Host preference of the Meliaceae shootborer *Hypsipyla*: further information from grafting *Cedrela odorata* and *Cedrela fissilis* on *Toona ciliata* (Australian red cedar)

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Revised manuscript received 14 August 2001

Summary

Exotic species of *Cedrela* (*C. odorata* C. de Canodolle and *C. fissilis* Vel.) grown on the New South Wales mid-north coast of Australia are not attacked by the shootborer *Hypsipyla robusta* whereas Australian red cedar (*Toona ciliata*) grown in plantation in the same location is heavily attacked. In an extension of our earlier study, further grafts were constructed using *C. odorata* and *C. fissilis* as scion and *T. ciliata* as rootstock. Tree height and degree of shootborer attack were monitored over a four-year period. Grafts of *C. odorata* on *T. ciliata* were attacked but not those of *C. fissilis* on *T. ciliata*, revealing further insights into the host preference of *Hypsipyla*. Possible reasons for the observed differences in susceptibility to attack between the two graft types are discussed.

Keywords: insect pests, pest resistance, hosts, grafting, Meliaceae, *Cedrela*, *Toona*, *Hisipyla*

Introduction

Attack by the shootborer *Hypsipyla* on Meliaceae species is the major factor thwarting establishment of plantations of this valuable timber tree species in the tropics and subtropics (Newton *et al*. 1993; Mayhew and Newton 1998). Given the extent to which this species has been destroyed over many decades in the tropical forests of Central and South America, Africa, South East Asia and Australia, shootborer attack has serious ecological and commercial implications. Control of attack by this insect is the key to establishing plantations of the species (references above and those in Floyd and Hauxwell 2001 and Griffiths 2001).

Numerous studies carried out on the biology of *Hypsipyla* have revealed much about their general behaviour and life cycle (Floyd and Hauxwell 2001). It is clear is that the adult female lays her eggs on the leaves of the host tree and the emerging larvae burrow into the growing parts of the tree, especially the dominant apical shoot, and eat out the pith. These shoots die and the tree compensates by growing lateral shoots. Aside from retarding apical growth, this leads to a tree with a stem of little commercial value.

Particularly relevant to shootborer attack is the host tree preference. *H. grandella*, the species isolated to tropical Central America and adjacent regions, has a particular preference for *Cedrela* and *Swietenia* (Mayhew and Newton 1998). *H. robusta*, on the other hand, apparently isolated to tropical Africa, South East Asia and Australia (but see Horak 2001), prefers *Toona* species such as *T. ciliata* (Grijpma and Gara 1970a, 1970b; Gripjma 1973, 1976; Edmonds 1993). Moreover, and especially relevant to this present research, Gripjma (1973) has observed that *T. ciliata* grown in Costa Rica is not attacked by *H. grandella*, and *Cedrela* spp. grown on the New South Wales mid-north coast are not attacked by the local shootborer, assumed to be *H. robusta* (Bygrave and Bygrave 1994, 1998).

In efforts to further understand the nature of the interaction of the local shootborer with its preferred host *T. ciliata*, we have made use of the above observations to construct grafts of *Cedrela* spp. as scion and *T. ciliata* as rootstock. Our initial report (Bygrave and Bygrave 1998) showed that when such grafts were planted in the field, the *Cedrela* spp. were rendered susceptible to attack. However, the relatively small number of grafts then tested, as well as the nature of their constructs, have been insufficient to establish conclusive differences in susceptibility to attack between *C. odorata* and *C. fissilis*.

In this paper we report on work that extends the earlier findings in several respects: (a) the number of grafted trees of each type examined in the field has been increased especially to test for suspected differences between the two types in susceptibility to attack, and (b) we have made a direct comparison of the degree of attack on the grafted trees with that on *T. ciliata* growing under similar conditions. These measurements have extended over a period of about four years. We also update progress on the growth and development of the grafted trees discussed in our earlier paper (Bygrave and Bygrave 1998).

Methods

Seedlings of *T. ciliata* were obtained from a private nursery on the New South Wales mid-north coast, the seed source being mature native *T. ciliata* growing in the Bellingen area. Seeds of *Cedrela* spp. were obtained from the Queensland Forest Service and germinated in a glasshouse in the Plant Culture Facility at The Australian National University; *C. odorata* was from seedlot No. 759 and *C. fissilis* from seedlot No. 2288.

Grafts of *C. odorata* and *C. fissilis* as scion with *T. ciliata* as root stock were made in August 1996; a V-graft was used. Both the *T. ciliata* rootstock and the *Cedrela* spp. scions were similar in size, generally a little less than 1 cm in diameter at the graft point. The grafted stock, comprising 6 of each graft type, were maintained in the glasshouse until the grafts proved successful as judged by general growth characteristics and budding on the scion. The young trees were planted in the field in late December 1996 on a private property near Macksville on the

December 1996 on a private property near Macksville on the New South Wales mid-north coast (30˚42'S, 152˚55'E). The field site is within 100 m of extensive mono-specific and mixed plantings of *T. ciliata* (ca. 500 trees) and within several hundred metres of extensive plantings of *C. odorata* and *C. fissilis* (ca. 200 trees) (Bygrave and Bygrave 1998). The area of the trial site is about 500 m^2. Non-grafted *T. ciliata* of a similar age were also planted at the site as controls. The height of all trees at planting ranged from 0.4 to 0.8 m. About 200 g of organic fertiliser (Dynamic Lifter) was added to the planting holes to encourage early growth. Before planting, the area was slashed and the herbicide glyphosate sprayed around the point of planting to a diameter of 2 m to minimise surrounding undergrowth. This later procedure was repeated on a regular basis in the course of the study.

Results and discussion

As mentioned, in the vicinity of the trial site, there is no evidence of shootborer attack on the *Cedrela* spp. following some eight years of growth whereas all *T. ciliata* trees have been attacked to varying degrees. Thus in our experience, the host preference for *Hypsipyla* in this location is *T. ciliata*. Despite such attack, however, many of the *T. ciliata* have reached a height of at least 8 m and have a DBH of about 20 cm.

Observations on the December 1996 plantings

In the present set of experiments, we were able to compare directly the degree of attack and rate of growth of *T. ciliata*, *C. fissilis* grafted on *T. ciliata*, and *C. odorata* grafted on *T. ciliata*. At the time of planting in the field all graft types were of a similar height, ranging from 0.4 to 0.8 m. Data in Table 1 show that by about 6 months after planting, three of the six *T. ciliata* had suffered mild attack as had one of the grafted *C. odorata*, but none of the grafted *C. fissilis*. By 14 months (i.e. mid-way through the second summer and thus during a period with high probability of shootborer attack in this location (Mo *et al.* 1997a,b), five of the six *T. ciliata* and four of the six grafted *C. odorata* had suffered attack. Although of a similar average height, none of the grafted *C. fissilis* had been attacked. Mild to severe attack was observed with both *T. ciliata* and grafted *C. odorata* at all other stages, but none with grafted *C. fissilis* to the present time.

As reported previously (Mo *et al*. 1997a,b; Bygrave and Bygrave 1998), attack on *T. ciliata* at this site is reflected in the presence of dead/dying shoots, tunnelling by larvae, accumulation of frass at tunnel openings and the presence of smaller brown or blue larvae at the base of the scours. These features were seen also in the infected shoots of the grafted *C. odorata*. In addition, the extent of tunnelling was generally greater in *C. odorata* grafts. Thus while tunnelling in *T. ciliata* would sometimes reach a depth of around 10 cm, it was not unusual to find tunnelling in the graft up to and sometimes exceeding twice this depth. Consequently the tree form of such grafts suffered to a far greater extent than that of *T. ciliata*. For example, on some grafts there were more than six branches at around 1.5 m. This multiple-branching was not seen with the *C. fissilis* grafts, most of which retained a single straight bole - some branching did occur at about 1.5 m but this did not appear to result from shootborer attack. Besides these features, at the time of the analysis in November 2000 (47 months after planting), the leaves of the *C. odorata* grafts were still yellow from the dry winter period but those of the *C. fissilis* grafts were deep green and lush. In addition one of the *C. fissilis* grafts had produced flowers. A statistical analysis of the data in Table 1 (not shown) indicated no significant difference in average mean height of each of the three tree types at 47 months.

Further observations on the December 1995 grafts previously described in 1998

The grafts of both *C. odorata* and *C. fissilis* scions on *T. ciliata* rootstock five years after planting out have been very successful, as reflected in the development of the trees in general (Table 2). All trees have attained heights approaching 10 m and where there were two grafts on the one rootstock, both scions have reached similar heights. We reported (Bygrave and Bygrave 1998) that in the early stages of the growth of the grafts, the main stem of the *Cedrela* scions immediately above the graft had increased in girth more rapidly than that of the stock to produce a 'bulge' in the stem. This was especially prominent after planting out. Over time, however, the bulges have been absorbed as the trees developed to the extent that only a slight difference in bole diameter is now evident. In the earlier report, it was a little difficult to distinguish between the degree of attack on the two (different) scions on the one rootstock stem; hence we saw the need to increase the number of grafts as well

Table 1. Tree height (m) and shootborer attack[1] in *Toona ciliata* and grafted trees

Tree number	Tree type[2]	Months after planting											
		6		14		20		25		37		47	
		Height	Attack[1]	Height	Attack	Height	Attack	Height	Attack	Height	Attack	Height	Attack
1	I	1.3	#	1.6	##	1.8	##	2.7	#	3	##	4	
2	I	1.2	#	1.7	#	2	##	3	#	4	#	4.2	
3	I	1.4	#	2.4	##	2.3	##	3.1	#	4	#	5	
4	I	0.6		1.6	##	1.6		2.7	#	3	#	3.5	
5	I	1		1.2	##	1.3	##	1.2	#	1.3	#	1.5	##
6	I	1.1		1.8	##	1.8	##	2.2	##	2.5	##	3.2	#
1	II	1.3		1.7	##	2.5		2.8	##	3	##	2.9	##
2	II	0.6		1		1.2	##	1.6	#	3	#	3.3	##
3	II	1		1.4	##	1.7	##	1.7	##	2.2	##	4	##
4	II	0.7		1.4		1.7	##	2	#	3	##	3.4	#
5	II	1.5		2	##	2.9		3	#	3	#	4	#
6	II	1.5	#	1.7	##	2.1	##	2.5	#	2	##	2.3	##
1	III	1		1.8		2		3.4		5		5	
2	III	0.3		1.1		1.1		1		1.5		1.8	
3	III	0.6		1.4		2		2.5		2.5		2.9	
4	III	0.8		1.9		2.7		3.5		5		5	
5	III	0.3		1.3		1.3		2		2.5		3.5	
6	III	0.2		0.6		0.9		1.3		1.6		2.5	

[1]# mild attack, ## = severe attack, blank = no attack

[2]I = *T. ciliata*, II = *C. odorata* graft, III= *C. fissilis* graft

as construct grafts with only single stems. Thus the differences in degree of attack on the two *Cedrela* spp. are now clearly evident. Of further interest is the production of flowers already by one of the *C. fissilis* scions.

Table 2. Summary of progress of the grafted trees reported in earlier work

Tree	Species / graft		DBH
no.	Stock	Scion	(cm)
1	*T. ciliata*	*C. odorata*	14
2	*T. ciliata*	*C. fissilis*	12
3	*T. ciliata*	*C. fissilis* and *C. odorata*	10, 8
4	*T. ciliata*	*C. fissilis* and *C. odorata*	9, 8
5	*T. ciliata*	*C. fissilis* and *C. odorata*	7, 17
6	*T. ciliata*	*T. ciliata* and *C. odorata*	10, 11
7	Non-grafted *C. fissilis*		14

Notes:

1. Measurements and observations were made on 10 November 2000, five years after planting out
2. All trees were of a similar height (ca. 8 m)
3. *C. fissilis* grafts were very green and in full leaf but *C. odorata* grafts had a semi-deciduous appearance and yellow leaves
4. *C. odorata* grafts were generally thinner and multi-branched with a smooth surface
5. *C. fissilis* grafts had a predominant main trunk with a rough surface

Concluding comments

Several features of the graft experiments are worthy of further comment. Among other things, they illustrate further the potential of this approach for learning more about the biology of *Hypsipyla* and, in particular, those factors that are involved in host preference with respect to both the adult insect and the larvae.

The first point is the evidence that, at least on the New South Wales mid-north coast, the local species of *Hypsipyla,* which we assume to be *H. robusta* (Horak 2001), has a clear preference for the grafted *C. odorata* over the grafted *C. fissilis*. Indeed 'preference' hardly seems an issue as there is no obvious evidence of any attack by the insect on the latter graft. Attack on *C. odorata* is reflected in the multiple-branching of the grafted trees (some now have up to as many as five or six branches) by contrast with the generally single, straight bole of the *C. fissilis* grafted trees. For the sake of these experiments, no pruning has been carried out on the *C. odorata* grafts.

The second point is the observation that the devastation produced by the larvae was reflected not only in the multiple-branching of the *C. odorata* grafts but also in the depth to which the larvae had penetrated in each individual branch. While this was not measured precisely, the evidence was abundant on all *C. odorata* grafts that the larvae had tunnelled far deeper, and often as much as twice as deep, as on *T. ciliata* trees. The extent to which the pith of grafted *C. odorata* shoots is eaten out by H. robusta larvae in these studies could also reflect the relative tenderness of the material in the early stages of shoot development. We have observed that shoot growth of the *C. odorata* grafts is also more rapid than that of *T. ciliata.*

In this context, the turn-around of insect preference from zero with non-grafted *C. odorata* trees to the extent described above with *C. odorata* grafts on *T. ciliata*, is of considerable interest. Presumably, and as already suggested (Gripjma 1976; Bygrave and Bygrave 1998), substances translocated across the graft from *T. ciliata* to *C. odorata* can attract *H. robusta.* The fact that this does not result in attraction with the *C. fissilis* graft indicates several possibilities, one being that there are substances in *C. fissilis* that mask those in *T. ciliata* which induce attraction. At the same time, the findings are consistent with the observations of others (Yamazaki *et al*. 1990, 1992; Carlos Navarro, Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), Turrialba, Costa Rica, *pers. comm.*) that in Central America, attack by *H. grandella* on *C. fissilis* is much less severe than on *C. odorata.*

Grafts of *T. ciliata* on *C. odorata* are being used in Brazil to analyse possible (chemical) factors that might be responsible for the attraction to *C. odorata* of *H. grandella*. While a number of secondary compounds, possibly limonoidal in nature, have been suspected as potential candidates (Agostinho *et al*. 1994; da Silva *et al*. 1999) there still is no clear evidence that any one or other might be directly responsible for attraction of the insect to the host. However, results from the chemical analyses being undertaken by this group, wherein particular key limonoids are distributed differently between *T. ciliata* and *C. odorata,* have also led members to believe that the taxonomic groupings of *Toona* and *Cedrela* require revision (da Silva *et al*. 1999).

Apart from the chemistry of the individual *Cedrela* species, the extent to which the adult female moth is or is not attracted to the different species is unknown. Since the texture, size and fine structure of the leaves and branching of *C. odorata* are quite different from those of *C. fissilis*, for example, the possibility exists, and needs to be examined, whether *Hypsipyla* actually lays eggs on *C. fissilis* grafts.

It is also noteworthy that the first flowers have appeared on several of the grafts. It will be of interest in due course to examine the nature of the offspring and to see the extent to which they are resistant or otherwise to *Hypsipyla* attack.

These studies thus reveal an interesting line of enquiry into Meliaceae/*Hypsipyla* interactions that hopefully will provide more useful information about the factors involved. The planting out in January 2001 of a further 25 grafted trees, to bring the total to some 60, has added to the resource base of the study.

Acknowledgements

We are grateful to George Serbov for carrying out the grafts and caring for the young grafts while establishing in the Plant Culture Facility, and to Dr John Banks (Forestry Department, Australian National University) for helpful discussions and expert advice. Suggested improvements to the text by the two reviewers were much appreciated.

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