Pruning quality affects infection of *Acacia mangium* and *A. crassicarpa* by *Ceratocystis acaciivora* and *Lasiodiplodia theobromae*

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Pruning (singling) is a common silvicultural practice in commercial *Acacia* plantations because these trees tend to have multiple stems. The wounds resulting from pruning are susceptible to infection by pathogens. *Ceratocystis acaciivora* and *Lasiodiplodia theobromae* have recently been shown to be important pathogens of *A. mangium* in Indonesia, where they are commonly associated with wounds on trees. The aim of this study was to determine the impact of different wound types on infection of *A. mangium* and *A. crassicarpa* by these two pathogens. Isolates of *C. acaciivora* and *L. theobromae*, found to be the most pathogenic in a prior study, were used to inoculate pruning wounds. Results showed that pruning conducted in a manner to reduce stem damage, resulted in lower levels of fungal infection. Where pruning resulted in tearing of the bark, there were greater levels of infection and disease occurred even without artificial inoculation.

Inoculation of pruning wounds on *A. mangium* and *A. crassicarpa* showed that both fungi have the potential to cause disease. However, *C. acaciivora* was most pathogenic. Results of this study showed conclusively that careful pruning will result in lower levels of disease in young *A. mangium* and *A. crassicarpa* plantations in Indonesia.

Keywords: Botryosphaeriaceae, *Ceratocystis*, disease management, silviculture, wound-infecting pathogens.

Introduction

Plantations of *Acacia mangium* and *A. crassicarpa* have expanded rapidly in Indonesia since the 1980's, specifically to provide raw material for Indonesian pulp and paper industries (Barr 2001, Anonymous 2004). These *Acacia* spp., however, tend to have poor stem form, with multiple stems and branches (Srivastava 1993, Lee and Arentz 1997). Pruning (singling) is thus carried out to improve tree form and to increase tree strength, reducing stem or branch breakage, particularly after strong winds (Beadle et al. 2007). Furthermore, the capacity of *A. mangium* trees to self prune is low in areas with high rainfall (Lee and Arentz 1997) and pruning is consequently necessary. These practices also reduce the density of stands so that optimum tree growth can be achieved (Neilsen and Gerrand 1999).

Wounds resulting from pruning activities provide infection sites for numerous pathogens (Glass and McKenzie 1989, Vartiamaki et al. 2009), including plantation grown *Acacia* spp. (Lee et al. 1988, Barry et al. 2005). For example, heart rot caused by a complex of *Phellinus noxius* and other unidentified basidiomycetes is common on *Acacia* spp. in Malaysia and Indonesia (Lee and Sikin 1999). A recent study to determine the cause of death of young *A. mangium* trees in Indonesian plantations (Tarigan 2008, Tarigan et al. 2010) has shown an association between pruning wounds and disease caused by *Ceratocystis acaciivora* and *Lasiodiplodia theobromae*.

In order to develop management guidelines for pruning *Acacia* spp. in Indonesia, a study was undertaken to consider the effect of the quality of pruning wounds on disease development. For this purpose, pathogenic isolates of *C. acaciivora* and *L. theobromae* were used in inoculations of pruning wounds of different quality on *A. crassicarpa* and *A. mangium*.

Materials and methods

One-year-old *A. mangium* and *A. crassicarpa* trees in Riau province, Indonesia were used in pruning wound inoculations. Trees ranged in diameter from 70 mm to 90 mm at approximately 1.3 m above ground level.

Isolates of *C. acaciivora* (CMW22563) and *L. theobromae* (CMW23003), identified as highly pathogenic in previous studies (Tarigan 2008, Tarigan et al. 2010) were selected for the inoculation experiments. Isolates were grown on 2 % (w/v) Malt Extract Agar (MEA) (Biolab, Midrand, South Africa) for 2 weeks prior to inoculation.

Two pruning methods were used. In one case, branches were pruned above the branch collar taking care not to tear the bark (Fig 1a). In the alternative pruning technique, branches were pruned on the branch collar and the bark was torn to create a flap (Fig 1b). Pruning wounds were made using a handsaw, similar to that used in routine pruning activities in Indonesia.

Pruning wounds were inoculated either by spreading inoculum over the wound surface, or alternatively by spraying a fungal spore/mycelium suspension onto the wounds. Where inoculum was spread over the wounds, 10 mm diameter agar plugs [2 % MEA (Malt Extract Agar, Biolab, Midrand, South Africa)], taken from the edges of actively growing fungal colonies, were spread over the surface of freshly made wounds. Alternatively, 1 ml of a fungal suspension (>10^{-4} spores per ml of sterile water) was sprayed onto the surface of freshly made wounds. For the controls, pruning wounds were sprayed with sterile water or inoculated with a sterile plug of 2 % MEA.

Twenty trees of *A. crassicarpa* and *A. mangium* were used in each of the four treatments. In all, 240 trees of each species were used. These included twenty trees of each species for each of the two wounding techniques, and twenty trees for each species inoculated by either spreading or spraying the inoculum onto the pruning wounds. Twenty control trees were included for each treatment, giving a total of 80 control trees for the entire experiment. After five weeks, the lengths of the lesions in the cambium associated with the pruning sites were measured. Data obtained were analyzed with analysis of variance (ANOVA) using SAS statistical analyses (SAS Version 8.2, 2001).

After measuring lesions, isolations were made from tissue associated with the pruning wounds. For *C. acaciivora,* pieces of symptomatic tissue from the areas associated with the inoculation points were collected and placed in moist chambers to induce sporulation. Spore masses were taken from the tips of fruiting structures and plated onto 2 % MEA to verify the presence of the inoculated fungus. For the *L. theobromae* and control treatments, pieces of tissue were taken from the pruning sites and these were plated onto 2 % MEA. The identity of the inoculated fungi was also confirmed by selecting representative isolates and subjecting them to DNA sequence comparisons based on the Internal Transcribed Spacer region (ITS1, ITS4) including the 5.8S rRNA operon as previously described (Tarigan et al. 2010).

Results

Inoculation of *A. mangium* and *A. crassicarpa* trees with *C. acaciivora* and *L. theobromae* resulted in lesions after five weeks. In general, the *C. acaciivora* isolate produced significantly larger lesions than those associated with *L. theobromae,* except on *A. crassicarpa* where inoculum was spread onto the wounds. The *L. theobromae* isolate did not produce lesions significantly different to those of the controls, except on *A. crassicarpa* where the inoculum was spread over the wounds and where the rough pruning was applied. In general, the lesions on *A. mangium* trees were longer than those on the *A. crassicarpa* trees (Fig 3).

Both the careful and the rough pruning methods produced lesions on *A. mangium* and *A. crassicarpa*. However, all treatments using the rough pruning method, including the control, produced much larger lesions than those associated with careful pruning (Figs 2, 3). The application techniques where inoculum was spread over or sprayed onto the pruning wounds both gave rise to large lesions on *A. mangium* and *A. crassicarpa* where rough pruning was applied. No, or only small lesions developed on *A. mangium* and *A. crassicarpa* where careful pruning was applied (Figs 2, 3).

Where careful pruning was used, the average lesion lengths associated with *C. acaciivora* inoculation was 81 mm and 69 mm on *A. crassicarpa* and 118 mm and 160 mm on *A. mangium* for the spray and spread techniques respectively (Fig 3). These were much smaller than those where rough pruning was applied and where lesion lengths were an average of 406 mm and 315 mm on *A. crassicarpa* and 450 mm and 343 mm on *A. mangium* for the spray and spread techniques, respectively (Fig 3). *Lasiodiplodia theobromae* inoculation gave rise to lesions with an average length of 16 mm and 16.3 mm on *A. crassicarpa* and 11 mm and 17 mm on *A. mangium* for the spray and spread techniques, where careful pruning was used. In contrast, where rough pruning was applied, mean lesions lengths were 240 mm and 372 mm on *A. crassicarpa* and 256 mm and 185 mm on *A. mangium* for the spray and spread techniques respectively (P values = 0.05; R-Square = 0.697; Coeff. Var. = 56.67; Root MSE = 9.74).

Re-isolation from lesions on trees inoculated with *C. acaciivora* consistently yielded the inoculated fungus. This fungus was never isolated from wounds on the control trees. Where trees were inoculated with *L. theobromae*, it was also re-isolated consistently, however, it was also isolated from some of the wounds on the control trees.

Discussion

Results of this study have shown clearly that the quality of pruning has a significant effect on the infection of *A. mangium* and *A. crassicarpa* by two fungal pathogens that are associated with stem disease development after pruning. These results have practical implications for the management of *A. mangium* and *A. crassicarpa* diseases in plantations in Indonesia where pruning is routinely used to improve stem form and growth. Furthermore, this study clearly supports the results of previous investigations where it has been shown that *C. acaciivora* and *L. theobromae* are important pathogens that kill *A. mangium* after pruning in Indonesia (Tarigan 2008, Tarigan et al. 2010).

Pathogenic isolates of *C. acaciivora* and *L. theobromae,* collected from diseased *A. mangium* in previous studies (Tarigan 2008, Tarigan et al. 2010), were used to inoculate pruning wounds on both *A. mangium* and *A. crassicarpa*. Both fungi produced significant lesions within five weeks of being applied to wounds created by rough pruning. These results, as well as those published by Tarigan et al. (2010) confirm that *C. acaciivora* and *L. theobromae* are important pathogens of *A. mangium*, causing disease and death of trees after infection via pruning wounds. We have also shown that after wounding, *A. crassicarpa* can be equally vulnerable to infection and disease, even though damage equivalent to that on *A. mangium* has not been observed in plantations.

Lesion lengths after inoculation on *A. crassicarpa* were smaller to those on *A. mangium*. These results confirm that *A. mangium* trees respond poorly to wounding, as previously described (Schmitt et al. 1995). Results of this study also confirmed those of previous inoculation experiments on *A. mangium* and *A. crassicarpa* (Tarigan et al. 2010) where lesions on *A. crassicarpa* were smaller than those on *A. mangium*. The results, furthermore, correlate well with field observations, where serious canker and die-back is more commonly found on *A. mangium* than on *A. crassicarpa* trees.

Wound type was shown to play an important role in lesion development on both *A. mangium* and *A. crassicarpa*. Results clearly showed that even in the absence of inoculation, significantly longer lesions develop on pruning wounds that have broken the branch collar and exposed larger areas of wood than on carefully pruned trees. This is most likely due to the fact that roughly pruned wounds allow more opportunities for opportunistic pathogens to infect and develop. Poorly pruned trees also have bark flaps under which fungi can develop and they are most likely more heavily stressed than undamaged trees. It has also been shown in other studies that the size of the pruning wound is directly related to the risk of infection (Chou and MacKenzie 1988, Heath et al. 2010).

Lasiodiplodia theobromae is a well known opportunist and latent pathogen (Burgess et al. 2006, Punithalingam 1976). The fungus can easily be isolated from the bark of healthy *A. mangium* and *A. crassicarpa* (Tarigan 2008, Wingfield unpublished) and it has the ability to cause disease on stressed tissues such as those that are found on roughly pruned trees. The fact that *L. theobromae* was isolated from pruning wounds on control trees supports this view. *L. theobromae* appears to be a common pathogen in Riau (Tarigan 2008), and is known to be a wound and stress-related pathogen (Punithalingam 1980). It commonly exists as an endophyte in healthy trees (Johnson et al. 1992). Isolation of *L. theobromae* from the controls during re-isolation was thus not un-expected.

No differences in lesion development were observed between the two different inoculation methods used in this study. Spreading mycelium over the wound surfaces or application of inoculum by spraying both gave rise to substantial lesions. However, application of the inoculum by spreading it over the wounds was preferable as it was easier to quantify the amount of inoculum being applied. This was particularly true in the case of *L. theobromae* that does not readily produce spores in culture.

Results obtained in our study on plantation grown *Acacia* spp., together with previously published results of wounding studies in other countries and on other tree species, could be used to significantly reduce the incidence of disease on these trees. For example, several studies have shown that wounds on trees are more likely to become infected during periods (i.e in spring) when trees are actively transporting sap (Biggs 1987), or in periods when wound healing and callus formation is slower, such as in late summer and autumn (Vartiamaki et al. 2009). Optimal periods for pruning should, however, be determined for each tree species and geographic locality, as variation has been found in the time of year most suitable for pruning activities (Vartiamaki et al. 2009).

Conclusions

Results of this study have shown clearly that careful pruning can reduce the incidence of stem disease in *Acacia* plantations in Indonesia. This, together with sound selection and breeding strategies, will ensure the success of future plantings. Poor silvicultural practices, such as excessive pruning and rough pruning should be actively avoided. Late pruning also results in large branches that are difficult to prune without significant damage to the stems and this practice should also be avoided.

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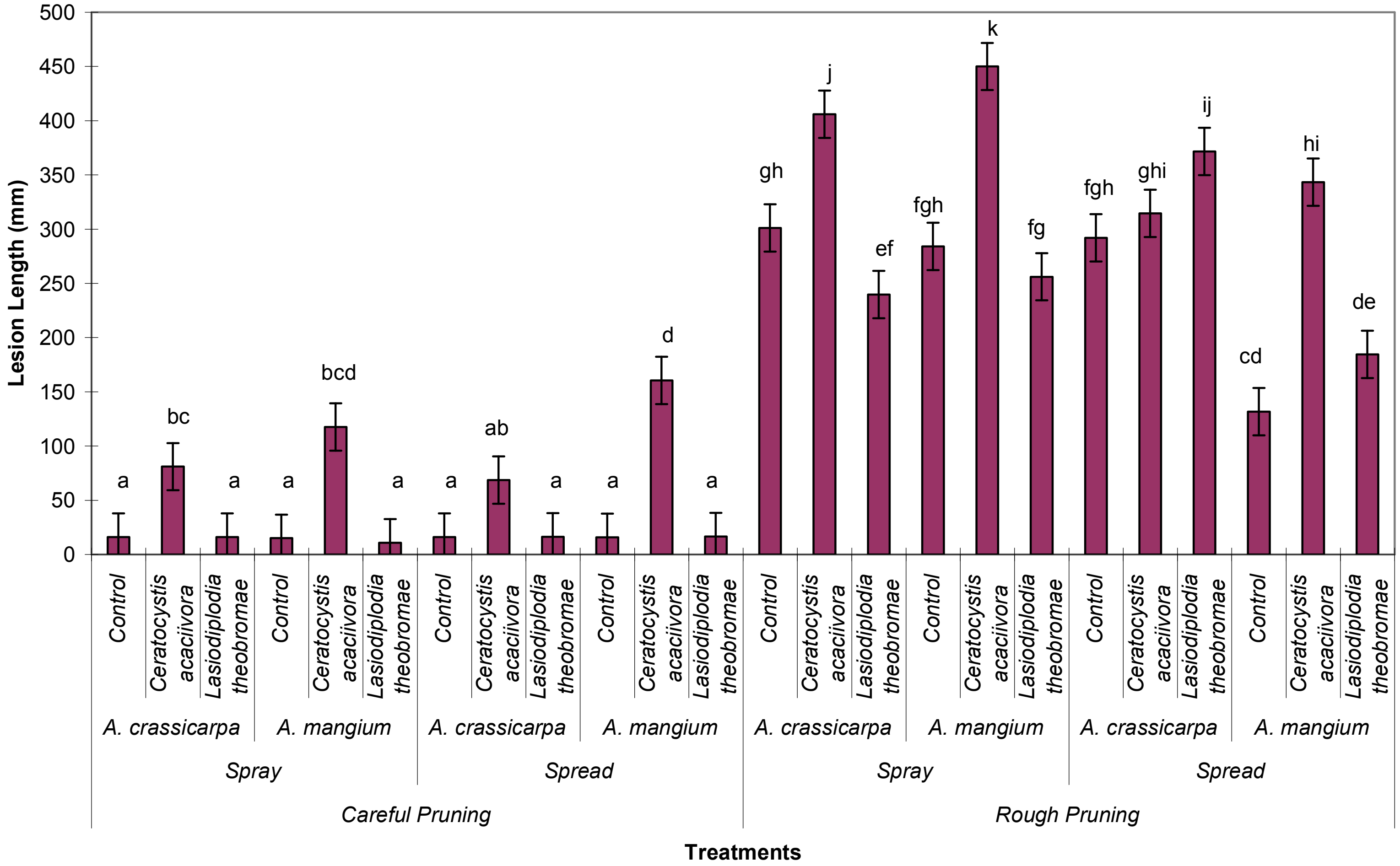
Fig 1. Pruning methods used and resultant lesions development for each. (a) Rough pruning where the branch collar was cut and the bark was torn, (b) extensive fungal stain developing from pruned branch (c) Careful pruning where the branches were pruned above the branch collar without tearing the bark, (d) no fungal stain/lesion developing at branch stub of a good pruning wound.

Fig 2. Lesion lengths on *Acacia mangium* and *A. crassicarpa* pruning wounds after inoculation with pathogenic isolates of *Ceratocystis acaciivora* (CMW22563) and *Lasiodiplodia theobromae* (CMW23003), five weeks after inoculation. Bars on the graph bearing the same letter are not significantly different from each other (P value = 0.05).


A
C
B
D



Lesion Length (mm)
500
450
400
350
300
250
200
150
100
50
0
a
bc
a
a
bcd
a
a
ab
a
a
d
a
gh
j
ef
fgh
k
fg
fgh
ghi
ij
cd
hi
de
Control
Ceratocystis acaciivora
Lasiodiplodia theobromae
A. crassicarpa
A. mangium
Spray
Spread
Careful Pruning
Rough Pruning
Treatments