

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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Flower bud formation in the field (1996-2001)

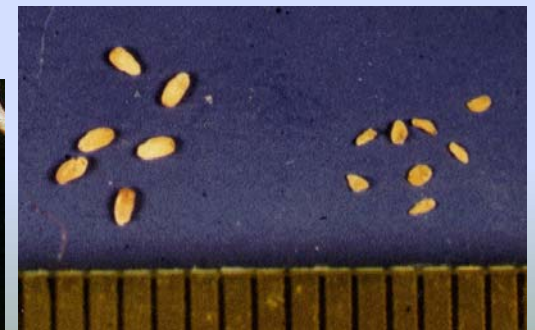
1998: 1 single *35S-rolC* tree
with two flower buds
(Age: 3)

1999: No flower buds

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

2001: No flower buds

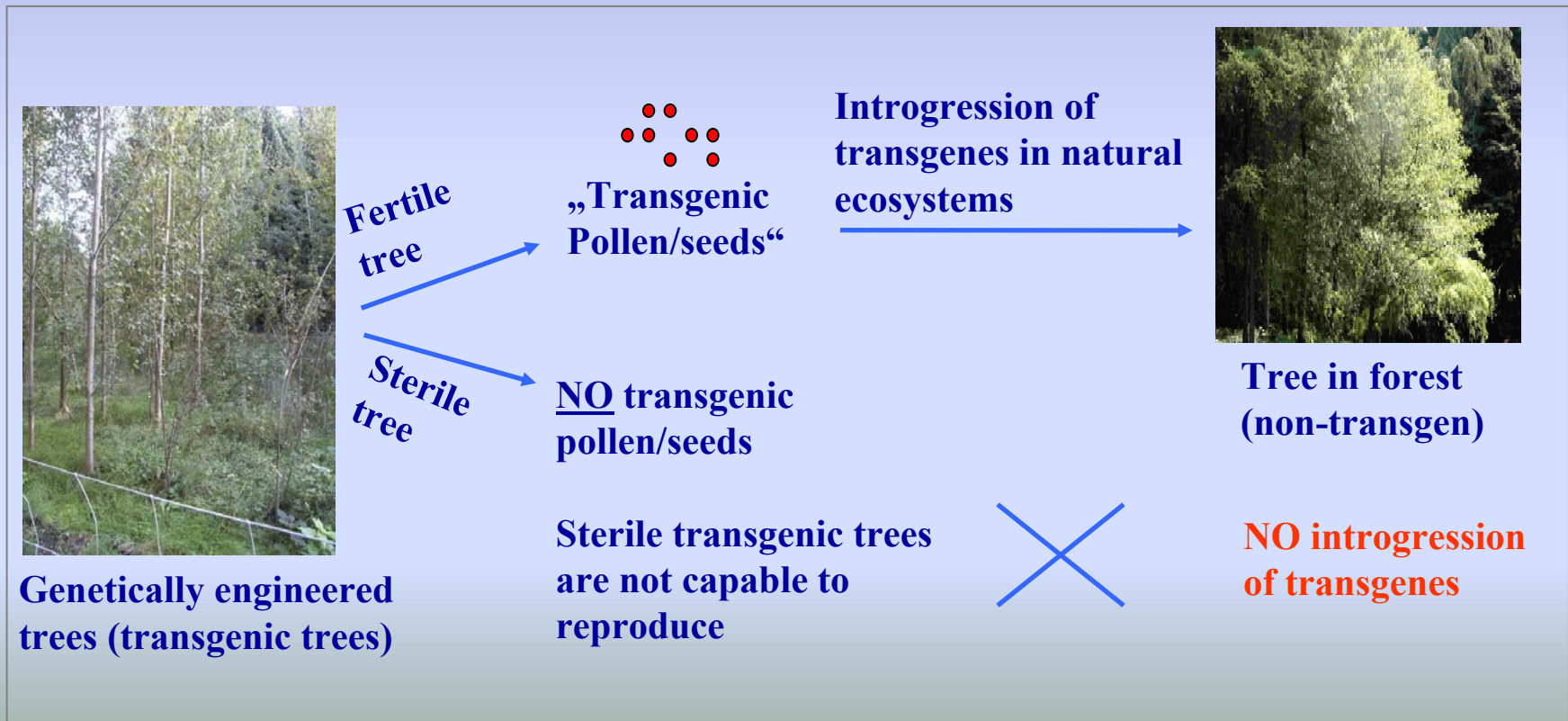
- ➔ Construct specific
- + Development of transgenic flower was followed in greenhouse
 - + Cross-pollination yielded 508 very small seeds
 - + No germination



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

Induction of sterility

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



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

Sterility gene constructs

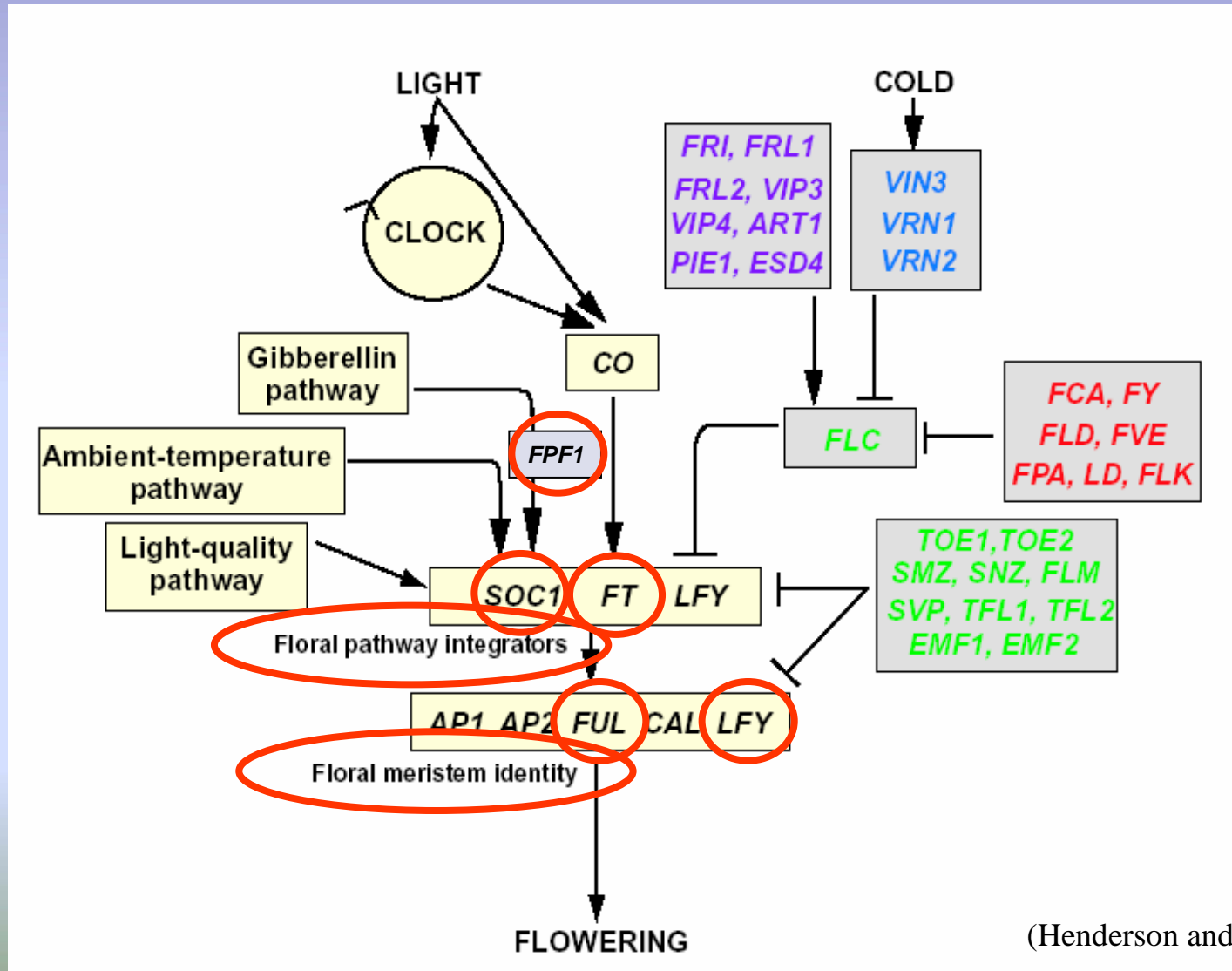
- TA29::*Barnase* (Debener, 2002)
 - TA29::*Vst1* (Debener, 2002)
 - CIGPDHC::*Barnase* (Debener, 2002)
 - CIGPDHC::*Vst1* (Debener, 2002)
 - MALE1::*STS* (Walter 2006)
 - TA29::*AS-PDH_E1alpha-1* (Kubo 2006)
- } (♂)
-
- BpMADS1::*Barnase* (Lemmetyinen *et al.*, 2001) (♂ / ♀)

Induction of early flowering in aspen

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

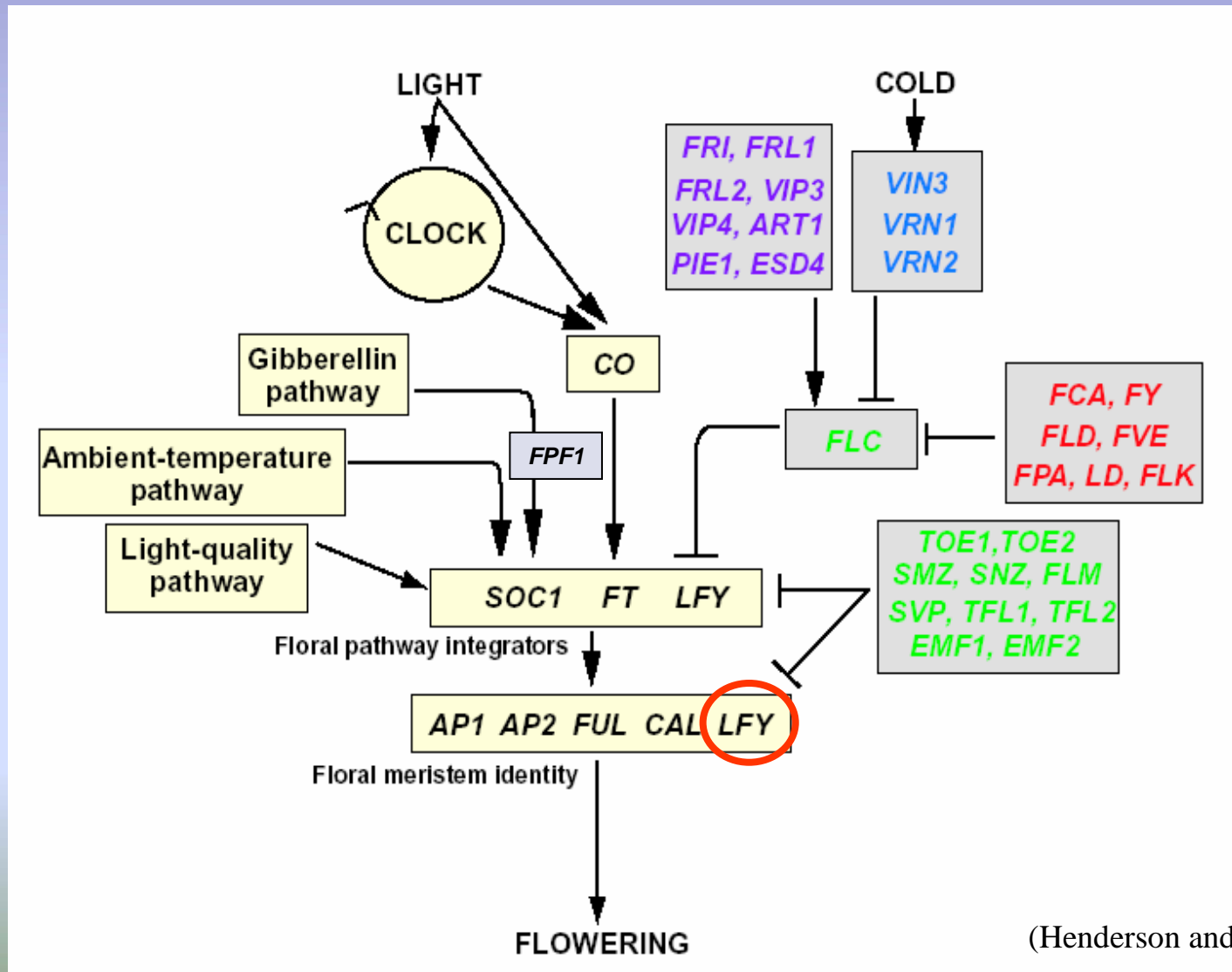
- Growth inhibitors
 - Daminozide. Flowering in Aspen after 2 years (Li pers. comm.)
 - Paclobutrazol **Not successful**
- Genetic transformation
 - *BpMADS4*, *Betula pendula* Roth. (Elo *et al.*, 2001)
 - *Rol C/Rol D*, *Agrobacterium rhizogenes* (White, 1985), in aspen (Fladung *et al.*, 2003)
 - *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*



35S::*Leafy*



Clone T89,
male



Clone Esch 5,
female

“Dwarf”-phenotype,
Single flowers instead of catkins

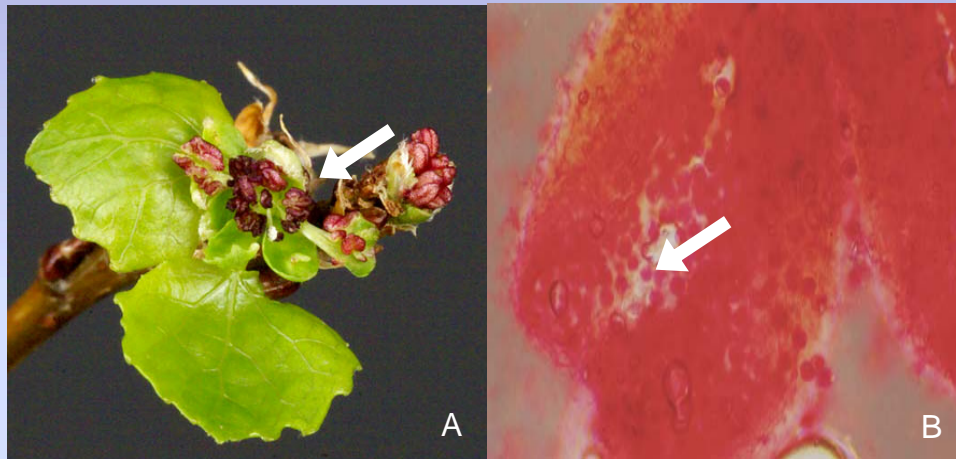
Weigel and Nilsson 1995
Hönnicka and Fladung, 2006

(Leafy was kindly provided by D. Weigel)

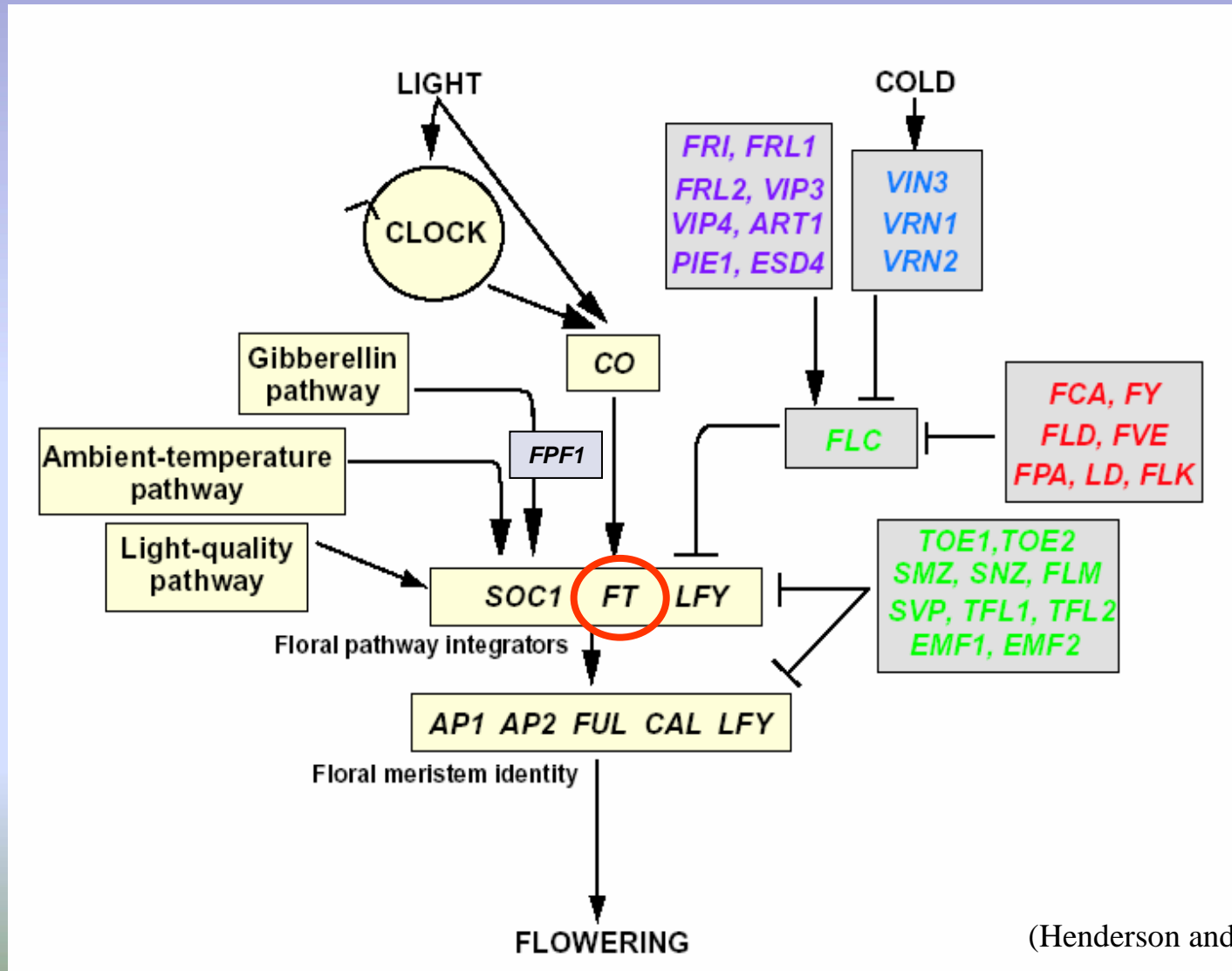
Are 35::Leafy flowers fertile?

- Yes, Flowers form pollen in greenhouse

Leafy
transgenics:

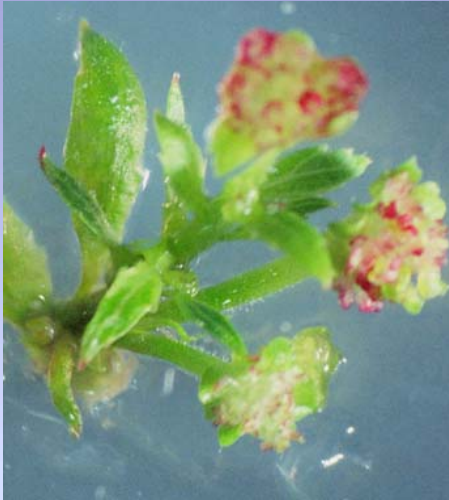


The flowering pathway in *Arabidopsis*



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

In vitro, male



In soil, male



(FT gene kindly provided by O. Nilsson)

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

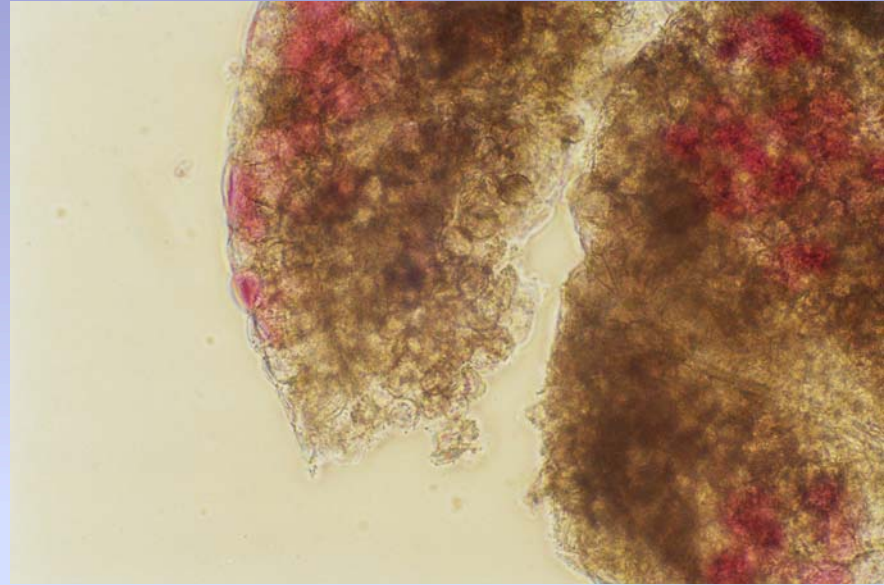
Fertility ?

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

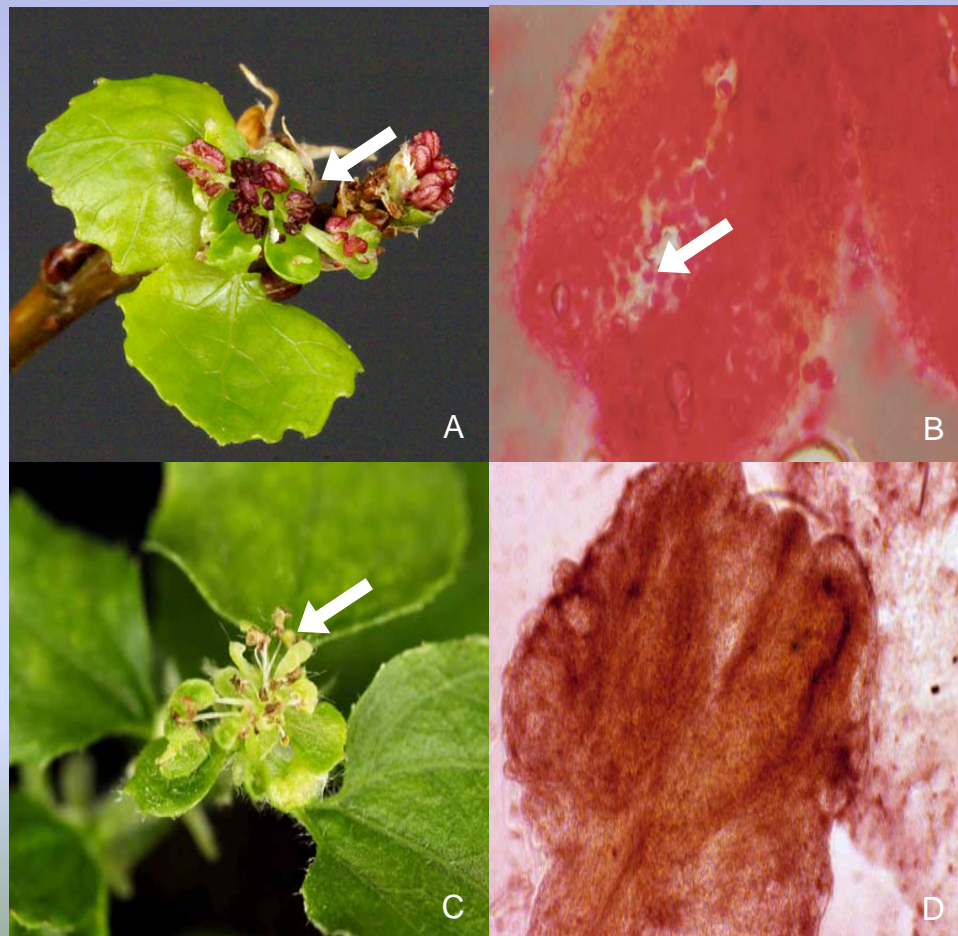


Transgenic approach

<u>Gene constructs</u>	<u>Early flowering in aspen</u>
35S:: <i>Leafy</i> (<i>Arab.</i>) <small>(Detlef Weigel, Max Planck Institute, Tübingen, Deutschland)</small>	Yes
35S:: <i>FT</i> (<i>Arab.</i>) <small>(Ove Nilsson, Swedish University of Agricultural Sciences, Umeå, Schweden)</small>	Yes
HSP:: <i>FT</i> (<i>Arab.</i>) <small>(Ove Nilsson, Swedish University of Agricultural Sciences, Umeå, Schweden)</small>	Yes
35S:: <i>rolC</i> (<i>A. rhiz.</i>) <small>(Angelo Spena, Max Planck Institute, Cologne, Deutschland)</small>	Yes
35S:: <i>rolD</i> (<i>A. rhiz.</i>) <small>(Maurizio Trovato, Universita di Roma, Rome, Italien.)</small>	So far no
35S:: <i>BpMADS4</i> (<i>Birch</i>) <small>(Toumas Sopanen, University of Joensuu, Department of Biology, Joensuu, Finland)</small>	So far no (disturbed senescence and dormancy)
35S:: <i>Soc</i> (<i>Arabidopsis</i>) <small>(Siegbert Melzer, Ghent University, Belgium)</small>	So far no
35S:: <i>Ful</i> (<i>Sinapis</i>) <small>(Siegbert Melzer, Ghent University, Belgium)</small>	So far no
35S:: <i>FPP1</i> (<i>Arabidopsis</i>) <small>(Siegbert Melzer, Ghent University, Belgium)</small>	So far no

Induction of sterility

- It was possible to test sterility constructs



35S::*Leafy*

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

Transformation of early-flowering poplar lines with sterility constructs

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

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

Many thanks to

- **Hans Hönicka**
- **Sandeep Kumar**
- **Michael Kaldorf**
- **Uwe Nehls**
- **Werner Gieffers**
- **Torsten Markussen**
- **Frank Deutsch**
- **Hans Muhs**
- **Technical co-workers**
 - # **Susanne Bein**
 - # **Olaf Nowitzki**
 - # **Doris Ebbinghaus**
 - # **Anke Schellhorn**
- **Greenhouse co-workers**

And:

**Raj Ahuja (USDA,
Placerville, USA)**

**Birgit Ziegenhagen
(Marburg, Germany)**

Angelo Spina, Italy

**Thomas Schmülling,
Berlin, Germany**

**Many thanks for your
attention !**