MANIPULATING ELMS: PROGRESS AND POTENTIAL

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SCOTLAND

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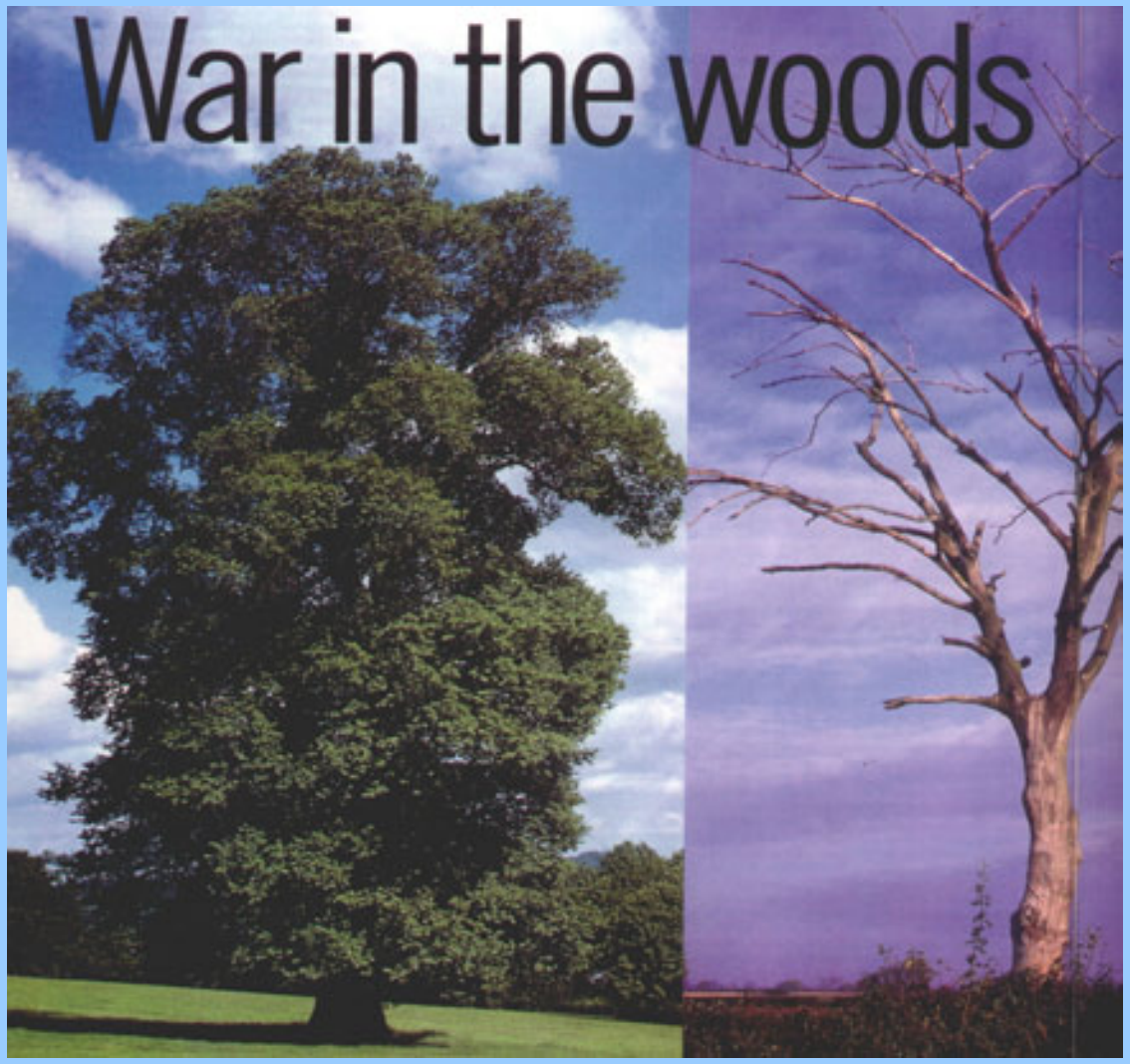
War in the woods

Cycles of *Ophiostoma novo ulmi* Spread

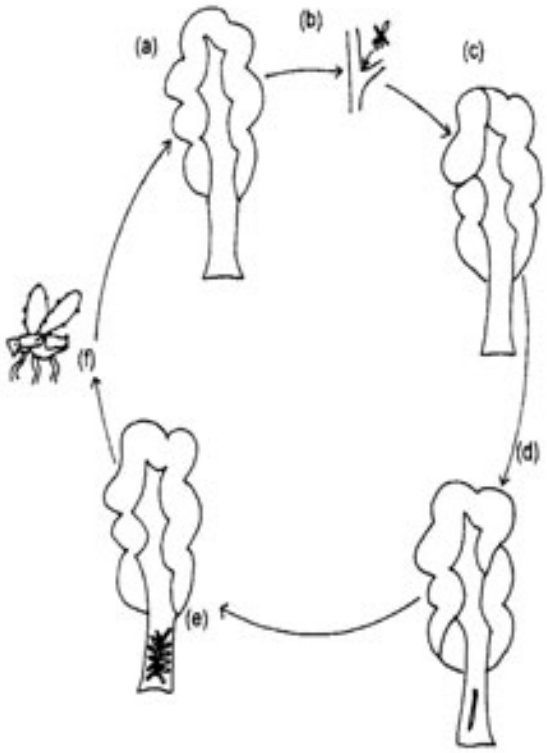

Figure 30.1 A simplified diagram representing the Dutch elm disease cycle. (a) Healthy elm tree; (b) Scolytus beetles feed in the two twig crotches of a healthy elm tree; (c) the tree becomes infected with *O. ulmi*; (d) Scolytus beetles breed in the weakened tree; (e) *O. ulmi* fruits in the larval galleries; (f) Adult beetles emerge carrying *O. ulmi* spores.

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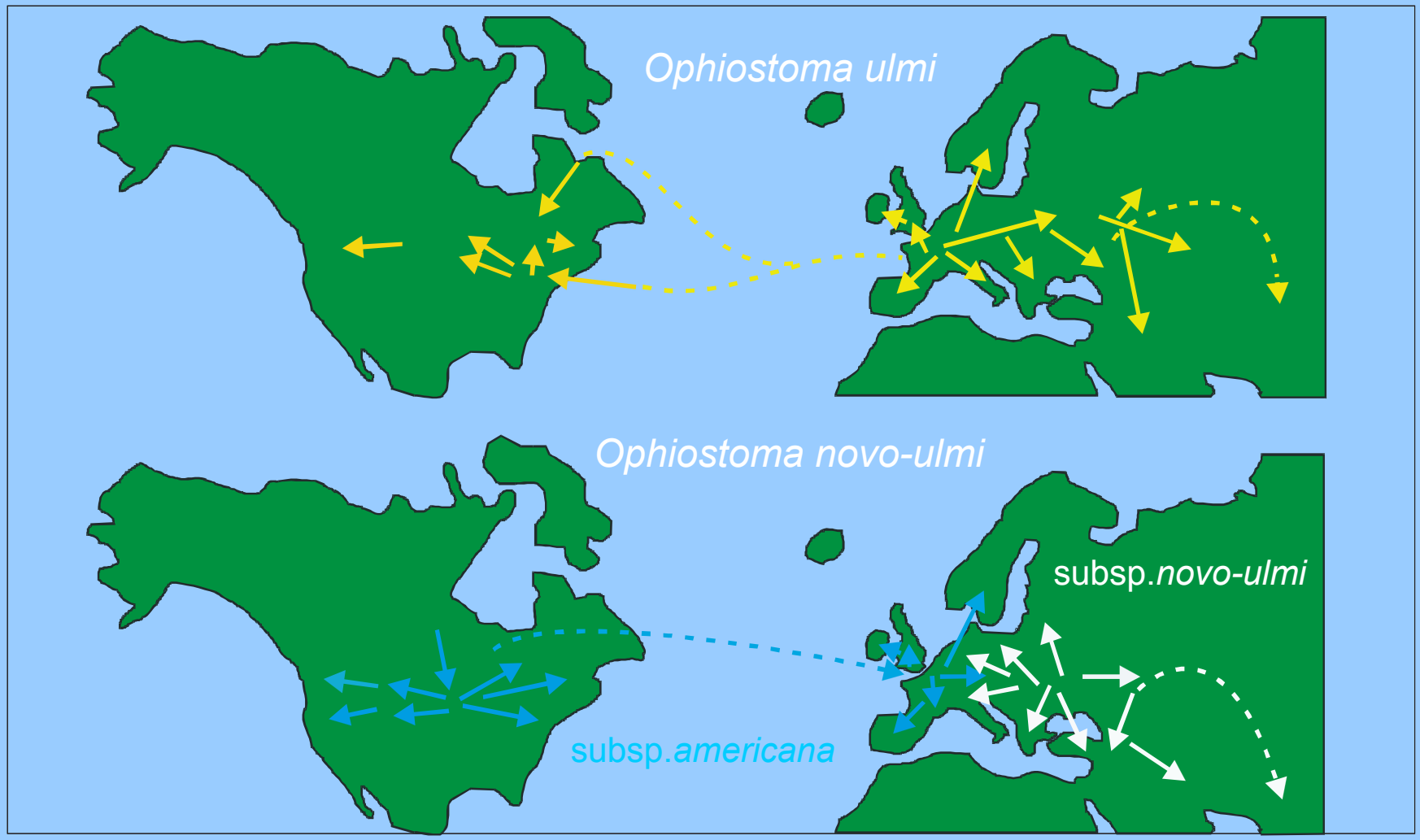

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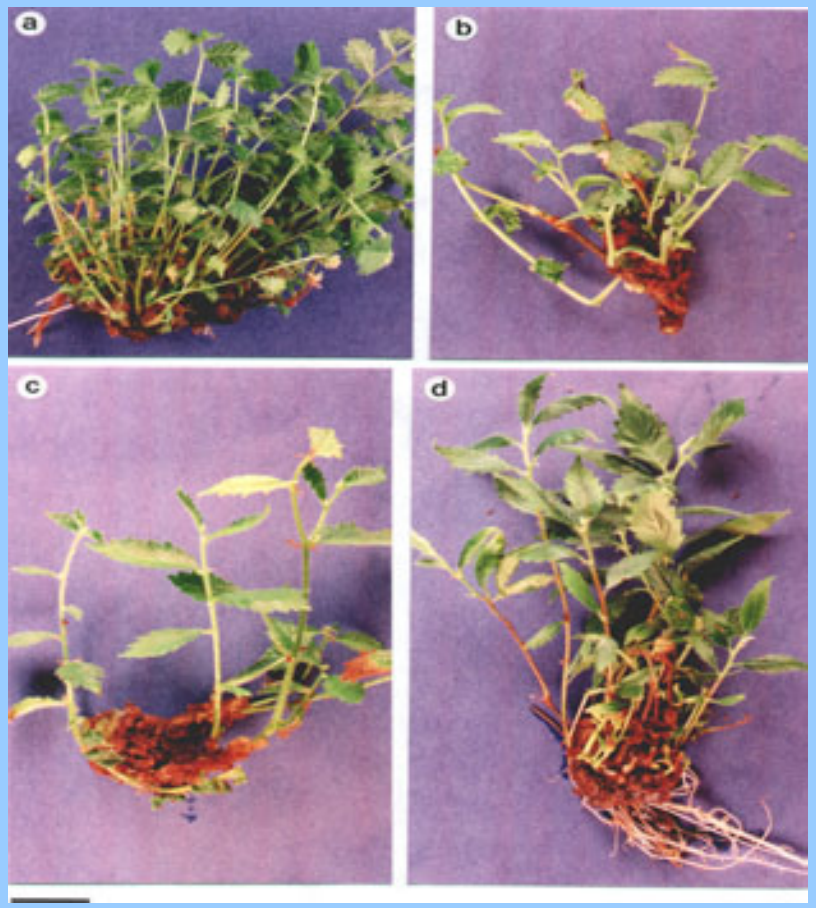
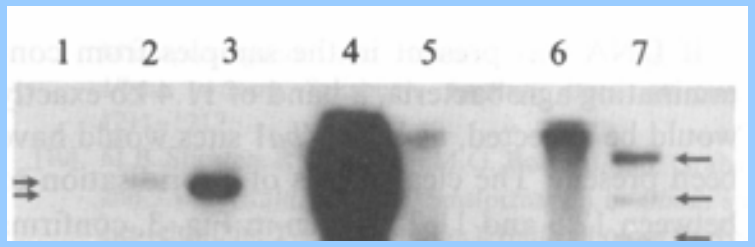
ELM BIOTECHNOLOGY

Tissue Culture and Plant Regeneration



- *Ulmus procera (a)*
- *Ulmus glabra (c)*
- *Ulmus americana (d)*
- *Ulmus parvifolia (b)*

Agrobacterium tumefaciens



- Aberrant shoots regenerated
- Genomic blot confirmed transgenic
- Low efficiency transformation & regeneration

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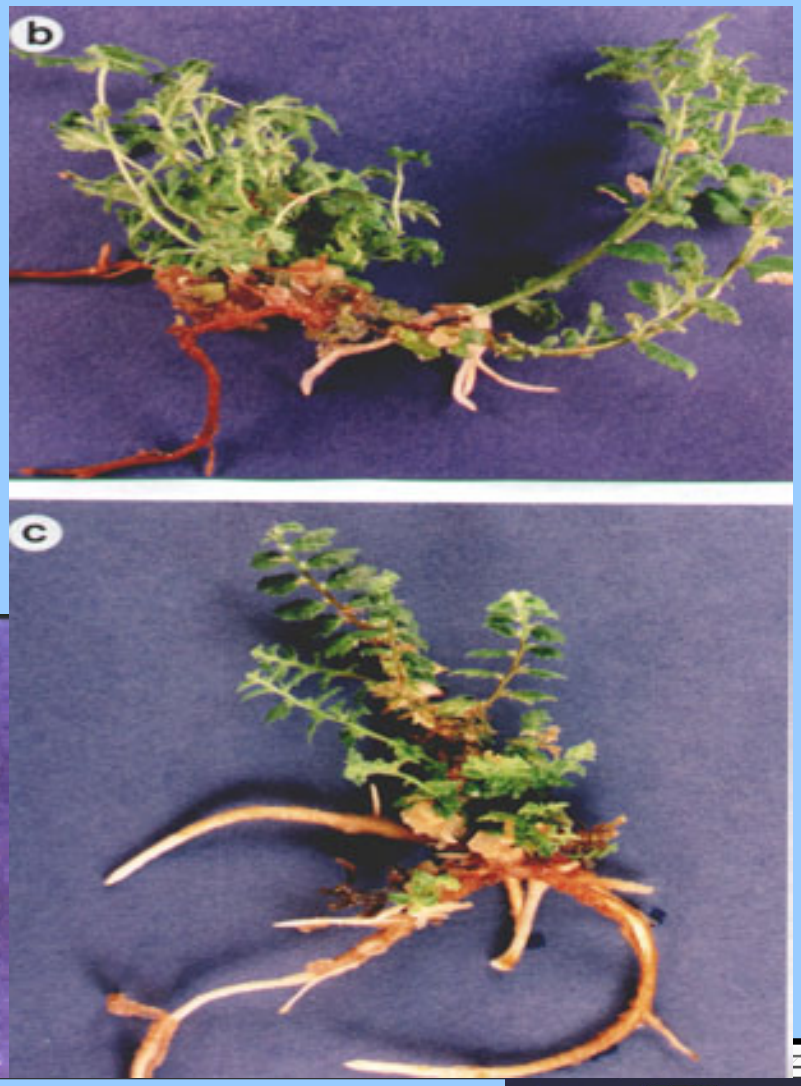
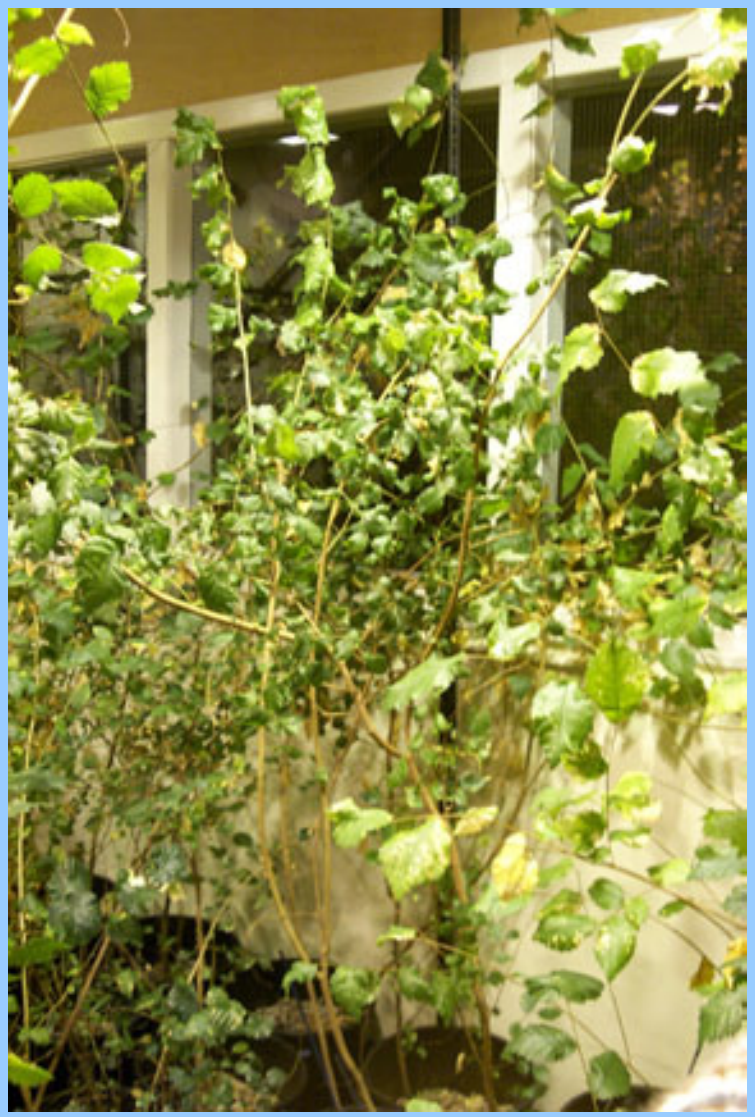
Agrobacterium rhizogenes

- SR4C1, SR4C2
- Dwarf shoots
- Reduced internode distance
- Reduced Leaf Area



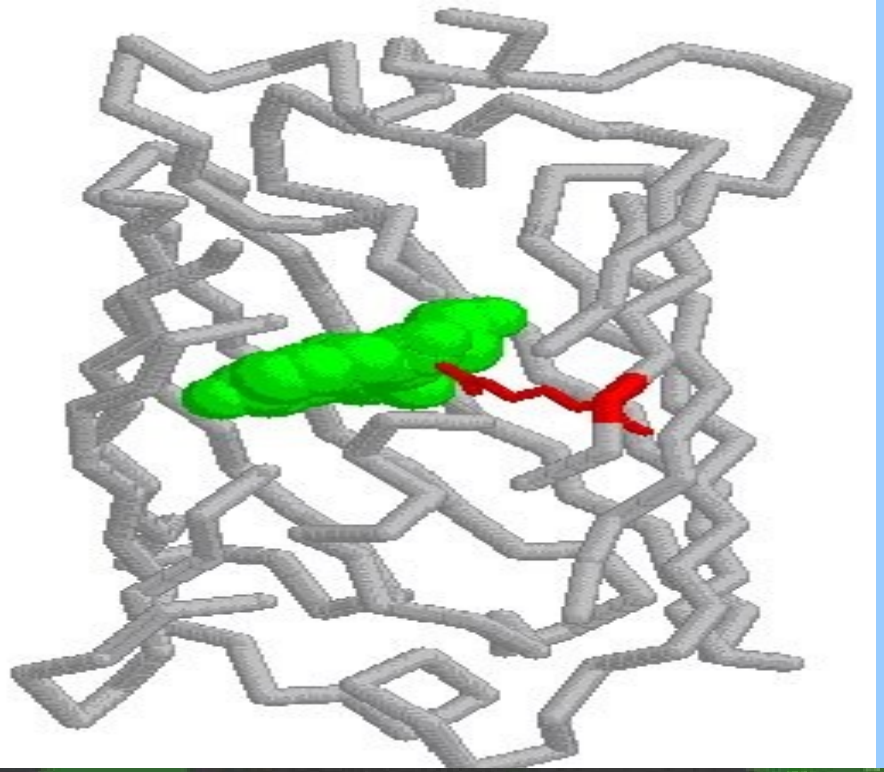


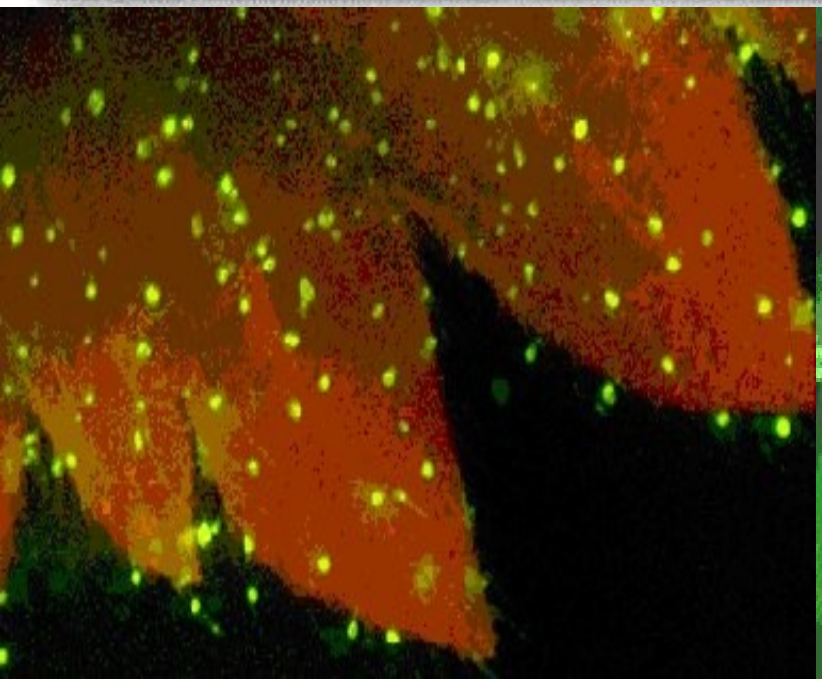
SR4C1 in Soil

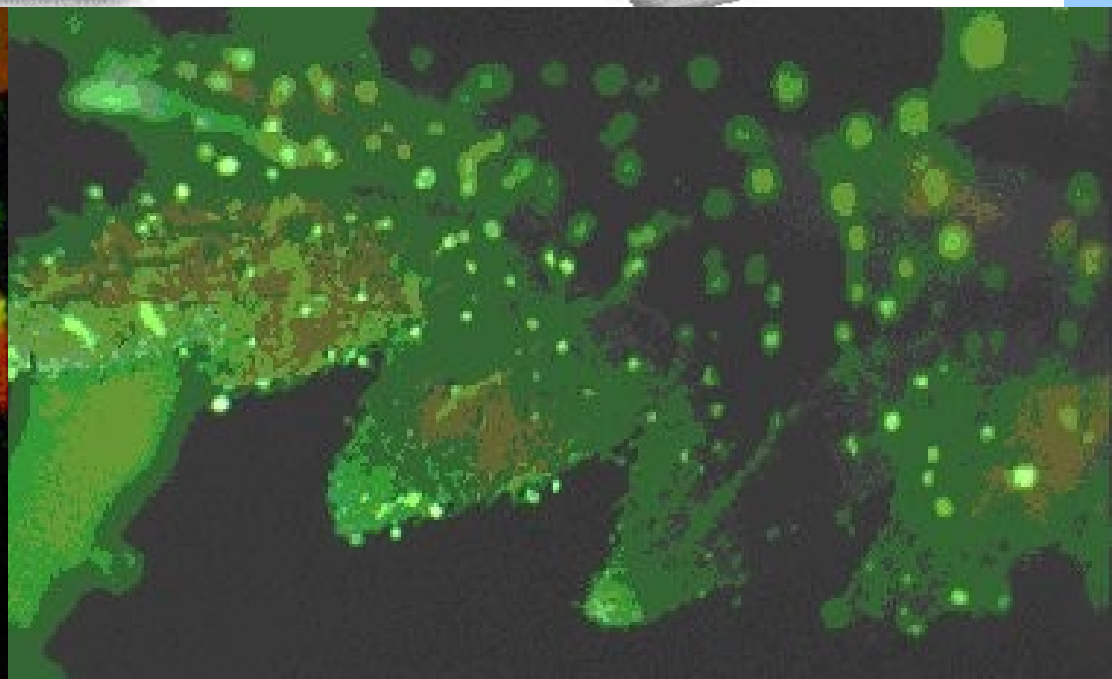
Biolistics

- CaMV35S::Jellyfish Green Fluorescent Protein
- CaMV35S::GUS Intron
- Construct screening tool


Aequorea victoria



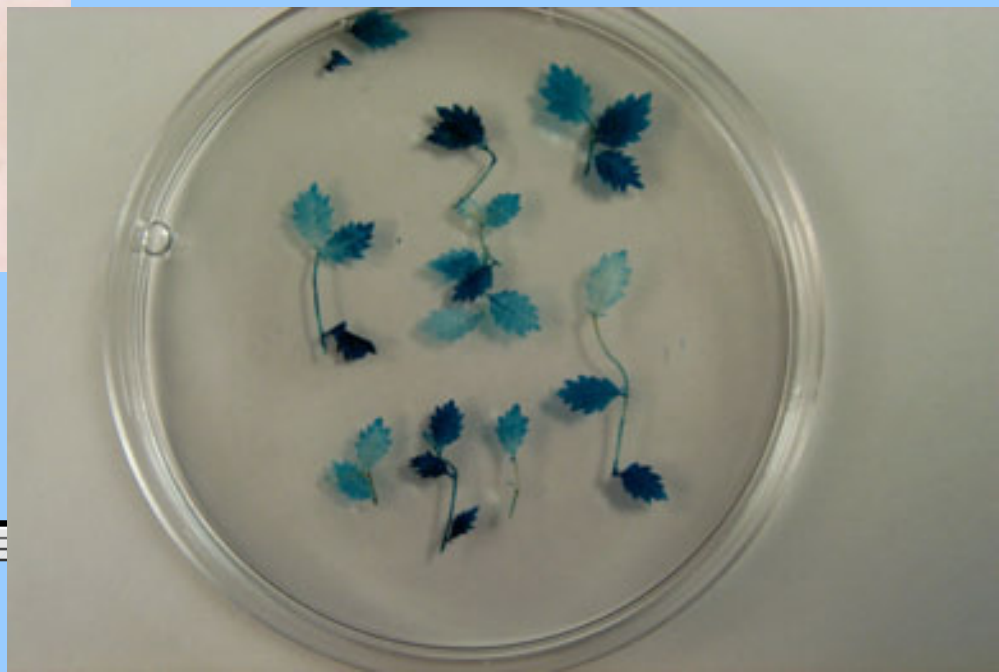




Gfp+ve Elm Growth

GM *U. procera* Phenotypically Normal Regenerants

- GUS expression in GM *U. procera* regenerants
- CaMV::35SGUS-Intron
- >100x increase GUS activity c.f. background

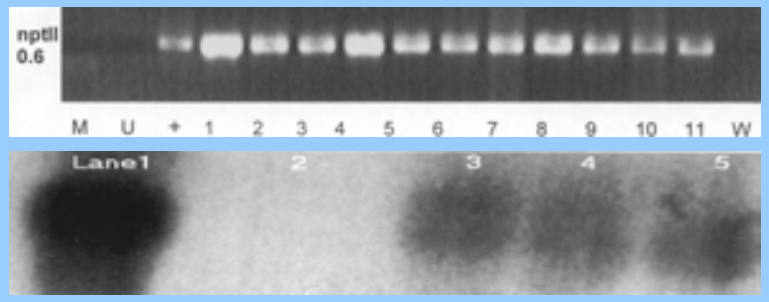


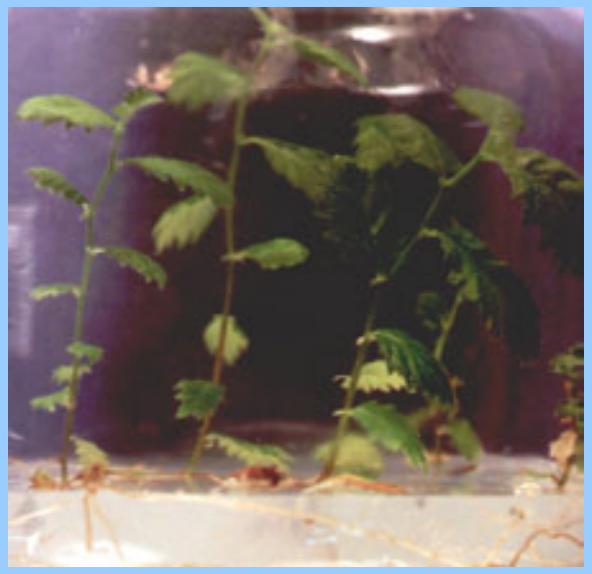
Variations in GUS Expression

Line	GUS Activity	+/-SD
2-5 Leaf	5.64	2.40
2-5 Stem	6.33	3.76
2-2 Leaf	6.31	3.71
2-2 Stem	7.52	4.39
3-7 Leaf	13.03	4.85
3-7 Stem	14.89	4.55
SR4 Leaf	0.00	0.02
SR4 Stem	0.00	0.02

GUS Act.: nMol MU/ug Protein/h; Mean 9 replicates

Characterisation GM Elms





- PCR analysis
- DNA-DNA Hybridisations
- Rooting on Kanamycin

Transfer to Soil

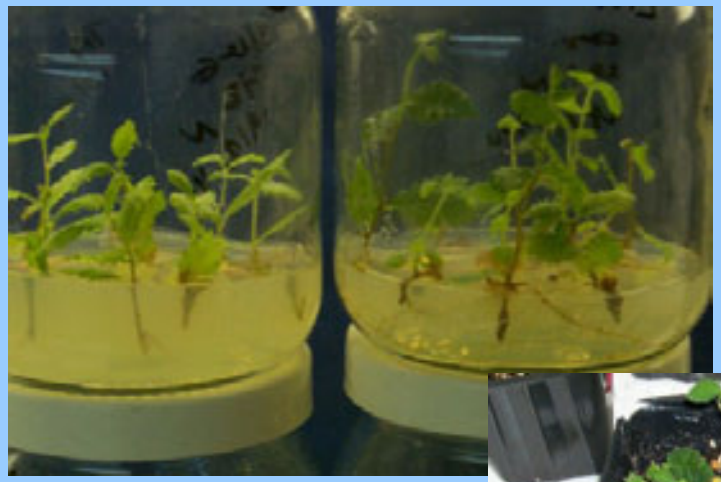
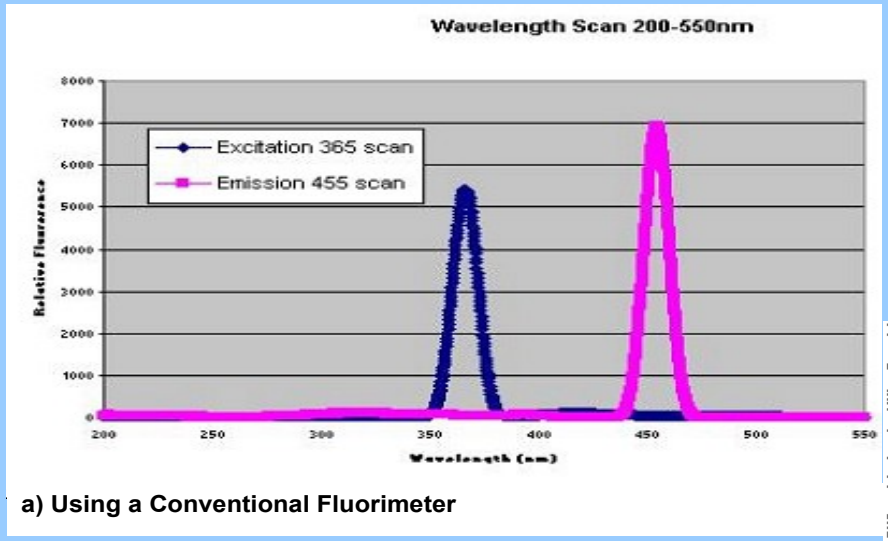
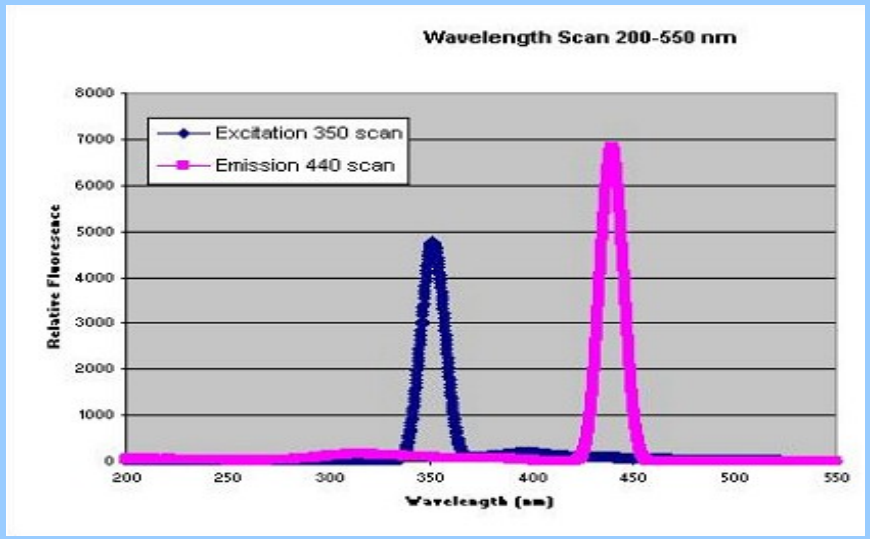


Figure 10 NaMU Fluorescence Spectra



a) Using a Conventional Fluorimeter



b) using RealTime ALEXAFilter

Figure 12 Real Time GUS analysis ZH4 and untransformed *Ulmus procera* SR4

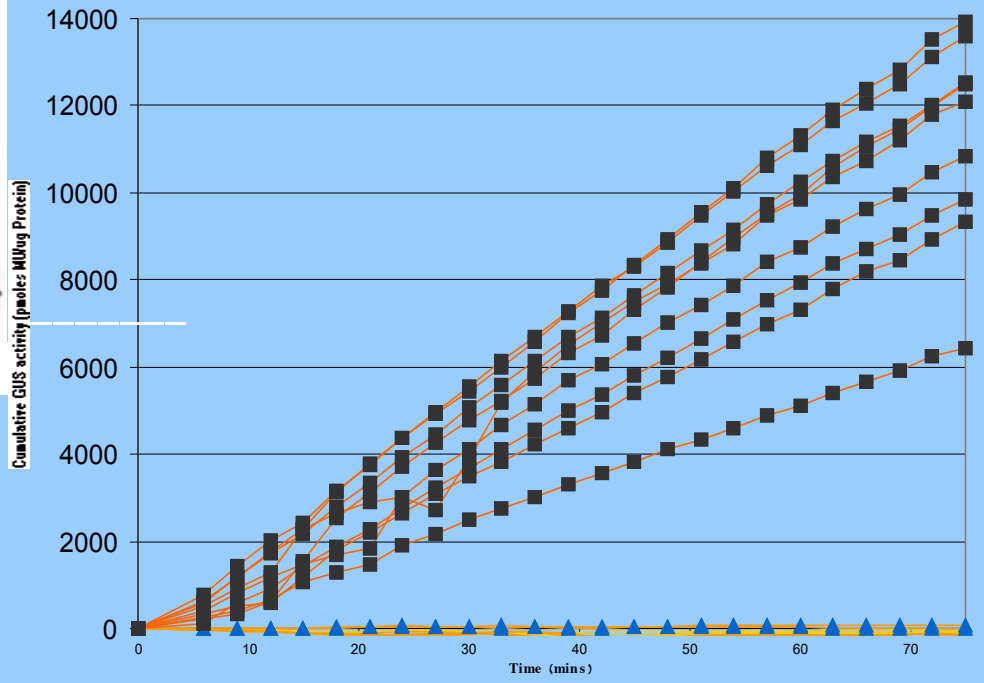


Figure 8 Seasonal *gus A* expression 2003/4

Error bars are standard errors of means from at least 9 independent replicates.

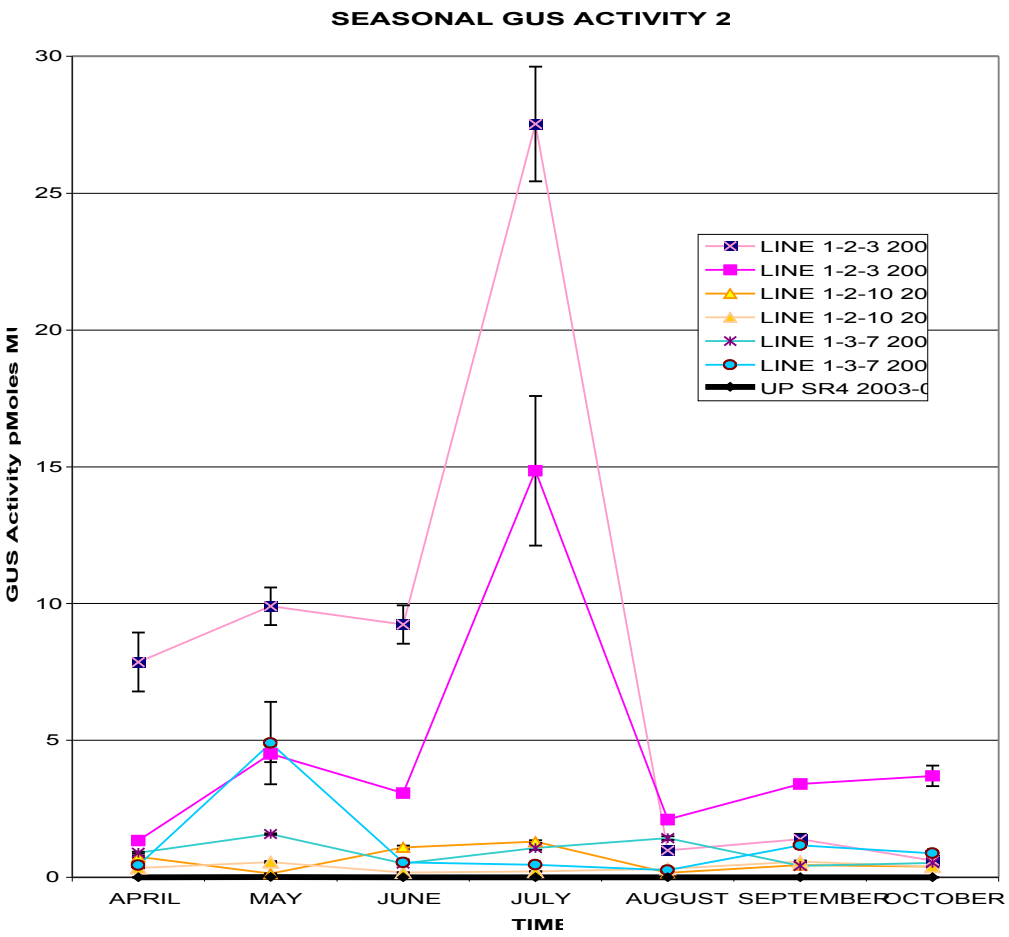
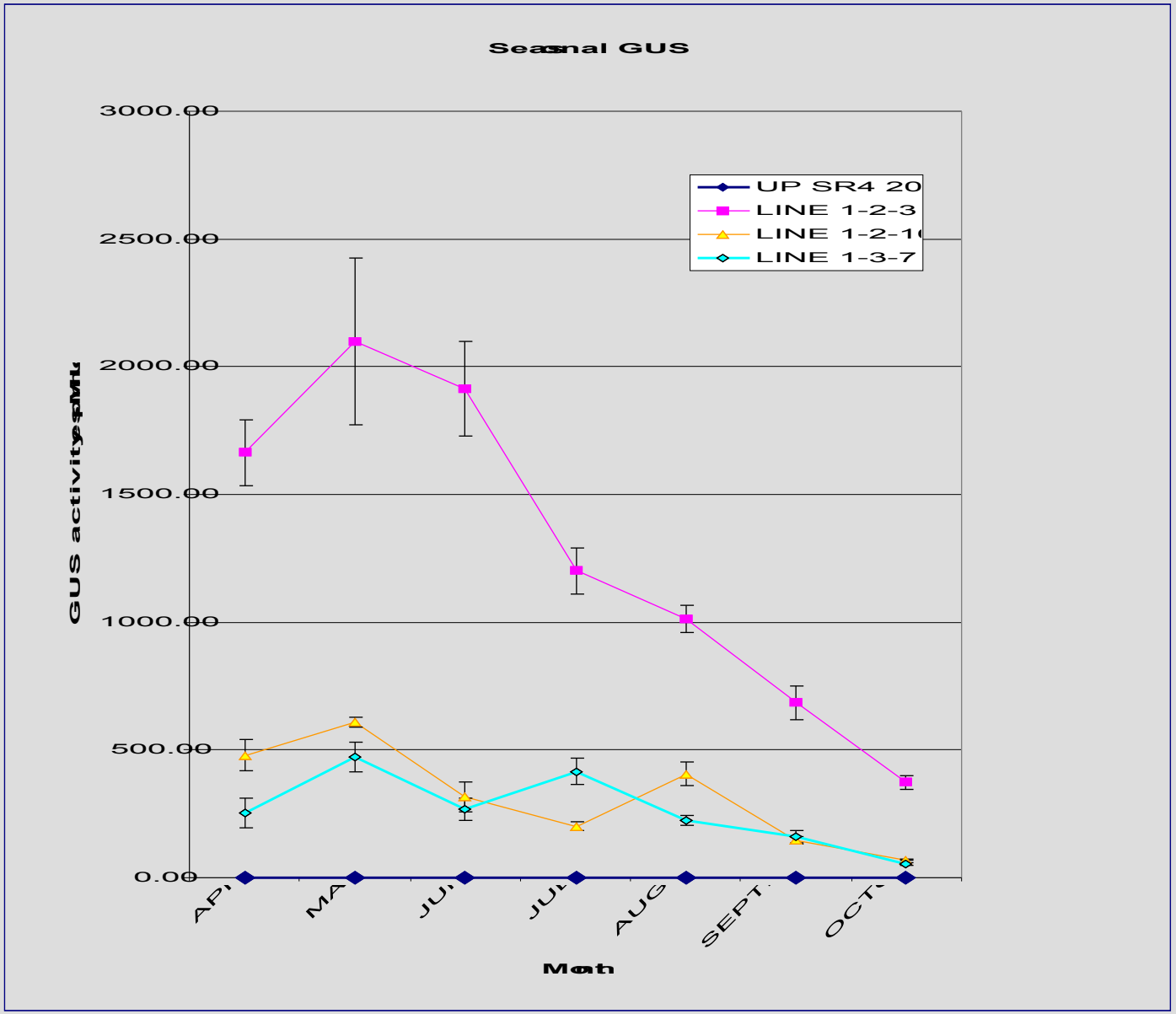


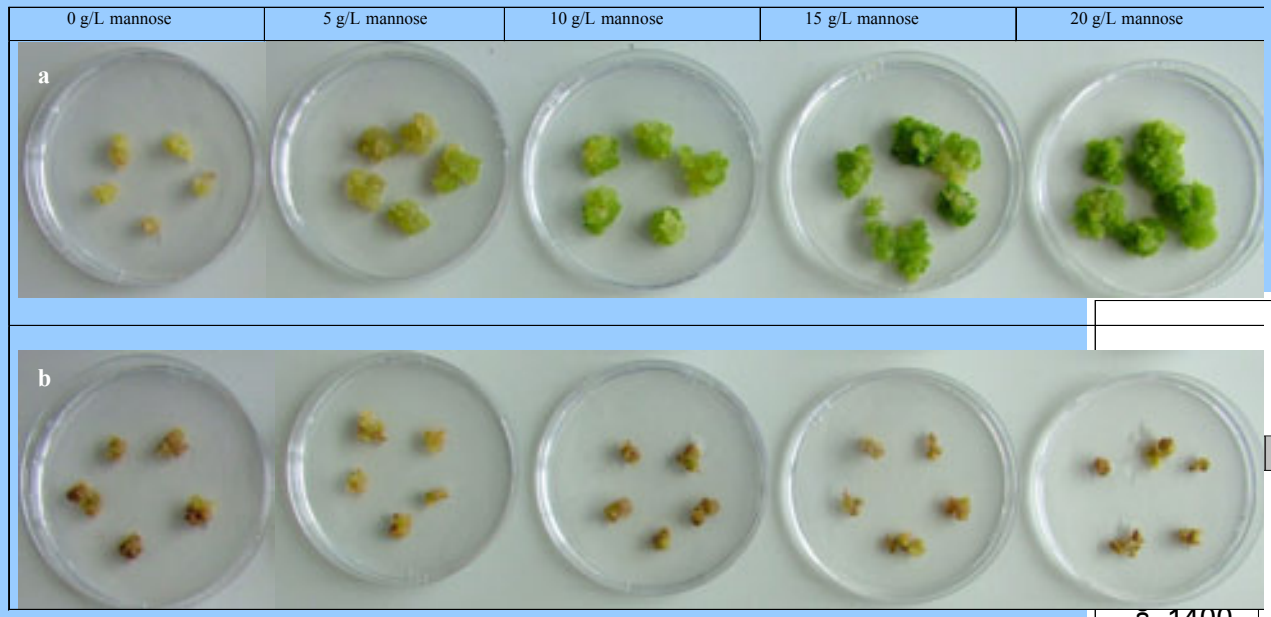
Figure 9 Seasonal expression 2005

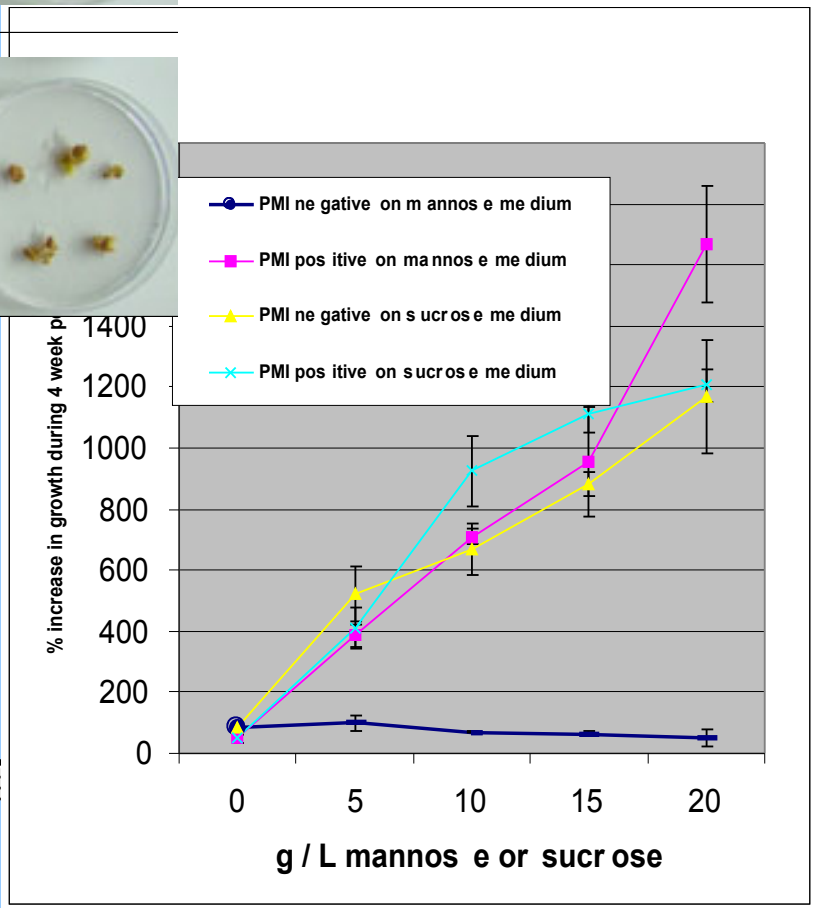


Other GM Elm Species

- *U. americana, U. glabra, U. parvifolia* shown to be suitable for *Agrobacterium* systems
- GUS histochemistry
- PCR products
- Shoot formation

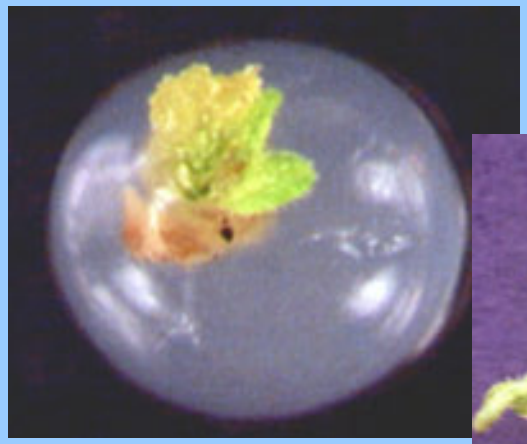
Figure 22 Growth of pmi positive transformant [(a)-upper] compared to pmi negative [(b)-lower] on phytagel solidified DKW media without sucrose but containing various levels of mannose.





Elm Cryopreservation

- Recovery from Freezing



- Shoot Regeneration

Elm Modifications

- Modify Internal Architecture: Ri-plasmid GM regenerants
- 3 Anti-Fungal Genes
- Urtica dioica Agglutinin UDA
- Dahlia Protein Dahlin D
- Radish Anti-Fungal Protein R
- GM Regenerants all three constructs
- Exploring alternative Anti-Fungals & Markers

AFP-Elm Regenerants

- *U.procera* plants obtained after AFP gene transfer

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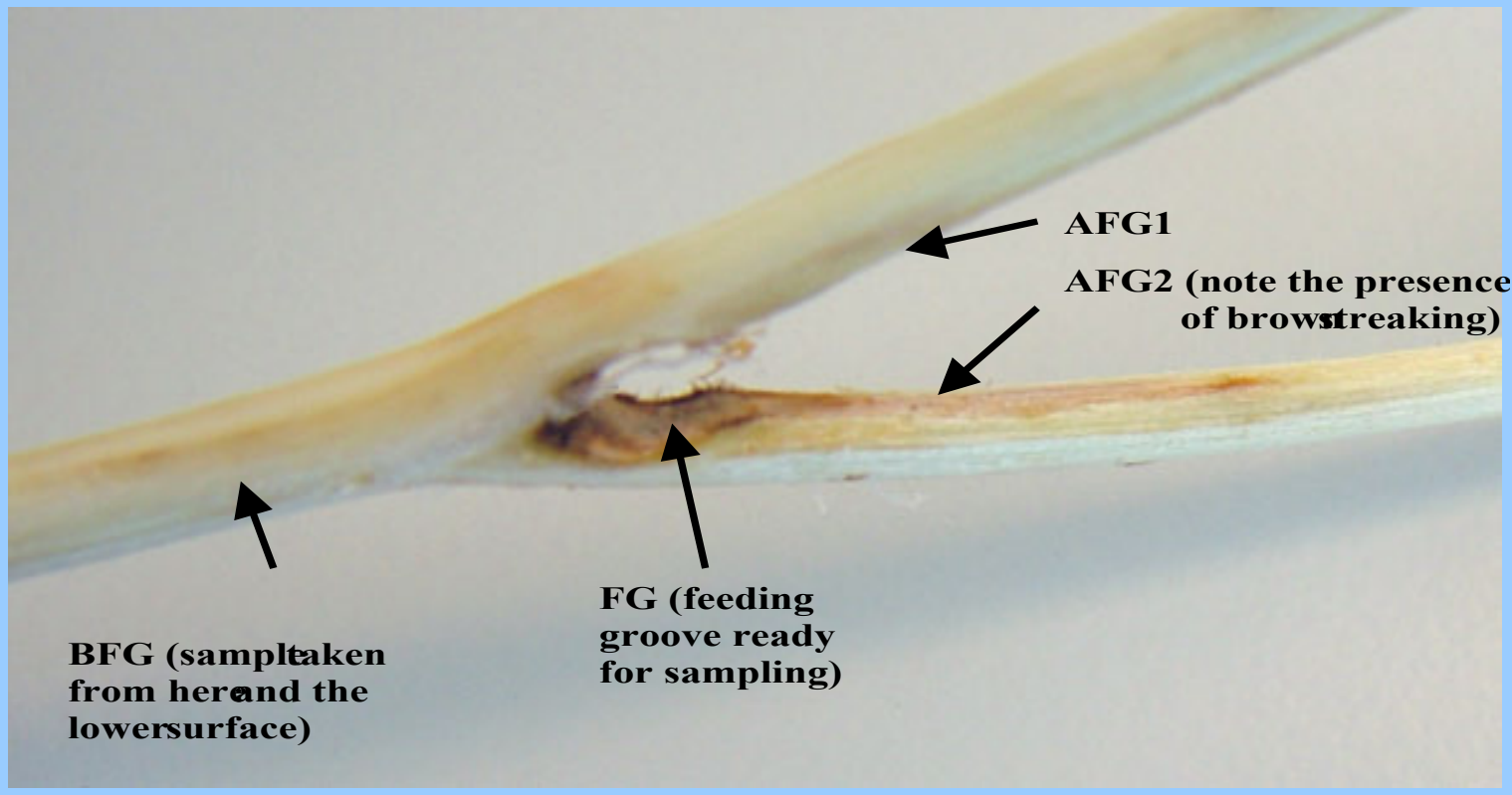
Fungal Challenge via Feeding Groove

2.3.4 Raw data for feeding groove trials 2005-2006

Table 6 Key to Tables 7a-7g & 8a-8e

Plant Line	*Ulmus procera* SR4 line Genotype
Groove No.	Name given to identify feeding groove (N.B. The experiment was performed double blind and this identity was only un-encoded once the results had been assessed)
Diam. mm below groove	Twig Diameter (in mm) below Feeding Groove
Beetle present Yes or No	Was the beetle still present at the end of the two day period when the paper was removed?
Growth of inoculum from paper	Fungal growth from filter paper post infection on Malt Extract Agar
FG1&2	Sample taken from Feeding Groove
AFG1&2	Sample taken from above Feeding Groove
BFG	Sample taken from below Feeding Groove
Xylem Streaking	Brown Streaking of xylem evident on cutting and removal of bark layer (Graded I for small amount of streaking to III for a lot of streaking)
?	*Ophiostoma* growth
Both streaking and fungal growth together	? I light streaking ? II medium streaking ? III dark streaking

Figure 6 Brown streaking and fungal re-isolation sampling points shown on a stem piece following removal of bark tissue sampling



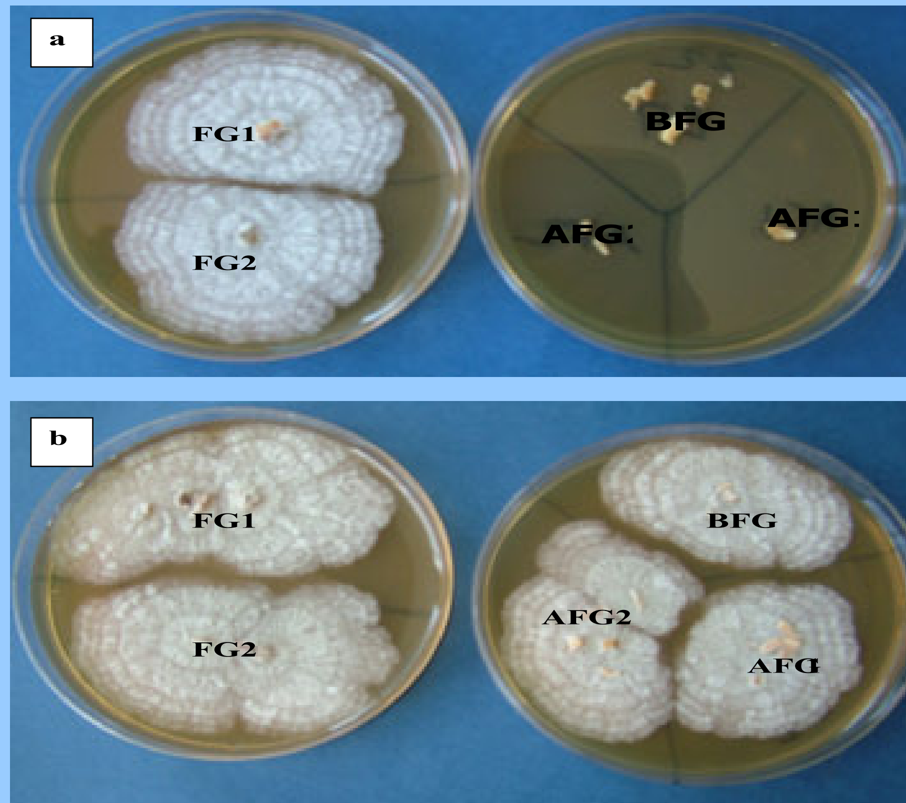


Figure 7 MEA plates containing chips from a) an antifungal gene containing line that exhibits resistance to the spread of the fungus compared with b) an untransformed, sensitive *Ulmus Procera* SR4

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Construct / Aspect	Fungus Isolated Above or Below FGroove	Brown Streaking Above or Below FGroove	Both Fungus Isolated & Brown Streaking	**NO** Fungus Isolated & **NO** Brown Streaking	Number Feeding Grooves
SR4	88.9%	44.4%	38.9%	19.4%	36
DP8	68.8%	27.1%	22.9%	29.2%	48
Dam6	63.9%	31.2%	23.0%	34.4%	61
U	52.6%	21.1%	15.8%	42.1%	19
R2425	45.5%	31.8%	13.6%	40.9%	22
C1(Ri)	60%	45%	35%	35%	20

Appendix 5

Genetic elements shown to be functionally effective

Cauliflower Mosaic Virus 35S promoter
Cestrum Mosaic Virus promoter
Nopaline synthase terminator
Phosphinothricin acetyl transferase/Bar
Neomycin phosphotransferase II
Ubiquitin 3 promoter
Green fluorescent protein (gfp5er)
Beta-glucuronidase (gusA)
GusA Intron
Rol A,B,C,D promoters
Rol AD
Nopaline synthase promoter
Nopaline synthase
Agropine synthase promoter
Agropine synthase
Agropine synthase terminator
Mannopoine synthase
Tryptophan-2-monooxygenase
Indole-3-acetamide hydrolase
Isopentenyl transferase
Atriplex canescens 3' untranslated region (UTR)
Cauliflower Mosiac Virus 35S polyA sequence

Genotpyes Available

- Ulmus procera SR4
- **SR4 + gusA, gfp, nptII, pmi**
- **SR4 + UDA**
- **SR4 + D**
- **SR4 + R**
- **SR4 C1**
- Ulmus glabra, parvifolia, americana
- Sorbus aucuparia
- Quercus robur
- Salix spp. Bowles' Hybrid, Jorr, Tora
- Salix spp. Dwarf Hybrids (x5)
- Alnus glutinosa
- Populus spp. hybrid

Elm Habitat Restoration

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- BioDundee
- Institute of Forest Biotechnology
- http://www.forestbiotech.org