Notes S3 – Research Needs

The goal of suggested research is to improve genetic methods, and to better understand the biodiversity impacts and social dimensions, of reproductively modified forest plantations

Genetic science and technology

1. Studies to improve methods for genetic transformation (gene transfer and plant regeneration) in diverse plantation genotypes and species. Current methods are slow, costly, and can cause significant somaclonal and insertion (pleiotropic) effects.
2. Building on genetic engineering advances, studies to improve methods for gene editing and directed gene insertion using gene editing methods. This includes methods for somatic removal of gene editing machinery, and possibly selectable markers, to improve stability and public/regulatory acceptance.
3. Studies of reproductive gene expression during floral and vegetative growth in target plantation genera. In depth transcriptomes for specific floral and vegetative tissues and cell types to build on growing genome and transcriptome databases.
4. Studies of reproductive gene and vegetative function of target sterility genes by RNAi, gene editing, and/or genome wide association studies (GWAS) through to field trials.
5. Development of gene editing methods that can produce stable male, female, and bisexual sterility (without negative pleiotropic effects) in field trials.
6. Development of gene editing methods that can produce stable male, female, and bisexual sterility (without negative pleiotropic effects) but allow for the production of reproductive structures that provide normal nutrition to dependent organisms, in field trials. The expected strategy would be to use gene editing to target late-acting genes during pollen germination/fertilization and embryo development.

Biodiversity

1. Stand level research on the impact of pollen, fruit and/or seed reduction on biodiversity. The goal of this research is to find out what organisms feed on reproductive structures, and to what extent they are locally dependent on them. The appropriate comparator for such research may be:
    a. GE- and non-GE plantations with unimpeded reproduction,
    b. Stands of similar structure but prior to and after the onset of reproduction,
    c. Mast vs. non-mast years, and
    d. Stands with differing genders in dioecious species like poplars.
2. Landscape research to scale-up the stand level studies cited above, but with attention to the degree to which (and whether) GE plantations (a) act as ecological traps, (b) are permeable to animal movement, and (c) when exotic species function as 'habitat' for native species.
3. Landscape-scale research on the proportion of the landscape that can occur in reproduction-modified GE plantations of various types before native species that use reproductive resources are expected to decline. Given that it is very unlikely that GE plantations will occupy large portions of any landscapes in the near future, research will likely take the form of simulation models based on known site-level behavior and demographic data for select indicator species (e.g., MacLean *et al.*, 2009). This includes continued research on what proportion of a landscape can be 'non-habitat' (reproductive resource free: pollen, fruit, and/or seed) before one would expect declines in species that feed on them.
4. Studies of the degree to which a suite of potentially sensitive taxa move through, "select" and use reproduction-modified GE stands. What cues are used? What are the risks of ecological traps?
5. Formal trade-off modeling for biodiversity at local and landscape scales as a function of different GE management strategies (proportions and spatial configurations of GE at the

landscape scale, proportions of protected areas and extensively managed native forests) (e.g., MacLean *et al.*, 2009).

6. Biodiversity surveys in plantations in general; understanding biodiversity involved in different flowering stages by explicit inclusion of reproductive tissues and times of flower, fruit, and seed production during annual cycles and stand development.
7. Estimates of the significance of reproduction to energy and nutrient cycling budgets, both in forests and associated aquatic systems.
8. Studies of the extent of seed predation in small- and/or wind-pollinated seeds, especially for insect/oviposition predation.
9. Studies of organisms that utilize multiple components of trees, including, but not limited to flowers? How will GE floral modifications impact those non-specialist organisms?
10. Studies of stand and landscape options for mitigation, such as use of borders, intensively flowering tree species in refugia, thinning, and density management, and provision of floral rich corridors.
11. Studies of whether reproductive modification has pleiotropic effects of significance to biodiversity, such as changes to normal secondary compound concentrations? Are these changes within or beyond the range seen within the species or in alternative plantation species?
12. Studies of the importance of wind-borne pollen as a source of organic cloud condensation nuclei relative to other organic and inorganic sources (e.g., dust, salt cyrstals)? At what temporal and spatial scale must pollen production be modified to have an impact? This will require both further environmental sampling, laboratory experiments, and environmental modeling.

Social dimensions

1. Surveys and other methods to investigate the extent that potential benefits of reproductive modification (i.e., containment) are perceived by different stakeholders (e.g., public, experts) as useful for mitigating gene flow from plantations to wild forests. Gene flow is a major concern voiced by opponents of genetic modification in forestry and agriculture, but the extent of public support for genetic methods for prevention are unclear, particular in light of the widespread concerns over similar "Terminator" technologies in agriculture (Lombardo, 2014).
2. Surveys and experiments to assess the relative influence of familiarity and knowledge in relation to acceptance of flowering modification. By associating GE methods with common features (e.g., human birth control, bread wheat as a source of genes, seedless grapefruits and bananas as kinds of plant reproductive modification), does this change acceptability? Are changes in acceptance based on heuristics or rational cognitive processes?
3. Almost all studies of perceptions associated with genetic modification in general, and forestry in particular, have been single cross-sectional studies at one point in time. Longitudinal research and panel design studies are needed to examine how perceptions may develop and change over time, and drivers of these changes.
4. Acceptance of biotechnological approaches such as flowering modification does not exist in isolation; it is influenced by many cognitive and social factors, and tradeoffs among these factors. Survey and experimental research is needed on tradeoffs and factors influencing these tradeoffs when making decisions about biotechnologies.
5. Survey and experimental research should assess whether trans-media risk/benefit communication frameworks (embraces multiple media outlets, including social media and mobile technologies), are effective means for increasing factual knowledge and reducing subjective perceived risks toward GE trees and reproductive modification.

6. Economic and cognition research on the degree to which “success” with GE reproductive modification will potentially affect uptake of GE and intensive plantation forestry in general, vs. added protection of native forests.

References

MacLean DA, Seymour RS, Montigny MK, Messier C. **2009**. Allocation of conservation efforts over the landscape: the TRIAD approach. Conservation Biology. Setting Conservation Targets for Managed Forest Landscapes. Cambridge University Press.

Lombardo L. **2014**. Genetic use restriction technologies: a review. *Plant Biotechnology Journal* **12**: 995–1005.