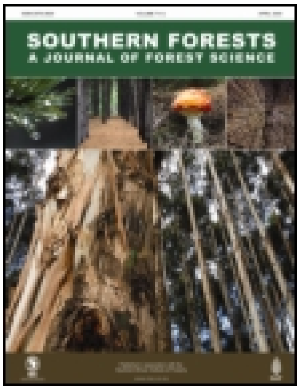

Southern Forests: a Journal of Forest Science

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tsfs20

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

M Tarigan [a] [b] , M J Wingfield [a] , M van Wyk [c] , B Tjahjono [b] & J Roux [a]

[a] Department of Microbiology and Plant Pathology, Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa

[b] PT Riau Andalan Pulp and Paper, Pekanbaru, Riau, Sumatra, Indonesia

[c] Department of Genetics, Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa

Available online: 11 Jan 2012

To cite this article: M Tarigan, M J Wingfield, M van Wyk, B Tjahjono & J Roux (2011): Pruning quality affects infection of Acacia mangium and A. crassicarpa by Ceratocystis acaciivora and Lasiodiplodia theobromae, Southern Forests: a Journal of Forest Science, 73:3-4, 187-191

To link to this article: http://dx.doi.org/10.2989/20702620.2011.639498

Southern Forests 2011, 73(3&4): 187–191
Printed in South Africa — All rights reserved

SOUTHERN FORESTS
ISSN 2070–2620 EISSN 2070–2639
doi: 10.2989/20702620.2011.639498

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

M Tarigan[1, 2], MJ Wingfield[1], M van Wyk[3], B Tjahjono[2] and J Roux[1*]

[1] Department of Microbiology and Plant Pathology, Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa
[2] PT Riau Andalan Pulp and Paper, Pekanbaru, Riau, Sumatra, Indonesia
[3] Department of Genetics, Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa
* Corresponding author, e-mail: jolanda.roux@fabi.up.ac.za

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 been shown recently 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 higher levels of infection and disease occurred even without artificial inoculation. Inoculation of pruning wounds with *C. acaciivora* and *L. theobromae* showed that both fungi have the potential to cause disease. However, *C. acaciivora* was most virulent. Results of this study showed conclusively that careful pruning will result in lower levels of disease in young *A. mangium* and *A. crassicarpa* plantations.

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

Introduction

Plantations of *Acacia mangium* and *A. crassicarpa* have expanded rapidly in Indonesia since the 1980s, specifically to provide raw material for Indonesian pulp and paper industries (Barr 2001, Anonymous 2004). These *Acacia* species, 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 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, Vartiamäki et al. 2009), also on plantation-grown *Acacia* species (Lee et al. 1988, Barry et al. 2005). For example, heart rot caused by *Phellinus noxius* and a complex of other unidentified basidiomycetes is common on *Acacia* spp. in Malaysia and Indonesia (Lee and Noraini Sikin 1999). Recent studies to determine the cause of death of young *A. mangium* trees in Indonesian plantations (Tarigan 2008, Tarigan et al. 2011) showed an association between pruning wounds and disease caused by *Ceratocystis acaciivora* and *Lasiodiplodia theobromae*.

In order to develop management guidelines for pruning *Acacia* species in Indonesia, a study was undertaken to investigate 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. Tree stems ranged in diameter from 70 to 90 mm at approximately 1.3 m above ground level. Inoculations were done in March 2007 and the trial was evaluated in May 2007.

Isolates of *C. acaciivora* (CMW22563) and *L. theobromae* (CMW23003), identified as highly pathogenic in previous studies (Tarigan 2008, Tarigan et al. 2011), were selected for the inoculation experiments. Isolates were grown on 2% (w/v) malt extract agar (MEA) (Biolab, Midrand, South Africa) for two 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 (Figure 1a). In the alternative pruning technique, branches were pruned on the branch collar and the bark was torn to create a flap (Figure 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 by spraying a fungal spore/mycelium suspension onto the wounds. Spore suspensions were chosen as they more closely imitate the situation where single spores would be introduced to wounds through insect activity and wind. Where inoculum was spread over the wounds, 10 mm diameter agar plugs (2% MEA), taken from the edges of actively growing fungal colonies, were spread over the surface of freshly made wounds so that the entire wound could be exposed to inoculum. Alternatively, 1 ml of a fungal suspension ($>10^4$ spores per ml of sterile water), prepared from actively growing fungal cultures of the same age as those used for the spreading technique, 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 20 trees of each species for each of the two wounding techniques, and 20 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 analysed with analysis of variance (ANOVA) using SAS statistical software version 8.2 (SAS Institute 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 (amplified with the primers ITS1 and ITS4) including the 5.8S rRNA operon as previously described (Tarigan et al. 2011).

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 the inoculum was spread onto the wounds. Lesions produced by the *L. theobromae* isolate did not differ significantly from 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 (Figure 2).

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 (Figures 1c and 2). No, or only small, lesions developed on *A. mangium* and *A. crassicarpa* where careful pruning was applied (Figures 1d and 2).

Where careful pruning was applied, the average lesion lengths associated with *C. acaciivora* inoculation were 81 mm and 69 mm on *A. crassicarpa* and 118 mm and 160 mm on *A. mangium* for the spray and spread techniques, respectively (Figure 2). These were much shorter 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 (Figure 2). *Lasiodiplodia theobromae* inoculation gave rise to lesions with average lengths of 16 mm for both inoculation techniques 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* with 256 mm and 185 mm on *A. mangium* for the spray and spread techniques, respectively (P values = 0.05; R^2 = 0.697; CV = 56.67; root MSE = 9.74).

Reisolation from lesions on trees inoculated with *C. acaciivora* consistently yielded the inoculated fungus. No fungal pathogens were isolated from wounds on the control trees. Where trees were inoculated with *L. theobromae*, the fungus was also reisolated consistently. It was, however, also isolated from some of the wounds on the control trees. No other fungi were obtained from the lesions.

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. 2011).

Pathogenic isolates of *C. acaciivora* and *L. theobromae,* collected from diseased *A. mangium* in previous studies (Tarigan 2008, Tarigan et al. 2011), 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. 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* under natural conditions has not yet been observed in plantations.

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

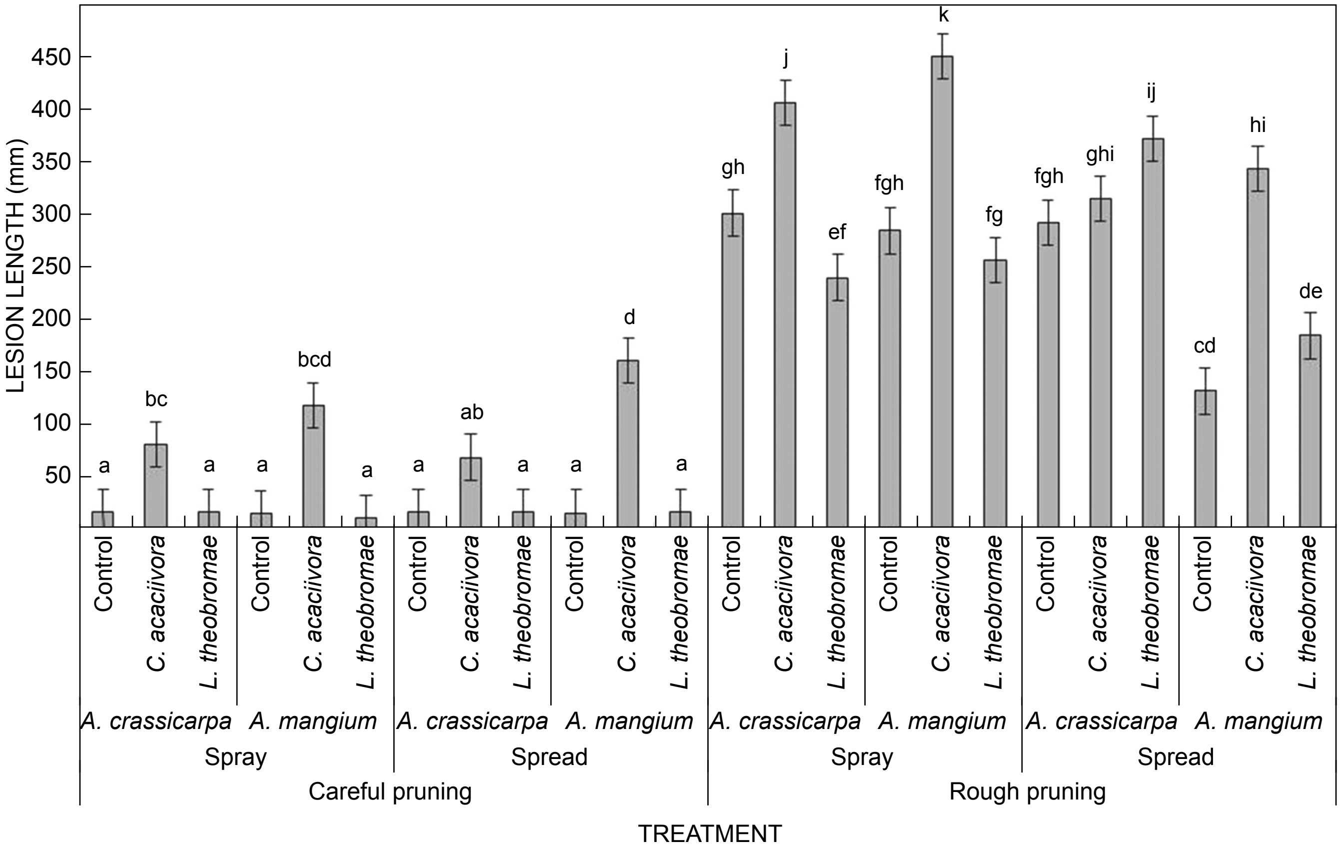

Figure 2: Lesion lengths on *Acacia mangium* and *A. crassicarpa* pruning wounds five weeks after inoculation with pathogenic isolates of *Ceratocystis acaciivora* (CMW22563) and *Lasiodiplodia theobromae* (CMW23003). Bars on the graph bearing the same letter are not significantly different from each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

Lesion lengths after inoculation on *A. crassicarpa* were shorter than 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* where lesions on *A. crassicarpa* were smaller than those on *A. mangium* (Tarigan et al. 2011). 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 present increased 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 trees with smaller wounds. 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 (Punithalingam 1976, 1980, Burgess et al. 2006). The fungus can easily be isolated from the bark of healthy *A. mangium* and *A. crassicarpa* (Tarigan 2008, MJ Wingfield unpublished data) 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. *Lasiodiplodia theobromae* appears to be a common pathogen in Riau (Tarigan 2008). It commonly exists as an endophyte in healthy trees (Johnson et al. 1992). Isolation of *L. theobromae* from the controls during reisolation was thus not unexpected.

No differences in lesion development were observed between the two different inoculation methods used in this study. Spreading mycelium over the wound surfaces and application of inoculum by spraying both gave rise to 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*, which 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, 1989), or in periods when wound healing and callus formation is slower, such as in late summer and autumn (Vartiamäki 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 (Vartiamäki 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.

Acknowledgments — The Tree Protection Co-operative Programme (TPCP) and the South African National Research Foundation are thanked for financial support to undertake this study. The first author also recognises the support of PT Riau Andalan Pulp and Paper for support to conduct postgraduate studies in South Africa.

References

Anonymous. 2004. Executive Summary. Current condition of forestry sector development. Statement on progress towards implementing sustainable forest management. The Mid-Term CGI Meeting held in Jakarta, 2 June 2004. Jakarta: Ministry of Forestry of Indonesia.

Barr C. 2001. Banking on sustainability: structural adjustment and forestry reform in post-Suharto Indonesia. Bogor: Center for International Forestry Research and WWF Macroeconomics for Sustainable Development Program Office.

Barry KM, Hall MF, Mohammed CL. 2005. The effect of time and site on incidence and spread of pruning-related decay in plantation-grown *Eucalyptus nitens. Canadian Journal of Forestry Research* 35: 495–502.

Beadle C, Barry K, Hardiyanto E, Irianto RJ, Mohammed C, Rimbawanto A. 2007. Effect of pruning *Acacia mangium* on growth, form and heart rot. *Forest Ecology and Management* 238: 261–267.

Biggs AR. 1987. Occurrence and location of suberin in wound reaction zones in xylem of 17 tree species. *Phytopathology* 77: 718–725.

Biggs AR. 1989. Temporal changes in the infection court after wounding of peach bark and their association with cultivars in infection by *Leucostoma persoonii*. *Phytopathology* 79: 627–630.

Burgess TI, Barber PA, Mohali S, Pegg G, de Beer ZW, Wingfield MJ. 2006. Three new *Lasiodiplodia* spp. from the tropics, recognised based on DNA sequence comparisons and morphology. *Mycologia* 98: 423–235.

Chou CKS, MacKenzie M. 1988. Effect of pruning intensity and season on *Diplodia pinea* infection of *Pinus radiata* stem through pruning wounds. *European Journal of Forest Pathology* 18: 437–444.

Glass BP, McKenzie H. 1989. Decay distribution in relation to pruning and growth stress in plantation-grown *Eucalyptus regnans* in New Zealand. *New Zealand Journal of Forestry Science* 19: 210–222.

Heath RN, van der Linde M, Groeneveld H, Wingfield BD, Wingfield MJ, Roux J. 2010. Factors influencing the infection of the wilt pathogen *Ceratocystis albifundus* on *Acacia mearnsii* in South Africa. *Forest Pathology* 40: 500–509.

Johnson GI, Mead AJ, Cooke AW, Dean JR. 1992. Mango stem end rot pathogens – fruit infection by endophytic colonisation of the inflorescence and pedicel. *Annals of Applied Biology* 120: 225–234.

Lee SS, Arentz F. 1997. A possible link between rainfall and heart rot incidence in *Acacia mangium*? *Journal of Tropical Forest Science* 9: 441–448.

Lee SS, Noraini Sikin Y. 1999. Fungi associated with heart rot of *Acacia mangium* trees in Peninsular Malaysia and East Kalimantan. *Journal of Tropical Forest Science* 11: 240–254.

Lee SS, Teng SY, Lim MT, Kader RA. 1988. Discolouration and heartrot of *Acacia mangium* Willd.—some preliminary results. *Journal of Tropical Forest Science* 1: 170–177.

Neilsen WA, Gerrand AM. 1999. Growth and branching habit of *Eucalyptus nitens* at different spacing and the effect on final crop selection. *Forest Ecology and Management* 123: 217–229.

Punithalingam E. 1976. *Botryodiplodia theobromae*. *CMI Descriptions of Pathogenic Fungi and Bacteria* no. 519. Kew: Commonwealth Mycological Institute.

Punithalingam E. 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat. *Bibiotheca Mycologica* 71. Vaduz: J Cramer.

SAS Institute. 2001. *SAS statistical software, SAS/STAT users guide, version 8.2*. Cary: SAS Institute.

Schmitt U, Liese W, Hong LT, Killmann W. 1995. The mechanism of wound response in *Acacia mangium*. *IAWA Journal* 16: 425–432.

Srivastava PBL. 1993. Silvicultural practice. In: Awang K, Taylor D (eds), *Acacia mangium growing and utilization. MPTS Monograph Series* no. 3. Bangkok: Winrock International and FAO. pp 113–147.

Tarigan M. 2008. *Ceratocystis* spp. and Botryosphaeriaceae on plantation *Acacia* species in Central Sumatra, Indonesia. MSc thesis, University of Pretoria, South Africa.

Tarigan M, Roux J, van Wyk M, Tjahjono B, Wingfield MJ. 2011. A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora sp. nov*. in Indonesia. *South African Journal of Botany* 77: 292–304.

Vartiamäki H, Hantula J, Uotila A. 2009. Susceptibility of silver birch pruning wounds to infection by white-rot fungus (*Chondrostereum purpureum),* a potential bioherbicide. *Silva Fennica* 43: 537–547.