Variability in the infestation of Leptocybe invasa on commecially grown eucalyptus germplasm in Kenya

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Variability in the Infestation of Leptocybe Invasa (Hymenoptera: Eulophidae) on Commercially Grown Eucalyptus Germplasm in Kenya

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Abstract

The growing of Eucalyptus trees in Kenya is widespread and there is high demand for their products. Infestation of Eucalyptus germplasm by *Leptocybe invasa* Fisher & LaSalle, a new pest, is causing a lot of worry since very little is known about the pest. Variability in the infestation of *L. invasa* on commercially grown Eucalyptus germplasm was examined in two trial sites established in western Kenya. The crown foliage gall density and gall count on a sample unit were used to determine the levels of infestation. Using analysis of covariance procedure and separation of means using least significant difference, the tested germplasm was classified into four susceptibility groups. The incidence and severity of *L. invasa* increased with age and were generally high in Eucalyptus hybrid clones as compared to improved Eucalyptus hybrids and species. However, *Eucalyptus henrii* and GC581 were found to be resistant to *L. invasa*. There was a significant positive correlation (corr. = 0.712) between the mean *L. invasa* gall count and the mean crown damage thus reinforcing use of the latter as a cost-effective method of assessing infestation of *L. invasa*. The use of resistant germplasm to *L. invasa* requires promotion for adoption as a management strategy. However, further research needs to be carried out on identifying the genes responsible for this resistance.

Key words: *Resistance, Leptocybe invasa, Eucalyptus germplasm, variability, clones.*

Introduction

Decrease in wood supply from natural forests and increasing demand for various wood products in the tropics imply that both plantation forests and on-farm tree growing will continue to be a major source of wood in the future. The introduction of exotic tree species that are fast growing and suitable to a wide range of agro-ecological areas is gaining momentum in eastern Africa. This is aimed at meeting the high demand for wood fuel and other wood products. An example of such introduced exotic trees is a wide range of *Eucalyptus* species, hybrids and clones to meet the high rural energy demand in tropical Africa. The exotic trees are generally being planted in woodlots and monoculture plantations. According to Wingfield *et al.,* 2001, such monocultures of genetically similar trees are highly vulnerable to insect pests and diseases attack. This is attributed to the increasing emergence of new insect pest problems on exotic plantation trees in the tropics (Nyeko *et al.*, *In press*). Recent examples of such pest outbreaks that raise serious concern to developers of tropical tree growing enterprises include; the conifer aphid, *Cinara cuppressivora* Watson and Voegtlin, on cypress in eastern and central Africa (Murphy, 1998; Day *et al.*, 2003), the sirex wood wasp, *sirex noctilio* Fabricius, on pines in South Africa (Hurley *et al.*, 2007), and now, *Leptocybe invasa* Fisher & LaSalle, on *Eucalyptus* species in many countries in Africa, Asia, the Middle East and Europe (Mutitu, 2003; Mendel *et al.*, 2004; Nyeko, 2004, EPPO, 2006).

Leptocybe invasa (Hymenoptera: Eulophidae), commonly known as Blue Gum Chalcid (BGC), is gradually and prominently gaining pest status in Kenya since it was first recorded on Eucalyptus tree seedlings in western parts of the country in November 2002 (Mutitu *et al.*, 2007a). It attacks mostly seedlings and field saplings causing damage on its host by forming massive typical bump-shaped galls on tree canopy, specifically on the leaf midribs, petioles and stems of new growths (Mutitu *et al.*, 2007b). Repeated attacks lead to twisted and knobbed appearance of leaves and the terminal leader shoot degenerates to a lateral shoot causing canopy to be umbrella shaped thus a deformed plant

(Mendel *et al.*, 2004). Severely infested trees show stunted growth, lodging, dieback and sometimes tree death (Mendel *et al.*, 2004; Nyeko, 2005). *Leptocybe invasa* infestation is more severe on nursery seedlings and young (1-3 year old) than on older trees (Nyeko, 2005; Mutitu *et al.*, 2005). Since its invasion in Kenya, within a period of four years, *L. invasa* has spread to all the provinces except Nairobi (Mutitu *et al*, 2007a). This fast spread and its devastating effect on the host tree threatens the growing of Eucalyptus species in the country. There is an urgent need to develop and implement management options to reduce the loss. However, very little is known about the pest and no specific management option exists, thus there is need to generate such information geared towards recommending suitable control method.

There is a considerable variation in the severity of attack on individual trees within affected Eucalyptus stand and it is common to find a completely healthy tree adjacent to a heavily infested neighbour. This field observation suggests that resistance to *L. invasa* may be an option for management. Host plant resistance is therefore a viable management option that can be attempted. According to Mutitu *et al.*, 2005, five different *L. invasa* preferred groups to *Eucalyptus* species, hybrids and clones were identified from a laboratory bioassay. The highly preferred was GC 10. Others like *E. camaldulensis, E. globules, E. grandis, E. nitens, E. paniculata, E. saligna, E. daniae* were found to be less preferred while GC12, GC14, GC522 and GC784 were moderately preferred. The non-preferred were *E. tereticornis* and *E. urophylla*.

The identification of Eucalyptus germplasm that are resistant to the *L. invasa* attack as well as the subsequent promotion of such planting materials for propagation by stakeholders is one of the possible options for the management of this pest. Therefore, the objective of this paper was to determine the variability of *L. invasa* attack on different widely commercially grown *Eucalyptus* germplasm. Such information heavily contributes to the development of host plant resistance as a management option for the pest.

Materials and Methods

The trial sites

The study was carried out in two sites, namely, Busia and Yala. Yala site was situated at Dominion Farm (34°9'E, 0°0'S) Yala division in Siaya district, at an altitude of 1156 meters above sea level (m.a.s.l.). The Busia site was situated at Dindi Farm (34°9'E, 0°21'N) Matayos division in Busia district, at 1196m.a.s.l. These sites were considered suitable because they are situated in the region of the initial *L. invasa* invasion area, hence pest population is high enough for natural infestation of the test germplasm. However, the Yala site was heavily destroyed by floods when the dykes on River Yala broke during heavy rains just 4 months after establishment. Thus only Busia site results are presented in this paper.

Experimental design and Eucalyptus germplasm

A total of twenty-four Eucalyptus germplasm (treatments) composed of seven Eucalyptus species, five improved Eucalyptus hybrids and twelve Eucalyptus hybrid clones (Table 1) were investigated for their resistance to attack by *L. invasa*. The experiment was set in Randomized Complete Block Design (RCBD) with two replicates at Busia. Each block consisted of 24 plots where the treatments were allocated at random and was surrounded by a guard row of local land races like *Eucalyptus camaldulensis, E. grandis* or *E. saligna*. Each plot was a single treatment composed of 12 trees.

Maintenance of trials

All the tested germplasm were obtained from Karura Tree Biotechnology Project nursery. The seedlings were planted-out at four months old at a spacing of 2.5 by 2.5 metres. Due to the large number of treatments being tested, the planting density per plot was reduced from the conventional one of sixteen trees to twelve to minimize variability within a block. The seedlings were watered for the first three months to enhance establishment and survival. Spot weeding around the tree base and slashing of weeds was done. The seedlings were protected against termite attack by applying a termiticide - Fipronil (Regent 3G) at the rate of 33g per tree at planting time.

Table 1: Eucalyptus Germplasm used in the trials

Groups of Eucalyptus Germplasm	No.	Eucalyptus germplasms (Treatments) used
Eucalyptus species	7	*Eucalyptus grandis* (EG), *E. saligna* (ES), *E. camaldulensis* (EC*), E. daniae* (ED), *E. urophylla* (EU), *E. henrii*(EH), *E. tereticornis* (ET).
Eucalyptus hybrid clones	12	GC3, GC10, GC12, GC14, GC15, GC16, GC522, GC581, GC584, GC642, GC785, GC796
Improved Eucalyptus hybrids	5	MAU1, MAU12, MAU16, MAG18, KMUG14.

GC = E. grandis x E. camaldulensis,

Assessments and Sampling procedures

A total of five assessments were carried out at a sampling frequency of two months intervals until the tree canopy closed (10 months after plant-out). Two parameters were assessed to determine the variability of attack by *L. invasa*. These were; (i) the number of galls per 20 cm sample unit, and (ii) the *L. invasa* gall density on the saplings crown foliage on a score of 1 to 4.

i) Assessment procedure for L. invasa gall numbers

In every plot, four trees were selected at random. In each of the selected trees, two branches were selected at random from the top five branches where the leader shoot was taken as the first branch. The number of *L. invasa* galls was counted using a tally counter on a 20 cm length from the branch tip. This is because *L. invasa* mostly attacks the young meristematic tissues of the sapling (Mendel *et al.,* 2004).

ii) Assessment of crown gall density as a score

To determine *L. invasa* crown damage, the whole tree foliage canopy was assessed for *L. invasa* galls density on the shoots. This was done on all the trees in the plot. This method is a modification of the one used for assessing cypress aphid by Day *et al.,* (2003). The modification was to accommodate the crown gall density as compared to the crown browning associated with cypress aphid attack. This was used to quantify the severity of attack (damage) and scored on a four-point scale as shown below:

Score 1 = no gall damage on crown foliage

Score 2 = presence of less than 25 per cent gall density on crown foliage

Score 3 = presence of between 25 and 50 per cent gall density on crown foliage, and

Score 4 = presence of more than 50 per cent gall density on crown foliage.

Data management and analysis

The data collected were managed using Microsoft Excel for Windows 2003. Mean damage score and mean number of galls were determined for each Eucalyptus germplasm (treatment) using summary statistics procedures. Pearson's correlation coefficient was computed to determine the relationship between mean damage score and mean number of galls per treatment. The number of galls were assumed to have a poisson distribution, which necessitated an appropriate transformation to normal distribution before statistical analysis. The natural logarithm transformation was employed for the purpose. An analysis of covariance was then applied on the transformed data (Loggalls) to test the effect of treatment on tree damage taking into account the effect of age (covariate) after planting using the following model:

$$Y_{ij} = \mu + t_i + b_j + c(x_{ij} - x) + \varepsilon_{ij} .$$

Where Y_{ij} = gall counts of the i^{th} Eucalyptus germplasm (treatment) in the j^{th} block

μ = Overall mean gall count

t_i = Effect of ith treatment

b_j = effect of jth block

c = the regression coefficient

x_{ij} = age of tree of treatment i in the jth block

x = mean of age

ε_{ij} = the error term

Least significant difference (LSD) procedure was used to separate the means. All analysis were done using Genstat 9th edition 2006.

Results and Discussions

Eucalyptus germplasm susceptibility

Through the use of LSD statistical procedures, the tested Eucalyptus germplasm were categorized into four highly significantly different groups ($p<0.001$). These were Resistant, Tolerant, Moderately susceptible and Highly susceptible groups based on the mean gall numbers (Table 2). These findings corroborate those of Mendel *et al.*, (2004), that *E. camaldulensis, E. grandis, E. saligna* and *E. tereticornis* are suitable hosts for *L. invasa.* Although Nyeko *et al.*, (In press) indicated that the clones susceptibility appeared to be influenced by parents it is surprising that GC581 is resistant. In addition to the known eucalyptus site-matching and growth performance information on Eucalyptus germplasm, this susceptibility information of *L. invasa* can be used by the stakeholders to promote what to plant in *L. invasa* infested areas.

Table 2. Eucalyptus germplasm susceptibility groups based mean number of galls

L. invasa **Susceptibility group**	**Eucalyptus germplasm**	**Mean number of galls**
Resistant	EH, GC581	0
Tolerant	EC, ED, EU, GC3, GC167, GC584, GC642, GC785, KMUG14, MAG18, MAU12	5
Moderately susceptible	EG, ES, GC12, GC522, GC796, MAU16	97
Highly susceptible	ET, GC10, GC14, GC15, MAU1	284

This classification concurs with the findings of Mutitu *et al*, 2005 although the previous research work was only limited to an insectary (laboratory bioassay). However due to the difference in behaviour of *L. invasa* in different agro-ecological zones and altitudes noted by Nyeko *et al.*, (In press), it is likely that the susceptibility grouping identified may not apply to all the zones. Therefore there is need for this research to be conducted in the entire host tree growing zones. The cause of this variable susceptibility of Eucalyptus germplasm to *L. invasa* requires further studies to establish the factors responsible for the resistance.

The results showed that treatments, within the first two months of establishment had naturally been infested by *L. invasa*. The rate at which the natural infestation occurred in the highly susceptible group within the first ten months of age was higher compared to the other groups. There was a slow infestation in the tolerant group compared to the moderately susceptible group (Figure 1). However, the resistant group (EH and GC581) remained uninfested (mean gall number = 0) during the ten months assessment duration. This fast natural infestation can be attributed to both the biology of the *L. invasa* and lack of its principal natural enemies. *Leptocybe invasa* has a thelytokous pathenogenetic form of reproduction and mulivotinous development (Mendel *at el*, 2004). These two traits help the pest population to increase very fast and with over-lapping generations. The absence of its principal natural enemies does not control the increase in population.

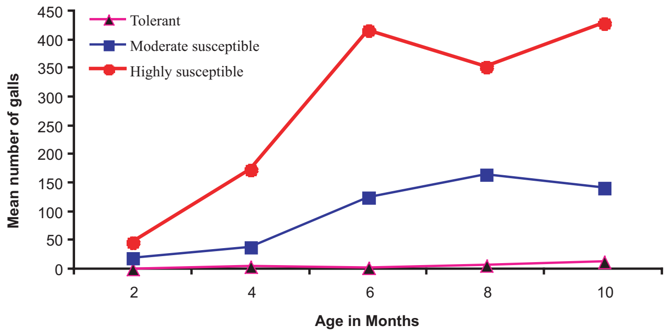

Figure 1. Mean gall numbers and tree age of the three different susceptible groups

Trend of *L. invasa* in the tested Eucalyptus germplasm (treatments)

The different tested Eucalyptus germplasm groups (Table 1) were significantly different at $p<0.05$. Generally more galls were observed in the Eucalyptus hybrid clones than in the species and the improved Eucalyptus hybrids. The Eucalyptus hybrid clones had a significantly ($p=0.05$) higher rate of infestation in the first six months and almost stabilizes thereafter. The improved Eucalyptus hybrid has an initial slow rate of infestation, however it abruptly shoots after eight months of age. The species group had a moderate rate of infestation that decreases after the age of six months.

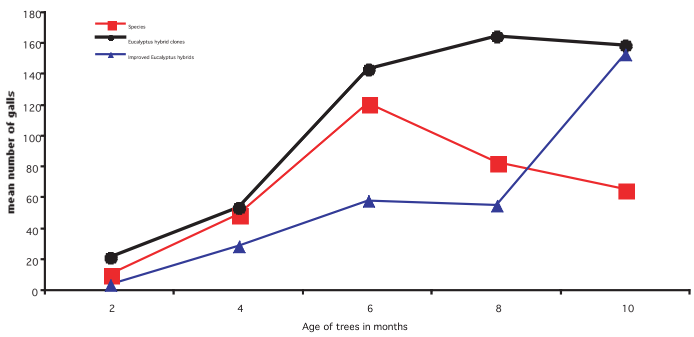

Figure 2. Comparison between Eucalyptus Germplasm groups

Relationship between damage scores and gall counts

Further analysis on the damage scores and gall counts results using Pearson's Correlation Coefficient showed that there was highly significant positive correlation (corr. = 0.712) between mean damage score and mean gall counts, implying that the higher the number of galls the higher the crown damage.

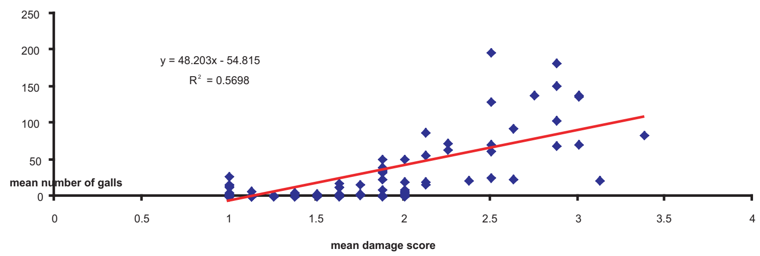

Figure 3. Relationships between mean damage score and mean gall count

The mean damage score number of galls accounted for about 57 per cent (figure 3) of the variation in mean gall numbers. The unaccounted variation of 43 per cent can be attributed to difficulties in counting of galls particularly in the susceptible group where there exists visible overlap of galls. This high positive correlation shows that the two methods (damage score and gall count) used in the assessment of the L. invasa infestation give almost similar results. However gall count method is labour intensive compared to the four-point scale crown damage scoring method. It is therefore economical to use the crown damage method to determine the L. invasa infestations.

Conclusions and Recommendations

- The study has shown that there is high variability of *L. invasa* infestation on the tested Eucalyptus germplasm. The most resistant was identified as EH and CG 581 while GC10, GC14, GC15, ET and MAU1 were found to be highly susceptible. This is an indication that host plant resistance strategy is a viable management option of this pest.
- Stakeholders can be able to use the susceptibility grouping of the Eucalyptus germplasm to determine the planting species in areas of *L. invasa* infestation. Similar research work should be carried in all major host tree growing areas (agro-ecological zones) to determine susceptibility groups for the areas.
- Gall damage scoring method can be used to assess the level of *L. invasa* infestation as compared to the labour intensive gall count method. However, further studies should be carried out to determine the accuracy.

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