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Faster evaluation of induced floral sterility in transgenic early flowering poplar

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Aspects of biosafety research

- *VGT*
  - *Possible flower formation during field release*
  - *Introduction of male/female sterility*
  - *VGT in non-transgenic aspen*
- *HGT*
  - *Plants, bacteria and fungi, Mycorrhiza fungi*
- *Vegetative propagation capacity (root suckering)*
- *Transgene stability*
- *Effects on target species*
- *Effects on non-target species*
- *Can wood be identified as „transgenic“?*

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Aspects of biosafety research

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Flower bud formation in the field (1996-2001)

1998: 1 single 35S-*rolC* tree
with two flower buds
(Age: 3)

1999: No flower buds

2000: 11 additional 35S-*rolC* trees
with 25 flower buds
(Age: 5)

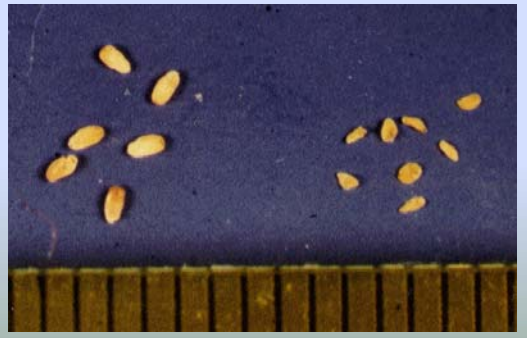
2001: No flower buds

→ Construct specific

+ Development of transgenic flower was follwed in greenhouse

+ Cross-pollination yielded 508 very small seeds

+ No germination



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Vertical gene transfer is a biosafety relevant problem when planting transgenic forest trees!!!

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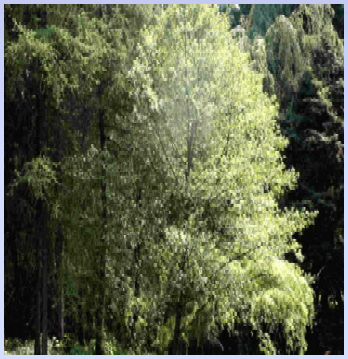
Induction of sterility

Prevention of an outcrossing of genes (vertical gene transfer) following induction of sterility in transgenic trees

Genetically engineered trees (transgenic trees)

Fertile tree → **„Transgenic Pollen/seeds“** → **Introgression of transgenes in natural ecosystems** →



Tree in forest (non-transgen)

Sterile tree → **NO transgenic pollen/seeds**

Sterile transgenic trees are not capable to reproduce ✕ **NO introgression of transgenes**

Sterility induced with gene technology

- Development of sterile lines
  - Male sterility
  - Female sterility
- RNA-degrading enzymes
  - E.g. barnase, stilbene synthase
  - Expressed in flower organs (anthers, ovary)
  - Different anther- and ovary-specific promoters
- Suppression of flowering

Literature: papers of S. Strauss, Hönicka & Fladung, 2006

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Sterility gene constructs

- TA29::*Barnase* (Debener, 2002)
- TA29::*Vst1* (Debener, 2002)
- CIGPDHC::*Barnase* (Debener, 2002)
- CIGPDHC::*Vst1* (Debener, 2002)
- MALE1::STS (Walter 2006)
- TA29::AS-PDH_E1alpha-1 (Kubo 2006)

(♂)

- BpMADS1::*Barnase* (Lemmetyinen *et al.*, 2001) (♂ / ♀)

Induction of early flowering in aspen

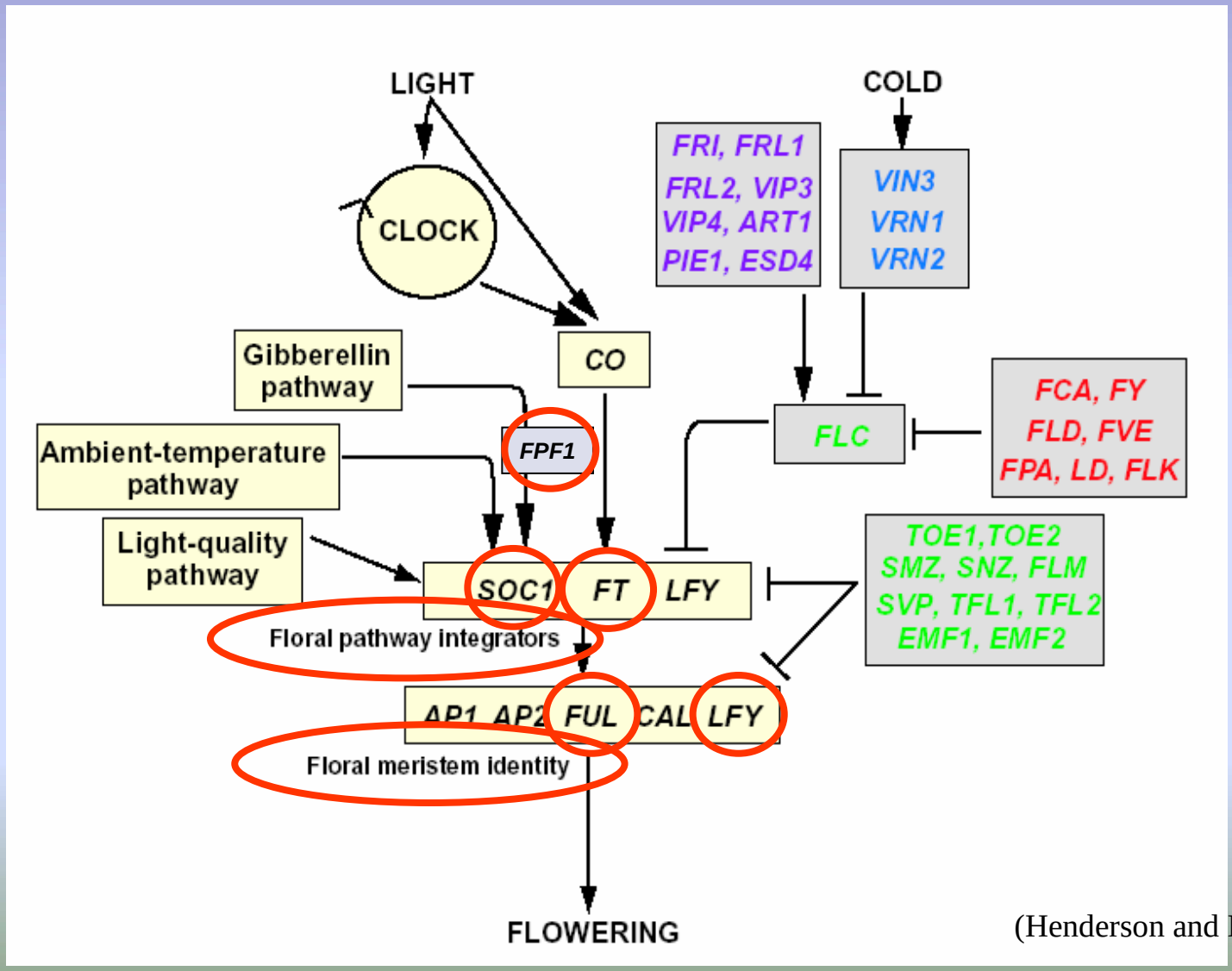
To proof sterility within a scientist‘s life → Early flowering

- Growth inhibitors
  - Daminozide. Flowering in Aspen after 2 years (Li pers. comm.)
  - Paclobutrazol

Not successful

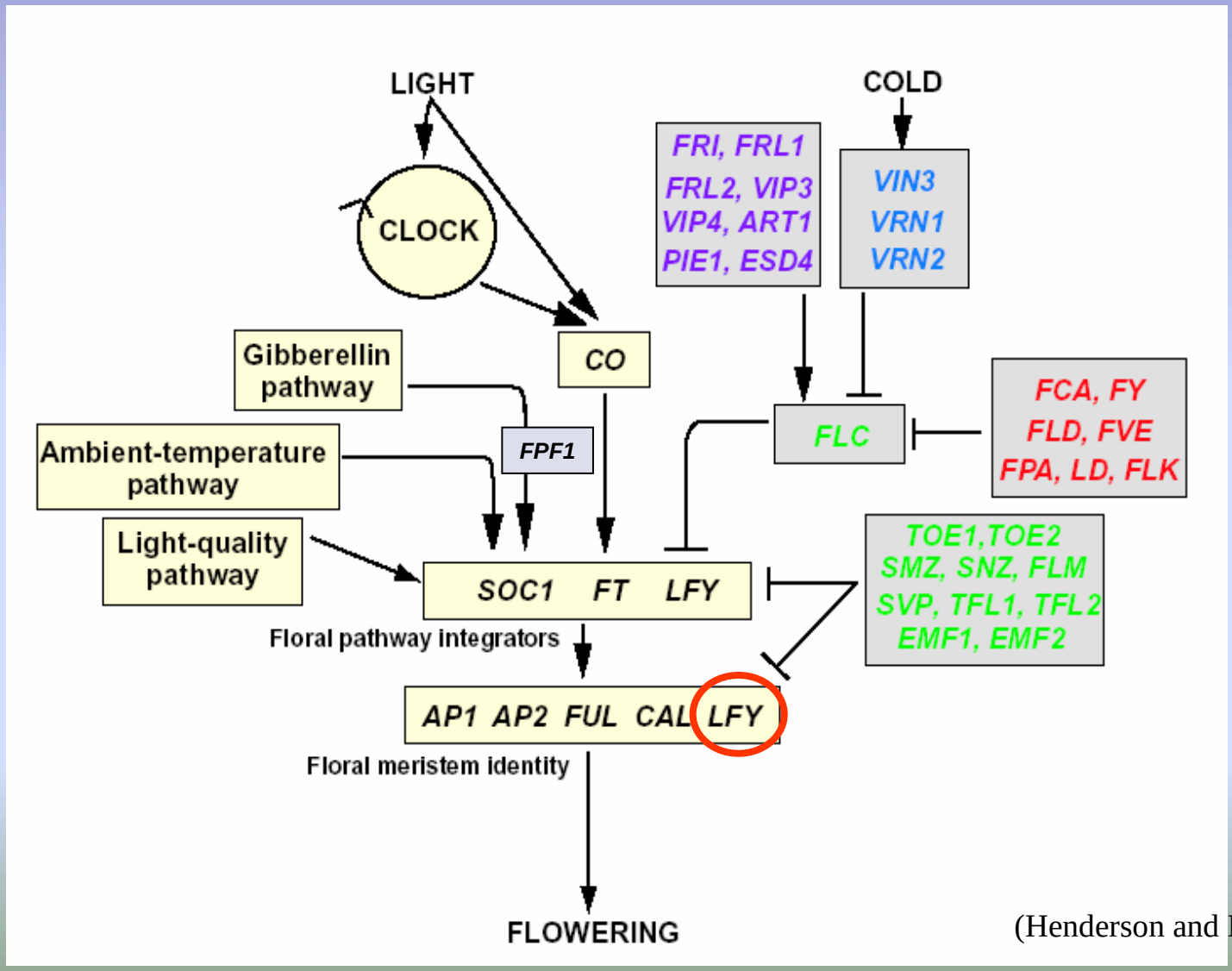
- Genetic transformation
  - *BpMADS4*, *Betula pendula* Roth. (Elo *et al*., 2001)
  - *Rol C/Rol D*, *Agrobacterium rhizogenes* (White, 1985), in aspen (Fladung *et al*., 2003)
  - *Leafy*, *Arabidopsis* (Schultz, 1991), in aspen (Weigel und Nilsson, 1995)
  - Other flowering genes

The flowering pathway in *Arabidopsis*



(Henderson and Dean 2004)

The flowering pathway in *Arabidopsis*



(Henderson and Dean 2004)

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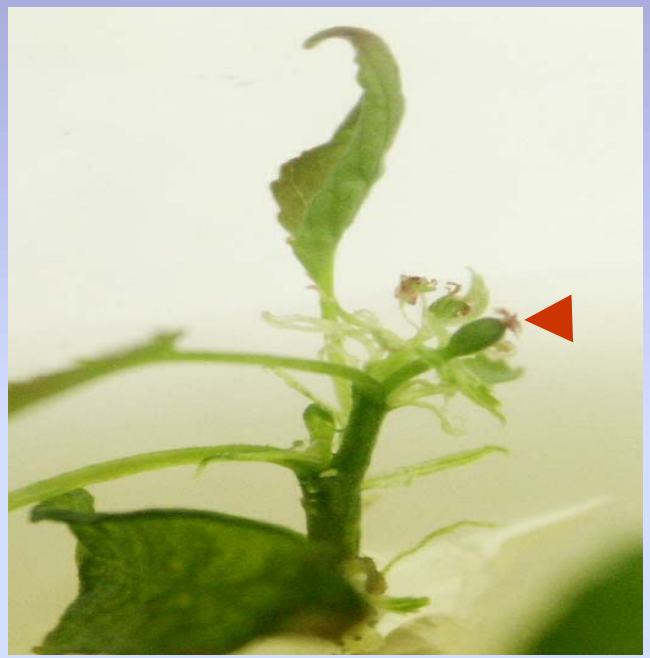
35S::*Leafy*

Clone T89,
male



Clone Esch 5,
female

"Dwarf"-phenotype,

Single flowers instead of catkins

Weigel and Nilsson 1995

Hönnicka and Fladung, 2006

(Leafy was kindly provided by D. Weigel

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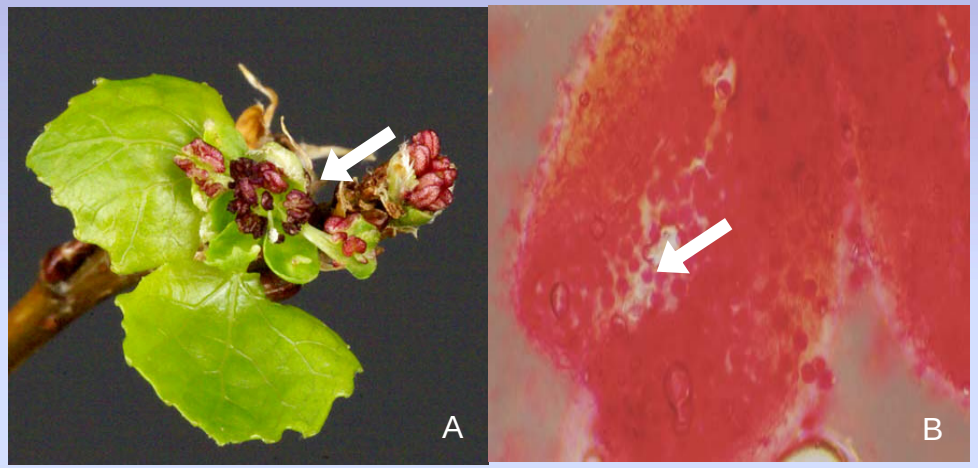
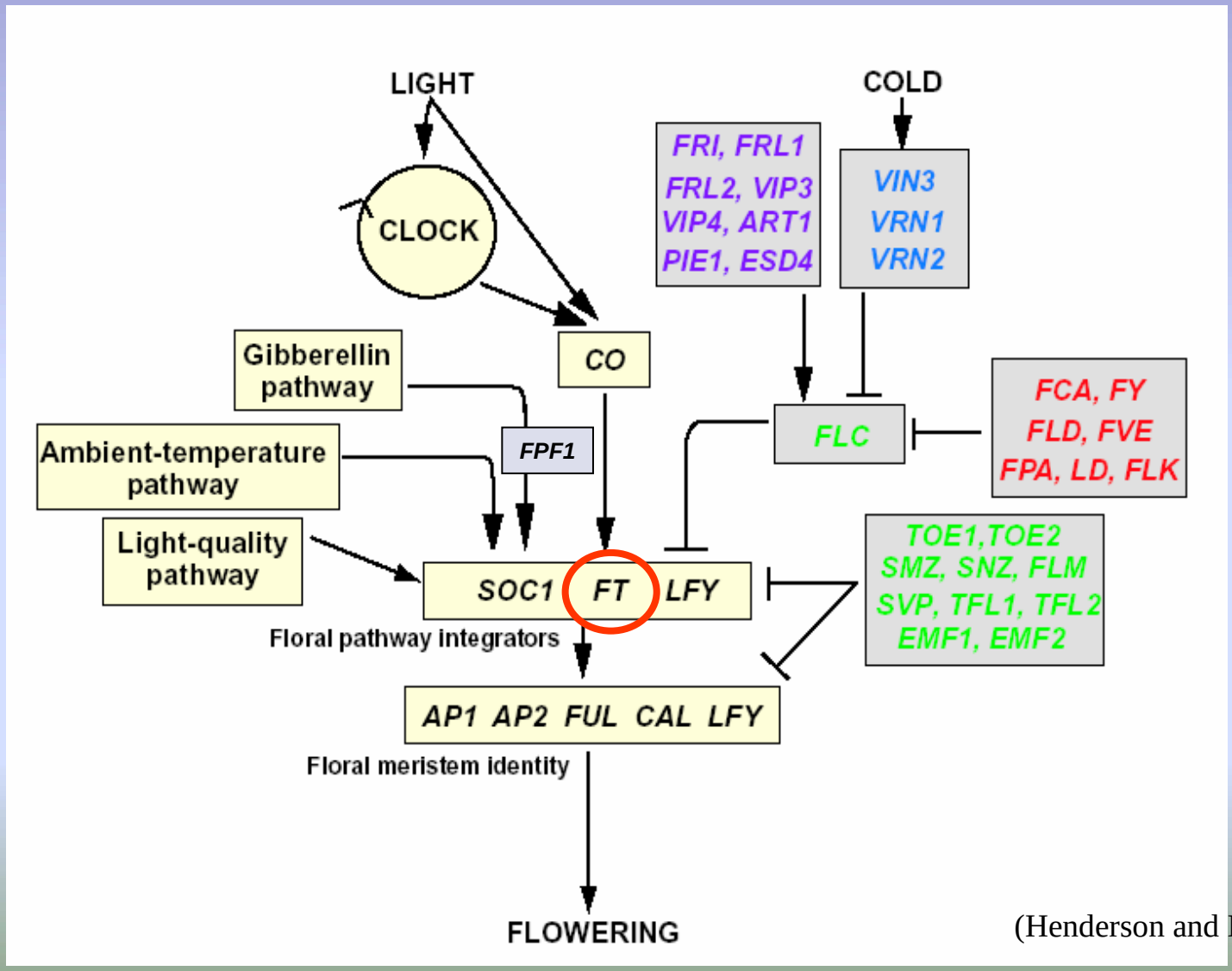
Are 35::*Leafy* flowers fertil?

- Yes, Flowers form pollen in greenhouse

Leafy transgenics:



The flowering pathway in *Arabidopsis*



(Henderson and Dean 2004)

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35S::*FT* und Hsp::*FT*

In vitro, male

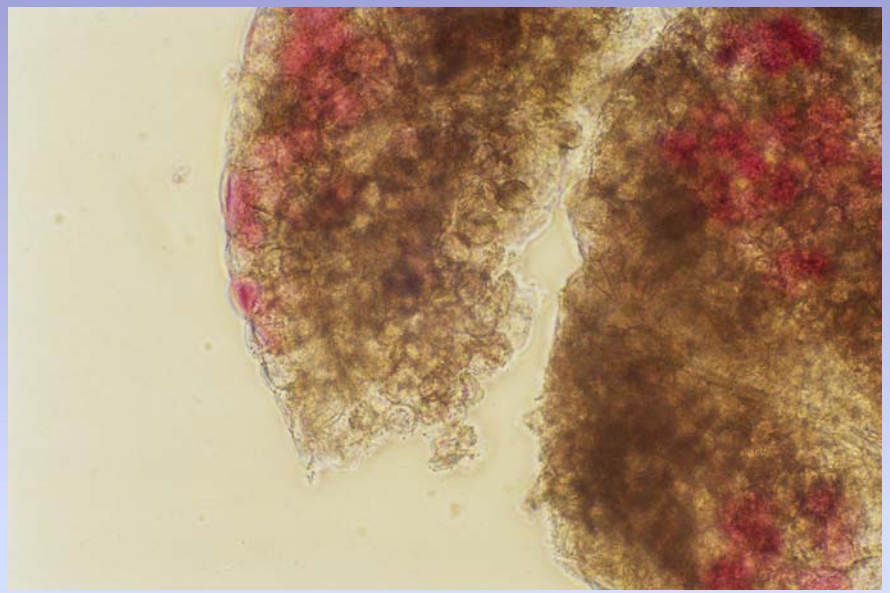
In soil, male

(FT gene kindly provided by O. Nilsson)

35S::*FT* und Hsp::*FT*

Fertility ?

Greenhouse



Climate chamber

YES (2007/08), but with varying amounts of pollen

(Seasonality: in winter/spring yes, in summer/autumn no)

In 35S::*FT* transgenics flowering can not be controlled

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Transgenic approach

Gene constructs	Early flowering in aspen
35S::*Leafy (Arab.)* (Detlef Weigel, Max Planck Institute, Tübingen, Deutschland)	Yes
35S::*FT (Arab.)* (Ove Nilsson, Swedish University of Agricultural Sciences, Umeå, Schweden)	Yes
HSP::*FT (Arab.)* (Ove Nilsson, Swedish University of Agricultural Sciences, Umeå, Schweden)	Yes
35S::*rolC (A.rhiz.)* (Angelo Spena, Max Planck Institute, Cologne, Deutschland)	Yes
35S::*rolD (A. rhiz.)* (Maurizio Trovato, Universita di Roma, Rome, Italien.)	So far no
35S::*BpMADS4 (Birch)* (Toumas Sopanen, University of Joensuu, Departament of Biology, Joensuu, Finland)	So far no (disturbed senescence and dormancy)
35S::*Soc (Arabidopsis)* (Siegbert Melzer, Ghent University, Belgium)	So far no
35S::*Ful (Sinapis)* (Siegbert Melzer, Ghent University, Belgium)	So far no
35S::*FPF1 (Arabidopsis)* (Siegbert Melzer, Ghent University, Belgium)	So far no

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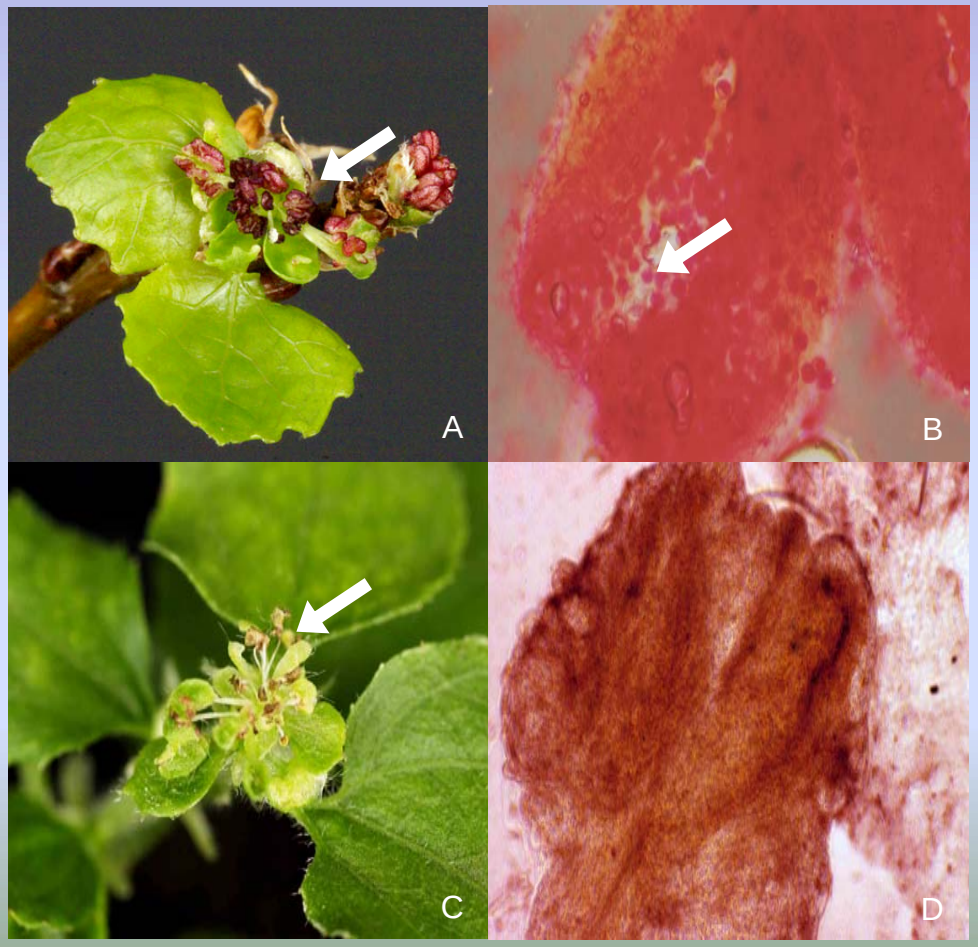
Induction of sterility

- It was possible to test sterility constructs



35S::*Leafy*

35S::*Leafy*
+
CGPDH-C::*Vst1*

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Transformation of early-flowering poplar lines with sterility constructs

Constructs	Hygromycin Resistance	PCR +	Southern +	Early flowering	Pollen in flower
TA29::*Barnase* (*5)	19	19	8 (3)**	Yes	No anther
TA29::Vst1(*5)	30	10	12 (7)**	Yes	Yes
CGPDHC::*Barnase* (*5)	15	9	10 (5)**	Yes	No anther
CGPDHC::*Vst1* (*5)	7	7	4 (3)**	Yes	No
MALE1::*STS* (*6)	25	20	n.t.	Yes	Yes
TA29::*AS-PDH-E1&1* (*7)	15	5	n.t.	Yes	No

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Summary

- Biosafety research includes a number of different aspects
- Avoidance of VGT is important, strategies have to be developed (containment)
- One possibility is to transfer “sterility-genes”
- To test these systems in trees within a few months after transformation early flowering has to be induced
- Few genes have been identified inducing successfully early flowering in poplar
- Few putative sterile lines have been produced, proof of long term sterility is presently under investigation

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Many thanks for your

attention !