Oecologia (1990) 85:257–265

Oecologia

The role of lignin as a defence against the spruce bark beetle *Dendroctonus micans:* effect on larvae and adults

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Received May 15, 1990 / Accepted in revised form August 5, 1990

Summary. The role of lignin as a physical defence against *Dendroctonus micans* was investigated in laboratory feeding experiments. The effect of lignin is dose-dependent, reducing larval survival, growth rate, and weight, as well as affecting gallery construction. Adults lay fewer eggs in lignified bark and also tend to construct abnormal galleries. The distribution of lignin in trees suggests a role in defence against bark beetles that feed in the thicker bark on the lower bole.

Key words: *Dendroctonus micans* – Defence – Lignin – Secondary chemicals – Host resistance

The spruce bark beetle, *Dendroctonus micans*, accidently introduced to Britain from mainland Europe (Bevan and King 1983), is now widely distributed in spruce plantations in Wales and the west Midlands. Norway spruce, *Picea abies*, is the main host species in Western Europe whereas in Turkey and Soviet Georgia, Oriental spruce, *P. orientalis*, is attacked and appears to be highly susceptible (Benz 1984). The North American Sitka spruce, *P. sitchensis*, which is extensively planted in Britain, is also susceptible, although less commonly attacked than Norway spruce (Evans et al. 1984; Grégoire 1988).

Most economically important bark beetle species coordinate a mass-attack on trees by release of aggregation pheromones and so can "overwhelm" a tree's natural defences. This process is usually aided by symbiotic fungi which may also enhance the nutritive quality of the bark which is rapidly utilized by the many larval broods that develop on trees that succumb to attack (Coulson 1979; Horntvedt et al. 1983; Raffa and Berryman 1983). In contrast, individual female *D. micans* may successfully establish a brood on a standing, apparently vigorous, tree without mass-attack (Grégoire 1988) and so this species can cause significant damage at relatively low population densities. Again, in contrast to most other species, *D. micans* larvae from a single brood feed *en masse* within a single large gallery, a behaviour that appears to be mediated by a larval aggregation pheromone (Grégoire et al. 1982).

As with other bark beetles, there is evidence that host resistance mechanisms are important in the population dynamics of *D. micans*, many attacks for example ending in failure to establish a brood (Evans et al. 1984; Grégoire 1984). Two resistance mechanisms against conifer bark beetles have been widely studied, both of which are active in defence against *D. micans*. The first is based on primary resin which flows from the bark and wood following insect attack or other damage. Primary resin, which contains largely monoterpenes and resin acids, may intoxicate beetles and can eject them from galleries or engulf and drown them (Vité and Wood 1961; Raffa and Berryman 1987). A second defensive mechanism, the dynamic wound response, is a reaction to fungal invasion of the damaged tissue around the gallery (Reid et al. 1967; Berryman 1969; Christiansen and Horntvedt 1983). This reaction is characterised by necrosis and the local synthesis and deposition of secondary resin and other materials that can prevent further fungal invasion and also render the tissues unpalatable to the beetles themselves (Russell and Berryman 1976; Bordasch and Berryman 1977; Raffa and Berryman 1987).

In this paper we report on a constitutive physical defence against *D. micans* resulting from the occurrence of lignified stone cell masses in bark tissues. The study is based on a series of laboratory experiments to determine the effect of lignin on larval development and on adult oviposition.

Methods

All host material for the experiments was obtained from adjacent compartments of Norway and Sitka spruce on the Long Mynd, Shropshire, planted at an elevation of 400 m (O.S. ref SO 412902).

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The compartments were planted in 1950–1952 and had been thinned once. Although *D. micans* was present in the area, only trees which were unattacked and free from other visible injury or disease were used in experiments. These experiments were done in a temperature controlled room at around 20° C, 60–70% RH and an 18-h photoperiod.

Larval experiments

Sitka spruce logs. Trees to provide logs for experiments were selected by estimating the amount of lignin, visible as brown "gritty" inclusions, in the cut end of a small bark section taken at about 1.3 m (= breast height [b.h.]) above the ground. Seven trees were selected to have either "low" or "high" lignification and were felled on 17 June 1986, the lower 50 cm of the bole discarded and two successive 80-cm lengths cut from the tree. Mid-log diameters ranged from 13 to 19.5 cm. The cut ends of the logs were sealed with wax to minimize drying during the 8–10 weeks of the experiments. To measure the amount of lignin in bark, three 1.5-cm diameter cores spaced along the log were taken from one randomly determined side. They were stored in an aqueous mixture of formalin, acetic and ascorbic acids, and methyl alcohol (FAA) prior to sectioning and staining described below. Eggs for this and other experiments were obtained from field-caught beetles capped onto fresh Norway spruce logs. Different egg broods were mixed together and stored for up to 7 days at 2–4° C. The logs were inoculated with 40 eggs on the day of felling at two positions on opposite sides of the log and approx 5 cm from the end. At each inoculation site, a 1.5-cm diameter core of bark was removed and replaced with a plug of adult frass and beetle-chewed bark and wood containing the eggs and was held in place by brown waxed paper stapled to the log. After inoculation the logs were enclosed in insect-proof nylon bags and suspended in an upright position on a rack, allocating positions at random but keeping the two logs from each tree together.

After 62 days, the galleries in each log were carefully dissected and the surviving larvae, pupae or adults counted and undamaged individuals weighed. The number and development stage of dead individuals was also recorded. An arbitrary score for the stage of development of each brood was calculated as the sum of the percent larvae, pupae, and adults multiplied respectively by 1, 2 or 3. Percent survival based on the number of eggs inoculated was determined.

Detached bark from Norway and Sitka spruce. Four separate experiments were done using bark from 5 or 7 trees selected to have a "high" or "low" lignin content. The trees ranged in b.h. diameter from 16.5 to 28 cm. The four experiments were started on the following dates: Sitka spruce (SS), experiment 1 14 May 1986, experiment 3 on 24 June 1986; Norway spruce (NS): experiment 2 on 3 June 1986, experiment 4 on 23 July 1986. Limited supplies of eggs from field-collected beetles precluded the simultaneous bioassay of bark from both species.

Bark for experiments was removed from living trees at intervals from April to September during which time the cambium separated cleanly from the xylem and bark remained in good condition for up to 3 weeks. Bark pieces (approx 13 × 23 cm) were taken at heights of 0.5, 1.25 and 2.0 m from the ground on each experimental tree. At each height a total of three pieces taken successively at approx 120° spacing round the circumference were usually required for development from egg to adult. Adjacent to the first bark piece at each height, a 1.5 cm diameter core was taken for estimation of lignin content and stored in FAA.

Bark pieces were inoculated with 30–40 eggs, usually on the day after collection, by folding back a triangular flap of phloem near one end and excavating the bark cortex below it to accommodate the eggs. The flap was repositioned and the bark sandwiched between two sheets of clear acrylic (6 mm thick) screwed together to hold the bark firmly without crushing. All bark pieces in an experiment were changed when food supply in one of them became limiting due either to larval feeding or fungal colonisation of the bark. Larvae were transferred to a cavity cut in the fresh bark so that the acrylic sheets could be reassembled without damaging the larvae. Changes were required at about 3-week intervals.

In experiments 1 (SS) and 2 (NS), adult weight and number of days from the start of the experiment to adult eclosion was determined. Experiments 3 (SS) and 4 (NS) were terminated when larvae had developed to the 5th instar or pupal stage because of the difficulty in removing bark from trees during late September. Larvae and pupae were weighed and percent survival calculated. A development score was determined during the larval stage after 70 days for experiment 3 and 28 days for experiment 4. It was computed from the sum of the percent larvae in the 1st/2nd, 3rd and 4/5th instar multiplied respectively by 1, 2 or 3.

Adult experiments

Detached bark from Norway and Sitka spruce. Adult beetles used in the experiment were collected from a single natural brood on Norway spruce. The beetles, which were still aggregated in or around the original larval gallery, were assumed not to have oviposited. They were stored in rehydrated Norway spruce bark powder together with a piece of fresh bark for 10 days at 2–4° C. Six trees each of Norway and Sitka spruce were selected as having bark > 3 mm thick 2 m from the ground and either a high or low lignin content. Bark pieces were taken from each tree at 0.5, 1.25 and 2.0 m on 6 June 1988. Each piece was divided into three (approx 8 × 13 cm) and a 1.5 cm diameter core removed from the centre to accommodate a single beetle. Beetles were weighed and those heavier than 25 mg (assumed to be female) were allocated to bark pieces at random. Each set of three pieces of bark was clamped between two sheets of acrylic as for the larval experiments and enclosed in a nylon mesh bag. A 1.5-cm diameter core of bark for estimation of lignin content was removed from experimental trees adjacent to each bark piece and stored in FAA.

During the experiment about a quarter of the beetles tunnelled out of the bark and these were returned to it daily. After 20 days, the number of eggs laid in each bark piece was determined by counting unhatched eggs and larvae. Bark cores were taken through adult galleries and stored in FAA before sectioning to reveal the position of galleries in relation to the distribution of lignin.

The amount and distribution of lignin in bark

A strip of bark approx 2–3 mm wide, oriented to give a longitudinal section, was taken from each bark core stored in FAA and heated in a water bath with a 5% agar solution for 6 h. Freezing microtome sections were then cut and stained in phloroglucinol solution which coloured lignin red. The percent lignin content and thickness of each bark section was estimated using a microcomputer image analysis system (Microscale II, Digithurst Ltd., Royston, Herts).

Results

The amount and distribution of lignin in bark

Lignin occurs predominantly in groups of stone cells. Although they can occur throughout the bark (Fig. 1a, b) they commonly form a distinct layer in the inner tissues (Fig. 1c, d). In the trees studied, a rhytidome was usually absent but the bark plates characteristic of parts of the lower stem of Sitka spruce form a thick rhytidome (Fig. 1b) that considerably reduces the thickness of the living bark.

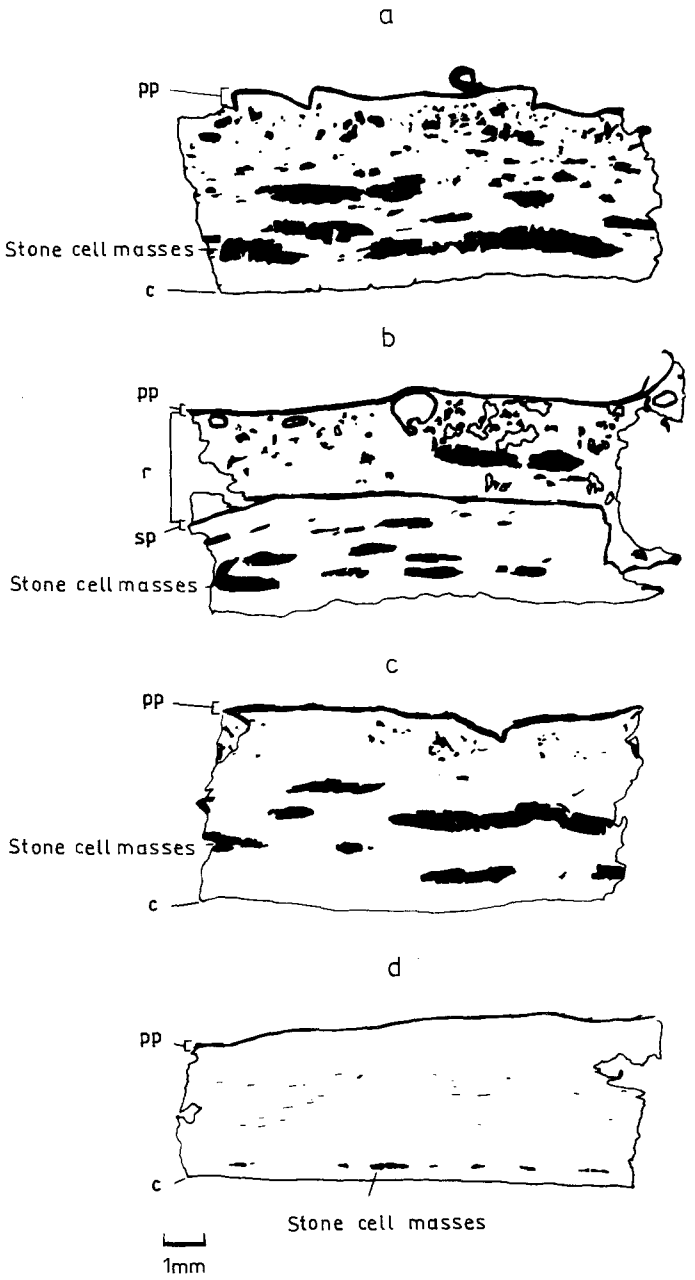

Fig. 1a–d. Examples of the distribution of lignified stone cells in spruce bark. **a, c, d** Norway spruce. **b** Sitka spruce. *pp* = primary periderm, *sp* = secondary periderm, *r* = rhytidome (largely dead bark), *c* = cambium

The lignin content of bark of trees used in the experiments is shown in Table 1. In general there was a decline in lignification of bark with increasing sample height (Table 2). In the larval experiment 4 (SS) and in both tree species in the adult experiment, lignin content and bark thickness were significantly correlated ($P<0.05$–0.001) (Fig. 2). When these two variables were used as covariates in separate analyses of variance of data from the adult experiment, lignin content explained much more of the variation in number of eggs laid between trees ($P<0.001$) than bark thickness ($P<0.05$) and where both were used as covariates in the same analysis, bark thickness was not significant. Bark thickness was not

Table 1. Mean percent lignin content of bark on trees used in experiments

	Tree rank no.						
	1	2	3	4	5	6	7
Larvae							
Sitka spruce logs*	0.4	3.0	4.0	4.3	14.5	15.9	21.0
Sitka spruce bark							
Expt 1	3.7	8.4	11.3	15.6	17.5		
Expt 3	0.8	3.7	8.4	10.3	11.4	15.5	22.3
Norway spruce bark							
Expt 2	8.2	8.6	10.4	12.0	14.3	16.7	18.4
Expt 4	1.0	3.5	4.3	5.3	12.6	14.0	18.2
Adults							
Sitka spruce bark	0.5	1.1	1.2	9.5	9.5	13.8	
Norway spruce bark	1.2	1.2	1.7	3.5	8.6	10.4	

Mean of 3 heights (* or 2 logs) per tree

Table 2. Mean percent lignin content of bark at 0.5, 1.25 and 2 m on trees used in detached bark experiments

		No. trees	Height (m)		
			0.5	1.25	2.0
Larvae					
Sitka spruce	Expt 1	5	15.2	12.0	6.6
	Expt 3	7	12.8	9.9	8.3
Norway spruce	Expt 2	7	15.1	13.8	9.1
	Expt 4	7	11.3	8.0	5.8
Adults					
Sitka spruce		6	8.0	5.9	3.8
Norway spruce		6	7.8	4.3	1.3

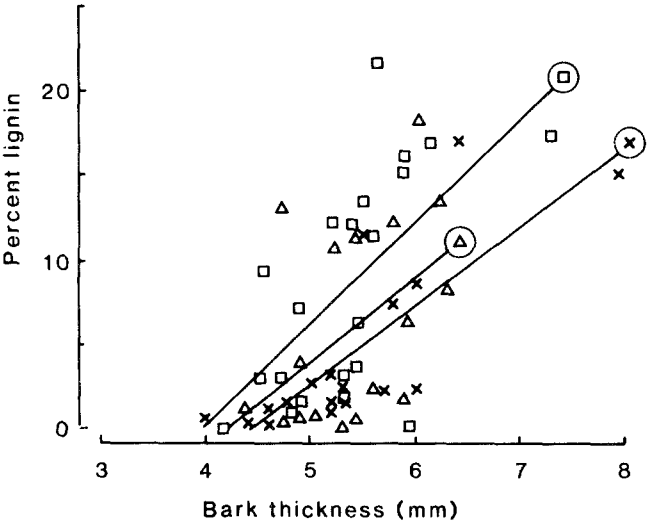

Fig. 2. Relationship between percent lignin and bark thickness in the adult experiment, X Norway spruce ($P<0.001$) and △ Sitka spruce ($P<0.05$), and larval experiment 4 □ ($P<0.01$)

therefore included as a covariate in any of the analyses reported here.

Larval experiments

Sitka spruce logs. At the end of the experiment, 54% of larvae had pupated. Most of the remaining larvae were feeding in bark of high lignin content suggesting that the presence of lignin slowed the development rate. Some of the bark on logs was found to be discoloured or colonized by fungi and this may have affected its nutritional quality for larvae. This problem was avoided in the detached bark experiments where bark was renewed periodically. Galleries made by young larvae in highly lignified bark tended to occur in the outer tissues, avoiding the lignified layer, whereas in parts of the gallery excavated by larger, probably 5th-instar larvae, bark was consumed down to the xylem.

Data on percent survival (after angular transformation), larval/pupal weight, and development score were analysed by covariance analysis, with percent lignin as the covariate. Survival appeared to be unaffected by variation in lignin but a significant amount of variation between trees in both mean larval/pupal weight ($P<0.01$) and development score ($P<0.001$) could be attributed to the amount of lignin in bark. A regression analysis of the weight and score variables on percent lignin, based on tree means (2 logs/tree) and weighted for the number of individuals, revealed that both larval/pupal weight ($b=-0.84\pm0.14$, $P<0.01$) and development score ($b=-5.8\pm0.56$, $P<0.001$) declined with increasing lignin content of the bark.

Detached bark from Norway and Sitka spruce. Larvae exhibited the typical aggregated feeding behaviour in bark pieces and in most cases excavated an apparently normal gallery (see Grégoire 1988, Fig. 2) (Fig. 3a). In highly lignified bark however, the feeding front was characteristically fragmented (Fig. 3b). We concluded that larvae were obliged to feed between the stone cell masses, either in small groups or even singly. Galleries formed by young larvae in lignified bark were often not visible on the inner phloem surface because they were excavated in the cortical tissue between the outer rhytidome and the inner lignified region.

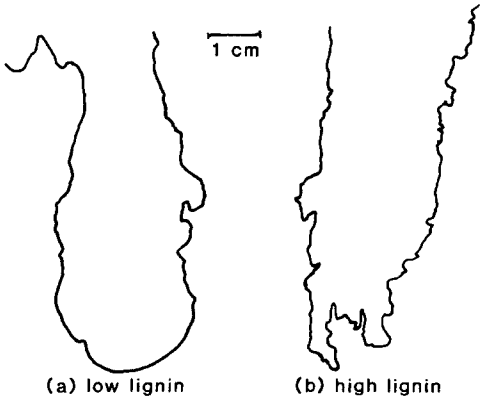

Fig. 3. a Normal and **b** disrupted feeding front in detached bark of Norway spruce viewed from the inner (cambial) surface of the bark phloem

The data were analysed by linear regression, to compare results from experiments 1 (SS) with 2 (NS) and 3 (SS) with 4 (NS). As the experiments were done consecutively, we assumed that species differences that were supported by both sets of experiments represented real differences between them rather than effects due to their different timing. For each data set, based on tree means, three regression models were fitted; a single line to the pooled data or separate lines for each species that were either parallel with differing intercepts or non-parallel. Data for survival were transformed to logits for analysis but were plotted on an untransformed scale. All variables, including percent lignin, were weighted for the number of individuals. The relationships between percent lignin and survival, development time/score and weight of larvae, pupae or adults based on the best fitting model in each case are shown in Fig. 4a–f.

In Norway spruce there was evidence of a small decrease in survival with increasing lignification (experiment 2 ns, experiment 4 $P<0.05$). Survival on Sitka spruce however showed a strong negative relationship with lignification ($P<0.001$) (Fig. 4a, d). In both sets of experiments the species × lignin interaction was highly significant ($P<0.01$, $P<0.001$).

The growth and development of survivors was also affected by lignin in bark. Development to adults (Fig. 4b) took significantly longer on Sitka spruce (mean 95.9 days) than Norway spruce (mean 81.2 days) ($P<0.05$) and in both species, it took around 10–15% longer in the most than in the least lignified bark in our experiments, although the common slope fitted was not statistically significant. In experiments 3 (SS) and 4 (NS), development score, an estimate of rate of development, was negatively correlated with lignin content ($P<0.01$) (Fig. 4e). Although the development scores for these two experiments are not strictly comparable, development in Sitka spruce was clearly much slower than in Norway spruce since the larvae had reached a similar developmental stage after about 70 and 28 days respectively when the development score was estimated. These results support the observations of the increase in development time to adulthood in Sitka compared with Norway spruce (experiments 1 and 2).

There was a small though significant ($P<0.05$) negative effect of lignin on adult weight which was similar in both species (Fig. 4c). Larval/pupal weight was also affected by lignin and there was a significant ($P<0.05$) species × lignin interaction, with weight declining more rapidly with increasing lignin content on Sitka spruce (Fig. 4f).

Analysis of variance of the individual experiments (1–4) with lignin as a covariate provided additional information on variation in survival and development within trees in relation to the three sample heights. Except for experiment 3 (SS), lignin differed significantly between the three sample heights and accounted for significant differences in some of the experiments in larval weight (experiment 3) ($P<0.05$), development score

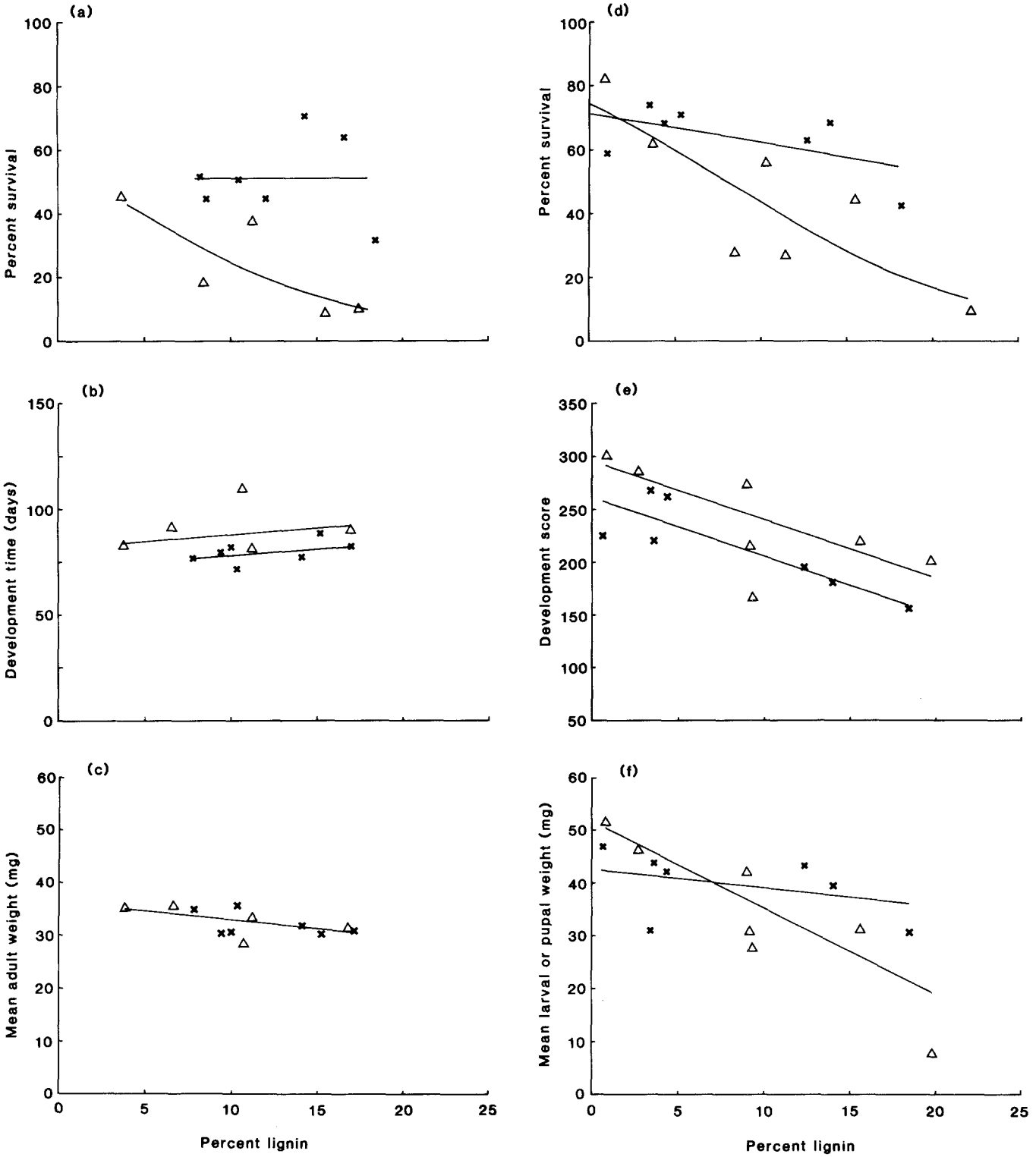

Fig. 4a–f. Comparison of results from **a–c** detached bark experiments 1 (SS) and 2 (NS) and **d–f** 3 (SS) and 4 (NS) on the effect of lignin in the bark of Norway (X) and Sitka (△) spruce on survival, development rate and weight of *Dendroctonus micans*. Values are means of 3 heights per tree

(experiment 4) ($P<0.05$) and survival (experiment 4) ($P<0.05$). Nevertheless, in three analyses, development time to adults (experiment 2) ($P<0.05$) and development score (experiments 3 and 4) ($P<0.01$, $P<0.05$), significant differences remained between the three sample heights even after allowing for the linear effect of lignin.

Adult experiments

Detached bark from Norway and Sitka spruce. A multiple regression analysis based on tree means and weighted for the number of beetles was performed on the number of eggs/beetle (transformed to square roots) in relation to

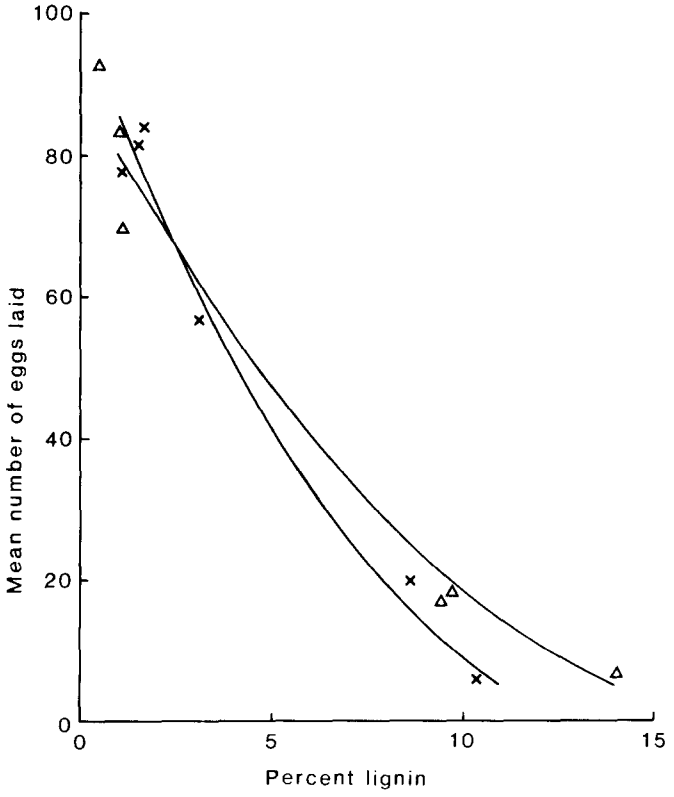

Fig. 5. Number of eggs laid in relation to the lignin content of bark. Values are means of 3 heights per tree and are adjusted for differences in initial weight of beetles. X Norway spruce △ Sitka spruce

lignin and initial weight. The following regression equation was obtained:

$$y = 9.5 + 0.5N - 0.52x_1 - 0.18x_1N - 1.1(x_2 - \bar{x}_2)$$

in which the effects of variation in species, lignin and initial adult weight have been kept distinct and where $y = \sqrt{\text{mean no eggs laid}}$; $N = 0$ for Sitka spruce, 1 for Norway spruce; $\bar{x}_1$ = percent lignin; x_2 = initial weight of adults in mg; $\bar{x}_2 = 35.8$ mg.

The data, plotted on an untransformed scale and adjusted for variation in initial weight (Fig. 5) show a significant negative relationship between oviposition and lignin content for each species ($P < 0.001$). A significant ($P < 0.05$) species × lignin interaction was also evident with more eggs laid on Sitka spruce at high lignin levels.

A covariance analysis was done on the data for each height, pooling the number of eggs/beetle from the three subdivisions of each bark piece. In the anova, percent lignin, a variate for the species × lignin interaction and initial weight of the beetles were included as covariates in an analysis of the mean number of eggs laid. There was no main effect due to species but the covariates accounted for a significant amount of variation both between individual trees ($P < 0.001$) and between the three positions on the tree ($P < 0.01$). The coefficients for the covariance regressions are given in Table 3 and indicate the importance of lignin both between and within trees. The significance of the species × lignin interaction suggests that the effect of lignin on beetle oviposition (Fig. 5) is more pronounced in Norway spruce. The negative coefficient of the initial weight covariate indicates that the number of eggs laid decreases with increasing beetle weight.

Table 3. Regression coefficients of the covariates for the analysis of oviposition in relation to lignification of bark

	Coefficient	t	P
Tree stratum			
Mean percent lignin	−0.58	16.01	<0.001
Species × lignin interaction	−0.10	2.75	<0.05
Initial adult weight	−0.94	7.58	<0.001
Tree/height stratum			
Mean percent lignin	−0.54	3.40	<0.01
Species × lignin interaction	−0.15	0.94	NS
Initial adult weight	−0.07	0.34	NS

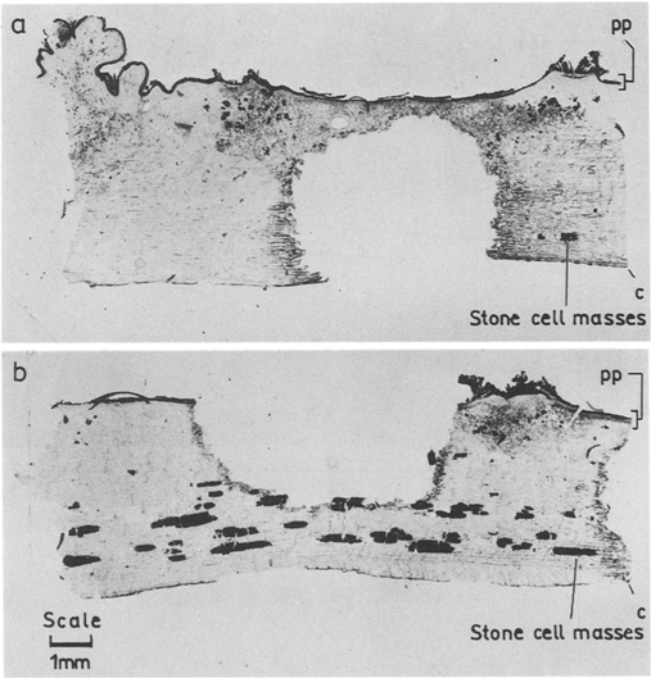

Fig. 6a, b. Tunnels made by female *Dendroctonus micans* prior to oviposition in bark with **a** low or **b** high lignin content. *pp* = primary periderm, *c* = cambium

Microtome sections through adult galleries revealed tunnels constructed in the relatively unlignified inner bark (Fig. 6a) but in highly lignified bark, adults clearly avoided the stone cell layer (Fig. 6b), producing a tunnel in the outer bark.

Discussion

Effect of lignin on D. micans

Our results show that survival, development rate and weight of larvae can be reduced when they feed on highly lignified spruce bark. The effect of lignin on adult weight,

and presumably fecundity, was relatively small in our experiments, probably because larvae were able to compensate for its effects by feeding for a longer period in highly lignified bark. An extended larval feeding period in lignified bark probably also explains the apparent species interaction between larval/pupal weight and lignin in experiments 3 (SS) and 4 (NS) (Fig. 4f). At the end of experiment 4, about half the larvae had pupated, most of them in bark with a low lignin content whereas the remaining feeding larvae were largely confined to highly lignified bark (see development score). Continued feeding by these larvae reduces the difference in weight between them and larvae that have already pupated and this "catching-up" process obscures the effect of lignin on larval growth (Fig. 4f). In experiment 3, on the other hand, 95% of individuals were still in the larval stage at assessment and the effect of lignin on larval weight is clearly shown. Thus the results in Fig. 4f reflect the different stages of development reached at the end of the two experiments and the different rates of development in bark of low or high lignin content rather than a species interaction in the effect of lignin on larval/pupal weight.

Adults are also affected by lignin, laying fewer eggs in lignified bark. Because beetles were collected from the field after some maturation feeding had occurred we are not able to determine whether the effect on oviposition was a nutritional one of feeding on our experimental bark during gallery construction or the result of altered beetle behaviour in response to lignin. It is clear however that lignification can affect tunnelling behaviour in adults (Fig. 6b) as well as larvae, so that on trees with highly lignified bark the outer bark tissues would often be too thin to allow adults to form complete galleries and larvae would be less protected by bark tissue and perhaps more exposed to natural enemies (e.g. Price et al. 1980).

In the adult experiments, the negative relationship between initial beetle weight and number of eggs laid (Table 3) is surprising and suggests to us that smaller beetles can utilize thinner unlignified bark tissues and so ultimately lay more eggs than larger beetles. However, selective pressures against smaller beetles are likely to be increased vulnerability to resin flow and a reduced capacity for dispersal. Interestingly, the effect of lignin on larvae is to produce smaller beetles so that where larvae are able to complete development on trees with lignified bark, the smaller adults produced may be more successful in re-attacking the tree.

In the field, the direct effects of lignin on *D. micans* should have a significant impact on population dynamics, but indirect effects may also be important. For example, slower development of broods may increase the chances of detection by natural enemies (Feeny 1976; Price 1986; van Emden 1986; Lawton and McNeill 1979), such as woodpeckers and the specialist predatory beetle *Rhizophagus grandis*, and also increase exposure of the larvae to induced defensive reactions in bark.

Sitka spruce bark itself appears to be inherently less suitable for larvae than that of Norway spruce, as suggested by the greater effect of lignin on survival (Fig. 4a, d) and the longer development time in Sitka spruce bark (Fig. 4b). However, the opposite effect is observed in the adult experiments in which lignin has a larger effect on adult oviposition in Norway spruce (Fig. 5). Field observations show that Norway spruce is usually attacked in preference to Sitka spruce (Bejer 1984; Bevan and King 1983), although interestingly, Sitka spruce may succumb more readily once broods are established (Evans et al. 1984; Bejer-Petersen 1976).

Mode of action of lignin and interaction with other defensive mechanisms

Lignins are among the most important defensive chemicals in plants and their role in preformed defence against fungi has long been recognised (e.g. Akai and Fukutomi 1980). Induced lignification in plants has also been studied in relation to attack by fungal pathogens (e.g. Vance et al. 1980). By contrast, there have been relatively few studies of the role of these ubiquitous chemicals in resistance to insect feeding. For bark beetles, their role in physical defence is disregarded by Cates and Alexander (1982) on the grounds that the beetles are well equipped to bore through wood and bark. Among other things, this seems to ignore the problem of establishment of very young larvae in lignified tissues. Our results show, on the contrary, that lignin can be a significant resistance factor limiting the exploitation of bark by *D. micans*.

Results from other studies show that lignin can provide both a physical and chemical defence against insects. In sorghum, for example, it has been specifically identified as a major factor in resistance to shoot fly *(Atherigona varia soccata)*. In this plant, lignification and thickening of the walls of cells enclosing the vascular bundle sheaths protects the central whorl of young leaves, physically preventing penetration by larvae (Blum 1968). In other plants, by contributing in a general way to "leaf toughness", lignin can influence the amount of plant material eaten. Toughness of mature leaves is often cited as one factor in their unsuitability for insect feeding (e.g. Feeny 1970) and Coley (1983) found leaf toughness to be the single most important factor correlated with herbivory among tropical rainforest trees. Leaf beetles *(Plagiodera versicolora)* feeding on mature *Salix* leaves suffer a dramatic increase in mandible wear in comparison to those feeding on young leaves (Raupp 1985) and this has the effect of reducing the rate of leaf consumption and beetle fecundity. Lignin can have chemical effects as well, for example reducing nutrient availability by hydrogen bonding to proteins and carbohydrates (Swain 1979) and in some cases it can be directly toxic. For example, when the tegument of *Phaseolus vulgaris* seeds was incorporated into an artificial diet for the bruchid *Acanthoscelides obtectus* the lignin present in the seed coat proved to be highly toxic to the young larvae and also increased development time of survivors and reduced fecundity of the emerging adults (Stamopoulos 1988).

We assume in our study that the major effect of lignin is physical protection of the nutritious inner bark and cambium and reduction in the nutritional quality of the bark. This is supported by the observation that larvae can compensate to some extent for slow growth by an

extended feeding period. In adults, the considerable wear that occurs to mandibles after a period of feeding in bark (D. Wainhouse, M. Ramsdale unpublished) again suggests a physical effect (c.f. Raupp 1985). By reducing the size of larval feeding groups (Fig. 3), lignin may have an indirect effect on larvae which seem to grow more slowly in small (<10) groups (A. Storer unpublished). Toxic effects may however contribute to the mortality of young larvae (Figs. 4a, d).

The existence of a dose-dependent physical defence against *D. micans* is consistent with current theories on evolutionary interaction between herbivores and plants (Feeny 1975, 1976; Rhoades and Cates 1976; Rhoades 1979). Adults of this highly specialised insect have a high tolerance of host resin or at least of its constituent monoterpenes (Everaerts et al. 1988), and the larvae, by continuous enlargement of a communal gallery, may reduce the effectiveness of the dynamic wound response (Grégoire 1985). In nature, these three defence mechanisms seem certain to interact in determining the overall resistance level of the tree. However, lignified tissues should be effectively protected from beetle attack during periods of environmental stress, such as drought, that are known to compromise the resin defence system in conifers (Vité 1961; Hodges and Lorio 1975).

Variation in lignification within and between trees

The greater degree of lignification of bark in the lower bole, although possibly providing some protection from mechanical damage, is consistent with its role as a defence against bark beetles that tend to attack this part of the tree, i.e. *D. micans* in Europe, and *D. rufipennis* and *D. punctatus* in North America. Although the upper parts of the tree are unprotected by lignin, here the bark may be too thin at least for adult galleries. Thus within-tree variation in the distribution of lignin appears to be related to the vulnerability of the tissues to attack.

Within our study area, there is wide variation between trees in the degree of lignification at a given height, with bark of some trees containing little or no lignin whereas adjacent trees may contain 20% or more. Observations in other stands support the view that there is considerable heterogeneity within the spruce population as a whole. The identification of provenance differences would offer considerable scope for the practical use of this important defensive trait.

Acknowledgements. Our thanks to Colin King and Nick Fielding for collecting beetles and suggesting the bark sandwich method and to Rosemary Fricker for help with monitoring the adult experiment. Our thanks to Drs. J.N. Gibbs and N.A. Straw for critical reading of the MSS.

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