Variations in abiotic defense within myrmecophytic and non-myrmecophytic species of *Macaranga* in a Bornean dipterocarp forest

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We examined the interspecific variations in intensity of total abiotic (chemical and physical) defenses in five sympatric *Macaranga* (Euphorbiaceae) species, including three myrmecophytic species. The intensity of the total abiotic defense for each *Macaranga* species was estimated by measuring inhibiting effects on the growth performance of the common cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) when the cutworm larvae were fed fresh leaves of each *Macaranga* species. Indices of the growth performance, number of dead larvae, pupal weight and length of larval period were obtained. We found that the intensities of total abiotic defense of the two non-myrmecophytic species were significantly stronger than those of the three myrmecophytic species, and that there was a significant difference in intensity even within the three myrmecophytic species. The former result supports the hypothesis that, unlike non-myrmecophytic species, myrmecophytic species cannot invest so many metabolic resources in abiotic defense, because they have to allocate nutrients to biotic defense (toward biotic defense agents). Moreover, the latter result suggests the possibility that the three sympatric myrmecophytes have different defense strategies, with a trade-off between abiotic and biotic defense, and/or with a trade-off between defense and other life-history traits such as growth and reproduction. Abiotic defense can be roughly separated into physical and chemical mechanisms. To assess the intensity of the physical defense of *Macaranga* leaves, we measured the leaf toughness of each species. In addition, to assess the intensity of the plant's general chemical defense, cutworm larvae were reared on an artificial diet containing dry leaf powder of each *Macaranga* species, and their growth performances were compared. The estimated orders of intensity of both leaf toughness and general chemical defense coincided with that of the total abiotic factors measured by the growth performance of cutworm on fresh leaves. This suggests the presence of both physical defenses, represented by leaf toughness, and a general chemical defense affecting the intensity of the total abiotic defense in similar ways.

Key words: ant-plant mutualism; Borneo; chemical defense; leaf toughness; myrmecophyte.

INTRODUCTION

Some plant species are known to depend on other organisms, mainly animals, for anti-herbivory defense (Vinson 1976; Faeth 1986; Buckley 1987;

Williams *et al*. 1988; Huxley & Cutler 1991; Farmer 1997). In this type of defense system, which is called biotic defense, plants attract or nourish other organisms so that they will protect them from herbivore attacks. In the tropics, ants flourish and some plant species use ants for anti-herbivory defense (Benson 1985; Davidson & Epstein 1989; Hölldobler & Wilson 1990; Huxley & Cuter 1991). These plants have mutualistic relationships with specific or non-specific ant partners in various ways; in order to attract ants that are expected to defend the nurse plants, they provide extrafloral nectar (EFN) for ants in some cases (Beattie 1985; Oliveira & Oliveira-Filho 1991;

Huxley & Cutler 1991), and nest sites and sometimes food in other cases (Janzen 1974; Beattie 1985; Davidson *et al*. 1991; Huxley & Cutler 1991). The plants that provide nest sites for ants and have symbiotic relationships with them are called myrmecophytes (Lincolon *et al*. 1982). It is expected that the myrmecophytes invest a considerable quantity of nutrients produced by their metabolic systems, which varies with the intensity of the mutualistic relationship, in order to attract ants and strengthen the relationship with them (Janzen 1966; Rher *et al*. 1973; Heil *et al*. 1997).

However, it is widely known that plants can defend themselves against herbivores without the help of other organisms, like ants, by using chemical defense mechanisms such as toxins and repellents, and by physical ones such as spines, hairs, thick cell walls and so on (Painter 1951; Atsatt & O'Dowd 1976; Feeny 1976; Rhoades & Cates 1976; Harris 1979; Grubb 1986; Marquis 1992; Pollard 1992; Zangerl & Bazzaz 1992; Bennett & Wallsgrove 1994; Harborne 1997). These types of defense mechanisms are called abiotic defense mechanisms, as opposed to biotic defenses such as ant defense. As well as biotic defenses, abiotic defenses require metabolic resources for producing secondary compounds including toxins and fibers such as lignin, cellulose and hemicellulose that affect the toughness and/or thickness of plant tissues (Barker 1989; Simms 1992). Thus, for abiotic defense, plants have to allocate photosynthetic products and nutrients to processes other than growth and reproduction (Simms & Rausher 1987; Simms 1992). Hence, abiotic and biotic defenses are difficult to reconcile in an individual plant under resource-limited conditions.

How do myrmecophytes balance these two cost-contradictory defense systems? So far, some chemical compounds, which appear to be effective as anti-herbivory agents, have been extracted from myrmecophytes (Rehr *et al*. 1973; Sultana & Ilyas 1986; Hnawia *et al*. 1990; Folgarait & Davidson 1994). Janzen (1966) hypothesized that abiotic defense has been lost in the myrmecophyte, possibly because maintenance of both biotic and abiotic defenses places an unnecessary metabolic burden on the plant. Rehr *et al*. (1973) provided evidence that supports this hypothesis by con-

ducting bioassays with a generalist herbivorous insect species on myrmecophytic and non-myrmecophytic *Acacia* plants. In their study, they compared the growth performance of the southern armyworm (*Prodenia eridania* Cramer, Noctuidae) when fed on artificial diets containing dry leaf powder from different *Acacia* species. In the *Acacia* species that they studied, the harmful effects on the growth of larvae were smaller in myrmecophytic species than in non-myrmecophytic species. However, they compared only myrmecophytic species with non-myrmecophytic species, and did not examine the differences between myrmecophytic species.

Macaranga is a genus consisting of mainly pioneer tree species with its center of diversity in New Guinea and Borneo, it includes many myrmecophytic species. (Whitmore 1969, 1975; Fiala *et al*. 1989). It has been reported that there is a wide variation in the intensity of mutual-dependency of ant–plant interactions from non-myrmecophytes through to facultative, transitional and obligate myrmecophytes in *Macaranga* (Fiala *et al*. 1989, 1994; Fiala & Maschwitz 1990). The variation in biotic defense levels in *Macaranga* has been partially described (Fiala *et al*. 1989), but the variation in abiotic defense levels has not been investigated.

The purpose of this paper is to examine whether Janzen's hypothesis (1966) is similarly applicable to sympatric *Macaranga* species with different levels of biotic defenses, and to examine whether there is any interspecific variation in the levels of abiotic defense even within typical obligate myrmecophytes. To assess the interspecific differences in the intensity of total abiotic defense, we compared the inhibitory effects on the growth of a species of polyphagous lepidopteran reared in the laboratory when fed on the fresh leaves of five sympatric *Macaranga* species. In addition, we divided the total abiotic defenses into physical and chemical components. We compared the leaf toughness of the *Macaranga* species to assess the intensity of their physical defenses, and compared the inhibitory effects on the growth of a polyphagous lepidopteran reared in the laboratory when dry leaf powder from the five *Macaranga* species were added to an artificial diet in order to assess the intensity of the plant's general chemical defense.

METHODS

Sampling site

All the leaves were sampled from November 1997 to May 1998 in a lowland, mixed dipterocarp forest in Lambir Hills National Park, Sarawak (4°2′N, 113°50′E, 50–150 m a.s.l.). The region is situated in the humid tropics with no pronounced dry season and little seasonal variation in temperature (Kato *et al.* 1995). At least 15 species of *Macaranga* occur in the study site and many are dominant pioneer trees, which are observed mainly at forest edges, treefall gaps and riversides in the forest (Davies *et al.* 1998).

Life history

Macaranga is a genus of approximately 280 species, which are distributed from West Africa to the South Pacific islands with the center of diversity in New Guinea and Borneo, and includes many myrmecophytic species (Whitmore 1969, 1975; Fiala *et al.* 1989). In Malaysia (Peninsula and Borneo), 23 of the 52 *Macaranga* species are myrmecophytes, and have symbiotic relationships with specific ant species (Fiala & Maschwitz 1991, 1992). Most of the ant species that are symbionts of *Macaranga* belong to *Crematogaster* (Hymenoptera: Formicidae: Myrmicinae), however a few species belong to *Camponotus* (Formicinae) (Maschwitz *et al.* 1996). Ants defend their host plant against herbivores and competitors (Fiala *et al.* 1989) and both the plants and their symbiont ants depend on each other for their survival. Non-myrmecophytic *Macaranga* species often attract non-specific ants by EFN (Fiala & Maschwitz 1991; Fiala & Linsenmair 1995).

In this study, we focused on five *Macaranga* species, *Macaranga winkleri* Pax. & Hoffm., *Macaranga trachyphylla* Airy Shaw, *Macaranga beccariana* Merr., *Macaranga gigantea* (Reichb. f. & Zoll.) Muell. Arg. and *Macaranga praestans* Airy Shaw. The former three species are obligate myrmecophytes, while the others are non-myrmecophytes. In obligate myrmecophytes, when seedlings are 5–10 cm in height, the internodes swell and the pith degrades so that ant queens are able to colonize the stems. Plants produce food bodies that are collected by the symbiont ants from inside the domatia-shaped stipules, and/or from the leaf surface. Symbiont ants utilize food bodies as their main source of food, and are often found attacking invading insects and vines of competing plant species. Three symbiont ants, *Crematogaster* spp., *Crematogaster borneensis* Andr. and *Crematogaster decamera* inhabit *M. winkleri*, *M. trachyphylla* and *M. beccariana* Merr., respectively (S. Yamane, pers. comm., 1999). Ant workers seldom leave their nurse plants, and plants without ant colonization suffer serious damage from herbivory. An ant colony occupies a host plant, and repeats the colony cycle until the host plant dies.

Macaranga gigantea and *M. praestans* are non-myrmecophytes, they do not have any fixed or obligate relationship with any particular ant species, and their stems do not swell. They often attract a range of ant species (which are not specific to that plant) ants with EFN, which are found on the leaf surface. Those ants that are attracted defend the plants from herbivores (Fiala & Maschwitz 1991), although the effectiveness and harshness of the defense is remarkably weaker than that in congeneric myrmecophytes. In *M. praestans,* only newly developing leaves have EFN, which then deteriorate as the leaves mature.

Although there are interspecific variations in habitat preferences with reference to light intensity, soil texture and levels of disturbance at microsites between the three myrmecophytic species, their habitats overlap closely and all three myrmecophytic species prefer moderate shade-intensity and clay-rich conditions (Davies *et al.* 1998). Although *M. gigantea* prefers slightly brighter conditions, its habitat also overlaps closely with that of the three myrmecophytic species (Davies *et al.* 1998). In contrast, *M. praestans* prefers much shadier conditions than do the other four species, such as the forest floor.

Herbivores

Chrysomelids, scarabaeids, weevils, some polyphagous lepidopteran larvae, grasshoppers and walking sticks were observed feeding on *Macaranga* leaves in the field. These insects are considered to be polyphagous and feed on many plant species other than *Macaranga*; therefore they could be categorized as generalist herbivores. Larvae of *Arhopala* spp. (Lepidoptera; Lycaenidae)

are considered to be adapted to myrmecophytic *Macaranga*, as they are able to appease ants by secreting honeydew (Maschwitz *et al.* 1984). In the study site, at least two species of *Arhopala* larvae were observed feeding on *Macaranga* seedlings, one was specific to *M. trachyphylla*, and the other was specific to *M. beccariana*. Galls were often observed being formed by gall midges (Diptera; Cecidomyiidae) on *M. beccariana* and *M. trachyphylla*.

Assessment of total abiotic defense levels

To determine the interspecific variation in the intensity of total abiotic defenses against generalist herbivore insects in the five *Macaranga* species, we conducted a bioassay using the common cutworm, *Spodoptera litura* (Lepidoptera; Noctuidae). We estimated the intensity of total abiotic defenses by comparing the inhibitory effects on the cutworm's growth performance when larvae were reared on the fresh leaves of each *Macaranga* species.

The common cutworm is polyphagous and widespread from Asia to Australia. Okamoto and Okada (1968) listed 77 species of dicotyledonous plants that act as its host. Therefore, we have assumed here that the total abiotic defensive effects against the generalist herbivores that may potentially feed on *Macaranga* plants could be represented by the effects on the common cutworm. Larvae of the common cutworm are able to complete their growth even when fed on an entirely artificial diet (Insecta LF, Nihon Nosankougyou Co., Yokohama, Japan). The strain used in this study had been maintained for 23 years in the laboratory.

A total of 720 larvae of the common cutworm were used in the bioassay. We prepared 72 plastic boxes (22 cm × 14 cm × 3.7 cm), and placed 10 first-instar larvae in each box less than 12 hours after the larvae had hatched. These larvae were reared at 28°C in the laboratory. The 72 plastic boxes were assigned to one of six treatments (12 boxes (replications) per treatment), within which the diets given to larvae were different; for the five experimental treatments, fresh leaves of each of the five *Macaranga* species were fed to the larvae, and, for the control treatment, the artificial diet cited above used. Except for *M. gigantea*, we randomly selected 1.5–2.2 m tall seedlings of each

Macaranga species to collect leaves that were used for the experimental diet. For *M. gigantea* leaves, we selected 3–8 m tall seedlings. The leaves used as feed were limited to five undamaged, mature apical leaves per plant. Every 2 days, the leaves and artificial feed were changed, and the larval survival was checked. The pupal weight was measured one day after pupation if larvae pupated. Pupae were stored at 28°C until eclosion.

Assessment of general chemical defense levels

The total abiotic defenses of a plant consist of both chemical and physical components. To assess the intensity of general chemical defenses separately, we conducted a bioassay similar to the previous one in which the common cutworm was also used. In this bioassay, instead of feeding larvae fresh leaves, we provided an artificial feed containing dry leaf powder of each *Macaranga* species in order to exclude the physical defense component of the leaves. The leaves for the dry leaf powder were sampled in the same way as for the bioassay with fresh leaves. The major veins were excluded from the sampled leaves, and were ground down by using an electric mill. The leaves were ground down to a fine flour, which was dried at 60°C in an electric oven, and stored in a container with silica gel at room temperature until its use. The protocols of larvae rearing and the number of replications were the same as for the previous bioassay, except that larvae were fed the mixture of ground leaves and artificial feed this bioassay.

The amount of photosynthetic products in the leaf correlates with leaf area. Thus, it is important to measure the abiotic defense level per leaf area. To standardize the amount of added dry leaf powder for interspecific comparison, we calculated the weight of leaf tissue per area (Table 1). We made the artificial feed with dry leaf powder of each species as follows: leaf powder equivalent to 320 cm^2 leaf area and 2 ml water were mixed well with 10 g artificial feed (Table 1). This mixture was stored in a plastic cup at 4°C until use in order to prevent desiccation and decay.

In the bioassay with dry leaf powder, the total feeding amount (FA) in each replication was also measured. FA was calculated as follows:

$$FA = 1/N \times (D_{initial} - D_{leftover} \times k), \qquad (1)$$

Table 1   The dry leaf weight of five *Macaranga* species and contents of the artificial feed for the bioassay with dry leaf powder

	Artificial diet (g)	Dry leaf powder (g)	Water (ml)	Dry leaf weight (g 10 cm^{-2} ± SD)
Macaranga winkleri	10	1.31	2	0.041 ± 0.014
Macaranga trachyphylla	10	0.80	2	0.025 ± 0.005
Macaranga beccariana	10	0.96	2	0.030 ± 0.005
Macaranga gigantea	10	1.73	2	0.054 ± 0.007
Macaranga praestans	10	1.76	2	0.055 ± 0.006
Control	10	—	2	—

The standardized amount of dry leaf powder (equivalent to 320 cm^2) was calculated based on the dry leaf weight per leaf area.

where N is the number of surviving insects in each replication, $D_{initial}$ is the fresh weight of the diet just before feeding, $D_{leftover}$ is the dry weight of the leftover diet, and k is the ratio of fresh weight to dry weight in the artificial feed containing the dry leaf powder. To calculate k, we measured the weight of the artificial diet containing dry leaf powder for each species, both when it was freshly mixed and when dry.

It is well known that pupal size correlates with fecundity in many insect species (Scriber & Slamsky 1981), and our preliminary study confirmed that the pupal weight of the common cutworm also correlates with fecundity ($y = 12\,874\,x - 1416$, $r = 0.79$, $P < 0.0001$, $n = 40$, pupal weight range: 0.258–0.430 g). Based on this regression, we calculated the expected fecundity for the entire initial larvae (EF) for each treatment in both bioassays as follows:

$$EF = 1/2 \times (12\,874 \times FPW - 1416) \times SR \quad (2)$$

where FPW and SR are the average weight of the female pupae and the average survival rate in each treatment, respectively.

Assessment of leaf toughness

To assess the intensity of the physical defense in each species, we measured leaf toughness, which is considered to be one of the most important agents of physical defense. Leaves were sampled for the measurement of leaf toughness in the same way as for the bioassay shown above. The toughness was measured by using a penetrometer (Aikoh Engineering Co., Tokyo, Japan).
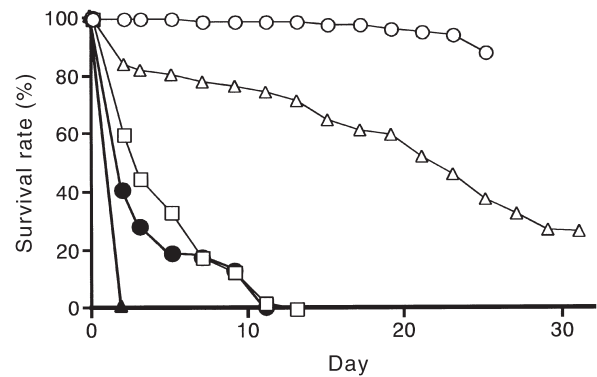
Fig. 1.   The hazard curves of the accumulative survival rates of common cutworm (*Spodoptera litura*) larvae fed on fresh leaves of five *Macaranga* species: (○) *Macaranga winkleri*, (△) *Macaranga trachyphylla*, (□) *Macaranga beccariana*, (●) *Macaranga gigantea* and (▲) *Macaranga praestans*. The hazard curves of accumulative survival rates were broken off on the day that all larvae were dead or reached pupal stage. The hazard curves of the accumulative survival rates of the five treatments were significantly different (Logrank-test, $\chi^2 = 390.545$, $P < 0.01$).

RESULTS

Effects of eating fresh leaves on the growth performance of the cutworm

Some larvae of the cutworm completed their growth on leaves of *M. winkleri* and *M. trachyphylla*, but all the larvae died within the early larval stage (almost first instar) on the leaves of other species (Fig. 1). The hazard curves of accumulative survival rates were significantly different

Table 2 Effects of a diet of fresh leaves of *Macaranga* on the growth performance of the common cutworm (*Spodoptera litura*)

Treatments	No. survivors until pupa	No. survivors until adult	Pupal weight (g ± SD)	Larval period (day ± SD)	Expected fecundity
Macaranga winkleri	113[a]	85[a]	0.19 ± 0.05	21.8[a] ± 3.4	358 ± 218
Macaranga trachyphylla	31[b]	15[b]	0.17 ± 0.05	27.1[b] ± 3.7	92 ± 66
Macaranga beccariana	0[c]	—	—	—	0
Macaranga gigantea	0[c]	—	—	—	0
Macaranga praestans	0[c]	—	—	—	0

See the text for the calculation of the expected fecundity.
Means followed by different letters are significantly different from each other (*t*-test, $P < 0.05$). For each treatment $n = 120$.

between the five *Macaranga* species (Logrank-test, $\chi^2 = 390.545$, $P < 0.01$; Fig. 1). The hazard curves of accumulative survival rates were not significantly different between *M. beccariana* and *M. gigantea*. However, all the larvae died by the third instar on *M. beccariana* and by the second instar on *M. gigantea*, although most larvae on both species penetrated the leaf surface with their mandibles and reached the inner tissues of the plants. Thus, the total abiotic defense levels of the two non-myrmecophytic species were higher than that of the three myrmecophytic species. On *M. praestans* leaves, larvae were not able to penetrate the leaf surface, and then all larvae appeared to have died of starvation. In the two treatments where some larvae reached the adult stage, the mortality on *M. winkleri* was significantly lower than that on *M. trachyphylla* (*t*-test, $P < 0.0001$; Table 2). The hazard curves of the accumulative survival rate did not differ between the sexes in any treatment. In addition, the sex ratio did not differ in any of the treatments. The pupal weights of larvae on *M. winkleri* and *M. trachyphylla* leaves were significantly different (Table 2). The pupal weights of larvae on *M. winkleri* and *M. trachyphylla* leaves were not significantly different between sexes. The variation in harmful influences on cutworm growth was emphasized in the expected fecundity of the 120 initial first-instar larvae on these two *Macaranga* species (Table 2).

Leaf toughness

There were significant differences in leaf toughness in the five *Macaranga* species (ANOVA, $F = 235.5$,

Table 3 Toughness of *Macaranga* leaves as measured by a penetrometer

	Weight at piercing (kg ± SD)
Macaranga winkleri	0.245[a] ± 0.026
Macaranga trachyphylla	0.292[b] ± 0.040
Macaranga beccariana	0.350[c] ± 0.034
Macaranga gigantea	0.430[d] ± 0.047
Macaranga praestans	0.563[e] ± 0.047

Weights followed by different letters are significantly different from each other (Scheffé's *F* test, $P < 0.01$). Weight was applied over a diameter of 3 mm.

d.f. $= 5$, $P < 0.0001$; Table 3). The leaf toughness was lower for the three myrmecophytic species than for the two non-myrmecophytic species. The order of leaf toughness from least tough to toughest was as follows: *M. winkleri*, *M. trachyphylla*, *M. beccariana*, *M. gigantea*, *M. praestans*. All pairing comparisons differed significantly (Scheffé's *F* tests at $P < 0.05$; Table 3).

Effects of eating dry leaf powder on the growth performance of the cutworm

The effects of dry leaf powder added to the artificial feed on the total mortality of the larval stage, pupal weight, larval period and total feeding amount, were all significantly different between the six treatments (ANOVA; summarized in Table 4).

The total mortality throughout the larval stage in the five experimental treatments was higher

Table 4 Effects of a diet of dry leaf powder of *Macaranga* on the growth performance of the common cutworm (*Spodoptera litura*)

Treatments	No. survivors until pupa	No. survivors until adult	Pupal weight (g)	Larval period (days)	Total feeding amount (g)	Expected fecundity
Control	117[a]	116[a]	0.39[a]±0.05	14.3[a]±1.1	4.28[a]±0.64	1898±357
Macaranga winkleri	111[a]	103[ab]	0.25[b]±0.04	18.2[b]±2.7	3.05[cd]±0.99	913±288
Macaranga trachyphylla	108[a]	91[ab]	0.27[b]±0.04	17.7[b]±2.4	3.86[ad]±0.98	919±241
Macaranga beccariana	95[a]	83[b]	0.26[b]±0.04	18.9[b]±1.8	3.69[bd]±1.02	832±207
Macaranga gigantea	48[b]	37[c]	0.20[c]±0.06	38.5[c]±6.7	2.87[bc]±1.13	229±109
Macaranga praestans	22[c]	15[c]	0.19[c]±0.04	37.5[c]±10.7	1.91[c]±1.05	90±58
Statistics through ANOVA						
F	49.1	48.4	167.8	384.2	19.7	—
d.f.	5	5	5	5	5	—
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	—

Means followed by the same letters are not significantly different from each other (Scheffé's F test, $P < 0.05$). Initially, 120 larvae were reared in each treatment. The initial average fecundity per larva was estimated in the same way as in Table 2. Data are given as mean±SD.

than that in the control treatment. The average larval mortality in larvae on each of the three myrmecophytic species was significantly lower than that in any of the non-myrmecophytic species (Scheffé's F test, $P < 0.01$; Table 4). The hazard curves of the accumulative survival rates of the three myrmecophytic species were significantly different (Logrank-test, $\chi^2 = 6.673$, $P < 0.01$; Fig. 2). The hazard curves of accumulative survival rate did not differ between the sexes in any of the treatments. In addition, the sex ratio did not differ in any of the treatments.

The pupal weights for the five experimental treatments were significantly less than those in the control treatment (Scheffé's F test, $P < 0.0001$; Table 4). The pupal weights for larvae raised on each of the non-myrmecophytic species were significantly lower than those for the myrmecophytic species (Scheffé's F test, $P < 0.0001$). There were no significant differences within the myrmecophytic species, or within the non-myrmecophytic species. The weights of pupae were not significantly different between the sexes. The variation in inhibitory effects on cutworm growth was emphasized in the expected fecundity of the 120 initial first-instar larvae fed on leaf powder from the five *Macaranga* species (Table 4).

The larval period in the five experimental treatments was significantly longer than that in the control treatment (Scheffé's F test, $P < 0.0001$;

Table 4). The larval period in the three treatments of myrmecophytic species was shorter than that in either of the two non-myrmecophytic species. There was no significant difference within the myrmecophytic species, or within the non-myrmecophytic species.

Throughout the larval stage in the four experimental treatments other than *M. trachyphylla*, FA was significantly less than in the control treatment (Scheffé's F test, $P < 0.05$; Table 4). The FA for the myrmecophytic species was larger than for the non-myrmecophytic species. There was no significant difference within the myrmecophytic species, or within the non-myrmecophytic species.

DISCUSSION

In many host plant–insect interactions, specialist herbivores tend to overcome abiotic defenses through coevolutionary processes (Ehrlich & Raven 1964; Feeny 1977). Since abiotic defense mechanisms are considered to have evolved mainly against generalist herbivores that have the potential to cause damage to the host plant, the efficiency of abiotic defense mechanisms in a particular plant species cannot be assessed if herbivorous insects that usually utilize the plant species in the field are used for the bioassay. The broad host range of the common cutworm, and the fact that the cutworm
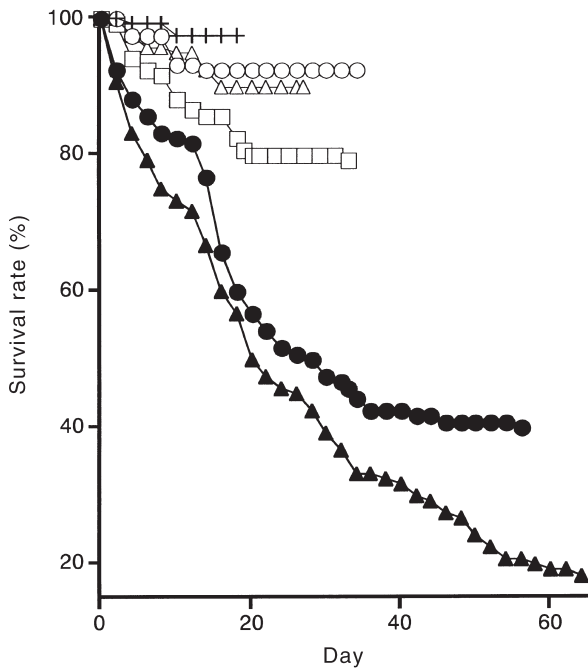
Fig. 2. The hazard curves of the accumulative survival rates of common cutworm (*Spodoptera litura*) larvae fed on artificial diets containing dry leaf powder of five *Macaranga* species: (○) *Macaranga winkleri*, (△) *Macaranga trachyphylla*, (□) *Macaranga beccariana*, (●) *Macaranga gigantea* and (▲) *Macaranga praestans*. (+) indicates the hazard curve in the control treatment (containing no dry leaf powder). The hazard curves of accumulative survival rates were broken off on the day that all larvae were dead or reached pupal stage. The hazard curves of accumulative survival rates of the three myrmecophytic species were significantly different (Logrank-test, $\chi^2 = 6.673$, $P < 0.01$).

can complete its life cycle on a few species of *Macaranga*, indicate that the moth is suitable for such a bioassay as a representative of generalist herbivores that may attack *Macaranga* trees. However, it is possibile that other polyphagous herbivores could respond differently. The details of abiotic defense need to be clarified through further research.

It is reasonable to say that the bioassay with fresh leaves in this study assessed the intensity of total abiotic defenses against generalist polyphagous herbivores in the five *Macaranga* species studied. This bioassay suggests that the intensities of the total abiotic defenses of the myrmecophytic species are significantly lower than those of the non-myrmecophytic species. This agrees with Janzen's (1966) hypothesis and studies

in *Acacia* (Rehr *et al*. 1973). Moreover, it is suggested that the intensity differs even within the three-myrmecophytic species. *Macaranga winkleri* and *M. beccariana* are armed with the weakest and strongest abiotic defenses within the three, respectively. The difference seems to be important in terms of anti-herbivory effects, because most cutworm larvae were able to complete their growth on *M. winkleri* and partly complete it on *M. trachyphylla*, while most larvae failed to mature on *M. beccariana*. Although *M. beccariana* is an obligate myrmecophyte, it has been revealed that it has an abiotic system by which at least attacks by generalist polyphagous herbivorous insects can be prevented.

Fiala *et al*. (1989, 1994) suggested that there was an interspecific variation in the intensity of ant (biotic) defenses between some obligate myrmecophytic species. An ongoing study of ours also suggests that in the obligate myrmecophytes studied here, there may be significant differences in the intensity of their biotic defenses. *Macaranga winkleri* appears to be the most intensively defended by ants, *M. trachyphylla* the second most intensively defended and *M. beccariana* is the least defended of the three myrmecophytic species (Itioka *et al*. in press). Thus, the intensity of total abiotic defense may correlate negatively with that of biotic defense in myrmecophytic species.

In this study, we sampled leaves for the bioassays from the same microhabitats where plants of all three myrmecophytic species were common. Therefore, it is assumed that the variation in abiotic defense intensity shown here is not simply caused by the variation in the environmental conditions of the microhabitat, but is based on species-specific genetic traits. That is, myrmecophytic *Macaranga* species may have different defense strategies in relation to the balance or trade-off between abiotic and biotic defense systems. This is strongly suggested when plants are at least 1.5–2.2 m seedlings, as the target plants for the sampling were all 1.5–2.2 m in height.

The three myrmecophytic species have similar life history traits, and their local distributions in gradients of micro-environmental conditions overlap closely (Davies *et al*. 1998). Defense strategies of a particular species may be constrained by its ecophysiological and life history traits, and may also impose constraints on these traits. To

understand the evolutionary processes and ecological conditions that cause and maintain such variations in defense strategies in sympatric species as shown here, it is necessary to clarify the nature of within-plant resource allocation, that is, how a particular plant species can optimally allocate its limited resources to defense (survival), growth and reproduction.

Abiotic defense mechanisms can be separated into physical and chemical components. In this study, we measured leaf toughness as a plausible major agent of physical defense, and we assessed the intensity of general chemical defense without physical effects by using the bioassay with dry leaf powder added to an artificial feed. In this bioassay, we compared the inhibitory effects of dry leaf powder of five *Macaranga* species on the growth performance of the cutworm in a similar way as for the bioassay with fresh leaves. This method is not able to measure the effects of chemicals that are volatile or easily broken down by heat treatment. However, constitutive chemical defense components, in this case condensed tannins, are thought to be ubiquitous in various plant species and effective against generalist herbivores (Coley & Aide 1991).

Leaf toughness was significantly different in the five *Macaranga* species studied, and the order of leaf toughness coincides with that of the intensity of total abiotic defense as estimated by the bioassays with fresh leaves. However, the variation in general chemical defense was not as large as the variation in leaf toughness. Especially between *M. winkleri* and *M. trachyphylla*, the interspecific difference in the intensity of general chemical defense was also similar to that of total abiotic defense. The inhibitory effects on the growth performance of the common cutworm, such as the number of dead, the pupal weight and the larval period of *M. winkleri* and *M. trachyphylla* were greater in the bioassay with fresh leaves than that with powdered leaves. These suggest that both the physical strength of leaf surfaces and general chemical defenses both contribute to the interspecific variation in the intensity of abiotic defense. In this study, we did not investigate other types of chemical defense mechanisms, such as those mediated by volatile or inducible chemicals. Although the details of chemical defense need to be clarified in the future, at least the intensity of general chemical defense can partly account for the intensity of the total abiotic defense.

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