PEST MANAGEMENT

Use of Grafting to Prevent *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) Damage to New World Meliaceae Species

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ABSTRACT - The susceptible species *Cedrela odorata* and *Swietenia macrophylla* to attack by *Hypsipyla grandella* (Zeller) larvae were grafted onto the resistant species *Khaya senegalensis* and *Toona ciliata*. Six-month-old grafted plants were then compared to their reciprocal grafts and to both intact (non-grafted) and autografted plants for damage due to *H. grandella* larvae and for their effects on larval performance. Two experiments were conducted: one in which the apical bud of the main plant shoot was inoculated with *H. grandella* eggs, and the other in which the bud was inoculated with third instars. Damage in each experiment was assessed by the number of frass piles, number and length of tunnels, number of damaged leaves, and damage to the apical bud. Larval performance was evaluated in terms of time to reach pupation and pupal weight and length. In both experiments, plant damage differed significantly among treatments ($P \leq 0.03$). Resistant rootstocks conferred resistance to susceptible scions. In both experiments, grafting by itself, regardless of the rootstock and scion combination, also reduced damage caused by *H. grandella* larvae. Scions of autografted susceptible species had similar resistance to susceptible scions grafted on resistant rootstocks. Few larvae reached pupation, and their pupal weight and length were similar.

KEY WORDS: *Cedrela*, *Swietenia*, *Khaya*, *Toona*, graft, mahogany shootborer

High-quality timber from Spanish cedar, *Cedrela odorata*, and mahogany *Swietenia macrophylla* (Meliaceae) are important for the economy of many neotropical countries (Newton *et al* 1993). Unfortunately, natural populations of these species are being reduced quickly due to selective harvest (Albert *et al* 1995). In addition, the mahogany shootborer, *Hypsipyla grandella* (Zeller), has limited their establishment in commercial plantations in Latin America, as its larva mainly feeds on apical shoots, inducing branching on the trees and rendering the timber unmarketable (Grijpma 1971).

Exotic Meliaceae species are less susceptible than indigenous ones to attacks by native *Hypsipyla* spp. (Cunningham *et al* 2005). For example, Australian red cedar *Toona ciliata* (Meliaceae), closely related to *Cedrela* spp., is heavily attacked by *H. robusta* Moore when growing in its Old World native habitats (Bygrave & Bygrave 1998), but is not attacked by *H. grandella* when planted in Central America (Grijpma 1976). Conversely, imported *Cedrela* spp. are not attacked by *H. robusta* in Australia (Bygrave & Bygrave 2001). Moreover, *S. macrophylla* and the African mahogany *Khaya senegalensis* (Meliaceae) have been reported to suffer less damage from either *H. robusta* or *H. grandella*, respectively.

This reduced susceptibility of exotic Meliaceae to native *Hypsipyla* species (Manso 1974, Grijpma 1976, Agostinho *et al* 1994) may allow production of resistant native Meliaceae trees to *H. grandella* by grafting susceptible scions onto resistant, exotic rootstocks. For instance, in Costa Rica, *C. odorata* shoots grafted onto *T. ciliata* were shown to be resistant, although this resistance was lower than that for *Toona* trees themselves (Grijpma 1976). Also in New South Wales, *C. odorata* and *C. fissilis* (Meliaceae) scions grafted onto *T. ciliata* differed in susceptibility to attack by *H. robusta*, with *C. odorata* scions being more susceptible than *C. fissilis* scions (Bygrave & Bygrave 2001). Resistance of *Cedrela* scions grafted to *Toona* rootstock scions strongly suggests that some defensive substances are translocated from root to grafted shoot (Grijpma & Roberts 1975). Translocation was later confirmed by Silva *et al* (1999) who found catechin (a phenolic compound synthesized by *T. ciliata*) in scions of *C. odorata* grafted onto *T. ciliata* rootstocks.

Autografting (a scion of a plant onto another part of the same plant or species) (Hartmann *et al* 2002) could potentially improve insect resistance of susceptible plant species by

inducing production of secondary defensive compounds in response to the wounding from making the graft union. The phenomenon of inducible resistance after wounding is widely demonstrated (Schoonhoven *et al* 2005), but the implications for grafting have not been examined. In addition, the effect of grafting per se, regardless of the combinations of species has not been taken into account in prior grafting studies involving *Hypsipyla* spp. In this study, autografts were included as additional controls to consider potential grafting effects. So far, grafting effects on *H. grandella* resistance have been examined only in combinations of *Cedrela* spp. and *T. ciliata*, neglecting other resistant and susceptible timber species within Meliaceae. In addition, the effects of reciprocal grafting and autografting are unknown, so that the basis for changes in scion resistance is unclear.

The objective of this study was to determine the effect of grafting different combinations of susceptible and resistant Meliaceae species in precluding damage by *H. grandella*. The hypotheses tested were: 1) exotic species (*K. senegalensis* and *T. ciliata*) are resistant to *H. grandella* attack, 2) resistant rootstocks confer resistance to susceptible scions, 3) susceptible rootstocks do not affect the resistance of exotic resistant scions, and 4) autografting confers resistance.

Material and Methods

Research was conducted at the Cabiria Experimental Station, on the premises of the Tropical Agricultural Research and Higher Education Center (CATIE), in Turrialba, Costa Rica. CATIE is located in the Caribbean watershed of this country, at 602 m altitude, within the premontane wet forest life zone (Tosi 1969). Average annual values for climatic variables are 2600 mm rainfall, 22ºC, 88% RH, and 17 MJ m^2 of solar radiation (Salas 2000).

All four species selected for study belong to the subfamily Swietenioideae (Pennington & Styles 1975). Seeds of the susceptible species *C. odorata* and *S. macrophylla* from Pococí, Costa Rica, as well as the resistant *K. senegalensis* from Burkina Faso, and *T. ciliata* from Australia, were provided by the Forest Seed Bank at CATIE.

Seeds were sown at CATIE's nursery, and the plants were kept inside a screenhouse used to acclimatize coffee plants. The wedge grafting technique (Bygrave & Bygrave 1998) was used to produce the various grafted combinations when the plants were 12 months old. The grafting union was 20-30 cm above the soil surface. Rootstocks were less than 1 cm in diameter at the grafting union, whereas the scions were slightly thinner. Grafted plants were maintained in the screenhouse until the scions developed at least 10 leaves (ca. six months).

Plant species susceptible to *H. grandella* were grafted onto resistant ones. Also, reciprocal combinations (i.e., resistant scion onto susceptible rootstock) and autografts (scion and rootstock from the same species) were completed. Intact (i.e., non-grafted) and autografted plants were used as controls (Table 1). The aim was to have a full set of 12 combinations, but grafts of *C. odorata* onto *K. senegalensis* and *K. senegalensis* onto *C. odorata*, for experiment I, or *S. macrophylla* onto *T. ciliata*, for experiment II, were unsuccessful due to incompatibility or were not ready when experiments were completed.

Table 1 Meliaceae species and their grafted combinations exposed to *Hypsipyla grandella* eggs (experiment I) and third instars (experiment II) in Turrialba, Costa Rica, 2004-2005.

Meliaceae species	Scion			
	Susceptible		Resistant	
Stock	C	S	K	T
Susceptible				
Cedrela odorata (C)	C/C[1]		K/C[2]	T/C
Swietenia macrophylla (S)		S/S	K/S	T/S
Resistant				
Khaya senegalensis (K)	C/K[2]	S/K	K/K	
Toona ciliata (T)	C/T			T/T

[1]For each plant combination, the left letter is the scion and the right letter is the rootstock; [2]Treatments not considered for experiment I.

Two experiments were completed with the main plant shoot inoculated either with eggs or third instars (8-16 mm long) of *H. grandella*. In this way, early instars emerging from eggs and the later instars were tested.

Eggs and larvae for experiments were taken from a colony maintained at the Entomology Laboratory at CATIE. The colony was established in 1998, and yearly renewed from field-collected larvae feeding on *C. odorata*. Larvae in the colony were normally fed with tender *C. odorata* leaves from first to third instars, and then placed onto an artificial diet (Vargas *et al* 2001) until pupation. The combination of leaves and artificial diet ease the management of the colony. Eggs hardly hatch on diet but easily on leaves. Feeding larvae only with leaves is hard since they are so voracious that they become cannibalistic if short in food. On another hand, tender leaves are scarce during the dry season. Pupae were moved to a metal framed cage covered with fine mesh, kept at a greenhouse for adult emergence, mate and oviposition. Eggs were collected and taken to the laboratory to sustain the colony.

Thirteen and fifteen treatments for experiment I (eggs) and II (third instars), respectively, were arranged in a completely randomized design with six replicates. Each replicate consisted of an individual plant with three eggs or two *H. grandella* larvae. Three eggs were used to ensure the presence of at least one larva per plant.

Experiment I: *Hypsipyla grandella* eggs. This experiment was conducted from 23 April to 15 June 2004. Plants were carefully inspected to preclude predation by ants, wasps or spiders. Bird predation was prevented by closing the sides of the greenhouse with a plastic shade net (50-60% of full sunlight), whereas ants were avoided by smearing a sticky substance, Tanglefoot (The Tanglefoot Co., Grand Rapids, MI), around tree stems 10-15 cm above the ground.

Four-day-old *H. grandella* eggs were placed on the main shoot by using a thin paintbrush between 16:30h and 17:00h,

which is the natural time for oviposition (Ramírez-Sánchez 1964). Age of *H. grandella* eggs was based on coloration; they were white just after oviposition and turned red before hatching on the fifth day (Taveras *et al* 2004).

Damage was appraised daily from day 2 to 15 after initiation by counting the number of frass piles (mounds of feces, 'sawdust' and silk), number and length of tunnels in the main or lateral buds and in shoots, number of damaged leaves due to larval feeding (whether on petioles or leaflets) and damage to the apical bud (scored at 0 for intact or 1 for either partially or fully consumed bud). Buds or shoots were then dissected and the length of all tunnels made by the larvae was measured; an average tunnel length per plant was calculated afterwards.

On day 15, the number of surviving larvae was recorded and individual larvae were transferred to vials with artificial diet (Vargas *et al* 2001). Vials were kept inside an environmental chamber (Percival I-35L, Boone, Iowa) at 25ºC, 80-90% RH, and 12:12 L:D, and the time to reach the pupal stage (days from oviposition to pupation), pupal length (mm) and weight (mg) were recorded. Pupation was considered completed when pupae turned dark brown, so that they could be weighed and measured without stress or injury.

Experiment II: *Hypsipyla grandella* third-instar larvae. This experiment was conducted from 25 November 2004 through 18 January 2005. Plants were 18 months old, including the period prior to grafting. Larvae were placed on the main plant shoot with a fine paintbrush. Variables examined and methods used were the same as those in experiment I.

Statistical analysis. Since the number of eggs or larvae was higher than required by *H. grandella* to cause damage, the number of surviving larvae was considered as a covariate for all the other variables. Data were examined for compliance of assumptions required for analysis of covariance (ANCOVA). If necessary, data were transformed by $Y = \text{sqrt}(Y + 0.5)$ to meet these assumptions.

Analysis of covariance was completed using the GLM procedure (SAS 2001). Orthogonal contrasts ($P \leq 0.05$) were used to test the species and graft combination effects on plant damage and larval performance. The contrasts were as follows: 1) intact susceptible vs. intact resistant species (C, S vs. K, T); 2) autografted susceptible vs. autografted resistant species (C/C, S/S vs. K/K, T/T); 3) autografted susceptible vs. susceptible grafted on resistant species (C/C, S/S vs. C/K, C/T, S/K); 4) autografted resistant vs. resistant grafted onto susceptible rootstock species (K/K, T/T vs. K/S, T/C, T/S). Apical bud damage was analyzed by a Chi-square test to examine the hypothesis that damage was similar among the species tested.

Results

Damage on plants. In both experiments, plant species, whether grafted or intact, significantly affected the number of frass piles, tunnel length and number of damaged leaves, whereas tunnel number differed only in experiment I (Table 2).

The number of frass piles was lower in both experiments for resistant species compared to susceptible ones (Table 2, Fig 1). The same result was obtained for autografted plants, but the effect was reduced for resistant species (Table 2, Fig 1). None of the other contrasts for treatment effects on frass piles was significant for either experiment (Table 2). In the first experiment, autografting susceptible species reduced the number of frass piles to a level as low as that of the susceptible species grafted onto the resistant ones (Fig 1a). In the second experiment, the number of frass piles was almost nil for both autografted resistant species and resistant species grafted onto susceptible ones. Even though the number of frass piles was almost nil on the resistant species, larvae occasionally attacked them, especially *K. senegalensis*. Also, although not significant, the number of frass piles tended to be consistently lower on *S. macrophylla* plants compared to *C. odorata* (Fig 1b).

The number of tunnels made in the various intact and graft combination plants was significantly affected ($P < 0.0384$) by only hatched larvae in experiment I (Table 2). Numbers tended to be lower in resistant plants compared to susceptible ones (both intact and autografted) (Fig 2), but contrasts revealed that the numbers were not statistically different (Table 2). In experiment I, however, intact and autografted resistant species completely lacked tunnels (Fig 2a).

Tunnel length differed significantly between susceptible and resistant intact plants in both experiments (Table 1, Fig 3), with *C. odorata* having much longer tunnels than *S. macrophylla*. The trend was similar among autografted plants, but was significant only in experiment II. None of the other contrasts for tunnel length was significant.

The number of damaged leaves differed substantially and significantly between susceptible and resistant plants for both experiments (Table 2, Fig 4), with *C. odorata* having more damaged foliage than *S. macrophylla* plants. Autografted susceptible plants had more damaged leaves than autografted resistant ones only in experiment II. None of the other contrasts for the number of damaged leaves was significant. In experiment I, autografted and intact *K. senegalensis* and *T. ciliata* plants lacked leaf damage (Fig 4a), although in experiment II some minor damage on *T. ciliata* leaves was observed (Fig 4a).

Apical bud damage differed among treatments ($X^2 = 41.13$, 39.29; $P < 0.0001$, 0.0003; d.f. = 13, 14) for experiments I and II, respectively. Both intact and autografted *K. senegalensis* and *T. ciliata* plants completely lacked apical bud damage (Fig 5), whereas intact *C. odorata* and *S. macrophylla* plants suffered 90% and 100% and 40% and 80% damage in experiments I (Fig 5a) and II (Fig 5b), respectively.

In experiment I, apical bud damage on autografted *C. odorata* was reduced by 78%, but autografting failed to reduce bud damage of *S. macrophylla*. When the latter was grafted onto *K. senegalensis*, damage was reduced by 50% with respect to intact *S. macrophylla* plants. Moreover, apical bud damage was absent from *C. odorata* scions grafted on *T. ciliata* with respect to damage on intact *C. odorata* (Fig 5a). In experiment II, apical bud damage was reduced by 50% and 16% on the respective autografts of *C. odorata* and *S. macrophylla* with respect to intact plants. Moreover, *C. odorata* grafted onto *T. ciliata* and *T. ciliata* grafted onto

Table 2 Probability values of orthogonal contrasts and analysis of covariance for variables evaluated in experiments with *Hypsipyla grandella* and four intact and grafted Meliaceae species in Turrialba, Costa Rica, 2004-2005.

Contrast between species	No. frass piles	Tunnel		No. damaged leaves
		No.	Length	
Experiment I: Eggs placed on plants				
	Probabilities			
Intact susceptible vs. intact resistant species	0.0009	0.0681	0.0053	0.0020
Autografted susceptible vs. autografted resistant species	0.0004	0.0566	0.1502	0.4416
Autografted susceptible vs. susceptible grafted on resistant	0.3869	0.6174	0.4018	0.5486
Autografted resistant vs. resistant grafted on susceptible	0.5959	0.3416	0.9521	1.0000
ANCOVA statistics P	0.0001	0.0384	<0.0001	0.0001
F value	4.12	2.03	4.78	4.16
Experiment II: Third-instar larvae placed on plants				
	Probabilities			
Intact susceptible vs. intact resistant species	< 0.0001	0.3727	< 0.0001	0.0002
Autografted susceptible vs. autografted resistant species	0.0309	0.8880	0.0035	0.0486
Autografted susceptible vs. susceptible grafted on resistant	0.9040	0.1891	0.2282	0.4603
Autografted resistant vs. resistant grafted on susceptible	0.8485	0.9949	0.9223	0.6650
ANCOVA Statistics P	<0.0001	0.3382	< 0.0001	0.0134
F value	4.21	1.17	3.90	2.28

The covariate for each variable was the mean number of surviving larvae per treatment. Degree of freedom (d.f.) = 12, 1 and 14, 1 for experiment I and II, respectively. Experiment I, intact susceptible species: *Cedrela odorata* (C), *Swietenia macrophylla* (S); intact resistant species: *Khaya senegalensis* (K), *Toona ciliata* (T); autografted susceptible species: C/C, S/S; autografted resistant species K/K, T/T; susceptible species grafted onto resistant species: C/T, S/K; resistant species grafted onto susceptible species: K/S, T/C, T/S. Experiment II, treatments identical as above plus C/K, K/C.

C. odorata, reduced the apical damage by up to 80% as compared with intact *C. odorata*. *Cedrela odorata* grafted onto *K. senegalensis* and *K. senegalensis* grafted onto *C. odorata* reduced the apical damage by 67% and 100%, respectively, compared to intact *C. odorata* (Fig 5b).

Larval performance. In experiment I, only larvae on intact *C. odorata* plants developed to the pupal stage (e.g., 29%, seven out of 24 surviving larvae), which required 32 days. In experiment II, 67% (i.e., six out of nine) surviving larvae on intact and autografted *C. odorata,* as well as on *C. odorata* or *S. macrophylla* grafted onto *K. senegalensis* plants developed to pupa requiring 30 days to do so. Therefore, the statistical analysis was completed only for pupal weight and length in experiment II, and these variables were similar among plant species (F = 0.26, 0.39; d.f. = 4, 1; P > F = 0.88, 0.81, respectively). Orthogonal comparisons were not completed due to high larval mortality mainly on *T. ciliata* plants.

Discussion

Although grafting has proven to be a successful propagation technique for a number of Meliaceae species (Bygrave & Bygrave 2005), some grafts failed. Grafts of *S. macrophylla* onto *T. ciliata* seemed to be incompatible under Turrialba, C. R. conditions. Intergeneric grafts such as these typically have low success rates (Hartmann *et al* 2002), as noticed by Bygrave & Bygrave (1998) trying to graft *T. ciliata* onto *C. odorata*. Fortunately, enough combinations of susceptible and resistant grafted species were produced to test the hypotheses regarding plant damage and larval performance.

In both experiments, the exotic species (*K. senegalensis* and *T. ciliata*) were clearly resistant to attack by *H. grandella* larvae, whereas the native species (*C. odorata* and *S. macrophylla*) were susceptible. These results possibly reflect the lack of coevolution between this New World insect species and Old World Meliaceae species, as was also demonstrated for neem *Azadirachta indica* (Meliaceae), whose metabolites showed either direct insecticidal or growth-disrupting effects on *H. grandella* (Mancebo *et al* 2002). Also, longer-term toxicity (Cornell *et al* 1998) apparently contributed to *H. grandella* mortality on resistant plants (based on the lower pupation observed in experiment I, since only intact *C. odorata* plants allowed development to pupation, whereas in experiment II more grafted plant combinations allowed pupation).

Cedrela odorata and *S. macrophylla* appeared to respond differently to grafting, as shown by their respective autografts. In both experiments, the number of frass piles was reduced by 66% by autografting *C. odorata*, whereas the reduction was only 51% for *S. macrophylla*. Autografting also diminished

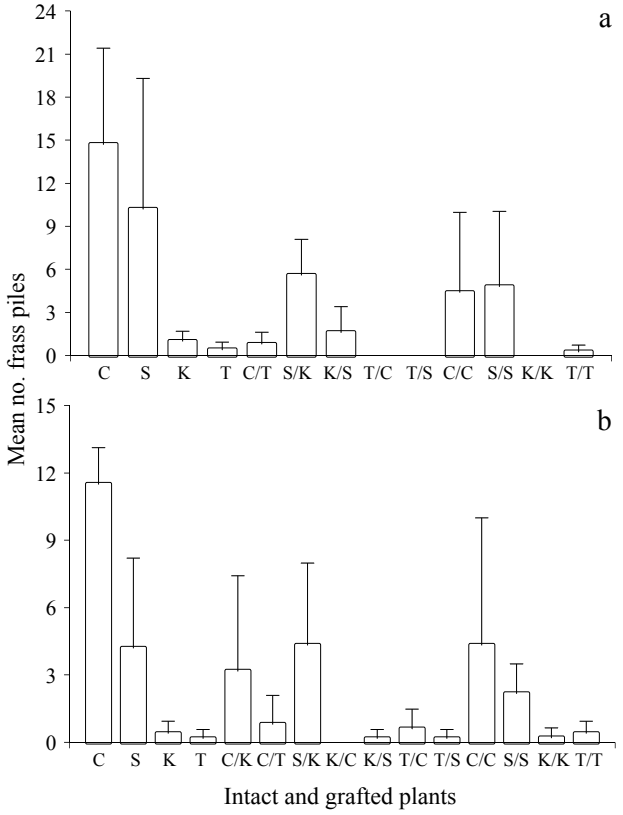

Fig 1 Mean number of frass piles per plant for intact and grafted Meliaceae species after inoculation with *Hypsipyla grandella* eggs (a) or third instars (b). Intact plants: C = *Cedrela odorata*, S = *Swietenia macrophylla*, K = *Khaya senegalensis*, T = *Toona ciliata;* grafted plants: C/K, C/T, S/K, K/C, K/S, T/C, T/S, C/C, S/S, K/K, T/T. Error bars indicate SE (n = 6).

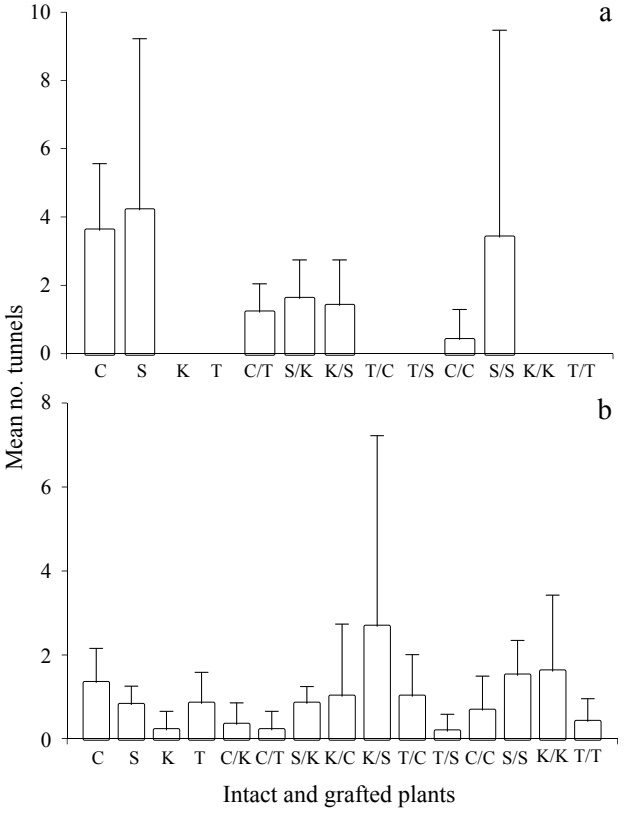

Fig 2 Mean number of tunnels per plant for intact and grafted Meliaceae species after inoculation with *Hypsipyla grandella* eggs (a) or third instars (b). Intact plants: C = *Cedrela odorata*, S = *Swietenia macrophylla*, K = *Khaya senegalensis*, T = *Toona ciliata;* grafted plants: C/K, C/T, S/K, K/C, K/S, T/C, T/S, C/C, S/S, K/K, T/T. Error bars indicate SE (n = 6).

tunnel length in *C. odorata* stems but not in *S. macrophylla* stems. Intact *S. macrophylla* plants are less susceptible than intact *C. odorata* (Speight & Wylie 2001), probably making the autografting effect to attack by *H. grandella* larvae more difficult to detect.

Intact susceptible plants had the most damaged apical buds in both experiments. In agreement with Speight & Wylie (2001), damage by *H. grandella* larvae was more severe on *C. odorata* than on *S. macrophylla* plants. An autografting effect was detectable based on the amount of apical damage, and this effect was most evident in experiment I for *C. odorata*. This finding is important since apical bud damage is the type of injury that leads to loss of apical dominance and causes branching of the main trunk, which results in a noncommercial tree (Grijpma 1976). The reduction of apical shoot damage on either autografted or any rootstock/scion combination plants is encouraging since it indicated that even attacked autografted trees could overcome shootborer damage and still grow into economically useful trees in commercial plantations.

Plant damage by *H. grandella* was noticeably influenced by larval age (neonates responses differed from those of third instars). For example, susceptible plant species had more damage as indicated by the number of frass piles and tunnels made by younger larvae than by older ones, whereas resistant species were free from damage indicated by both tunnel number and length in experiment I. In this experiment, resistant intact plants, and grafted plants using *T. ciliata* either as rootstock or scion had only shallow perforations, which were soon sealed by the plant. These patterns were consistent with expectations based on prior studies showing that *H. grandella* neonates first feed on petiole and leaf surface or shoot surface, and then on the apical bud (Grijpma 1971). Therefore, the 21.7% and 30.8% of apical damage for experiment I and II, respectively, were expected.

Hypsipyla grandella has limited the establishment of commercial plantations of *C. odorata* and *S. macrophylla* species in Neotropics. Due to the damage threshold of one larva per plant and the susceptibility of native species (Hilje & Cornelius 2001), losses from *H. grandella* infestations approach 100% in many plantations (Cornelius *et al* 2004). Therefore, the resistance detected in exotic species and in *C. odorata* grafted onto *T. ciliata*, or *S. macrophylla* grafted onto *K. senegalensis*, as well as on the autografted susceptible species, could be economically important and exploit internal defenses of the trees against *H. grandella*.

Our results indicate that resistance from *K. senegalensis* and *T. ciliata* can be transferred to scions of native species by grafting. This resistance may have been due to toxins or feeding deterrents translocated from rootstocks across

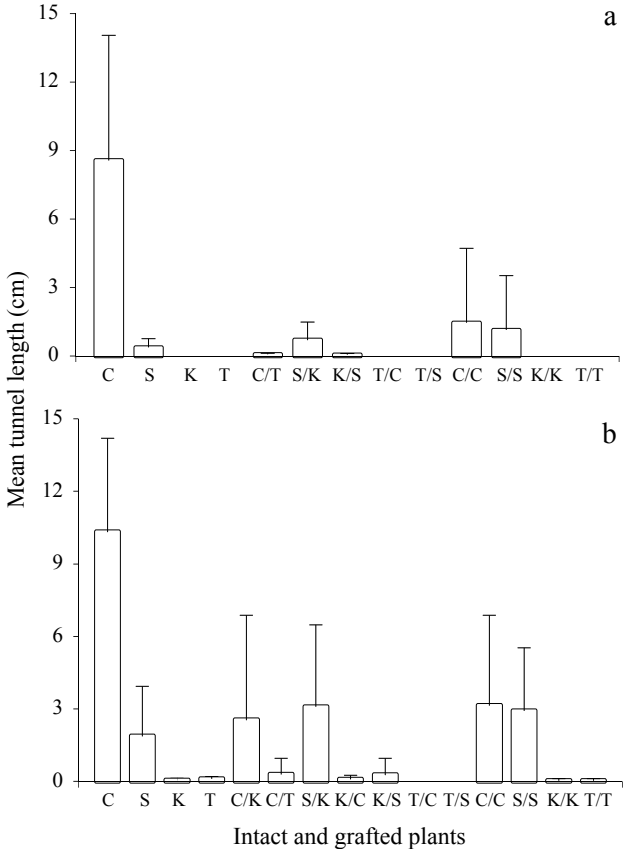

Fig 3 Mean tunnel length per plant for intact and grafted Meliaceae species after inoculation with *Hypsipyla grandella* eggs (a) or third instars (a). Intact plants: C = *Cedrela odorata*, S = *Swietenia macrophylla*, K = *Khaya senegalensis*, T = *Toona ciliata;* grafted plants: C/K, C/T, S/K, K/C, K/S, T/C, T/S, C/C, S/S, K/K, T/T. Error bars indicate SE (n = 6).

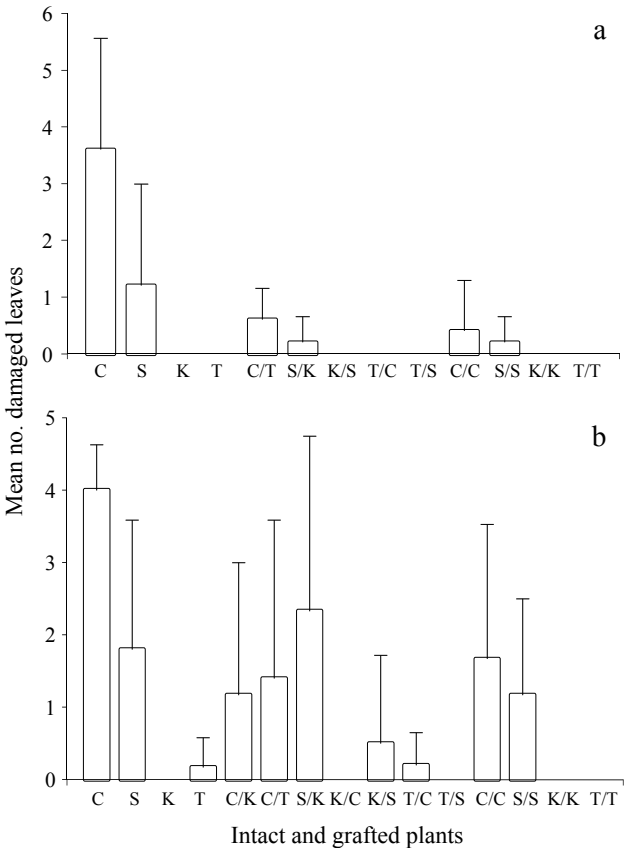

Fig 4 Mean number of damaged leaves per plant for intact and grafted Meliaceae species after inoculation with *Hypsipyla grandella* eggs (a) or third instars (b). Intact plants: C = *Cedrela odorata*, S = *Swietenia macrophylla*, K = *Khaya senegalensis*, T = *Toona ciliata*; grafted plants: C/K, C/T, S/K, K/C, K/S, T/C, T/S, C/C, S/S, K/K, T/T. Error bars indicate SE (n = 6).

the graft union. Candidate bioactive compounds include alkaloids (Smolenski *et al* 1974), limonoids (Koul & Isman 1992, Maia e*t al* 2000), or phenolics (Newton *et al* 1999, Silva *et al* 1999), all of which are produced in these species and have been implicated as defensive compounds (Schoonhoven *et al* 2005).

After contact with *T. ciliata* main shoots, third instars ballooned to lower leaves and tried to feed on them instead of the main shoot, but this response resulted in the death of the larvae. If they bit a lateral bud, an exudate was produced, in which larvae became entrapped. Both susceptible species grafted onto resistant rootstocks, autografted susceptible species, and resistant *K. senegalensis* plants lacked an exudate on lateral buds that could trap larvae. The exudates produced by *T. ciliata* were apparently part of the defenses of this species, as are resinous exudates in other plant species (Lewinshon 1991, Phillips & Croteau 1999), but fail to be transmitted in the grafting procedure.

In addition to the expected resistance to *H. grandella* by intact (Bygrave & Bygrave 2001) and autografted resistant plants, grafting alone provided some degree of resistance even for the susceptible species. This improved resistance could be attributed to plant-induced defenses resulting from the mechanical damage (wounding to make the graft). Plants respond to mechanical wounding by the induction of numerous genes (Reymond *et al* 2000) and may prevent insect feeding by decreasing nutritional value or increasing concentrations of defensive secondary compounds in new foliage (Schoonhoven *et al* 2005) which could be toxic for insects. This result seems to diminish the potential importance of defensive substances unique to the resistant plant species and also may explain the high mortality of larvae in both experiments including the susceptible species. Mortality was higher for later *H. grandella* instars (experiment II) than for neonates (experiment I). Although neonates may be more susceptible to specific resistance factors in the plants involved in this study, neonate lepidopterans can detect diets that could be toxic to them, whereas later instars seem to lack that ability (Zalucki *et al* 2002), so behavioral effects could contribute more to the results in experiment I than those from experiment II.

Whatever the mechanisms, resistant trees could be deployed in two ways. First, entire plantations of *C. odorata* grafted onto *T. ciliata* plants could be established. This approach has been successful for *T. ciliata* grafted onto *C. fissilis* in Australia, where the trees maintained their resistance

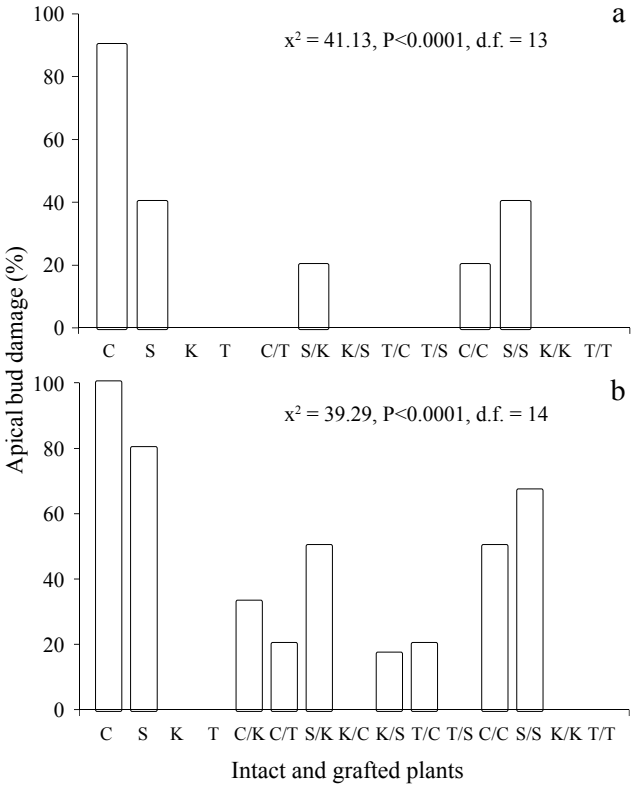

Fig 5 Percentage of apical bud damage per treatment for intact and grafted Meliaceae species after inoculation with *Hypsipyla grandella* eggs (a) or third instars (b). Intact plants: C = *Cedrela odorata*, S = *Swietenia macrophylla*, K = *Khaya senegalensis*, T = *Toona ciliata;* grafted plants: C/K, C/T, S/K, K/C, K/S, T/C, T/S, C/C, S/S, K/K, T/T.

against *H. robusta* after eight years (Bygrave & Bygrave 2005). Taking into account that, depending on the site, five to eight years are required by *C. odorata* and *S. macrophylla* plants to achieve a commercially valuable bole (Cibrián *et al* 1995), long-term evaluation of the grafted plants should be completed to determine if resistance is maintained and if they are adapted to field conditions.

Second, autografted *C. odorata* and *S. macrophylla* providing some level of resistance could lead to simple methods for enhancing tree resistance based only on wounding. This avenue needs to be explored more thoroughly, with additional studies to test the effects of wounding on insect resistance. One question that must be investigated is the duration of the wounding effect on elevating the plant's resistance to *H. grandella* larvae. Wounding trees may have a positive effect on their growth, since *T. ciliata* trees damaged by *H. robusta* (Cunningham & Floyd 2006) or *S. macrophylla* and *C. odorata* damaged by *H. grandella* (Pers. obs.) grow more quickly and produce more biomass than non-damaged trees.

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