# RNAi suppression of two *AGAMOUS* homologs in sweetgum (*Liquidambar*) impairs male and female reproductive development under field conditions

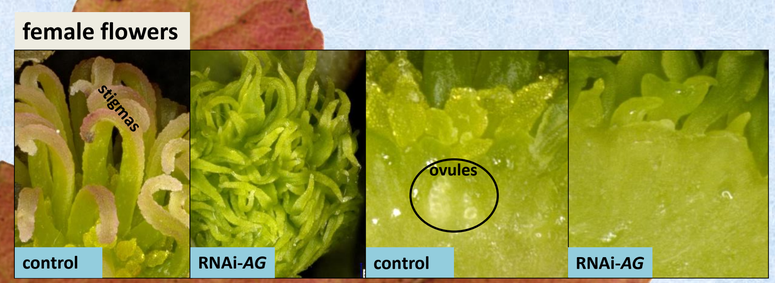
Amy L. Klocko[1], Elizabeth Etherington[1], Kori Ault[1], Amy M. Brunner[1], Thomas Lockwood[1], Nichole Covarrubias[1], Cathleen Ma[1], Manzhu Bao[1], and Steven H. Strauss[1]
[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA Amy.Klocko@oregonstate.edu

Hipster forest scientist & lead author

## Abstract

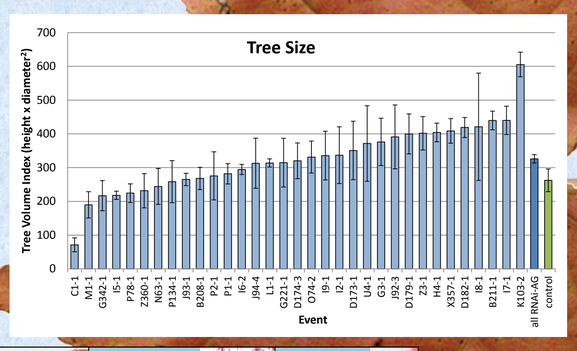
Many woody ornamentals are grown as exotics outside of their native range, where they have the potential to become invasive. As street trees, their fruits often create sanitation problems and their pollen exacerbates allergies. A reduction in fertility of these ornamental plants would help to mitigate these problems. We used RNA interference (RNAi) to suppress the expression of a key floral development gene, *AGAMOUS* (*AG*), in sweetgum trees. Sweetgum is a popular ornamental tree in the United States known for its vibrant fall foliage, however, it is also known for producing copious amounts of hard, rough fruits termed gum-balls or burr-balls. Shed fruits are long-lasting and are an unwelcome nuisance on streets, roofs, and often clog gutters. We used *Agrobacterium* to transform sweetgum variety 'Worplesdon' with a double-stranded RNAi-inducing transgene that targets the two distinct *AG* orthologs in sweetgum. A total of 33 independent transgenic events, plus 12 non-transgenic controls, were planted in the field in 2006. RNAi-*AG* sweetgum trees maintained normal growth and vivid fall coloration during 9 years of study. We found that 8 events had highly modified floral morphology, failed to produce seeds or pollen, and had anthers and carpels that were converted to flat leaf-like structures . The female flowers from these events developed into dry papery fruits lacking seeds. Traits were stable across multiple growing seasons. RNAi of *AG* is highly effective at modifying fertility and burr-ball development.

## RNAi-*AG* flowers lacked stigmas, ovules or anthers

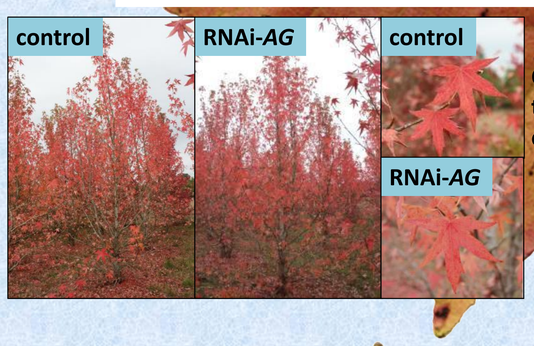



The female portions of control flowers were covered in numerous pink stigmas. By contrast, RNAi-*AG* flowers had smooth green leaf-like projections. Sectioned control flowers had ovules, which were absent from RNAi-*AG* flowers. Male control flowers had well-formed anthers while RNAi-*AG* flowers had flat leaf-like structures.

## RNAi-*AG* sweetgum trees grew well and maintained vivid fall foliage coloration

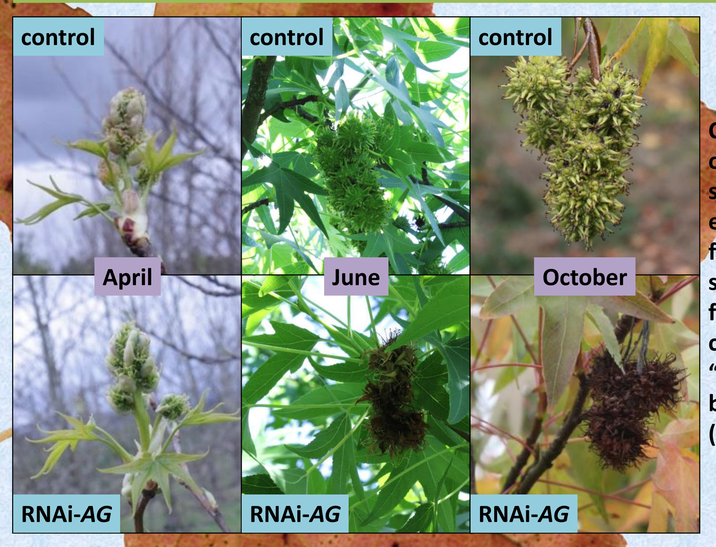



Measurements of tree growth showed that, on average, RNAi-*AG* events grew as well as control trees. Means given at right. Bars show growth by insertion event. Error bars show one standard error of the mean.




Control and RNAi-*AG* trees had brightly colored fall leaves.

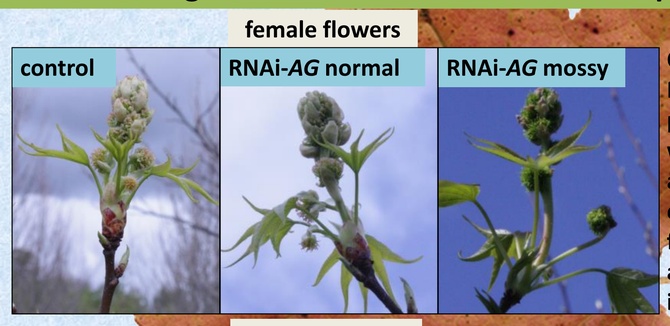
## Replicated flowers matured into brown papery fruits




Control flowers opened in spring (April), expanded into fruits over the summer (June) and formed characteristic "spikey" green balls by fall (October).

RNAi-*AG* trees formed replicated flowers in spring (April), formed fruits which were brown by summer (June) and remained so through fall (October).

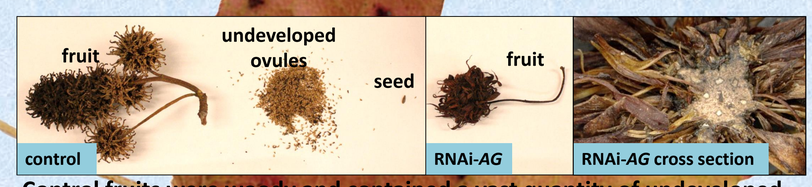
## RNAi of sweetgum *AG* resulted in "moss-like" replicated female flowers




Control trees and most RNAi-*AG* events produced female flowers with similar overall appearances. However, 8 events had female flowers that were green and mossy (fuzzy green) in appearance.

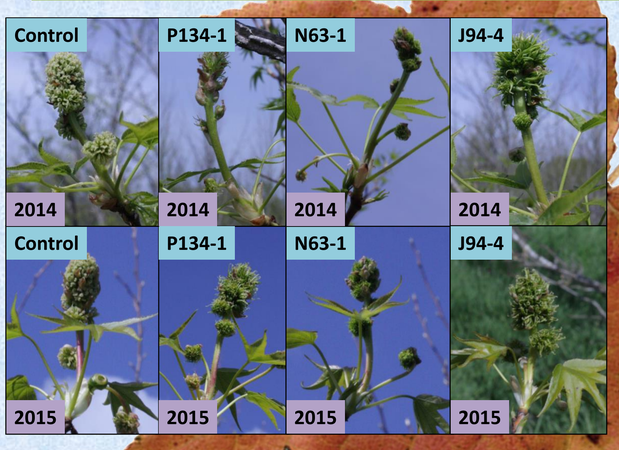
The male portions of bisexual flowers did not have obvious differences at the macroscopic level.

## Fruits from replicated RNAi-*AG* flowers were small and seedless

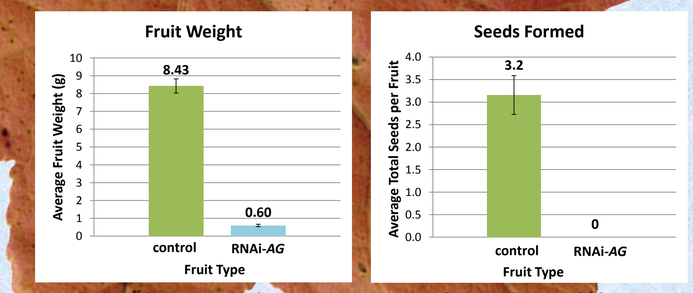



Control fruits were woody and contained a vast quantity of undeveloped ovules and a small number of well-formed seeds. RNAi-*AG* fruits were papery and had a small solid core that lacked both undeveloped ovules or seeds.

## Floral phenotypes were stable across growing seasons




Three events with replicated flowers bloomed in both 2014 and 2015, with similar floral phenotypes in both growing seasons.




Analysis of 32 control and 65 RNAi-*AG* fruits showed that RNAi-*AG* fruits were significantly smaller than control fruits and did not contain any seeds.

## Conclusions

* RNAi of sweetgum *AG* led to replicated flowers
* RNAi-*AG* flowers had strongly reduced female fertility
* RNAi-*AG* fruits were small and seedless
* RNAi-*AG* trees maintained attractive fall foliage and normal vegetative growth
* *AG* suppression or mutation was effective for reducing the potential for invasiveness, and the messiness, of sweetgum trees

## Acknowledgments

We thank Arborgen for providing *AG* gene sequence data, and for help with transformation, rooting and propagation. This project was funded by the J. Frank Schmidt Family Charitable Foundation who also provided plantation border trees, and by the USDA Nursery Crops Nursery and Floriculture Initiative (1230-21000-051-06S).