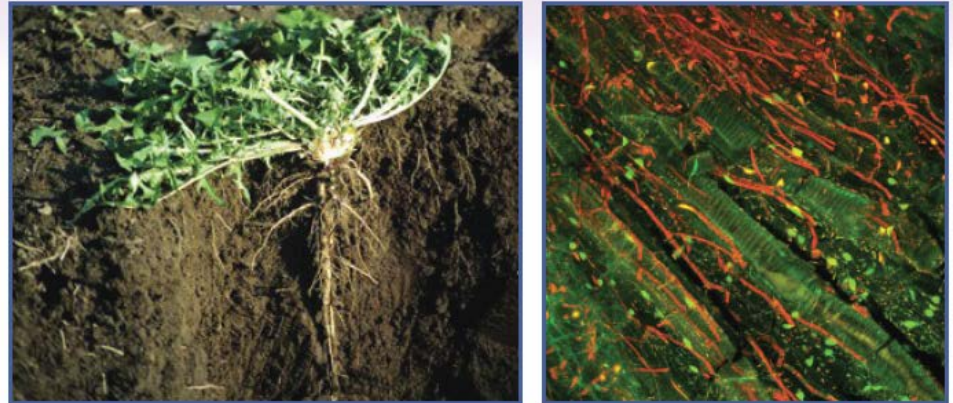
## Fungal wood-rooters



www.shutterstock.com · 31583797

- *Chondrostereum purpureum* Silverleaf has been found in dead wood from grey willow and *Tremetes versicolor* has been found in dead wood of Chinese Privet (Ward et al. 1999)
- *work by secreting wood-digestive enzymes*

## Fungal root-rotters (overseas)



Once in the soil, *Phoma macrostoma* enters the roots and then the fungus (red) grows towards the plant's vascular system (green), as shown by microscopic imaging.



# Cut stump control

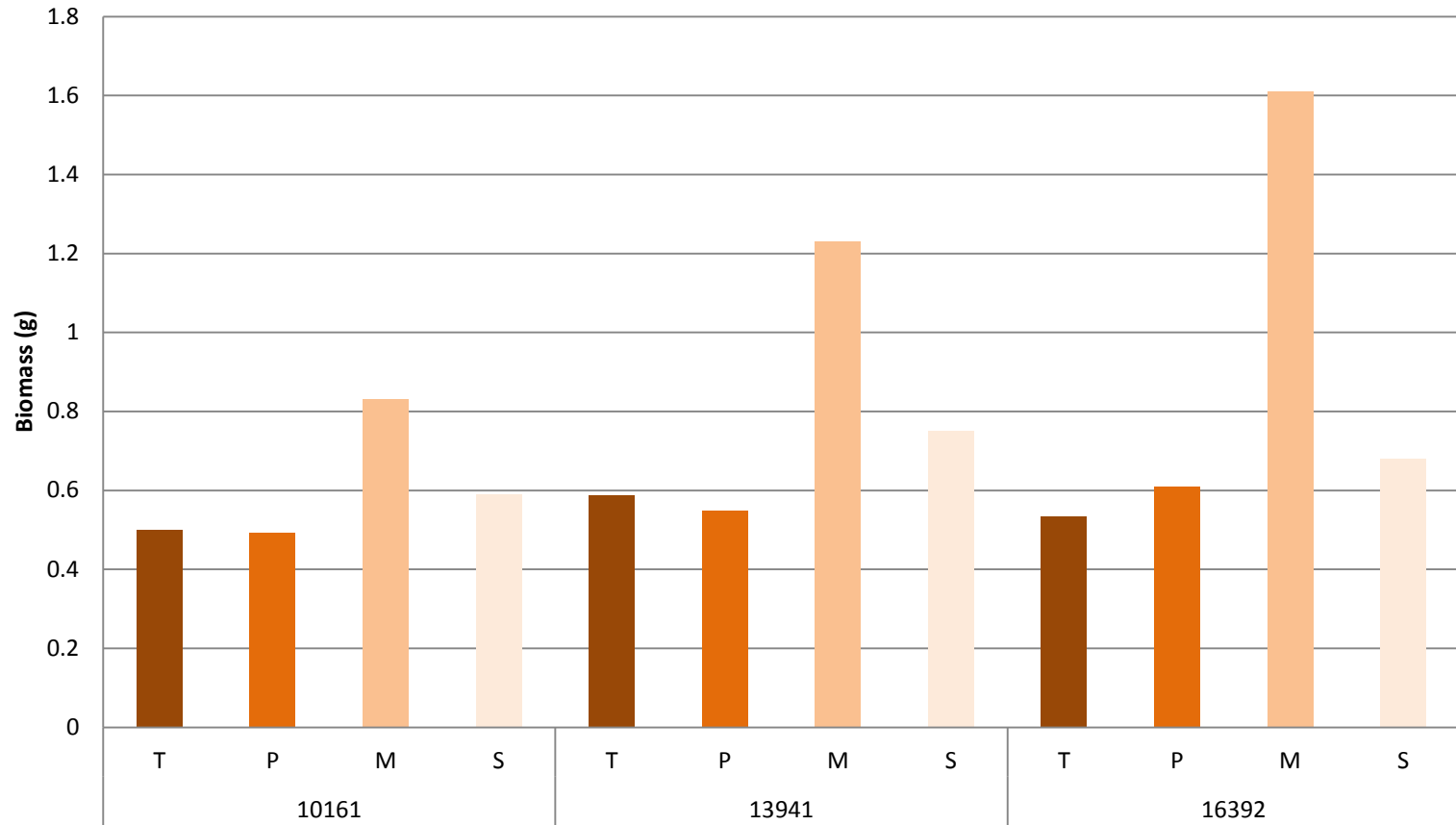
- *Hunua Catchment* trials 2009
  - Re-sprouting-willow suppressed
  - Using inoculum –plugs of *Chondrostereum*
  - Also with combinations of other wood-rot fungi
  - Durable, systemic control after 2-years
  - Low-tech approach → success!
  - Therefore if we invested more time and money, we would get a greater success?
  - Needed to consider a higher-tech approach



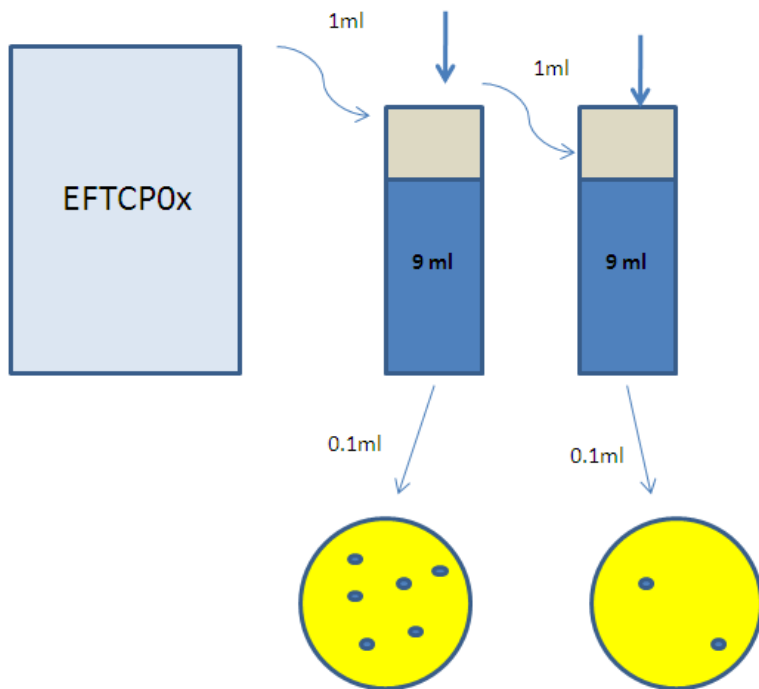
*In-field trial on willow at Hunuas  
Thanks to Sue Bunning, AC, Water Care for access*

# Biomass studies - substrates

Biomass of Cp



# Colony Forming Units (CFU)



- Mean CFU/ml – EFT 13  
=  $3.3 \times 10^7$  ( $1.3 \times 10^4$  SE)
- Mean CFU/ml – EFT 14  
=  $2.9 \times 10^7$  ( $1.5 \times 10^4$  SE)
- *Chontrol Paste™ CFU*  
*registered product*  
*available at  $10^5$ - $10^7$ .*

# EnForm Tech Formulation technology

- Proprietary Surface Fermentation Technology platform named Surface Technology (ST)
- Biofungicide: Monitor™ (*Trichoderma viridie*)
- Bioinsecticide: Biosoft™ (*Beauveria bassiana*)
  - Formulation of *Serratia entomophila* for biological control of grass grubs (Johnson et al. 2001).
- Bionemeticide: Yorker™ (*Paecilomyces liacinus*)
- Agriland Biotech Ltd (IND) now works for commercial launching of Metasoft™ (*Metarhizium anisopliae*), Vertisoft™ (*Verticillium lecanii*) and N-shield™ (*Pochonia clamydosporia*) using ST

# Glasshouse cut-stump trial

Woody host	Formulation trialled	Season
Poplar	EFT 13 EFT 14	Summer
Grey willow	EFT 13 EFT 14	Summer Winter
Crack willow	EFT 13 EFT 14	Summer Winter



# Glasshouse application



# Glasshouse results

Species	TT	Mean stump mass	T-value	s.d.
Grey	EFT 14	44.64	0.49	63.9
Grey	Gly-360	36.89	0.024*	32.9
Grey	Water	46.70	n/a	66.1



# Glasshouse results



Species	TT	Live shoots
Crack	EFT 13	0 <sub>a</sub>
Crack	EFT 14	0 <sub>a</sub>
Crack	Gly-360	0 <sub>a</sub>
Crack	Control	87 <sub>b</sub>



# Cut-stump field-application



# Cut-stump field-results

Species	TT	Mean number of live shoots	t-value
Crack	EFT 13	5.2	0.031*
Crack	EFT 14	3.5	0.0010*
Crack	Water	21.7	n/a



# Drill and inject field-application





# Field-trial results



**50% of all inoculated stumps have fruiting bodies after 1.5 years**

# Micro-gel: Stage 1 - Prototype

- Efficacy against range of species
- Shelf-life >12 months
- Summer and winter efficacy
- Two types of delivery system:
  - Cut-stump (paste)
  - Drill and inject
- Comparable to-greater number of CFU's compared to commercial product



# Stage 2: Product development

- Developed an efficient way of bulk production (>20 kg)
- Ensure stability and shelf life with appropriate formulation
- Test in the field and make improvements if necessary
- Commence application technology development
- Commence patenting
- Commence registration for product, and market and distribute product.





# Acknowledgments

- Kate Leggett, Greg Hoskins
- Nick Waipara, Holly Cox (AC)
- WaterCare
- Landcare Research  
Beating Weeds (II)  
“Expanding the toolbox” for funding 2011-2013
- Sue Bunning
- Jane Barton

