Diversity in *Eucalyptus* susceptibility to the gall-forming wasp *Leptocybe invasa*

Gudrun Dittrich-Schröder[1, 2], Michael J. Wingfield[1], Brett P. Hurley[1, 2] and Bernard Slippers[1,3]

[1] *Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, 0002, South Africa*

[2] *Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002, South Africa*

[3] *Department of Genetics, University of Pretoria, Pretoria, 0002, South Africa*

Abstract

1 Extensive variation to damage by the invasive gall-forming wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae) is known to exist amongst *Eucalyptus* genotypes.

2 In this study, thirty of the fifty tested genotypes were susceptible to gall formation and development of the wasp. Gall development on the petiole and leaves of plants was compared to calculate percentage of infestation per plant and per genotype.

3 A positive correlation between galls on petioles and leaves indicated an absence of specificity at this level and that either leaves or petioles could be used to obtain an accurate estimate of the level of infestation.

4 Genotypes of *E. nitens x E. grandis* and *E. grandis* x *E. camaldulensis* were most susceptible with the maximum damage index value for leaves and petioles being 0.52 and 0.39, respectively. *Eucalyptus dunii, E. nitens, E. smithii, E. urophylla* and *E. saligna* x *E. urophylla* showed little or no infestation.

5 The results suggest that selection and planting of resistant / less susceptible genotypes will be an important aid in managing damage from the *L. invasa* invasion.

Keywords: Invasive pest, forest entomology, genotypic resistance, Hymenoptera, Eulophidae

Correspondence: Bernard Slippers, Tel: +27124205822; Fax: +27124203960; E-mail: Bernard.Slippers@fabi.up.ac.za

Running title: *Eucalyptus* susceptibility to *Leptocybe invasa*

Introduction

Eucalyptus plantations in South Africa and other parts of the world have recently become threatened by the invasive gall-forming wasp, *Leptocybe invasa* Fisher & LaSalle (Hymenoptera: Eulophidae) (Mendel *et al*., 2004). *Leptocybe invasa* was first discovered on species of *Eucalyptus* in the Middle East and Mediterranean region in 2000 (Mendel *et al*., 2004). This wasp is native to Australia, but it was only found there after it infested trees in introduced environments (Mendel *et al*., 2004). Since the initial reports, the wasp has spread extremely rapidly and it now occurs in the *Eucalyptus* planting areas of the Mediterranean basin, southern Europe, southern Asia from Iraq to India and Vietnam, and parts of northern, eastern and southern Africa and South America (Mendel *et al*., 2004; Basavana Goud *et al*., 2010; Thu *et al.,* 2010; Nyeko *et al*., 2010; Wilken *et al*., 2010). *Leptocybe invasa* was first reported in South Africa in 2007 (Neser *et al.* 2007).

Leptocybe invasa is not a pest in its native environment, Australia, but *Eucalyptus* plantations in other countries have experienced significant damage (Nyeko *et al*., 2010; Thu *et al.,* 2010; Basavana Goud *et al*., 2010). *Leptocybe invasa* attacks new growth of all ages of *Eucalyptus*, including nursery stock (Mendel *et al*., 2004). Galling occurs on the petioles and leaves (mainly mid-ribs) of trees, causing leaf-curl and early senescence of the leaves (Mendel *et al*., 2004). Heavy galling causes malformation and stunted growth of trees and in extreme cases, tree death (Mendel *et al*., 2004). Infestations by *L. invasa* in its introduced range affect the productivity of commercial *Eucalyptus* plantations, ultimately adversely affecting the revenue generated from the forestry sector.

Various strategies are being pursued for the management of *L. invasa* in its introduced range. Basavana Goud *et al* (2010) and Kulkarni (2010b) showed that chemical control is generally ineffective to control the pest. However, biological control is a preferred strategy and it has shown much promise. For example, the parasitic wasps, *Quadrastichus mendeli* Kim & La Salle (Hymenoptera: Eulophidae) and *Selitrichodes kryceri* Kim & La Salle (Hymenoptera: Eulophidae) have been introduced to Israel from Australia in an effort to control *L. invasa* in the Mediterranean region (Kim *et al*., 2008). An Australian *Megastigmus* species (Hymenoptera: Torymidae) (Doğanlar & Hassan, 2010), two *Megastigmus* species native to Israel and Turkey (Protasov *et al*., 2008), and a range of parasitoids native to India (Kulkarni *et al* 2010a), have also been used. Detailed work on the biology of *S. kryceri* and *Q. mendeli* has shown parasitism levels of 52 % and 73 %, respectively (Kim *et al*., 2008).

Other than biological control, planting *Eucalyptus* material resistant to *L. invasa* represents an additional option for an integrated management strategy. This is based on the fact that variation in susceptibility between *Eucalyptus* genotypes to infestation by *L. invasa* has been shown in various studies. Mendel *et al.* (2004) stated that *E. camaldulensis* and other members of the Exsertaria section were most susceptible. The list of susceptible and resistant genotypes has since been expanded by studies in other countries (Nyeko *et al*., 2005; Thu *et al*., 2009; Javaregowda & Prabhu, 2010). These studies have shown variation between *Eucalyptus* genotypes, but interestingly, also within certain genotypes. Thus, the potential exists to use host resistance, together with biological control and other control methods, in an integrated approach to reduce the impact of *L. invasa*.

In this study we examined the phenomenon of variation in susceptibility within *Eucalyptus* genotypes to infestation by *L. invasa*, which has not been quantified in previous trials. This is done in a South African context by considering the extent to which the currently planted genotypes will be susceptible to *L. invasa* through a representative set of genotypes.

In the process, the study also identifies potentially resistant or less susceptible genotypes that could be planted in the future.

Materials and Methods

Study location and plant material

The study was conducted at the Forestry and Agricultural Biotechnology Institute (FABI) nursery, University of Pretoria, Pretoria, South Africa (S 025° 45.155' E 028° 15.386'). *Leptocybe invasa* was recorded at the FABI nursery in 2008 and a natural population of *L. invasa* has since become established at the nursery. Fifty *Eucalyptus* genotypes from five different species and five different hybrids were used. These genotypes were supplied by South African forestry companies. A clone of the *E. grandis* x *E. camaldulensis* hybrid (GC 540) which was known from previous work to be highly susceptible to *L. invasa* (Nyeko *et al*. 2010) was considered as a positive control.

Plants were 30 – 50 cm in height with approximately 16 – 127 leaves (depending on the clone – some clones have many smaller leaves whereas others have few but larger leaves) were established in 5 litre plastic bags in potting medium and placed outside under hail netting to allow natural infestation by *L. invasa*. The plants were exposed to *L. invasa* from October 2009 to April 2010. This time period was specifically chosen to ensure that *L. invasa* would complete its life cycle (approximately 132 days) (Mendel *et al*., 2004).

Trial layout

The trial consisted of 50 treatments (*Eucalyptus* genotypes), with 14 replicates of each treatment, and 700 plants in total. A randomised block design with fourteen blocks was used. The blocks were stratified by space (seven different positions in the nursery) and edge effect (outer and inner 'block' for each position) (Figure 1). Each of the blocks was separately

randomized using random numbers without replacement. Five litre potting bags with sand were used as spacers between the plants to reduce crowding and ensure that each plant was accessible to *L. invasa*.

Data capture and statistical analyses

Every plant was scored for damage by *L. invasa*. Only two methods were used to score damage with both assessments occurring on the same day. In one assessment the number of leaves on each plant that had galls on the mid-ribs was scored. In the other assessment, the number of leaves on each plant with galled petioles was quantified. The number of galled leaves and petioles were recorded as a percentage of the total leaves on the plants. A damage index was calculated for leaves and petioles as the product of incidence (proportion of plants infested) and mean severity (percentage infestation / 100). Research conducted by Nyeko *et al*. (2010) showed that there was a strong positive correlation between the number of galls and the damage index eliminating the need to count individual galls.

Due to the large number of zero values in the data an integer of one was added to the data to enable it to be log transformed. A t-test was used to test for significance between the level of infestation of the leaves and petioles of the 50 genotypes resulting in a table containing p-values for the pairwise least squares means (LS mean). If the p-values for the model, p-values for the effect and R-squared values were less than or equal to 0.05 the standard error p-values were used to determine significance. A generalized linear model (GLM) analysis was used to compare the percentage of galled leaves and galled petioles between treatments, between outer and inner blocks and between blocks. Clones where seven or more replicate plants showed no galling were discarded from the analysis to decrease the zero count in the data set. Twenty-one clones remained for analysis by means of the GLM. The residuals from the transformed data of the 21 clones showed acceptable symmetrical

distribution to continue with the GLM. A Kendall Tau correlation coefficient was calculated to examine the interaction between percentage infestation of the leaves and percentage infestation of the petioles. SAS version 8.2 (SAS Institute, 2001) was used for all statistical analyses. The 21 genotypes that showed damage were presented in a tabular form to indicate whether the levels of damage were significantly different.

Results

Originally 50 genotypes were included in the study. During data collection identities of clones were verified. For one clone the genetic identity was unknown and this clones' data was thus excluded from the results. Therefore results of forty-nine and twenty clones were presented. Twenty-two of the forty-nine *Eucalyptus* genotypes (44.9 %) were susceptible to some degree to gall formation, on the leaves and petioles, induced by *L. invasa* (Tables 1 and 2). A significant correlation between percentage infestation of leaves and percentage infestation of petioles was observed ($r^2 = 0.66$; $P < 0.0001$). The position of the genotype in the nursery or whether it was in an inner or outer block did not significantly affect infestation levels (petioles: $P = 0.38$, $F_{6, 246} = 1.07$; $P = 0.46$, $F_{1, 246} = 0.55$ respectively; leaves: $P = 0.17$, $F_{6, 246} = 1.52$; $P = 0.82$, $F_{1, 246} = 0.05$ respectively). Significant differences were, however, observed between different *Eucalyptus* genotypes ($p < 0.0001$ for both petioles and leaves; for the selected 21 genotypes analysed after eliminating clones with 0 – 6 plants showing galls).

There were significant differences in infestation of genotypes both between and within *Eucalyptus* hybrids and species (Figure 2, Tables 1, 2, 3 and 4). The damage index for both the petioles and leaves showed that *E. nitens* x *E. grandis* (genotypes 36 – 39) were the most heavily infested, followed by *E. grandis* x *E. camaldulensis* (genotypes 7 – 15) (Tables 1 and 2). The incidence of infestation on *E. nitens* x *E. grandis* (genotypes 36 – 39) was 100 % for all except genotype 38 where the incidence value was 0.93 for the petioles. The damage index

was more variable amongst *E. grandis* x *E. camaldulensis* (genotypes 7 – 15) genotypes. The damage index within genotypes of this hybrid ranged from 0 – 0.27 (petioles) and 0 – 0.37 leaves (Tables 1 and 2).

The genotypes *E. grandis* x *E. nitens* (genotypes 16 – 20), *E. grandis* x *E. urophylla* (genotypes 1 – 4, 6, 21 – 33) and *E. saligna x E. urophylla* (genotype 50) showed lower levels of susceptibility to *L. invasa* than *E. nitens* x *E. grandis* (genotypes 36 – 39) and *E. grandis* x *E. camaldulensis* (genotypes 7 – 15) (Figure 2). Of all the *E. grandis* x *E. nitens* (genotypes 16 – 20) and *E. grandis* x *E. urophylla* genotypes (genotypes 1 – 4, 6, 21 – 33), only *E. grandis* x *E. urophylla* 27 was not significantly less susceptible to all the *E. nitens x E. grandis* (genotype 36 – 39) and the more susceptible *E. grandis* x *E. camaldulensis* genotypes (genotypes 7, 8 and 12) (Table 3 and 4). Four of five and 16 of 18 genotypes showed little to no infestation by *L. invasa* for the *E. grandis* x *E. nitens* (genotypes 16 – 20) and *E. grandis* x *E. urophylla* (genotypes 1 – 4, 6, 21 – 33) hybrids respectively (Figure 2, Table 1 and 2). The *E. saligna* x *E. urophylla* genotype 50 showed no infestation to *L. invasa*.

Of the presumably pure *Eucalyptus* species tested, *E. grandis* (genotypes 44 – 49), *E. dunii* (genotypes 40 – 41), *E. nitens* (genotype 43), *E. smithii* (genotype 42) and *E. urophylla* (genotype 5 & 35), all except *E. grandis* (genotypes 44 – 49), showed little to no gall formation (Figure 2, Table 1 and 2). Gall formation on *E. grandis* (genotypes 44 – 49) genotypes ranged from nil to moderate, with the most susceptible genotype having a damage index of 0.19 (petioles) and 0.12 (leaves). The *E. dunii* (genotypes 40 – 41) and *E. nitens* (genotype 43) genotypes showed no infestation, and only slight infestation was observed on the *E. smithii* (genotype 42) and *E. urophylla* (genotype 5 & 35) tested (Figure 2, Table 1 and 2).

Discussion

This study clearly showed that resistance in *Eucalyptus* planting material has much potential to reduce damage by invasive populations of *L. invasa.* Amongst the 49 genotypes tested there was significant variation in susceptibility to *L. invasa*. This finding is of considerable importance to commercial *Eucalyptus* forestry around the world.

Of the genotypes tested, *E. nitens* x *E. grandis* and *E. grandis* x *E. camaldulensis* were the most susceptible to attack by *L. invasa*. Similar results were displayed on *Eucalyptus grandis* in Vietnam where high levels of infestation were observed (Thu *et al*., 2009). Moderate to high levels of susceptibility were observed in South Africa, Kenya and Uganda (Nyeko *et al*., 2010) on *E. grandis* x *E. camaldulensis* genotypes.

Genotypes of *E. saligna* x *E. urophylla, E. grandis* x *E. urophylla* and the species *E. dunii*, *E. nitens*, *E. smithii* and *E. urophylla* showed lower susceptibility to *L. invasa* than *E. grandis* (genotypes 44 – 49)*, E. grandis* x *E. camaldulensis, E. grandis* x *E. nitens,* and *E. nitens* x *E. grandis*, although there was variation in susceptibility. Some of these genotypes have also been previously shown to be resistant, or at least tolerant, to *L. invasa*. For example, in Vietnam *E. smithii* and *E. urophylla* showed low susceptibility in the nursery and field respectively (Thu *et al*., 2009). Our study showed less than 5% infestation and a damage index of less than 0.1 for *E. smithii* and *E. urophylla.* Basavana Goud *et al* (2010) also reported that *E. urophylla* in India showed little damage or only damage after oviposition. A similar result was recorded for *E. urophylla* clones in Kenya (Nyeko *et al*., 2010).

In South Africa, *Eucalyptus* genotypes are commonly made between *E. grandis* and *E. camaldulensis, E. urophylla* or *E. tereticornis* (Denison & Kietzka, 1993). The commercial use of *Eucalyptus* genotypes is also increasing due to their many favourable characteristics (Denison & Kietzka, 1993). These characteristics include adaptation to particular sites and an ability to select for tolerance to pests and diseases, as well as a range of climatic variables

(Denison & Kietzka, 1993). Selection of resistant genotypes thus provides a potential opportunity to reduce the damage due to *L. invasa.*

Of particular interest and importance is the variation of susceptibility within genotypes of *Eucalyptus*. No genotypes selected from the cross between *E. nitens* x *E. grandis* or *E. grandis x E. camaldulensis* were equally susceptible. Similarly, although most *E. grandis* x *E. urophylla* and *E. grandis* x *E. nitens* genotypes included in this trial were not susceptible, three of the twenty-three genotypes showed relatively high levels of susceptibility. This variation in susceptibility illustrates the fact that there is a multiplicity of possible combinations arising from hybridisation between species and that these do not necessarily reflect the broad susceptibility. Thus, every genotype will likely have to be screened for resistance prior to commercial deployment.

In this study, genotypes of the hybrid *E. nitens* x *E. grandis* showed more than twice the percent infestation than plants representing the *E. grandis* group. This would suggest that genotypes resulting from a cross where the one parent (pure species, in this instance the *E. nitens* parent) shows high levels of susceptibility may show reduced levels of susceptibility when crossed with a less susceptible species (such as *E. grandis*). Fritz (1999) suggested that the level of susceptibility or resistance of a genotype is determined by which parent is dominant in the genotype. Should the genotype be similar to the parent, a susceptible parent would yield a genotype, which is dominant for susceptibility and a resistant parent would yield a genotype, which is dominant for resistance (Fritz, 1999). In most instances the parent that is susceptible is dominant in the cross resulting in a susceptible genotype. Most commonly, the susceptible trait is dominant, as seen in studies on moths, scale insects, bruchid weevils, leaf beetles and adelgids (Fritz, 1999). Paige & Capman (1993) and Fritz *et al* (1996) showed that dominance of resistance traits is a rare occurrence.

An interesting trend was observed when comparing the groups of trees in terms of their genetic make up and the level of infestation. Genotypes of the hybrids of *E. grandis x E. camaldulensis* generally showed higher levels of infestation when compared to the *E. grandis* group. This could possibly indicate that the *E. camaldulensis* component is a driving factor in the susceptibility in the genotype of *E. grandis* x *E. camaldulensis*. This is substantiated by research conducted in India, Israel, Kenya, Uganda and Vietnam (Table 5) where *E. camaldulensis* or its genotypes were amongst the most susceptible to *L. invasa* infestation.

The resistance of a particular genotype can be influenced by surrounding environmental factors (Maddox & Cappuccino, 1986). This is evident from work conducted by Mutitu *et al*. (2007) where the susceptibility of *E. grandis* trees to infestation by *L. invasa* differed depending on whether they were planted in low or moderate / high rainfall areas. Caution is required when extending nursery trial results from one location to various locations in the field, as environmental factors may differ substantially. In addition, faster growing genotypes, at the time of peak emergence and oviposition of *L. invasa*, are potentially more susceptible to gall-formation, as they provide an abundance of new growth and thereby greater success of gall formation (Anderson *et al.,* 1989). Tree age may also influence susceptibility as demonstrated by Thu *et al* (2009) who showed that nursery seedlings were more susceptible to damage by *L. invasa* than plants older than two years of age.

It is unknown which cues are utilised by *L. invasa* to detect its host. Examination of plants used in this experiment showed oviposition scarring on all plants irrespective of genotype. Not all genotypes used in the experiment were suitable hosts for the development of *L. invasa*, which was evident by the absence of gall development on some genotypes. This, however, suggests that *L. invasa* does not respond to genotype specific cues, but rather to genus specific cues.

Comparisons of the susceptibility of *Eucalyptus* gentoypes to *L. invasa* between countries are difficult as different parameters are used to quantify the amount of damage. In India, damage was assessed based on the number of galls per plant thereby broadly categorizing plants as ungalled, low, moderate or severe (Javaregowda & Prabhu, 2010). In Vietnam a severity scale was established using percent infestation of leaves and twigs of the crown to categorise the amount of damage caused by *L. invasa*. These damage indices were then used to assign levels of damage severity to clones categorizing them as nil, low damage, medium damage, severe damage and very severe damage (Thu *et al*., 2009). In the present study, as well as in studies in Kenya and Uganda, the damage index was calculated as the result of the severity multiplied by the incidence of *L. invasa* calculated for each plot (Nyeko *et al*., 2010). We suggest that an effort should be made to standardize the technique used to determine damage in such susceptibility trials so that comparisons between countries can be made with more accuracy. To standardize such a technique it is important to take into consideration the ease with which this technique can be applied to avoid unnecessary errors due to variation (e.g. *L. invasa* galls are multi-chambered and in severe infestations galls may develop adjacent to one another making it very difficult to determine the exact number of galls and developing hymenopterans present). It is recommended that a damage index is used to determine *L. invasa* damage where the severity (which has been calculated similarly in all above cases) is multiplied by the incidence.

Results of this study showed a high correlation between damage to leaves and damage to petioles. This result suggests that either petioles or leaves can be used to calculate damage, as opposed to using both. However, recent observations in the field (Brett Hurley, personal communication) have shown that some genotypes (not tested here) are highly susceptible to gall formation on petioles, but not on leaves, or vice versa. The relationship between gall

formation on leaves and petioles and the factors that influence this phenomenon require further investigation.

This study and others clearly show that *Eucalyptus* genotypes display considerable variation in susceptibility to damage by *L. invasa*. Some genotypes are generally more susceptible than others and although not absolute, this can be reflected in the hybrids between species. However, even in seemingly more susceptible species or hybrids, the potential exists for resistant genotypes to emerge. Likewise, highly susceptible genotypes may also occur in apparently resistant species and this is further complicated when hybrids are made. While additional susceptibility trials are needed across different environments and tree ages, further research is also needed to better understand the mechanisms governing resistance to *L. invasa* and thus be able to better predict the susceptibility of new genotypes or current genotypes planted in new areas.

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Table 1. *Eucalyptus* genotypes tested in the study showing the incidence and mean severity of *L. invasa* induced galls on the petioles and the associated damage index. Genotypes are ranked in descending order according to damage index. The rank based on the damage index of the leaves is given for comparative purposes.

Eucalyptus genotype	Genotype number	Incidence	Mean Severity (% infestation)	Damage Index	Rank*	
					Petioles	Leaves
E. nitens x E. grandis	37	1.00	39.47	0.39	1	3
	39	1.00	30.20	0.30	2	4
	36	1.00	29.38	0.29	3	2
E. grandis x E. camaldulensis	8	0.93	29.57	0.27	4	7
E. nitens x E. grandis	38	0.93	25.92	0.24	5	1
E. grandis x E. camaldulensis	12	0.86	27.60	0.24	6	5
E. grandis	48	0.93	20.10	0.19	7	12
E. grandis x E. camaldulensis	7	0.93	19.46	0.18	8	6
E. grandis x E. urophylla	27	0.86	11.77	0.10	9	8
	3	0.71	8.03	0.06	10	9
E. grandis x E. camaldulensis	13	0.86	7.39	0.06	11	10
E. grandis x E. nitens	17	0.64	5.57	0.04	12	7
E. grandis	45	0.50	6.27	0.03	13	15
E. grandis	47	0.64	2.96	0.02	14	13
E. grandis x E. camaldulensis	15	0.57	3.71	0.02	15	14
E. urophylla	5	0.36	3.06	0.01	16	16
E. grandis	44	0.29	1.39	0.00	17	18
E. grandis x E. urophylla	21	0.29	0.00	0.00	18	17
E. grandis x E. camaldulensis	11	0.07	1.33	0.00	19	19
E. grandis x E nitens	20	0.00	0.00	0.00	20	20
	19	0.00	0.00	0.00	21	21
	16	0.00	0.00	0.00	22	22
E. grandis x E. camaldulensis	10	0.00	0.00	0.00	23	23
E. grandis x E. urophylla	31	0.07	0.00	0.00	24	24
E. smithii	42	0.07	0.00	0.00	25	25
E. grandis x E. urophylla	1	0.00	0.00	0.00	26	26
E. urophylla	35	0.07	0.00	0.00	27	27
E. nitens	43	0.00	0.00	0.00	28	28
E. grandis x E urophylla	30	0.00	0.00	0.00	29	24
	2	0.00	0.00	0.00	30	30
	4	0.00	0.00	0.00	31	31
	6	0.00	0.00	0.00	32	32
E. grandis x E. camaldulensis	9	0.00	0.00	0.00	33	33
	14	0.00	0.00	0.00	34	34
E. grandis x E nitens	18	0.00	0.00	0.00	35	35
E. grandis x E. urophylla	22	0.00	0.00	0.00	36	36
	23	0.00	0.00	0.00	37	37
	24	0.00	0.00	0.00	38	38
	25	0.00	0.00	0.00	39	39
	26	0.00	0.00	0.00	40	40
	28	0.00	0.00	0.00	41	41
	29	0.00	0.00	0.00	42	42
	32	0.00	0.00	0.00	43	43
	33	0.00	0.00	0.00	44	44
E. dunii	40	0.07	0.05	0.00	45	45
	41	0.00	0.00	0.00	46	46
E. grandis	46	0.00	0.00	0.00	47	47
	49	0.00	0.00	0.00	48	48
E. saligna x E. urophylla	50	0.00	0.00	0.00	49	49

* Not all the clones are in the same ranking order in the table showing petiole and leaf damage

Table 2. *Eucalyptus* genotypes tested in the study showing the incidence and mean severity of *L. invasa* induced galls on the leaves and the associated damage index. Genotypes are ranked, in descending order, according to damage index. The rank based on the damage index of the petioles is given for comparative purposes.

Eucalyptus genotype	Genotype number	Incidence	Mean Severity (% infestation)	Damage Index	Rank*	
					Leaves	Petioles
E. nitens x E. grandis	38	1.00	52.34	0.52	1	5
	36	1.00	51.45	0.51	2	3
	37	1.00	48.18	0.48	3	1
	39	1.00	42.41	0.42	4	2
E. grandis x E. camaldulensis	12	0.93	40.23	0.37	5	6
	7	0.93	38.75	0.36	6	8
	8	0.93	37.55	0.35	7	4
E. grandis x E. urophylla	27	0.93	21.18	0.20	8	9
	3	0.93	20.95	0.19	9	10
E. grandis x E. camaldulensis	13	0.93	20.27	0.19	10	11
E. grandis x E. nitens	17	0.79	14.01	0.13	11	12
E. grandis	48	0.93	15.48	0.12	12	7
	47	0.64	8.48	0.07	13	14
E. grandis x E. camaldulensis	15	0.50	7.79	0.05	14	15
E. grandis	45	0.36	4.83	0.02	15	13
E. urophylla	5	0.43	2.17	0.01	16	16
E. grandis x E. urophylla	21	0.29	1.45	0.01	17	18
E. grandis	44	0.29	1.58	0.00	18	17
E. grandis x E. camaldulensis	11	0.29	1.10	0.00	19	19
E. grandis x E. nitens	20	0.29	0.79	0.00	20	20
	19	0.14	0.43	0.00	21	21
	16	0.14	0.36	0.00	22	22
E. grandis x E. camaldulensis	10	0.14	0.34	0.00	23	23
E. grandis x E. urophylla	31	0.07	0.33	0.00	24	24
E. smithii	42	0.07	0.26	0.00	25	25
E. grandis x E.urophylla	1	0.07	0.24	0.00	26	26
E. urophylla	35	0.07	0.21	0.00	27	27
E. nitens	43	0.07	0.18	0.00	28	28
E. grandis x E. urophylla	30	0.07	0.06	0.00	29	29
	2	0.00	0.00	0.00	30	30
	4	0.00	0.00	0.00	31	31
	6	0.00	0.00	0.00	32	32
E. grandis x E. camaldulensis	9	0.00	0.00	0.00	33	33
	14	0.00	0.00	0.00	34	34
E. grandis x E. nitens	18	0.00	0.00	0.00	35	35
E. grandis x E. urophylla	22	0.00	0.00	0.00	36	36
	23	0.00	0.00	0.00	37	37
	24	0.00	0.00	0.00	38	38
	25	0.00	0.00	0.00	39	39
	26	0.00	0.00	0.00	40	40
	28	0.00	0.00	0.00	41	41
	29	0.00	0.00	0.00	42	42
	32	0.00	0.00	0.00	43	43
	33	0.00	0.00	0.00	44	44
E. dunii	40	0.00	0.00	0.00	45	45
	41	0.00	0.00	0.00	46	46
E. grandis x E. camaldulensis	46	0.00	0.00	0.00	47	47
	49	0.00	0.00	0.00	48	48
E. saligna x E. urophylla	50	0.00	0.00	0.00	49	49

* Not all the clones are in the same ranking order in the table showing petiole and leaf damage

Table 3. Differing levels of significance between infestations of petioles observed between genotypes

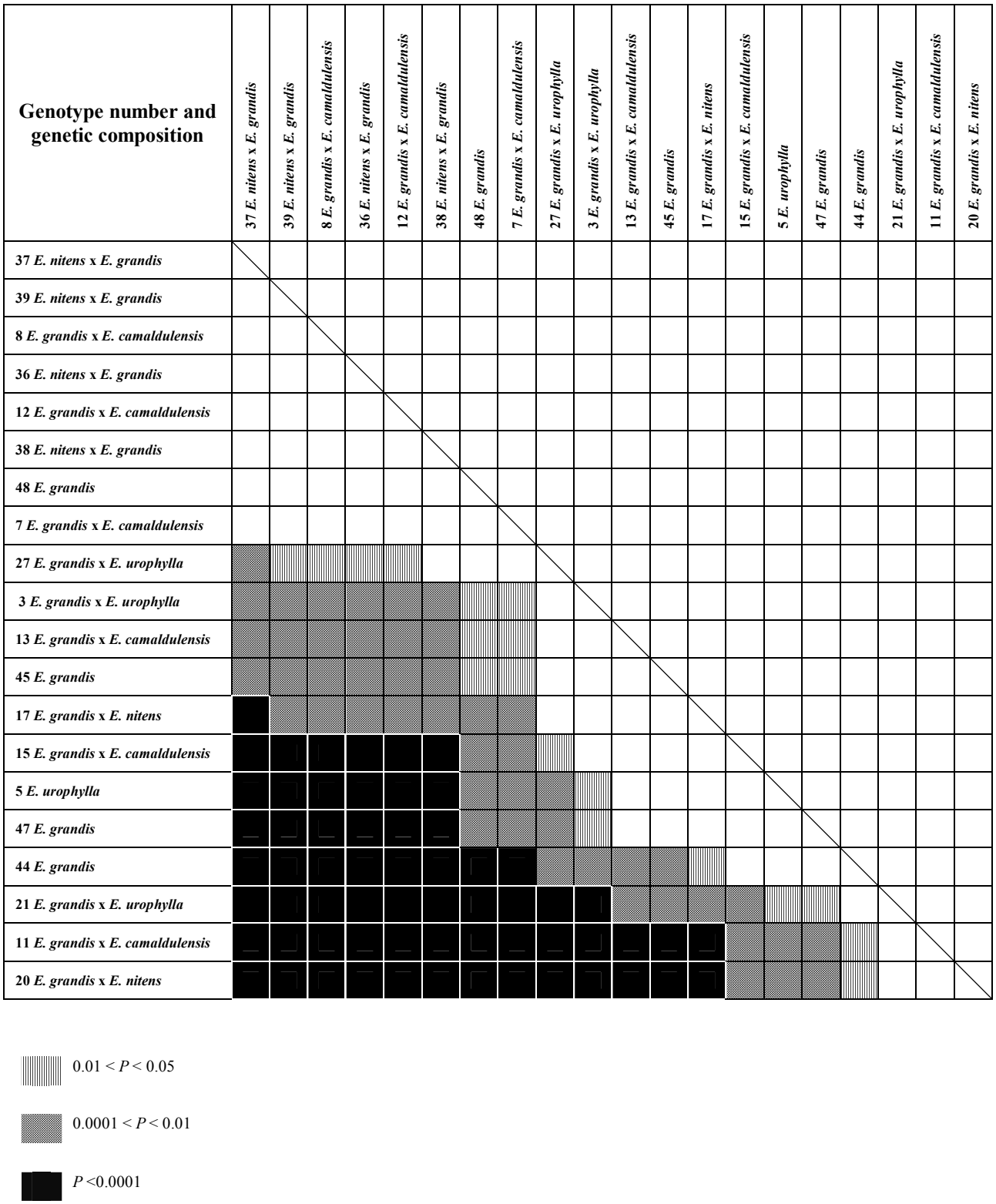

Table 4. Differing levels of significance between infestations of leaves observed between genotypes

Genotype number and genetic composition	**38 *E. nitens* x *E. grandis***	**36 *E. nitens* x *E. grandis***	**37 *E. nitens* x *E. grandis***	**39 *E. nitens* x *E. grandis***	**12 *E. grandis* x *E. camaldulensis***	**7 *E. grandis* x *E. camaldulensis***	**8 *E. grandis* x *E. camaldulensis***	**27 *E. grandis* x *E. urophylla***	**3 *E. grandis* x *E. urophylla***	**13 *E. grandis* x *E. camaldulensis***	**17 *E. grandis* x *E. nitens***	**48 *E. grandis***	**47 *E. grandis***	**15 *E. grandis* x *E. camaldulensis***	**45 *E. grandis***	**5 *E. urophylla***	**44 *E. grandis***	**21 *E. grandis* x *E. urophylla***	**11 *E. grandis* x *E. camaldulensis***	**20 *E. grandis* x *E nitens***
38 *E. nitens* x *E. grandis*																				
36 *E. nitens* x *E. grandis*																				
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39 *E. nitens* x *E. grandis*																				
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21 *E. grandis* x *E. urophylla*																				
11 *E. grandis* x *E. camaldulensis*																				
20 *E. grandis* x *E nitens*																				

$0.01 < P < 0.05$

$0.0001 < P < 0.01$

$P < 0.0001$

Table 5. The *Eucalyptus* species and clones from India, Israel, Kenya, Uganda and Vietnam most susceptible to infestation to *L. invasa*

Country	***Eucalyptus* genotype**	**Source**
India	*E. camaldulensis, E. grandis, E. tereticornis*	Basavana Goud *et al*., 2010
Israel[1]	*E. botryoides, E. bridgesiana, E. camaldulensis, E. globulus, E. gunii, E. grandis, E. robusta, E. saligna, E. tereticornis, E. viminalis, E. grandis* x *E. camaldulensis*	Mendel *et al*., 2004
Kenya	MAU1*, *E. grandis x E. camaldulensis* 14, *E. grandis x E. camaldulensis* 15, *E. grandis x E. camaldulensis* 10	Nyeko *et al*., 2010
Uganda	*E. camaldulensis*, *E. grandis x E. camaldulensis* 540, *E. grandis x E. camaldulensis* 784	Nyeko *et al*., 2010
Vietnam	*E. camaldulensis, E. grandis, E. tereticornis*	Thu *et al*., 2009

[1] *Eucalyptus* species evaluated do not indicate severity of infestation but only suitability for oviposition and development
* *Eucalyptus urophylla*

Figure 1. A diagram showing the layout of an outer and inner block. There were seven such outer – inner block combinations in the study. Black shaded cells indicate sand bags used as spacers. The demarcated grey area indicates the inner block whereas the remaining plants comprise the outer block. The white cells with numbers indicate the placement of the different *Eucalyptus* genotypes.