

Nicotiana hybrids for the contained production of plant-made materials

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Kentucky Tobacco R&D Center (KTRDC)

UK College of Agriculture

- Plant genetic engineering research (45,000 sq ft lab and office, greenhouses, farm and farm equipment)
- Mission to develop crop opportunities for KY farmers
- Provide facilities & grants to researchers
 - Tobacco analytical facility, plant growth facilities, reference cigarette program, field trials, regulatory assistance, plant transformation, etc.
- Technology development
 - Genetic promoters, gene switches, expression technologies, enzymes for biofuels, etc.
- House a business incubator (translational emphasis)

KTRDC Field Research Program





Hybrid 57: Breeding option #2
Limited fertility, quick early growth
73,912 kg/ha green biomass

Hybrid 63: Breeding option #1
Sterile, ID preserved, blue mold tolerant, horn worm res.
77,639 kg/ha green biomass

Hybrid 90: Breeding option #1
Sterile, ID preserved, blue mold tolerant, horn worm res.
94,060 kg/ha green biomass

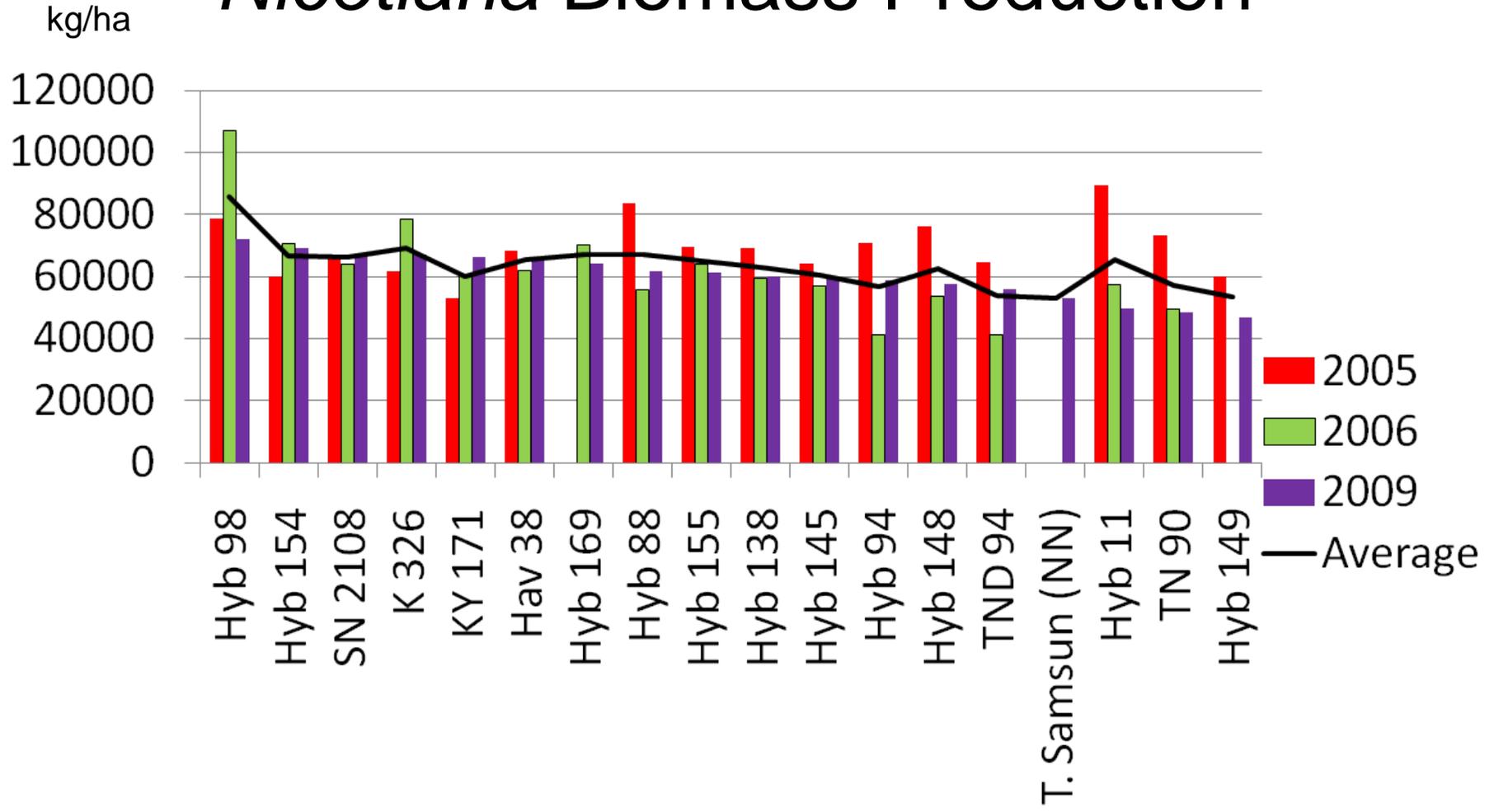
Hybrid 98: Breeding option #3
Sterile, Blue mold and TMV tolerant, delayed flowering
95,915 kg/ha green biomass







Nicotiana Biomass Production



USDA Biotechnology Risk Assessment Grant (BRAG)

3 year grant to U of KY and U of TN

Objectives

- Produce an interspecific hybrid *Nicotiana* plant expressing genes for fluorescent-protein 'tags' that will facilitate tracking of its pollen and whole plant expression (TN 90 x *N. glauca*)



GFP-1 hybrid cross TN 90 x *N. glauca* (both parents transformed for whole plant GFP expression)

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Objectives

- Produce an interspecific hybrid *Nicotiana* plant expressing genes for fluorescent-protein 'tags' that will facilitate tracking of its pollen and whole plant expression (TN 90 x *N. glauca*)
- Perform field-level gene-flow experiments using a modified Nelder-wheel design monitoring the gene traffic in both directions (pollen dispersal from the hybrid and seed-set on the hybrid)

Modified - Nelder Wheel Plot (UKY site)

Groups of 5 male sterile MSTN 90 plants

54 m

38 m

Beehive

23 m

9 m

50 fertile SN 2108 plants + 50 hybrid GFP-1 plants

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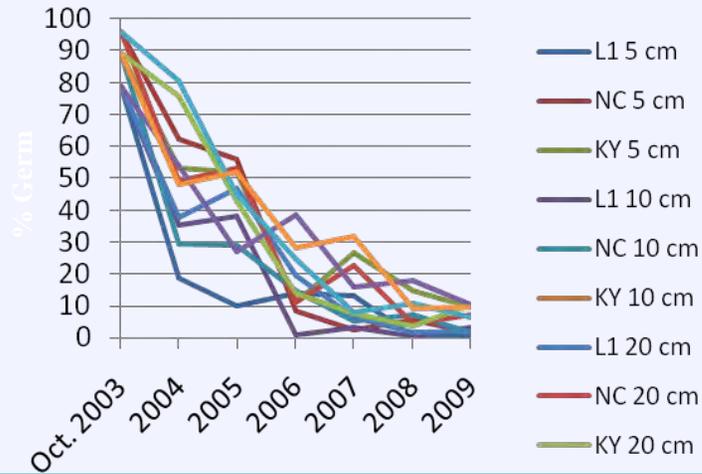
Preliminary summary of data from 2010 greenhouse crosses and field experiment

Location	Kentucky				Tennessee	
Material Source	MSTN 90 x hybrid GFP-1 (greenhouse)	hybrid GFP-1 x SN 2108 (greenhouse)	hybrid GFP-1	MSTN 90	hybrid GFP-1	MSTN 90
Total # of pods	11	23	73	155	263	118
Total # of seeds	445	12	16	11170	84	5968
# of seeds germinated	3	5	9	7340	38	74
Total # surviving	2	3	5	7340	42	73
# of GFP + seedlings	2	3	4	0	31	1
# of GFP – seedlings	0	0	1	7340	1	73
# of surviving GFP + plants confirmed with spectrofluorometer	2	3	4	0	31	0

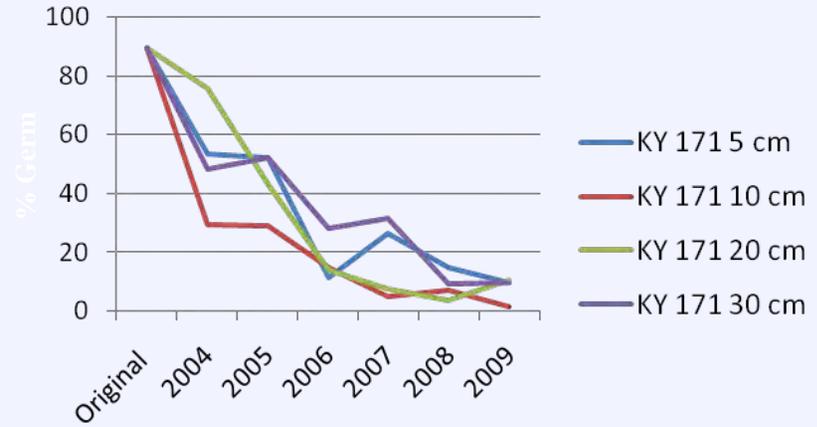


Seed Germination Over Time

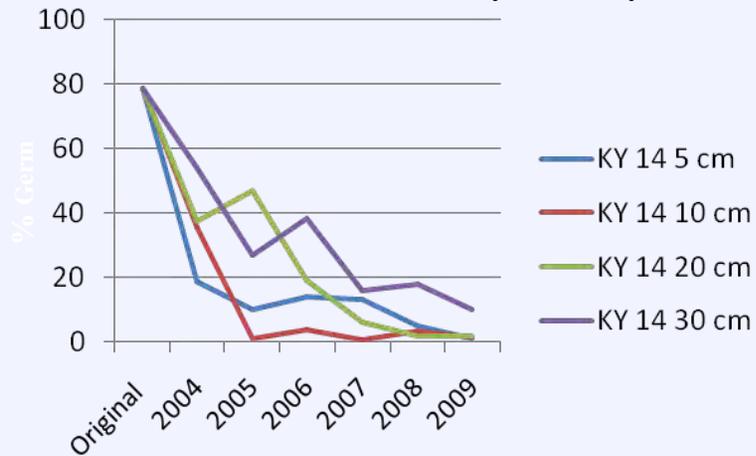
Germination % over time (All entries)



Germination % over time for Kentucky 171 (Dark Fired)



Germination % over time for Kentucky 14 (Burley)



Germination % over time for North Carolina 297 (Flue Cured)





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