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Safe Importation of *Eucalyptus* Germplasm into Hawaii

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Background:

Recently, interest has been building for establishing or expanding *Eucalyptus* plantations in Hawaii. Such plantations could be useful for the production of fiber, construction materials, bioenergy, biochar, or other purposes. Such plantations would benefit from the importation of new *Eucalyptus* germplasm to Hawaii for tests to determine what species and clones are optimally suited for the Hawaiian sites and desired end products. A major concern is whether *Eucalyptus* germplasm can be imported safely without introducing new pathogens that could threaten other native and exotic Myrtaceous species in Hawaii. The introduction of a new, more virulent race of the eucalypt-guava-ohia- myrtle rust pathogen (*Puccinia psidii*) is a major concern; however, other unknown pathogens pose additional threats. For example, *Chrysoporthe cubensis* (Hodges et al. 1979; Gryzenhout et al. 2004), *Erytricium salmonicolor*, *Ceratocytis fimbriata* (Fig. 1), *Ralstonia solanacearum*, and leaf disease pathogens have also caused several

disease outbreaks on *Eucalyptus* plantations in Brazil (Alfenas & Zauza 2007; Alfenas et al. 2009; Alfenas et al. 2011).

Fig. 1. Ceratocystis wilt of *Eucalyptus*, caused by *Ceratocystis fimbriata*, is an example of a *Eucalyptus* disease that could be transmitted by improper transfer of eucalypt germplasm.

In Brazil, each *Eucalyptus* clone for commercial plantations is selected from thousands of clones derived from breeding programs that use diverse germplasm and interspecific and intraspecific crosses. Clones are screened for site adaptation, growth rate and form, disease resistance, and the desired commercial traits for wood (e.g., fiber length, wood density, microfibril angle, etc), and new clones are deployed each year. Although the top clones selected for deployment in Brazilian plantations depend, in large measure, on the planting site (climate, soils, etc) and desired wood, such massive screening programs are the basis of all successful *Eucalyptus* plantations in Brazil. This issue of being able to select optimal eucalypt germplasm is also relevant to Hawaii, which has distinct environmental conditions compared to Brazil, and where the optimal wood properties are likely different for the desired products in Hawaii. However, the general principal

remains applicable in that great gains in growth rates and wood properties can be achieved by selecting the optimal genetic materials.

It is well recognized that the introduction of exotic insect and pathogen pests represent a great risk to native and managed ecosystems of Hawaii, where a long-term geographic isolation from other global land masses have restricted the native insect and pathogen pests. Thus, unrestricted importation of eucalypt germplasm from numerous global regions would likely introduce a myriad of insects and pathogen pests that could impact *Eucalyptus* species, other 80 Myrtaceous species growing on these islands, or even other unrelated host plants. Thus, strict phytosanitary measures are necessary to safely import eucalypt germplasm.

It might seem that risks of introducing exotic pathogens could be minimized by using *Eucalyptus* germplasm sources, such as *E. grandis, E. saligna, E. robusta, E. urophylla, E. globulus,* and hybrids (Whitesell et al. 1992) that are already established in Hawaii. More than 90 *Eucalyptus* species from diverse provenances have been tested in Hawaii since the 1860s (Skolmen 1986). Some authors (Aradhya and Phillips 1993) have argued that eucalypt germplasm may be sufficiently variable for use in breeding/selection programs to produce optimal seedlings and/or clones that are adapted to the planting site. Although locally available genetic resources should not be neglected, new introductions of eucalypt material would likely contribute substantially to the development of optimal germplasm for use in Hawaii. Thus, interest will likely remain high to import new eucalypt germplasm onto the Hawaiian islands by any entity considering the establishment of eucalypt plantations for commercial purposes.

Risks of introducing new pathogen and insect pests that could accompany new eucalypt germplasm can be reduced by limiting the number of eucalypt germplasm sources that are introduced and the geographic locations from which they are imported. Of the 700 species of eucalypts, many do not grow at rates or have the form that would be required to make a commercial tree species, and of all the *Eucalyptus* species have been tested in Hawaii, relatively few have the growth rates, form, and wood properties that are potentially acceptable for commercial forestry (e.g., Aradhya and Phillips 1993). To help limit potential *Eucalyptus* species and sources for new trials, species climate modeling approaches, such as those proposed by Whitesell et al. (1992) could be considered. In this manner, the risk of introducing new pests and pathogens can be reduced by simply limiting imported germplasm to those eucalypt species that show the greatest promise. Additional approaches can be used to narrow eucalypt germplasm sources for genetic trials in Hawaii. One approach is to characterize the Hawaiian climate and soil conditions where commercial eucalypt plantations are planned, then the best germplasm sources for those conditions can be identified or selected among available global sources.

For example, Brazil has climatic and soils conditions that correspond somewhat closely with areas in Hawaii where commercial plantations might be considered. Furthermore, Brazil has done extensive testing to identify exotic and artificially bred eucalypt germplasm with the greatest potential for fast growth and desirable wood properties. It is estimated that millions of different eucalypt genotypes have been evaluated within well-replicated experiments in Brazil. Thus, one efficient and economical approach to obtain suitable eucalypt germplasm for Hawaii could focus on the importation of limited (e.g., 50-150) different potentially suitable eucalypt genotypes from an area in Brazil with similar climatic, edaphic, and other environmental

conditions. Such approaches to narrow numbers and sources of imported germplasm selections will also reduce the risk of introducing exotic pathogens and insects. However, significant risk of importing exotic pathogens and insects remains whenever any exotic eucalypt germplasm is imported.

The remainder of this document will focus on designing suitable phytosanitary measures and policies that should minimize risks of introducing exotic pathogens and insects on eucalypt germplasm imported to Hawaii.

Some Suggested Protocols to Import Pathogen- and Insect-free *Eucalyptus* Germplasm:

Tissue Culture

The safest method to avoid the introduction of pathogens is to establish plants in axenic (sterile) conditions, and ensure the absence of pathogens before release to the environment. This can be accomplished via surface-sterilized seeds or rooted plantlets derived from tissue cultures of axillary buds or somatic embryogenesis. Methods are available for collecting and storing field-grown materials for *Eucalyptus* micropropagation (Watt et al. 2003a). Seedlings or plantlets can be produced and grown *in vitro* to ensure that pathogens are not present. Significantly, *in vitro* growth conditions (25 °C and ~100% humidity) are ideal for the development of *Eucalyptus* rust and most other pathogens, and infections caused by any of these pathogens, if they are present, will be easy to observe (Fig. 2).

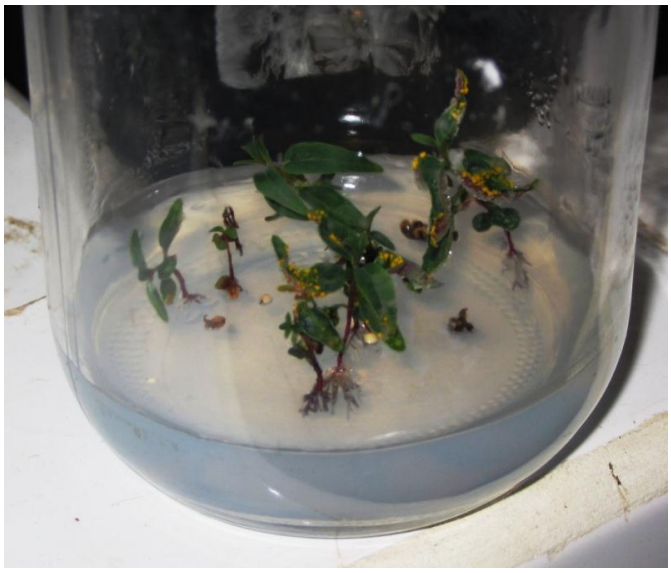

Fig. 2. Eucalypt rust appearing on *Eucalyptus* grown *in vitro*.

To be absolutely safe, a phytosanitary procedure that should determine if any rust or any other pathogens are present could begin during tissue culture, and include the following steps:

1) Transfer the *Eucalyptus* tissue culture plants (germplasm) to a plastic/glass tubes containing medium under *in vitro* (sterile) conditions;
2) Seal the container with parafilm;
3) Wait a minimum of 3 weeks; if there is no appearance of the rust/rust pustules or any other pathogens (e.g., bacteria and fungi), that plantlet can be declared "rust or pathogen free";
4) Surface sterilize the "rust free" container and put them in a hermetically sealed shipping container under a laminar hood;
5) When the shipping container arrives in Hawaii, mail or hand carry this container to the quarantine lab in Hawaii in accordance with APHIS PPQ and other state permit guidelines;

6) Place the container in a biocontainment facility at ca. 25°C for 3 weeks; if the tissue cultured *Eucalyptus* plantlets show no symptoms or signs of the rust pathogen or other microbes, they can be deemed "disease free" and released to the intended receiver.

Seed

Although tissue culture would be an ideal option, methods are not readily available to produce tissue cultures of many *Eucalyptus* spp. and clones within species (Watt et al. 2003b), including some genotypes with great potential value. Because of this, the germplasm pools for commercially valuable, tissue-cultured *Eucalyptus* clones are somewhat limited. Therefore, seeds of *Eucalyptus* spp. should also be considered for safe germplasm importation to Hawaii. *Eucalyptus* seed are different from tissue culture. *Eucalyptus* seeds can provide diverse germplasm, but the value of germplasm in seed is largely unknown until the resulting tree is grown. Furthermore, *Eucalyptus* seeds vary in their ability to be stored and remain viable; fortunately adequate methods for *Eucalyptus* seed handling are largely known and have been summarized (Krugman and Whitesell 2008).

Several surface-disinfestation techniques have been developed to help rid eucalypt seed of pathogens and other pests. The most common protocol listed is to soak the seed in an approximately 1% sodium hypochlorite solution for about 20 minutes, followed by washes in sterile water. The exact concentration and duration of the dip may vary slightly and in direct proportion to the thickness of the seed coat. A detergent, such as Tween 20® may be used to reduce surface tension of the solution and thereby improve penetration of the seed coat. Unfortunately, this technique and others are not always effective, and some established

techniques are proprietary. *Eucalyptus* seeds are quite small, often less than 1-2 mm in length and they are sometimes variable in shape (Fig. 3). This may account for some of the variability in the effectiveness of some seed sterilization techniques.

Fortunately, additional steps can be followed that should assure pathogen/insect-free, seed-derived germinants. After surface disinfestation, eucalypt seeds can be germinated aseptically on nutrient medium *in vitro* and with a parafilm seal and allowed to growth for at least 3 weeks. If there are no signs or symptoms of pathogens are evident after 3 weeks, these germinants can be considered free of pathogens. After appropriate phytosanitary permits are obtained, eucalypt germplasm deemed "pathogen free" could be express mailed to Hawaii following phytosanitary permit guidelines. In Hawaii, steps 5 and 6 from the tissue culture transfer procedures (see above) would be necessary.

Fig. 3. Seed capsules, chaff and seeds (the dark black particles) of *Eucalyptus tereticornis* – forest red gum (Photo by Forest and Kim Starr)

For rooted cuttings of *Eucalyptus*, the same concentration of sodium hyporchlorite can also be used although the duration of the dip time should be increased to 30 minutes followed by rinsing with sterile water. However, in general, importation of rooted cuttings with soils poses a great threat to Hawaii and/or elsewhere due to many unknown potential pathogens. Because numerous introductions of invasive pathogens have occurred on plant/soil materials in spite of rigorous inspection processes, the importation of rooted cuttings should be avoided unless safer methods are developed to eliminate risks.

***Ex vitro* Propagation of *Eucalyptus*:**

After seedlings or plantlets are established in green house conditions in Hawaii, they can be multiplied by cuttings. Even during this stage, monitoring is necessary to help ensure that no exotic, latent pathogens develop. Techniques to multiply clones by rooted cuttings are well established for many *Eucalyptus* spp. (Figs 4, 5, and 6).

Fig. 4. Clonal multiplication of *Eucalyptus* clones. Right: cuttings are taken from hedge plants in greenhouse or shadehouses. Left: cuttings are rooted in a mist bed. (Photos courtesy of CENIBRA®, Belo Oriente, MG, Brazil)

Fig. 5. Preparing Eucalptus clones for planting. Right: rooted cuttings are sorted and repotted. Left: rooted cuttings are prepared for growth outside. (Photos courtesy of CENIBRA®, Belo Oriente, MG, Brazil)

Fig. 6. *Eucalyptus* clones ready for planting (Photo courtesy of CENIBRA®, Belo Oriente, MG, Brazil)

SUMMARY

Significant risks of introducing exotic pathogens and insects are associated with the importation of eucalypt germplasm to Hawaii. These risks can be reduced by using locally available materials and limiting the species, germplasm, and geographic sources of imported eucalypt material. Risks are minimized by importing axenic tissue cultures or seed-derived germinants to a containment facility in Hawaii, where plantlets and seedlings are monitored before release into the environment. In general, importation of rooted cuttings with soils is not recommended due to many unknown potential pathogens. The continued monitoring is necessary after establishing seedlings or plantlets in greenhouse so that no exotic, latent pathogens develop. *Eucalyptus* may provide an important natural resource for Hawaii, therefore it is critical to establish strict phytosanitary guidelines and regulation to allow 'clean and safe' importation of *Eucalyptus* germplasm. Different regulatory branches, such as USDA-APHIS, Hawaii Department of Agriculture-Plant Quarantine, and Hawaii's potential *Eucalyptus* industries, must coordinate and collaborate to prevent potential invasive pathogen and insects on *Eucalyptus* spp.

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