Forest Pathology WILEY-BLACKWELL

For. Path. 43 (2013) 153–164 doi: 10.1111/efp.12013

Spatial–temporal patterns of Ceratocystis wilt in *Eucalyptus* plantations in Brazil

By M. A. Ferreira[1,7], T. C. Harrington[2,6], C. C. Gongora-Canul[3], R. G. Mafia[4], E. A. V. Zauza[5] and A. C. Alfenas[1]

[1]Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG Brazil; [2]Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011, USA; [3]Pioneer Hi-Bred Int. Mexico, Jalisco, Mexico; [4]Fibria Celulose S.A. Technology Center, Aracruz, ES Brazil; [5]Suzano Papel e Celulose S.A., Teixeira de Freitas, BA Brazil; [6]Email: tchaarrin@lastate.edu (for correspondence); [7]Present Address: Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, MG Brazil

Summary

Ceratocystis wilt, caused by *Ceratocystis fimbriata*, has become the most important disease in eucalyptus (*Eucalyptus* spp. and hybrids) plantations in Brazil. To further our understanding of the epidemiology of this disease, we surveyed eucalyptus plantations in the states of Minas Gerais and Bahia that were known to have Ceratocystis wilt or were thought to have been planted with infected rooted cuttings. There was generally higher disease incidence in the Minas Gerais plantations, which were on former Cerrado forest sites and likely had soilborne inoculum prior to planting eucalyptus. In such plantations, disease incidence was not evident before 20 months after planting but slowly increased up to 50% at 74 months. The symptomatic and killed trees were aggregated, perhaps from uneven distribution of inoculum in the soil. Also, the progression of cumulative disease incidence best fit a monomolecular model, which is typical of soilborne diseases (fixed level of initial inoculum with little or no secondary inoculum during the crop rotation). However, plots where some trees had been harvested during the rotation showed very high levels of disease incidence in the sprouts that arose from stumps, suggesting secondary spread of the pathogen on harvesting tools or machinery. Most of the Bahia plantations were on pastureland prior to eucalyptus cultivation, and the pathogen was likely introduced with infected nursery stock. In such plots, symptoms were evident as soon as 7 months after planting, and most of the mortality occurred within 12 months. The diseased trees on former pastureland sites were sometimes aggregated within planting rows, suggesting that bunches of infected nursery stock were planted together within the rows. Care should be taken in planting disease-free planting material and spreading the pathogen on tools, but on sites with soilborne inoculum, use of resistant clones may be the only management option.

1 Introduction

Ceratocystis wilt is arguably the most important disease in clonally propagated eucalyptus (*Eucalyptus* spp. and hybrids) plantations in Brazil, where nearly 5 million hectares have been planted with eucalyptus (Alfenas et al. 2009; ABRAF, Associação Brasileira de Produtores de Florestas Plantadas 2012). As the industry has expanded, Ceratocystis wilt has become an increasing problem. The disease is caused by *Ceratocystis fimbriata* Ellis & Halst., a native of South America with a broad, but highly variable, host range (Baker et al. 2003; Thorpe et al. 2005; Ferreira et al. 2010; Harrington et al. 2011; Harrington 2013). Some consider the name *C. eucalypticola* M. van Wyk & M.J. Wingf. to be more appropriate for the causal agent of Ceratocystis wilt on eucalyptus (van Wyk et al. 2012). However, previous work (Ferreira et al. 2010; Harrington et al. 2011) showed that Brazilian isolates from eucalyptus and other hosts are interfertile with each other and with strains from sweet potato (*Ipomoea batatus*), the original source material for the description of *C. fimbriata*. Furthermore, Brazilian strains are not host-specialized nor distinguished morphologically or phylogenetically. Therefore, the eucalyptus strains in Brazil are considered herein as *C. fimbriata sensu stricto*. Although other strains of the pathogen and other *Ceratocystis* spp. have been reported on eucalyptus around the world (Nkuekam et al. 2012; van Wyk et al. 2012), Brazilian strains of *C. fimbriata s.s.* have been the most damaging in causing mortality and reducing yield (Ferreira et al. 2007; Alfenas and Ferreira 2008).

Ceratocystis wilt was first observed in eucalyptus in 1997 in the state of Bahia (Ferreira et al. 1999) and has been most studied there and in the adjacent state of Minas Gerais (Ferreira et al. 2011). It is also known in the states of Espírito Santo, Mato Grosso do Sul, São Paulo, Maranhão and Pará (Alfenas et al. 2009; Ferreira et al. 2011). Genetic analyses of isolates of *C. fimbriata* suggest that Brazilian genotypes have been moved to other regions of South America, Africa and Asia in infected eucalyptus cuttings (Roux et al. 2000; Barnes et al. 2003; van Wyk et al. 2006, 2012, 2012; Ferreira et al. 2010, 2011; Harrington et al. 2011).

Infections typically start in the roots or base of the tree and move up into the stem, causing a dark staining of the woody xylem, primarily in a radial pattern in cross-section, following the ray parenchyma. Ceratocystis wilt also is characterized by slightly sunken cankers, wilting and drying of lateral branches or the tops of trees, or death of the whole tree (Ferreira et al. 2006; Alfenas et al. 2009; Harrington 2013). The epidemiology of Ceratocystis wilt in eucalyptus is not fully understood, but recent genetics studies have found Brazilian populations of *C. fimbriata* to be highly variable in aggressiveness and have suggested that the pathogen is soilborne and native to certain forest types, such as the Mata Atlântica and Cerrado forests of Brazil (Ferreira et al. 2010, 2011; Harrington 2013). The two forest types differ in that the former is coastal, generally with higher rainfall and densely stocked, while Cerrado forests are more interior, typically have an extended dry season during the cooler winter months, and are more open.

Populations of *C. fimbriata* introduced on contaminated tools or in clonally propagated material show little or no genetic variation (Ferreira et al. 2011; Harrington et al. 2011). Harvesting trees may increase levels of soilborne inoculum due to

Received: 11.6.2012; accepted: 30.9.2012; editor: D. Zhou

sawdust contaminated with aleurioconidia (pigmented, thick-walled conidia produced in wood), and the pathogen may spread on cutting tools to other stumps, which may be managed for coppice or for collecting cuttings for rooting in a nursery (Ferreira et al. 2011; Harrington 2013). Contaminated nursery stock is a major source of inoculum, especially on former pastureland sites, which may have little or no soilborne inoculum (Ferreira et al. 2011; Harrington 2013). Although there is a possibility that the fungus is carried by insects (Heath et al. 2009; Nkuekam et al. 2012), insect vectors have not been identified in Brazil and may not be important (Harrington 2013). Thus, the epidemiology of the disease is complex and our knowledge incomplete. A better understanding of the sources of inoculum and progression of the disease over time may lead to more effective management.

To further our understanding of the epidemiology of Ceratocystis wilt in eucalyptus, we surveyed eucalyptus plantations in Minas Gerais and Bahia that were known to have Ceratocystis wilt or were thought to have been planted with infected rooted cuttings. We compared the incidence and spatial distribution (degree of aggregation) of the disease in plantations on formerly forested sites (presumably with soilborne inoculum) and former pastureland (with little or no soilborne inoculum). We also studied the progression of disease over time to determine whether the disease follows a simple or compound interest model.

2 Material and methods

2.1 Data collection and study areas

Disease incidence was assessed for all trees in temporary or permanent plots established from 2003–2008 within 27 commercial eucalyptus plantations in the states of Minas Gerais and Bahia. Each plantation was of a clonally propagated hybrid of *Eucalyptus grandis* × *E. urophylla* (urograndis), except plantations BA8H and BA9H, in which the planted clone (H) was a natural hybrid of *E. grandis* (open pollinated) and plantations of clone G, which was *E. urophylla* × *E. camaldulensis* (urocam). Each plantation was designated by state, plantation number and clone designation (Table 1). In most cases, the location of each dead, symptomatic and asymptomatic tree was mapped, and the distribution of diseased trees was analysed for the presence or absence of aggregation. Planters generally start at one end of the area to be planted, usually along a roadside, and plant trees in a row perpendicular to the road. The orientation of the planting rows was noted, as well as tree spacing within and between rows.

In all of the selected plantations, the presence of Ceratocystis wilt was confirmed by assessment of foliar and xylem symptoms. Symptomatic trees typically have many wilted or dead leaves attached to the affected branches, and the dark brown to black discoloration in the woody xylem, especially if in a radial pattern, is unique to Ceratocystis wilt (Ferreira et al. 2006; Alfenas et al. 2009). The pathogen was isolated from discoloured xylem taken from at least some of the killed or symptomatic trees in most of the plantations (Ferreira et al. 2011). Elongated cankers were also present on some affected trees. Within each plot, all trees showing wilt symptoms (dead foliage hanging throughout the tree or on some major branches) were noted as symptomatic for Ceratocystis wilt, although some trees also were checked for xylem discoloration. Dead or missing trees in an area with confirmed Ceratocystis wilt were classified as killed by Ceratocystis wilt unless there was some other obvious cause of death.

2.1.1 Minas Gerais plots

The surveyed plantations in Minas Gerais were in natural Cerrado forest type prior to eucalyptus cultivation (Table 1). Plantations of three different clones (C, G and I) were sampled near the towns of Curvelo and João Pinheiro (Ferreira et al. 2011). Nine plantations were sampled before harvest. The plots in MG2C, MG9C, MG11C, MG14I and MG15I were purposely placed in areas of the plantation where Ceratocystis wilt was known to occur. The plots in MG16G, MG17G, MG18G and MG19G were purposely placed in a portion of the plantation with a high incidence of Ceratocystis wilt. Plots MG1C-s, MG9C-s and MG10C-s were randomly placed in areas of three plantations where the trees had been harvested and stump sprouts were used for collection of propagation cuttings to be taken to a nursery for rooting (letter ‘s’ designates that regenerated sprouts were surveyed for disease incidence). In most cases, the sprouts had been thinned to a single sprout per stump by the time of sampling.

2.1.2 Bahia plots

Plantations of four eucalyptus clones (A, B, D and H) were surveyed in Bahia (Ferreira et al. 2011). Most of the surveyed plantations were on former pastureland with no woody vegetation prior to planting eucalyptus (Table 1). However, plantations BA8H and BA9H were planted on a former pastureland site that had advanced, natural woody regeneration, which had been removed prior to eucalyptus planting in 2003. Plots in BA8H and BA9H were placed in areas of the plantations where Ceratocystis wilt was known to occur.

Plantation BA1A-s was a site of a small farm before clearing and eucalyptus planting in 1996. The plantation was heavily damaged in a windstorm in 2000, and in 2001, the damaged trees were cut and sprouts from the stumps were a source material for the production of rooted cuttings that were planted elsewhere in south Bahia, including plantations BA2A, BA3A, BA4A and BA5A. The identified genotypes of *C. fimbriata* from the latter plantations were mostly identical to the genotypes found in the source of the cuttings, plantation BA1A-s (Ferreira et al. 2011). Plantations BA2A, BA3A, BA4A and BA5A were visited because it was known that they were planted with cuttings taken from BA1A-s, and plots in those young

Table 1. Disease incidence and spatial autocorrelation analysis of Ceratocystis wilt in eucalyptus plantations planted on former Cerrado forest sites, former pastureland with woody regeneration, a former small farm with woody plants, and former pastureland without woody regeneration.

Vegetation before eucalyptus plantations	Plantation and clone[1]	City	Number of consecutive plantings of eucalyptus	Size of plots (m)	Plantation age at time of survey (months)	Per cent symptomatic and dead trees at time of survey	Spacing of trees within rows × across rows (m)	Autocorrelation proximity pattern of disease distribution[2]		
								Within rows	Across rows	Diagonal
Cerrado Forest	MG1C-s[3]	Curvelo	ND[4]	40 × 40	56/18[3]	50.1	2 × 3	12*	6*	3**
	MG2C	Curvelo	ND	40 × 40	74	12.8	2 × 3	3**	0	0
	MG9C	Curvelo	ND[4]	40 × 40	74	2.7	2 × 3	0	0	0
	MG9C-s[3]	Curvelo	ND	40 × 40	56/18[3]	39.7	2 × 3	20*	7**	7**
	MG10C-s[3]	Curvelo	ND	40 × 40	50/12[3]	22.6	2 × 3	4**	0	1**
	MG11C	Curvelo	ND	40 × 40	62	15.6	2 × 3	5**	1**	1**
	MG16G	João Pinheiro	First	50 × 10	33	45.8	2 × (6/3)[5]	47**	3**	5*
	MG17G	João Pinheiro	First	50 × 10	33	51.7	2 × (6/3)[5]	3*	1**	0
	MG18G	João Pinheiro	First	50 × 10	33	62.9	2 × (6/3)[5]	11*	7*	6*
	MG19G	João Pinheiro	First	50 × 10	33	46.9	2 × (6/3)[5]	5*	1**	1**
	MG14I	Curvelo	ND	50 × 40	50	5.7	2 × 3	0	0	0
	MG15I	Curvelo	ND	50 × 40	74	12.3	2 × 3	11**	1**	1**
	MG20G	Bocaiúva	First	10 × 10	20–53	13.4–44.4	2 × 3	ND	ND	ND
Pastureland with Woody Regeneration	BA8H	Eunápolis	First	13 × 52	65	48.5	2.4 × 5	2**	0	0
	BA9H	Itabela	First	18 × 52	62	40.0	2.4 × 5	5**	2**	0
					74	51.0	2.4 × 5	4**	2**	0
Small Farm with Woody Plants	BA1A-s[3]	Eunápolis	First	10 × 39	48/24[3]	22.3	2.4 × 5	0	0	0
Pastureland without Woody Regeneration	BA2A	Eunápolis	First	50 × 20	17	1.2	2.4 × 5	12**	3**	2**
	BA3A	Eunápolis	First	50 × 25	19	1.4	2.4 × 5	8**	0	0
	BA4A	Eunápolis	Second	50 × 25	12	1.5	2.4 × 5	12**	1**	1**
	BA5A	Eunápolis	Second	56 × 52	17	2.9	2.4 × 5	5**	1**	1**
	BA10B-s[3]	Teixeiras de Freitas	First	50 × 40	66/6[3]	12.8	3 × 3	5**	2**	0
	BA11B	Teixeiras de Freitas	Second	50 × 42	6	4.1	3 × 3	0	0	0
	BA12B	Teixeiras de Freitas	Second	50 × 42	6	4.0	3 × 3	2**	0	0
	BA13B	Teixeiras de Freitas	Third	50 × 10	12	24.2	3 × 3	2**	2**	0
	BA6D	Caravelas	Second	35 × 30	17	1.4	3 × 3	3**	0	0

[1]The first two letters designate the state (MG = Minas Gerais, BA = Bahia), and the letter after the plantation number designates different clones of *Eucalyptus*.
[2]Number of positive continuous spatial lag positions (SL+) within rows, across rows and diagonally that are contiguous with the origin [0.0] lag in the core cluster. Significant autocorrelation coefficients at the $p = 0.05$ and $p = 0.01$ levels are indicated by single and double asterisks, respectively.
[3]Plantations designated with a 's' were harvested, and then stump sprouts were assessed for disease incidence. The number before the slash is the age of the plantation at the time of harvest, and the number after the slash is the age of the stump sprouts at the time of survey.
[4]Not determined.
[5]2 × (6/3) spacing consisted of rows alternating between 6 and 3 m across.

plantations were placed in the few areas where Ceratocystis wilt was evident. The plot in plantation BA1A-s was placed in a storm-damaged area that was heavily diseased and had been used for collection of cuttings.

Clone B was the first eucalyptus clone to be recognized with Ceratocystis wilt, and the Teixeira de Freitas area (plantations BA10B-s, BA11B, BA12B, BA13B, BA14B, and BA15B) was the first location where the disease was recognized in Brazil (Ferreira et al. 2011). *Ceratocystis fimbriata* had been recovered from infected cuttings of clone B at a large nursery in Bahia, and a limited number of genotypes of *C. fimbriata* had been identified in this and other clones in Bahia and São Paulo, suggesting that the nursery was a source of infected cuttings (Ferreira et al. 2011). Plantations BA10B-s was in its first rotation of eucalyptus, the stand was harvested at 66 months in 2002, and the sprouts from those stumps were assessed for disease incidence 6 months later. Plantations BA11B and BA12B were in the second rotation of eucalyptus, and BA13B was in its third rotation. Eucalyptus had been grown at site BA12B since 1976, and the vegetation before eucalyptus was probably pastureland. All plots in plantations of clone B were placed in areas where the disease was known to occur, except that the plots in BA14B were placed at random.

The cuttings for plantation BA6D came from a nursery in another state, Espírito Santo, but the genotype found in that plantation was identical to a common genotype of *C. fimbriata* recovered from clone B plantings (Ferreira et al. 2011). Previous to eucalyptus, the BA6D site was in pastureland (Table 1). Plots were placed where the disease was known to occur.

2.2 Disease progress within plantations

Disease incidence (number of diseased trees divided by the total number of trees assessed × 100) was assessed over time in plantations that were believed to have had soilborne inoculum prior to planting eucalyptus (MG20G, BA8H and BA9H) or in plantations that were believed to have been planted with infected rooted cuttings of clone B (Ferreira et al. 2011). In each plot, all trees were classified as asymptomatic, symptomatic or dead using a binary scale (0 = apparently healthy and 1 = diseased, both symptomatic and dead). Unless there was evidence of another cause of mortality, dead trees were considered killed by Ceratocystis wilt.

Five permanent plots (10 × 10 trees) of 100 trees each in plantation MG20G (near the town of Bocaiúva) were assessed 18 times from 20 to 53 months after planting to a former Cerrado forest. Similarly, three permanent plots of 100 trees (10 × 10 trees) in BA8H and four permanent plots of 100 trees (10 × 10 trees) in BA9H were surveyed four times at 34–74 months after planting.

The proportion of diseased trees in the MG20G plots was compared over time using various temporal population growth curve models. Absolute rates of disease progress were calculated for each plot after transformation of the proportion of diseased trees to four population growth curve models in their linearized form: monomolecular ($\ln[1/(1-y)] = \ln[1/(1-y_0)] + r_M t$), exponential ($\ln[y] = \ln(y_0) + r_E t$), logistic ($\ln[y/(1-y)] = \ln[y/(1-y_0)] + r_L t$) and Gompertz ($-\ln[-\ln y] = -\ln[-\ln(y_0)] + r_G t$) (Campbell and Madden 1990), where y_0 is the initial proportion of trees diseased, y is the proportion of trees diseased and t is time; r_M is the rate for the monomolecular model, r_E is the rate for the exponential model, r_L is the rate for the logistic model and r_G is the rate for the Gompertz model. The models were compared and the best model was selected based on the highest coefficient of determination (R^2), statistical significance ($Pr > F$), lowest mean squared error (MSE) and absence of undesirable trends in residuals (Nutter 2007).

Proportions of diseased trees were determined in five plantations (BA11B, BA12B, BA13B, BA14B and BA15B) of clone B in southern Bahia. In each plantation, the cuttings were believed to have been infected in the nursery (Ferreira et al. 2011), and the cuttings were planted on former pastureland. Plantations BA11B and BA12B were assessed once, at 6 months of age, while plantation BA13B was assessed at 12, 18, 25 and 34 months, and plantation BA15B was assessed at 12 and 18 months. Disease levels in plantation BA14B were assessed in randomly distributed, temporary plots at 7 months, and a second set of randomly distributed plots were used for the 33-month assessment.

2.3 Spatial pattern of disease

Binary (presence/absence) spatial maps of diseased trees (symptomatic and killed) were prepared for 42 plots in 25 plantations. The degree of aggregation and the directionality of the aggregation of diseased trees were determined within planting rows, across rows and diagonally by spatial autocorrelation analysis, using the software LCOR2 (Gottwald et al. 1992). The spatial location [x, y] of each tree and the disease status (z) of the plants were used as input data for the analysis. The numbers of contiguous and discontinuous positive spatial lag correlations (SL+) from the lag position [0,0] was estimated for the three directions (within row, across rows and diagonally) with statistical significance levels of $p = 0.01$ and $p = 0.05$ (Campbell and Madden 1990).

3 Results

3.1 Disease incidence and temporal progress

The sampled plantations varied greatly in disease incidence (Table 1). Most of the plots were purposely established in diseased portions of plantations, so it is difficult to compare disease levels. Nonetheless, there was generally higher disease incidence in the Minas Gerais plantations, which were on former Cerrado forests sites (Ferreira et al. 2011). Of the three eucalyptus clones surveyed in Minas Gerais, the highest disease incidence was seen in clone G, which was the only *E. urophylla* × *E. camaldulensis* clone in the study. The plots of stump sprouts of clone C (MG1C-s, MG9C-s and MG10C-s) had higher disease incidence than unharvested eucalyptus plantations of clone C of approximately the same age (Table 1). At 74 months after planting, the unharvested section of plantation MG9C had only a 2.7% disease incidence, but disease incidence was 39.7% in stump sprouts in the area of the plantation that had been harvested at 56 months (Table 1).

The Bahia plantations BA8H and BA9H also had high disease levels (Table 1), and both plantations had natural woody regeneration prior to clearing and planting of clone H (Ferreira et al. 2011). The disease incidence also was relatively high (22.3%) in the 2-yr-old sprouts that came from the stumps created 4 years after planting BA1A-s, which was formerly a small farm (Table 1).

There was a low incidence of disease in the plantations of clone B surveyed at 6 or 7 months (Tables 1 and 2, Fig. 1), but plantations of clone B had higher disease levels at 12 months of age or older. A plantation of stump sprouts (BA10B-s) had disease incidence of 12.8%. Disease incidence was also higher in plantation BA13B (Table 1), which was in its third rotation of eucalyptus and may have been planted to a susceptible eucalyptus clone in previous rotations. Plantations of clone A and D on former pastureland sites had low disease incidence, although the plots were purposely placed in diseased areas of the plantations (Table 1).

Table 2. Disease incidence and spatial autocorrelation analysis of Ceratocystis wilt in 18 plots within eucalyptus plantation BA14B at 7 months or 33 months after planting on a former pasture site at a 3 m × 3 m spacing.

			Autocorrelation proximity pattern of disease distribution[1]		
Plantation age at time of survey (months)	Plot number	Disease incidence (%)	Within rows	Across rows	Diagonal
7	P1	5.0	0	2*	0
	P2	2.8	0	0	0
	P3	4.0	0	0	0
	P4	5.2	0	0	0
	P5	6.0	0	0	0
	P6	3.0	0	0	0
	P7	1.0	0	0	0
	P8	8.8	0	0	0
33	P9	24.4	2*	0	0
	P10	18.8	2**	0	2**
	P11	12.8	2**	0	0
	P12	15.0	2**	0	0
	P13	17.2	0	2**	0
	P14	27.2	0	0	0
	P15	19.6	0	0	0
	P16	2.2	0	0	0
	P17	31.2	0	0	0
	P18	36.2	0	0	0

[1]Number of positive continuous spatial lag positions (SL+) within rows, across rows and diagonally that are contiguous with the origin [0.0] lag in the core cluster. Significant autocorrelation coefficients at the p = 0.05 and p = 0.01 levels are indicated by single and double asterisks, respectively.

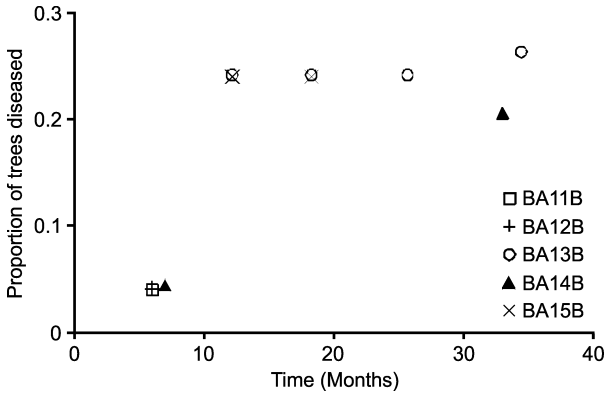

Fig. 1. Proportion of trees diseased in five eucalyptus plantations on former pastureland sites planted with rooted cuttings of clone B that were likely infected with *Ceratocystis fimbriata* in the nursery.

3.2 Disease progress within plantations

In contrast to Bahia plantations, Ceratocystis wilt was not observed in young (<12 months old) plantations in Minas Gerais, and the surveyed plantations there were 33–74 months old (Table 1). In MG20G, disease incidence was low at 20 months and slowly increased to 44.4% at 53 months (Fig. 2a). Just below 10% of the living trees were symptomatic throughout most of this period, but newly killed trees were still evident and the disease incidence still appeared to be increasing at the 53-month assessment. The disease progressed similarly in all five plots within plantation MG20G, although there were some minor differences in disease levels among the plots (Fig. 2b).

When the proportion of symptomatic and dead trees in MG20G was transformed to test for the best fit of various temporal models, each of the four tested growth curve models fit the disease progress data (Table 3). However, the linear monomolecular model provided the best fit for the data, with the R^2 for the five plots ranging from 0.87 to 0.97 (the model explaining 87–97% of the variation in transformed disease incidence over time), the MSE ranging from 0.020 to 0.055 and rates of disease progress ranging from 0.011 to 0.031 units of disease/month. Two Bahia plantations on sites that were formerly wooded, BA8H and BA9H, were each sampled at four times, and the disease levels and progress of disease in these plantations were similar to that in plantation MG20G (Fig. 2c). In both plantations BA8H and BA9H, the incidence of disease still appeared to be increasing at the time of the 74-month assessment.

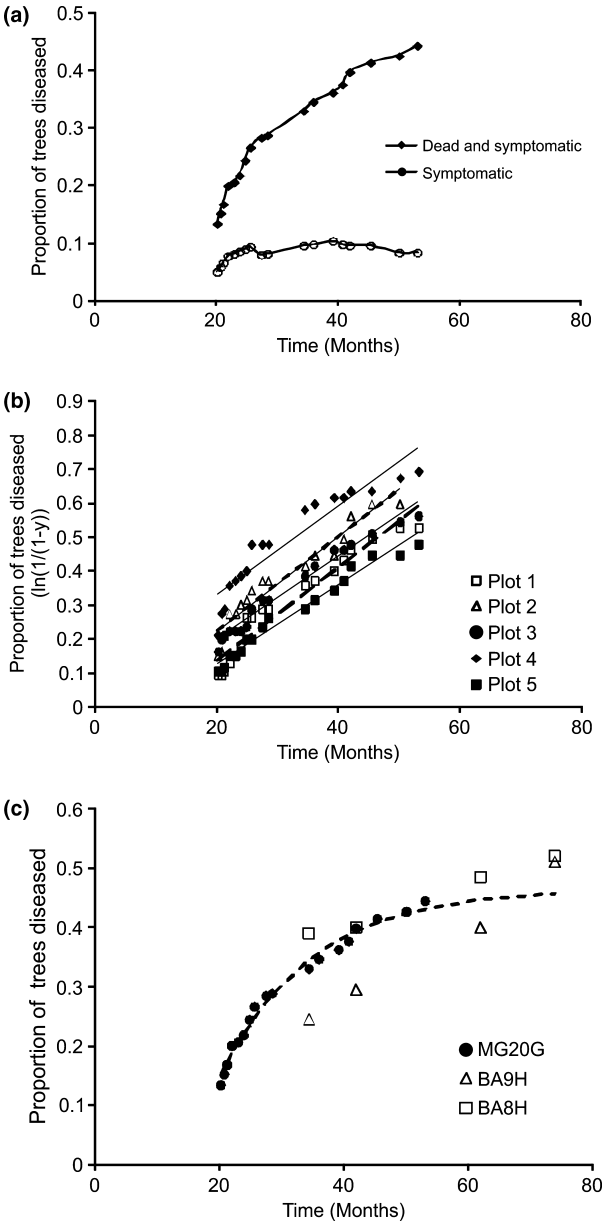

Fig. 2. Proportion of eucalyptus trees diseased after planting on sites with likely soilborne inoculum of *Ceratocystis fimbriata*. (a) Proportion of symptomatic and dead trees in plantation MG20G from 20 to 53 months after planting to a former Cerrado forest site (means of five permanent plots). (b) Linearized (monomolecular model) proportion of total diseased trees in the five plots within plantation MG20G. (c) Proportion of diseased trees in plantation MG20G and two plantations on former pastureland with advanced woody regeneration (BA8H and BA9H). Dashed line indicates estimated values in plantation MG20G following the monomolecular model for linearization.

In contrast to the plantations on formerly wooded sites, the plantations of clone B on former pastureland showed low levels of disease at a relatively young age, but then disease levels rose sharply to a fixed level within 1 year of planting. Plantations BA11B and BA12B had 4% of the trees diseased at 6 months (Table 1). When plotted together, the plots purposely placed in diseased portions of plantations BA11B, BA12B, BA13B and BA15B appeared to reach a maximum disease level of 24% as soon as 12 months after planting (Fig. 1). The randomly placed, temporary plots in plantation BA14B showed variation, with 1–8% of the trees dead or symptomatic at 7 months and up to 36% of the trees dead or symptomatic at 33 months (Table 2).

Plantations BA2A, BA3A, BA4A and BA5A were also on former pastureland, and the plots purposely placed in diseased areas of the plantings had disease incidence of only 1.2–2.9% at 12–19 months after planting (Table 1). Similarly, plantation BA6D on a former pastureland site had a disease incidence of 1.4% at 17 months (Table 1).

Table 3. Summary of regression analysis parameters and statistics for linear transformation of four population growth models (y = proportion of trees diseased) over time (months) of Ceratocystis wilt in five plots of eucalyptus planted to a former Cerrado Forest site (plantation MG20G).

Model[1]	Plot number	R squared[2]	p Value[3]	MSE[4]	y Intercept	Rate[5]
Monomolecular	R1	0.94	<0.0001	0.0391	−0.142	0.014
	R2	0.94	<0.0001	0.0385	−0.061	0.014
	R3	0.97	<0.0001	0.0204	−0.033	0.012
	R4	0.87	<0.0001	0.0554	0.066	0.031
	R5	0.97	<0.0001	0.0225	−0.108	0.012
Logistic	R1	0.82	<0.0001	0.2958	−3.064	0.058
	R2	0.85	<0.0001	0.2039	−2.225	0.044
	R3	0.94	<0.0001	0.1067	−2.314	0.042
	R4	0.79	<0.0001	0.2029	−1.662	0.036
	R5	0.90	<0.0001	0.1852	−2.958	0.051
Exponential	R1	0.78	<0.0001	0.2580	−2.922	0.044
	R2	0.79	<0.0001	0.1680	−2.192	0.030
	R3	0.02	<0.0001	0.6514	−2.266	0.030
	R4	0.73	<0.0001	0.1483	−1.728	0.022
	R5	0.87	<0.0001	0.1652	−2.850	0.040
Gompertz	R1	0.87	<0.0001	0.1270	−1.326	0.030
	R2	0.89	<0.0001	0.0970	−0.994	0.026
	R3	0.96	<0.0001	0.0520	−1.007	0.024
	R4	0.83	<0.0001	11.130	−0.692	0.022
	R5	0.94	<0.0001	0.0760	−1.264	0.026

[1]Linear transformation of population growth models: monomolecular ln[1/(1-y)], logistic = ln[y/(1-y)], exponential = ln(y) and Gompertz's = -ln[-ln(y)] (Campbell and Madden 1990).
[2]Coefficient of determination.
[3]Significance level.
[4]Mean squared error.
[5]Apparent infection rate is the increase in proportion of trees diseased (y) per month after transformation.

3.3 Spatial pattern of Ceratocystis wilt

Plots in Minas Gerais plantations showed some aggregation of diseased trees, especially within rows, if the disease incidence was >6% (Table 1, Fig. 3). The within-row spacing in these plantations was only 2 m, while the between row spacing was 3–6 m, so this could partially explain the generally greater spatial autocorrelation values within rows than across rows or diagonally. In plantation MG9C, the area that had been cut had a high disease incidence in sprouts that arose from the harvested stumps, and MG9C-s had much greater spatial autocorrelation values than the uncut portions of MG9C (Table 1, Fig. 3b). The Bahia plots on former pastureland sites that had advanced woody regeneration (BA8H and BA9H) also had disease trees that were aggregated.

Plantations BA2A through BA5A had very low levels of disease, but the diseased trees were highly aggregated within rows (Table 1). In contrast, the plantation from which the cuttings for these plantations originated (BA1A-s) had a high disease incidence but no statistically significant aggregation of disease (Table 1, Fig. 4).

Most of the other Bahia plantations had low disease incidence and low spatial autocorrelation values (Table 1). The clone B and clone D plantations in Bahia showed generally low levels of aggregation, and although the within row and across row spacings were both 3 m, the aggregation within rows tended to be stronger than across rows, and there was no diagonal aggregation (Table 1). In plantation BA14B, there was little aggregation evident at 7 months, but there was somewhat more aggregation when disease levels were higher at 33 months (Table 2, Fig. 5).

4 Discussion

Some of the highest incidences of disease were found in plantations that probably had high levels of soilborne inoculum from native forest trees or fruits trees that had been cleared before planting eucalyptus. Disease incidence in these plots increased slowly after 20 months, and then the disease progress gradually tapered off near the end of the stand rotation. In contrast, disease incidence in plantations on former pastureland sites was detected as early as 6 months, and disease incidence quickly reached a maximum level at 12 months, suggesting that the rooted cuttings were infected prior to planting. Disease incidence was high in sprouts arising from harvested eucalyptus stumps, suggesting that harvesting tools can spread the pathogen.

There was generally higher incidence of disease in the Minas Gerais plantations, which were formerly Cerrado forests and likely had high levels of soilborne inoculum (Ferreira et al. 2011). Disease levels differed among the Minas Gerais clones, but lack of randomly placed survey plots prevents drawing strong conclusions. Clone G, which was the only *E. urophylla* × *E. camaldulensis* clone surveyed, appeared to be highly susceptible to the disease.

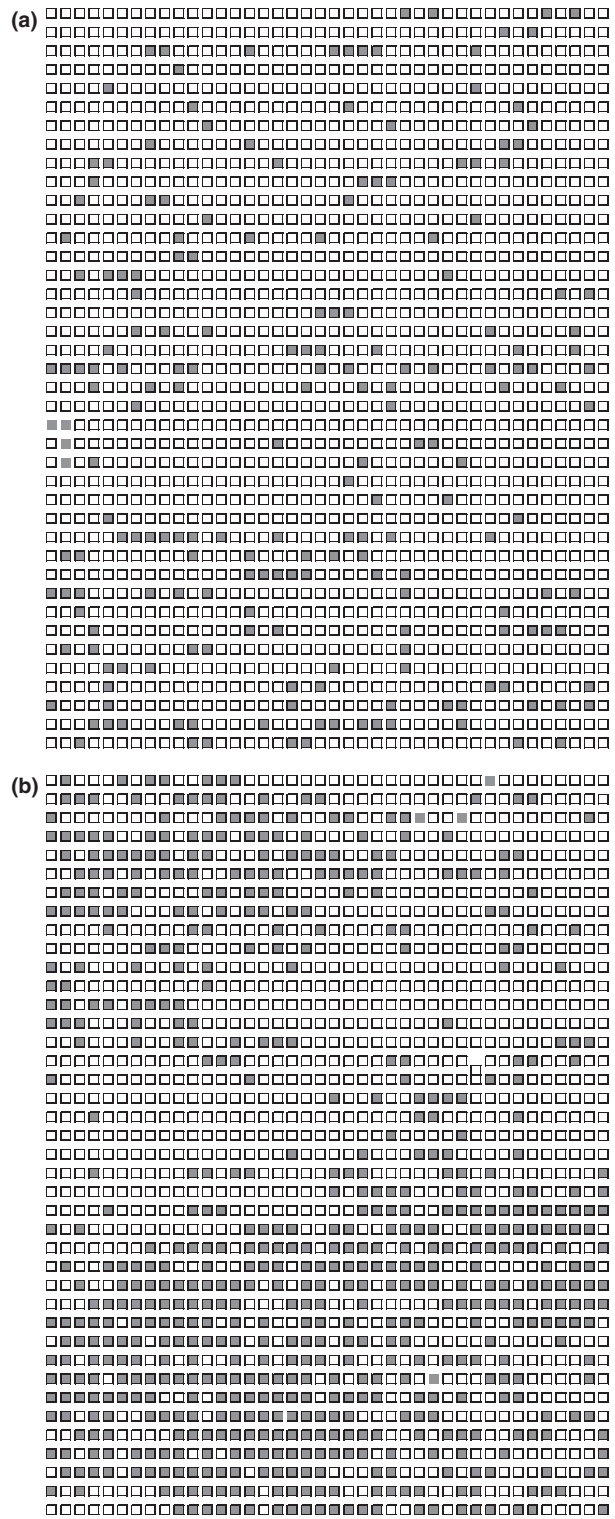

Fig. 3. Distribution of eucalyptus trees killed or with symptoms (filled in squares) of Ceratocystis wilt in plantings of clone C on former Cerrado forest sites at 2 × 3 m spacing (within planting rows × between rows). Horizontal rows in figures were planting rows. (a) Plantation MG2C at 74 months after planting, with significant autocorrelation of diseased trees within planting rows (horizonatally) but not across or diagonally. (b) Plantation MG9C-s, which was harvested after 56 months and stump sprouts managed for another 18 months before surveyed. Diseased trees showed significant autocorrelation within rows, across rows and diagonally (autocorrelation values shown in Table 1).

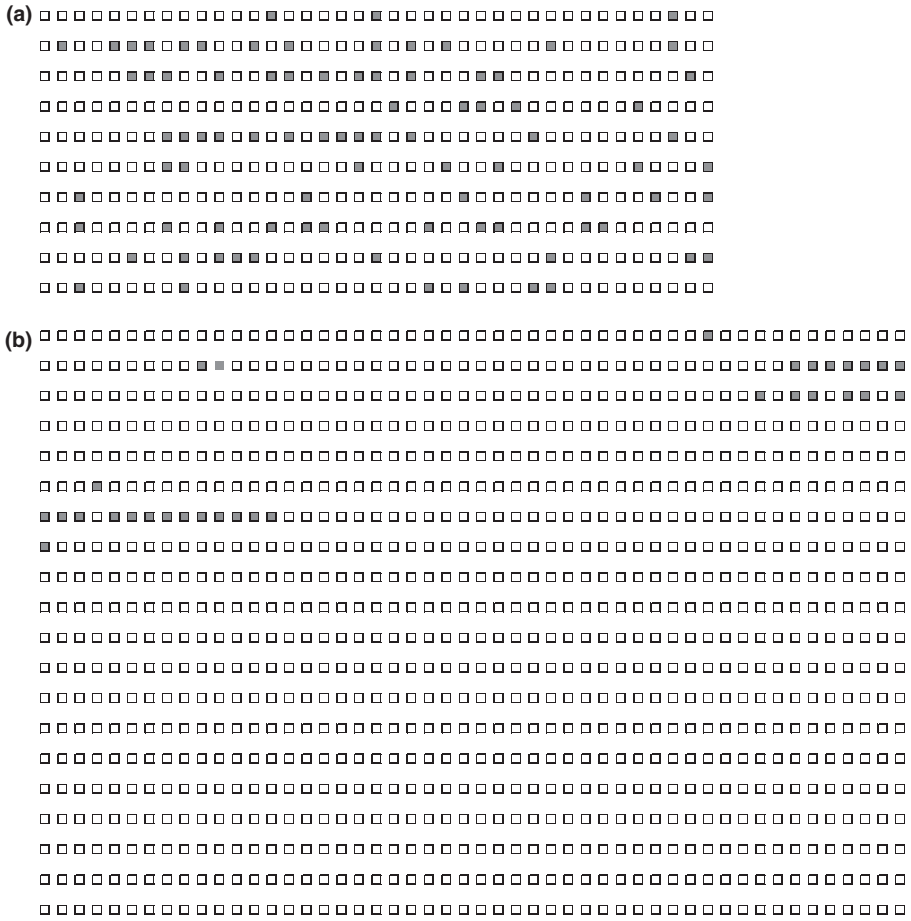

Fig. 4. Distribution of eucalyptus trees killed or with symptoms (filled in squares) of Ceratocystis wilt in plantings of clone A at 2.4 × 5 m spacing (within planting rows × between rows). Horizontal rows in figures were planting rows. (a) Plantation BA1A-s, which was planted on a former small farm site, cut 48 months later after storm damage, and then the sprouts were harvested for rooting in a nursery or managed for another 24 months before surveyed for disease. There was no significant autocorrelation of diseased trees. (b) Plantation BA2A, which was a former pastureland before planting with infected cuttings collected from plantation BA1A-s and surveyed after 17 months. Diseased trees showed significant autocorrelation within rows, across rows and diagonally (autocorrelation values shown in Table 1).

In general, Ceratocystis wilt has not been noted in young plantations in Minas Gerais, probably because most trees there are infected by soilborne inoculum, and some time is needed for the fungus to infect the root systems and colonize the tree sufficiently to cause foliar symptoms and mortality. This incubation period was estimated to be about 18–20 months after planting by extrapolating the disease progress curve data from plantation MG20G. In this plantation, the number of dead trees increased steadily at first and then tapered off, with the disease progress data most closely fitting a monomolecular model. The epidemics in plantations BA8H and BA9H also fit closely the monomolecular model for the MG20G epidemic. The disease level in those Bahia plantations still appeared to be increasing at the age of the last samplings, at 74 months.

Soilborne diseases are typically monocyclic, (Gilligan 1983; Campbell 1986; Campbell and Madden 1990; Madden et al. 2007; Nutter 2007), where there is little secondary spread of the pathogen during the rotation of the stand. In such diseases, there is typically a fixed initial level of inoculum at the time of planting, and root infections increase steadily at a simple interest rate as root systems grow and encounter inoculum. However, as a higher proportion of trees are infected, more root infections may lead to only modest increases in the proportion of diseased trees, and the number of newly infected and symptomatic trees decreases steadily when most of the trees are already infected.

Of the survey plots in Minas Gerais plantations and the Bahia plantations BA8H and BA9H, all but two showed some aggregation of diseased trees. Plots in MG9C and MG14I showed the lowest disease incidence in Minas Gerais, 3 and 6% infection, respectively, and no aggregation was detected. The aggregation found in other plots was likely due to non-random distribution of soilborne inoculum prior to planting (Campbell and Noe 1985), perhaps with high concentrations of inoculum in the vicinity of cleared trees that had been infected by *C. fimbriata*. It is assumed that soilborne inoculum is in the form of aleurioconidia in sawdust or the frass of wood-boring insects (Harrington 2013). Aggregated patterns are more typically associated with local sources of inoculum and limited dispersal, whereas random patterns often result when the

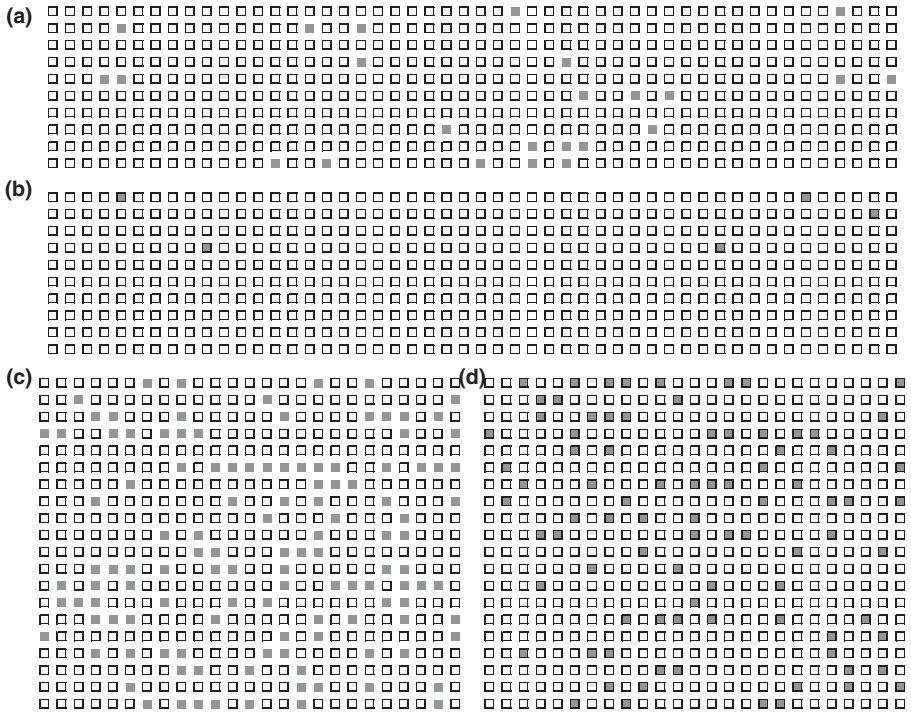

Fig. 5. Distribution of eucalyptus trees killed or with symptoms (filled in squares) of Ceratocystis wilt in plots of plantation BA14B of clone B at 3 × 3 m spacing (within planting rows × between rows) on former pastureland. Horizontal rows in figures were planting rows. (a, b) Plots P1 and P7 at 7 months after planting. (c, d) Plots P9 and P13 at 33 months after planting. Diseased trees showed significant autocorrelation across rows in P1 and P13 and within planting row in P9 (autocorrelation values shown in Table 2).

initial inoculum reaches a population of plants from a distant source (Burdon 1987). Aggregation in plots with putative soilborne inoculum was particularly strong within rows, perhaps because the trees were more closely spaced within rows than across rows or diagonally, but mechanical site preparations prior to planting may have also dispersed inoculum within rows.

Portions of plantations in Minas Gerais and Bahia that had been harvested and then managed for sprouts had relatively high levels of disease. Plantations of clones C (MG1C-s, MG9C-s and MG10C-s) and A (BA1A-s) were managed for coppice growth (a second rotation of trees from stump sprouts) or for generation of cuttings that could be brought to the nursery for rooting. Aleurioconidia in sawdust from harvested trees may be moved on tools to nearby stumps during harvest, and sprouts from those stumps may become infected. Although there is little experimental evidence of spread of *C. fimbriata* on cutting tools, such spread is known to be important with closely related species, such as *C. platani*, *C. cacaofunesta* and *C. variospora*, and likely is important in the epidemiology of Ceratocystis wilt in eucalyptus (Harrington 2013).

In contrast to the plantations where there was likely soilborne inoculum and disease was not evident until 18 months after planting, the plantations of clones A, B and D on former pastureland sites had symptomatic trees as soon as 6 months after planting. This relatively quick disease development is likely due to the infection of the cuttings prior to planting, and mortality may begin in weeks or months after planting. Isolates of *C. fimbriata* from some of these plantations were analysed genetically and found to be nearly uniform, suggesting that one or a few genotypes from the nursery were infecting the planting material (Ferreira et al. 2011). The infected cuttings may be expected to show symptoms and die early, with few surviving beyond 12 months, especially if it is a highly susceptible clone (Ferreira et al. 2011). In the case of cuttings of clone B, some symptomatic plants were seen at 6 months, but by 12 months, about 24% of the young trees were dead, and disease incidence was no higher at 36 months. Disease incidence in the plantings of clones A and D were much lower than in clone B, and low levels of disease might be expected in plantings of infected cuttings. However, clone B is highly susceptible to Ceratocystis wilt, and there appears to be a high level of aggressive genotypes in nurseries where clone B is produced (Ferreira et al. 2011; Harrington et al. 2011).

In spite of relatively low disease incidence, there was significant within-row aggregation of diseased trees in most of the plantations on former pastureland sites. All but one of the former pastureland plantations (BA11B) had aggregation within the planting row. In plantation BA14B, there was little aggregation evident at 7 months, but there was evidence of somewhat more aggregation when disease levels were higher at 33 months. One possible explanation for within-row aggregation on former pastureland sites is that as the planters moved down the row, they sometimes had bunches of rooted cuttings that were infected by *C. fimbriata*, and this resulted in a high incidence of the disease within some rows. During the process of clonal propagation, bunches of infected cuttings could be collected and maintained together from the time of collection from a diseased mother plant, through the rooting process, and into the boxes dispatched to the planters. Also,

bunches of infected cuttings could arise through mechanical transmission of the pathogen on scissors and other tools. Thus, infected cuttings could be planted in a linear fashion as the planter moves down a planting row.

The results of this study have several management implications. Where sites are cleared of diseased, woody vegetation and there may be high levels of soilborne inoculum, planting of resistant clones may be the only feasible way to manage Ceratocystis wilt (Ribeiro et al. 1984; Rossetto et al. 1997; Zauza et al. 2004; Sanches et al. 2008; Alfenas et al. 2009; Guimarães et al. 2010). Eucalyptus clones vary greatly in susceptibility to Ceratocystis wilt, but resistance and susceptibility depends on the particular strains of the pathogen tested, which vary greatly in aggressiveness, and selection of resistant clones may be site specific (Zauza et al. 2004; Guimarães et al. 2010; Harrington et al. 2011; Mafia et al. 2011). Secondly, the dramatic increase in disease levels in sprouts from stumps of harvested trees means that diseased stands cannot be managed for coppice growth or as a source of cuttings, although it is not known if there is a build-up of soilborne inoculum after harvesting diseased eucalyptus plantations (Ferreira et al. 2011). Lastly, care must be taken to plant disease-free material, as there is potential for high levels of mortality early in the planting, and there is also a risk of the introduction and establishment of soilborne inoculum in new areas (Ferreira et al. 2011; Harrington et al. 2011). Disease-free mini-hedges are considered to be the best source of cuttings, but Ceratocystis wilt is often overlooked in the nursery, and cutting tools there can also be contaminated with the fungus (Ferreira et al. 2011). Thus, disease monitoring and disinfecting tools are another part of the integrated management of Ceratocystis wilt (Harrington 2013).

Acknowledgements

This study was funded by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPEMIG (Fundação de Amparo a Pesquisa no Estado de Minas Gerais) and SIF-Empresas (Sociedade de Investigações Florestais). We thank Daniel Henrique Breda Binoti, who provided invaluable assistance in collection of data, and Forrest W. Nutter, Jr., who provided many helpful comments on data analysis.

References

ABRAF, Associação Brasileira de Produtores de Florestas Plantadas, 2012: No base 2011. Brasília: Anuário Estatístico da ABRAF.

Alfenas, A. C.; Ferreira, E. M., 2008: Emerging diseases in eucalyptus plantations. Trop. Plant Pathol. **33**, S25–S28.

Alfenas, A. C.; Zauza, E. A. V.; Mafia, R. G.; Assis, T. F., 2009: Clonagem e doenças do eucalipto, 2nd ed. Viçosa, Minas Gerais, Brazil: Editora UFV. 500p.

Baker, C. J.; Harrington, T. C.; Kraus, U.; Alfenas, A. C., 2003: Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. Phytopathology **93**, 1274–1284.

Barnes, I.; Roux, J.; Wingfield, B. D.; O'Neill, M.; Wingfield, M. J., 2003: *Ceratocystis fimbriata* infecting *Eucalyptus grandis* in Uruguay. Australas. Plant Pathol. **32**, 361–366.

Burdon, J. J., 1987: Diseases and plant population biology. Cambridge: Cambridge University Press.

Campbell, C. L., 1986: Interpretation and uses of disease progress curves for root diseases. In: Population dynamics and management. Ed. by Leonard, K. J.; Fry, W. E. New York: Macmillan Publishing Company, pp. 38–54.

Campbell, C. L.; Madden, L. V., 1990: Introduction to plant disease epidemiology. New York: John Willey & Sons.

Campbell, C. L.; Noe, J. P., 1985: The spatial analysis of soilborne pathogens and root diseases. Annu. Rev. Phytopathol. **23**, 129–148.

Ferreira, F. A.; Demuner, A. M.; Demuner, N. L.; Pigatto, S., 1999: Murcha-de-*Ceratocystis* em eucalipto no Brasil. Fitopatol. Bras. **24**, 284.

Ferreira, F. A.; Maffia, L. A.; Barreto, R. W.; Demuner, N. L.; Pigatto, S., 2006: Sintomatologia da murcha de *Ceratocystis fimbriata* em eucalipto. Rev. Árv. **30**, 155–162.

Ferreira, E. M.; Alfenas, A. C.; Binoti, D. H. B.; Machado, P. S.; Santos, C. A. G. dos., 2007: Influência da murcha-de-ceratocystis sobre o crescimento de um clone híbrido de *Eucalyptus* spp. Fitopatol. Bras. **32**, S216.

Ferreira, E. M.; Harrington, T. C.; Thorpe, D. J.; Alfenas, A. C., 2010: Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. Plant. Pathol. **59**, 721–735.

Ferreira, M. A.; Harrington, T. C.; Alfenas, A. C.; Mitzubuti, E. S. G., 2011: Movement of genotypes of *Ceratocystis fimbriata* within and among *Eucalyptus* plantations in Brazil. Phytopathology **101**, 1005–1012.

Gilligan, C. A., 1983: Modeling of soil-borne pathogens. Annu Rev. Phytopath. **21**, 45–64.

Gottwald, T. R.; Richie, S. M.; Campbell, C. L., 1992: LCOR2 – Spatial correlation analysis software for the personal computer. Plant Dis. **76**, 213–215.

Guimarães, L. M. S.; Titon, M.; Lau, D.; Rosse, L. N.; Oliveira, L. S. S.; Rosado, C. C. G.; Christo, G. G. O.; Alfenas, A. C., 2010: *Eucalyptus pellita* as a source of resistance to rust, Ceratocystis wilt and leaf blight. Crop Breed. Appl. Biotechnol. **10**, 124–131.

Harrington, T. C. 2013: Ceratocystis diseases. In: Infectious forest diseases. Ed. by Gonthier, P.; Nicolotti, G. Wallingford, UK: CAB International, (in press).

Harrington, T. C.; Thorpe, D. J.; Alfenas, A. C., 2011: Genetic variation and variation in aggressiveness to native and exotic hosts among Brazilian populations of *Ceratocystis fimbriata*. Phytopathology **101**, 555–566.

Heath, R. N.; Wingfield, M. J.; van Wyk, M.; Roux, J., 2009: Insect associates of *Ceratocystis albifundus* and patterns of association in a native savanna ecosystem in South Africa. Environ. Entomol. **38**, 356–64.

Madden, L. V.; Hughes, G.; Van Den Bosch, F., 2007: The study of plant disease epidemics. St. Paul: APS.

Mafia, R. G.; Alfenas, A. C.; Ferreira, E. M.; Binoti, D. H. B., 2011: Método de seleção e identificação de fontes de resistência à murcha do eucalipto causada por *Ceratocystis fimbriata*. Rev. Árv. **35**, 817–824.

Nkuekam, G. K.; Wingfield, J. M.; Mohammed, C.; Carnegie, A. G.; Pegg, G. S.; Roux, J., 2012: *Ceratocystis* species, including two new species associated with nitidulid beetles, on eucalypts in Australia. Antonie Van Leeuwenhoek **101**, 217–241.

Nutter, F. W. Jr, 2007: The role of plant disease epidemiology in developing successful integrated disease management programs. In: General concepts in integrated pest and disease management. Ed. by Ciancio, A.; Mukerji, K. G. Springer: Netherlands, pp. 45–79.

Ribeiro, I. J. A.; Lourencao, A. L.; Paradela Filho, O.; Soares, N. B., 1984: Seca da mangueira: VII. Resistência de cultivares de mangueira ao fungo Ceratocystis fimbriata Ell. & Halst. Bragantia **43**, 237–243.

Rossetto, C. J.; Ribeiro, I. J. A.; Gallo, P. B.; Soares, N. B.; Sabino, J. C.; Martins, A. L. M.; Bortoletto, N.; Paulo, E. M., 1997: Mango breeding for resistance to diseases and pests. Acta Hortc. **455**, 299–304.

Roux, J.; Wingfield, M. J.; Bouillet, J. P.; Wingfield, B. D.; Alfenas, A. C., 2000: A serious new wilt disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. For. Pathol. **30**, 175–184.

Sanches, C. L. G.; Pinto, L. R. M.; Pomella, A. W. V.; Silva, S. D. V. M.; Loguercio, L. L., 2008: Assessment of resistance to *Ceratocystis cacaofunesta* in cacao genotypes. Eur. J. Plant Pathol. **122**, 517–528.

Thorpe, D. J.; Harrington, T. C.; Uchida, J. Y., 2005: Pathogenicity, phylogenetics and human dispersal of *Ceratocystis fimbriata* on the family Araceae. Phytopathology **95**, 316–323.

van Wyk, M.; van der Merwe, N. A.; Roux, J.; Wingfield, B. D.; Kamgan, G. N.; Wingfield, M. J., 2006: Population genetic analyses suggest that the Eucalyptus pathogen *Ceratocystis fimbriata* has been introduced into South Africa. S. Afr. J. Sci. **102**, 259–263.

van Wyk, M.; Roux, J.; Nkuekam, G. K.; Wingfield, B. D.; Wingfield, M. J., 2012: *Ceratocystis eucalypticola* sp. nov. from *Eucalyptus* in South Africa and comparison to global isolates from this tree. IMA fungus **3**, 45–58.

Zauza, E. A. V.; Alfenas, A. C.; Harrington, T. C.; Mizubuti, E. S.; Silva, J. F., 2004: Resistance of Eucalyptus clones to *Ceratocystis fimbriata*. Plant Dis. **88**, 758–760.