



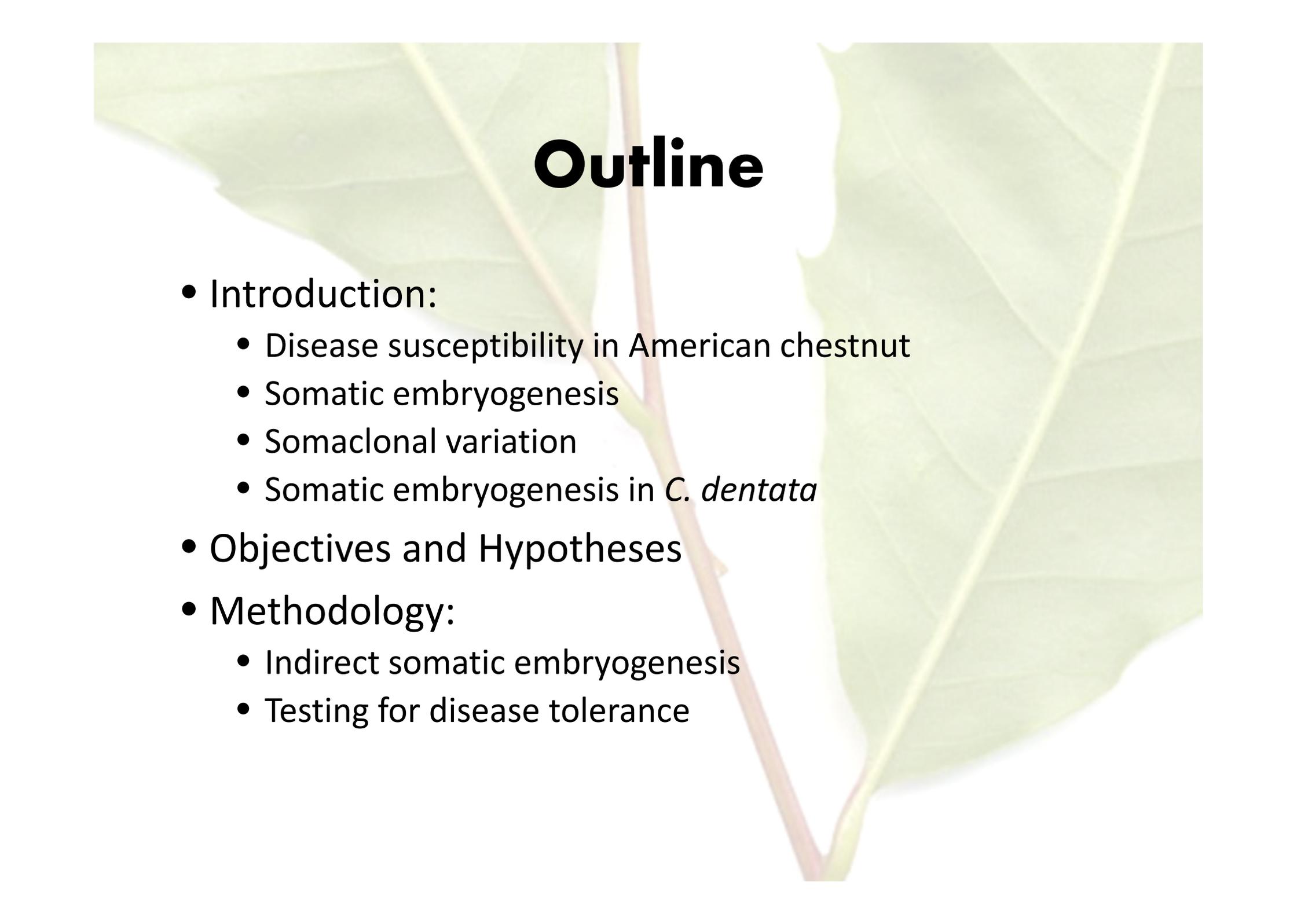
Somaclonal Variation as a Breeding Tool in American Chestnut

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Outline

- Introduction:
 - Disease susceptibility in American chestnut
 - Somatic embryogenesis
 - Somaclonal variation
 - Somatic embryogenesis in *C. dentata*
- Objectives and Hypotheses
- Methodology:
 - Indirect somatic embryogenesis
 - Testing for disease tolerance

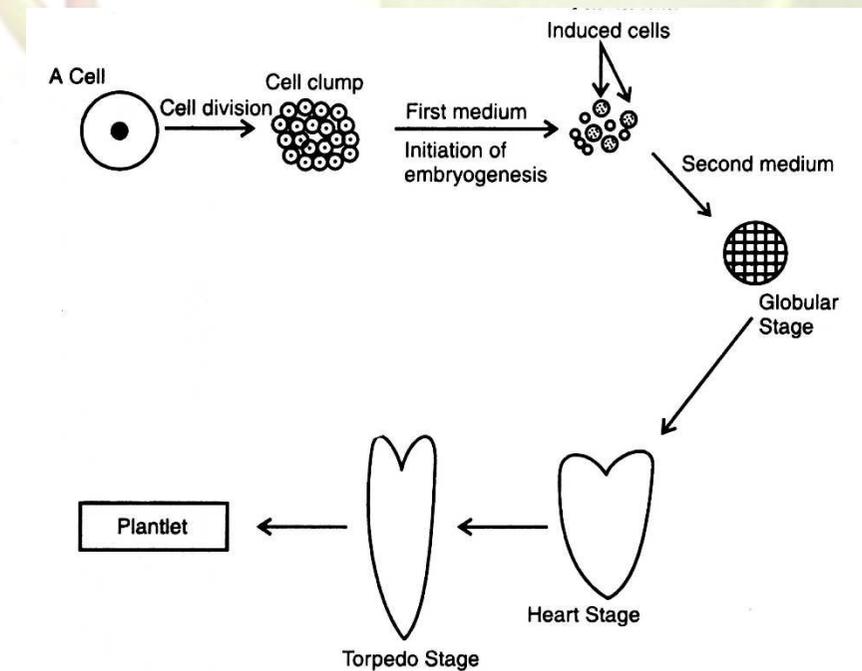
Disease Susceptibility

- Chestnut blight
 - *Cryphonectria parasitica*
- Ink disease
 - *Phytophthora* sp.
 - Principally *P. cambivora* and *P. cinnamomi*
- *C. dentata* is highly susceptible to both diseases.
 - Asian *Castanea* spp. are tolerant
 - Disease tolerance in *C. dentata* pursued by backcross breeding to Asian *Castanea* spp.

(Burnham 1988; Jeffers 2009)

Somatic Embryogenesis

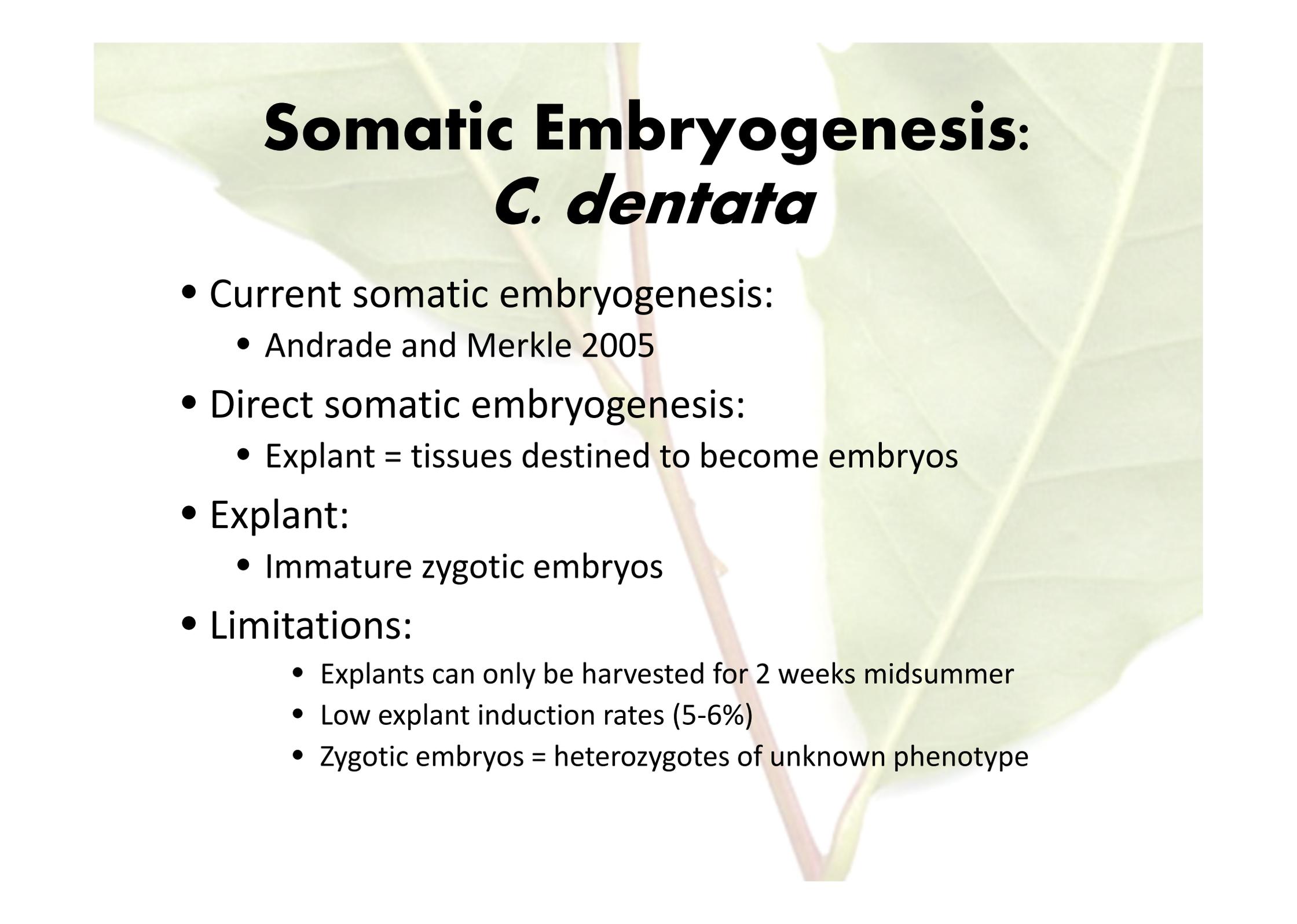
- Small explants of plant tissues → meristematic tissues → embryos → complete plantlets.
- “Somatic plantlets”



Somaclonal Variation

- Product of somatic embryogenesis.
- Regenerated plantlets have a wide range of phenotypic variations.
 - Known as somaclonal variants.
 - Includes chromosome alterations, epigenetic changes, one time deletions or insertions, transposable elements, etc.
- Disease tolerance is commonly affected in somaclonal variants.
- It's a numbers game:
 - Undirected changes

(Scowcroft 1985; Wang and Wang 2012)



Somatic Embryogenesis: *C. dentata*

- Current somatic embryogenesis:
 - Andrade and Merkle 2005
- Direct somatic embryogenesis:
 - Explant = tissues destined to become embryos
- Explant:
 - Immature zygotic embryos
- Limitations:
 - Explants can only be harvested for 2 weeks midsummer
 - Low explant induction rates (5-6%)
 - Zygotic embryos = heterozygotes of unknown phenotype

Somatic Embryogenesis: *C. dentata*

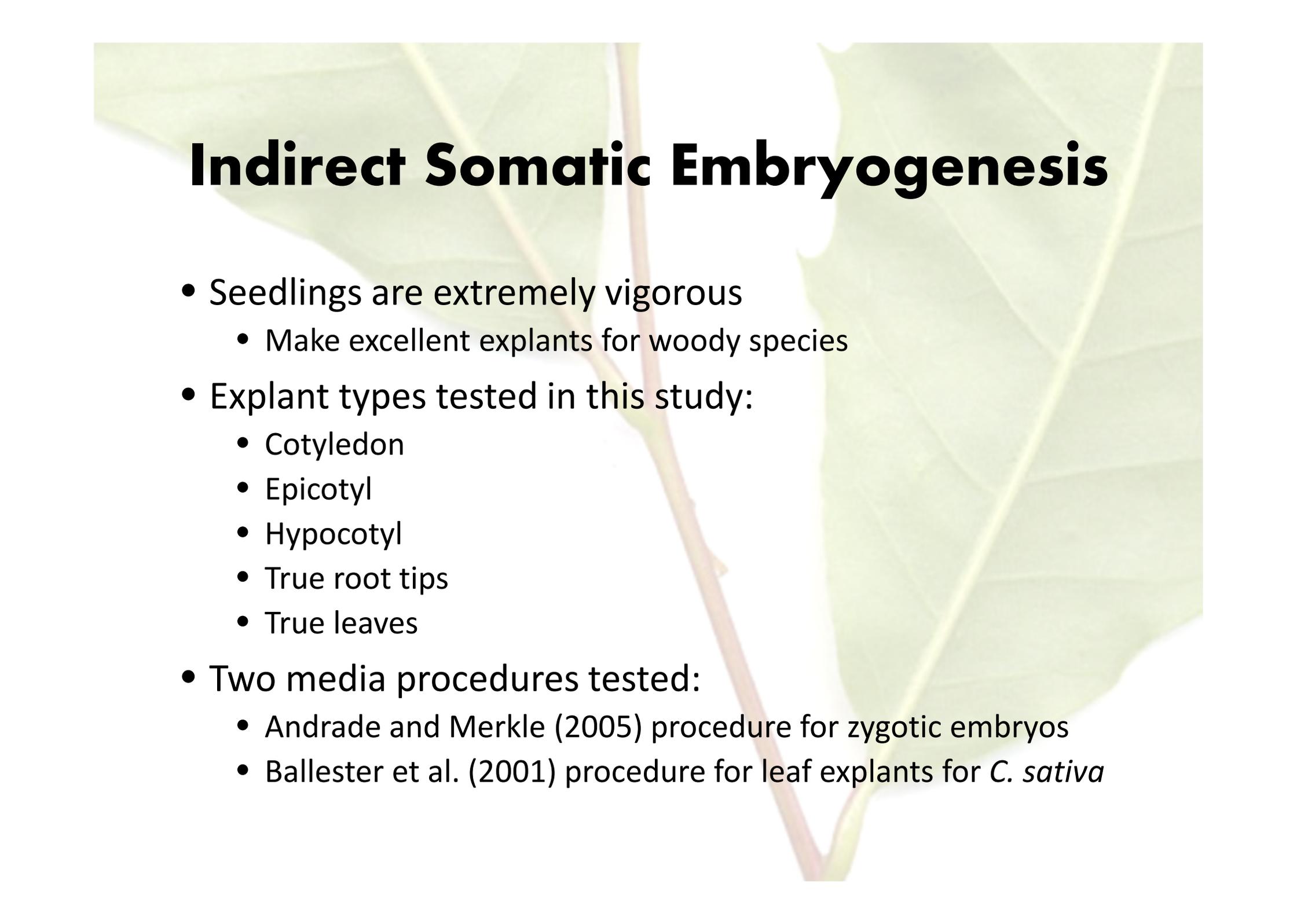
- Indirect somatic embryogenesis:
 - Somatic embryogenesis from vegetative tissues
 - Leaves, stems, etc.
 - Possible with *C. sativa*
 - Never investigated with *C. dentata*
- Benefits:
 - Year-round somatic embryogenesis for *C. dentata*
 - Possible higher induction rates (29% in *C. sativa*)
 - Vegetative tissues = possible to use mature trees as explants
 - Somatic embryogenesis with elite trees

(Ballester et al. 2001; Corredoira et al. 2005)

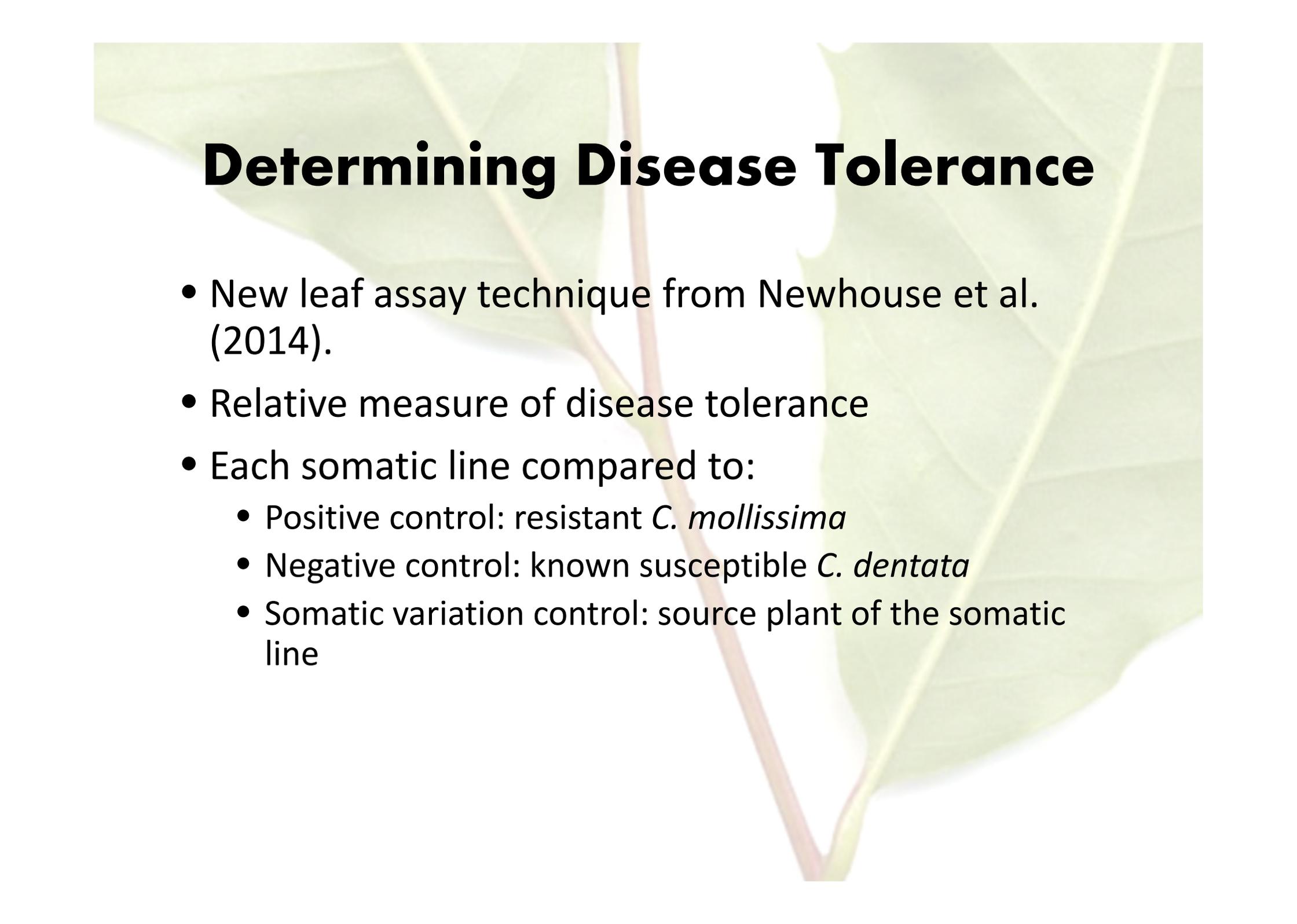
Objectives and Hypotheses

- Objectives:
 - To develop a methodology for indirect somatic embryogenesis of *C. dentata*.
 - To increase through somaclonal variation, the disease tolerance of *C. dentata* to chestnut blight and ink disease.
- Hypotheses:
 - Ideal explants will be identified for indirect somatic embryogenesis in *C. dentata*.
 - Somaclonal variants with increased disease tolerance will be generated.

Indirect Somatic Embryogenesis

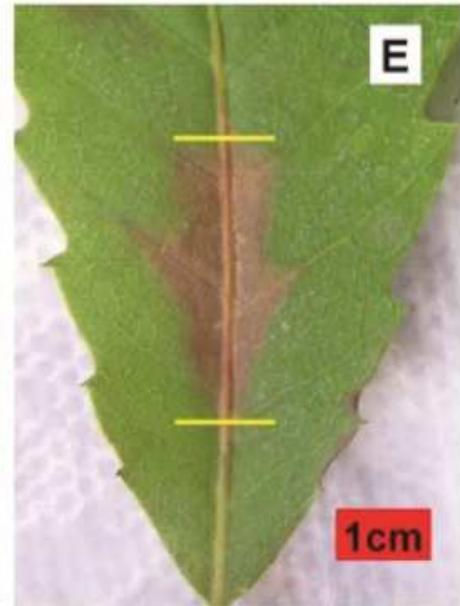
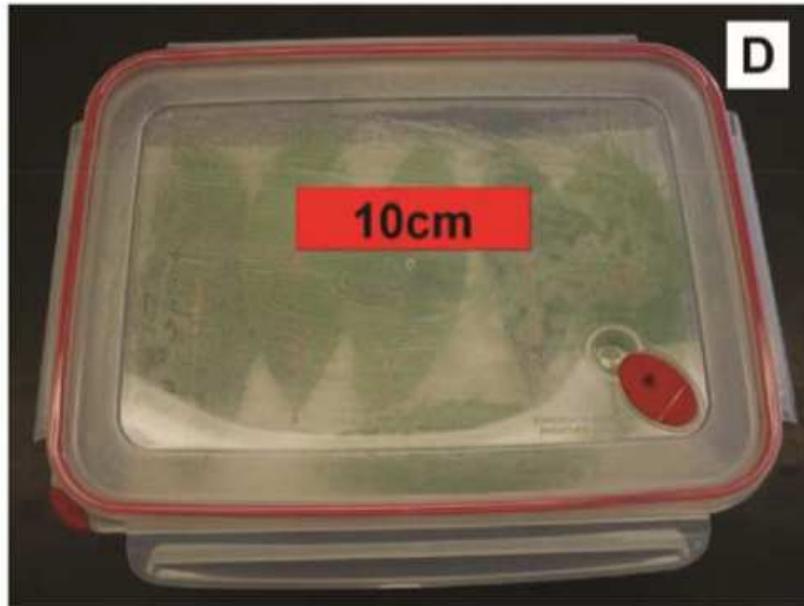
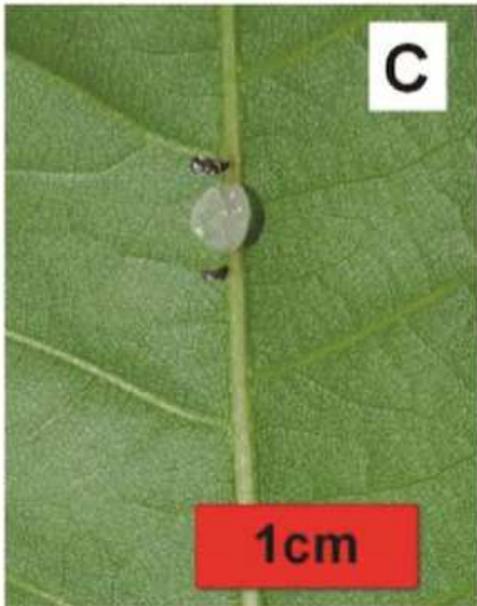
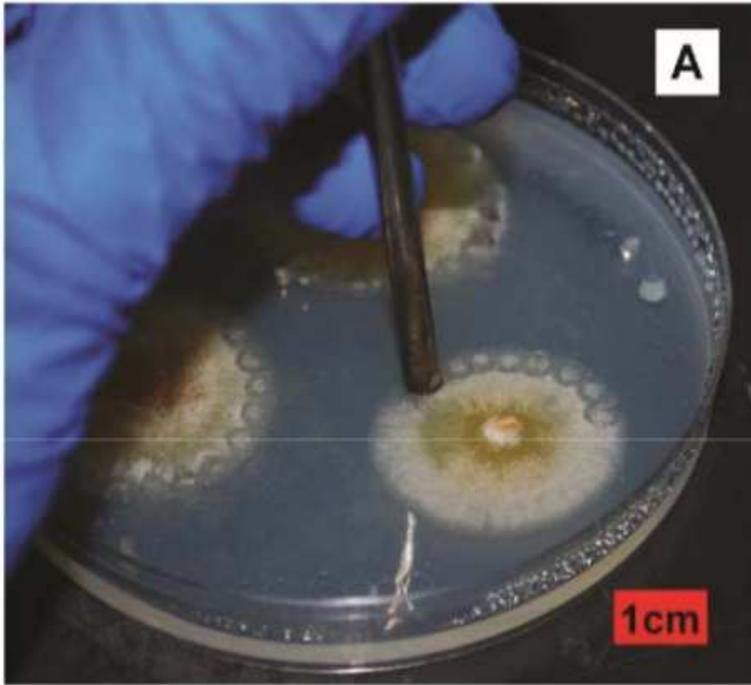


- Seedlings are extremely vigorous
 - Make excellent explants for woody species
- Explant types tested in this study:
 - Cotyledon
 - Epicotyl
 - Hypocotyl
 - True root tips
 - True leaves
- Two media procedures tested:
 - Andrade and Merkle (2005) procedure for zygotic embryos
 - Ballester et al. (2001) procedure for leaf explants for *C. sativa*



Determining Disease Tolerance

- New leaf assay technique from Newhouse et al. (2014).
- Relative measure of disease tolerance
- Each somatic line compared to:
 - Positive control: resistant *C. mollissima*
 - Negative control: known susceptible *C. dentata*
 - Somatic variation control: source plant of the somatic line



(Newhouse et al. 2014)

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*Fonds de recherche
sur la nature
et les technologies*

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Questions?

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