RESISTANCE OF *MILICIA* SPECIES TO *PHYTOLYMA LATA* (PSYLLIDAE): THE ROLE OF LEAF ANATOMICAL AND MORPHOLOGICAL STRUCTURES

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ABSTRACT

*The anatomical and morphological structures responsible for variation in resistance in **Milicia** progenies to **Phytolyma lata** attack were investigated. Differences in cell types and cell frequency of eight progenies from the moist and dry semi-deciduous forest zones were determined using fresh leaves from a 3.5-year old **Milicia** progeny trial plot. Considerable differences in distribution of cell types per square millimetre (mm^2) were found in both lines. Resistance was highly related to the presence of tannin localization cells, whiles susceptibility was largely influenced by the relative abundance per square in millimetre of parenchyma, phloem and trichome cells. Tannin localization cells occurred two times more in the resistant **Milicia** progenies. However differences in tannin localization cells were observed among resistant lines, indicating the variability among individual progenies. It is apparent from our study that the level of resistance in **Milicia** species may be influenced by cell characteristics of individual progenies. This observation offers a good opportunity to explore the technique of breeding naturally resistant **Milicia** progenies to **Phytolyma.***

Keywords: *Milicia* spp.; *Phytolyma lata*; resistance; susceptible; Iroko

INTRODUCTION

Milicia spp. (*Milicia excelsa* (Welw) C.C. Berg. and *Milicia regia* (A. Chev.) C.C. Berg) known in the timber trade as Iroko is a valuable hardwood tropical African species (Ofori and Cobbinah, 2007; Oteng-Amoako, 2006; Taylor, 1960). Iroko timber is important for decorative and structural purposes and has high resistance to fungal decay (Hawthorne, 1995). Even though these species are wide spread in tropical Africa (Omolokun and Oladele, 2010; Ouinsavi *et al.,* 2005), their natural distribution overlaps only in Ghana and Cote D'Ivoire (Taylor, 1960). In Ghana both species are found in the high forest zone, with the population density increasing proportionally along a gradient of rainfall amount from Wet Evergreen to the Dry Semi-deciduous forest zones (Nichols *et al.,* 1998). Although these species have over the years been harvested from the natural forest, overexploitation coupled with paucity in natural regeneration threatens their long-term existence. Attempts to establish *Milicia* spp. in plantations have however been hampered by attacks from gall-forming insects in the genus *Phytolyma* (Hemiptera: Psyllidae) the most prominent being

Phytolyma lata (Wagner *et al*., 2008; Bosu *et al.,* 2006; Nichols *et al.,* 1999; Cobbinah, 1986; White, 1966). The insect prefers young seedlings, and induces the formation of galls on buds and young leaves at the terminal ends of the shoots (Cobbinah and Wagner, 1995).

The nymphal stages feed within the gall tissue, breaking down the epidermal cells which eventually cause fermentation of the leaf parenchyma (Wagner *et al.,* 2008). When the galls eventually open to release the adults, the accompanying terminal dieback from fungal decay causes growth reduction and seedling mortality in many cases (Cobbinah, 1986). Previous studies have however indicated that there is considerable variation in resistance among different *Milicia* progenies (Cobbinah and Wagner, 1995). The variability in resistance may be due to diversity and distribution of both chemical and morphological structures of the leaves which affect palatability (Castro-Diez *et al.,* 2000; Gutschick, 1999; Hartley, 1998; Cobbinah, 1986). Coley (1986) noted that the distribution of defences among plant tissues can have significant influence on feeding patterns and population dynamics of insect herbivores. For a better understanding of the interaction between *Milicia* spp. and *P. lata*, knowledge of the chemical, anatomical and physical structures implicated in the relationship is essential. This study was purposely designed to examine the role of morphological and anatomical traits in leaves of susceptible/resistant *Milicia* progenies.

MATERIALS AND METHOD

Experimental Site and Design

The *Milicia* spp. progeny trial was established in 1993 at Mesewam forest nursery (06°44'N and 06°3'W) about 15km from Kumasi, Ghana. The site is in the moist-semi deciduous forest zone (Hall and Swaine, 1981), and has a mean annual rainfall between 1500-1700mm per year. Mean monthly minimum and maximum temperatures vary between 20°C and 29°C. Before planting the area was an open field with a gentle slope from east to west. The site is heavily degraded from decades of rigorous site preparation for slash and burn agriculture and, has been used for research trials which often included exotic timber species such as *Tectona grandis, Pinus caribbea, Cedrela odorata, and Leucaeana leucocephala.* The plot was established with twenty-one (21) *Milicia* progenies each represented by 25 plants, and with a spacing of 1.22mx1.22m between individual seedlings (Cobbinah and Wagner, 1995). The *Milicia* seedlings were raised from seeds collected from seven locations in *Milicia* distribution belt in Ghana (Hall and Swaine, 1981).Within the experimental plot, progenies were randomly arranged. More details about the site and trial design are provided by Bosu *et al*. (2006) and Cobbinah and Wagner (1995).

Collection of Plant Material

Fresh mature leaves were collected from saplings when plantation was 3.5-year old. For the purposes of this study, eight rows each containing ten *Milicia* saplings representing eight progenies of *Milicia* spp. from five locations (Table 1) were chosen from the plot. Six trees were randomly marked from each row, three of which were observed to show resistance to *P. lata* attacks. Ten fresh leaves were subsequently collected from each of the saplings. The grouping of *Milicia* spp. into resistant or susceptible progenies was according to classifications suggested by Cobbinah and Wagner (1995) based on the ratio of incidence of small galls to large galls following *P. lata* attack.

Small galls were less than 3mm in size, hard and rarely opened to release adult *P. lata* (resistant). On the other hand, saplings with predominantly large galls between 5mm and 10mm in diameter, fleshy that frequently burst open to release adult psyllids for re-infestation to occur were described as susceptible.

Leaf Treatment and Isolation of Epidermal layers

Fresh mature leaves collected were fixed in 50 percent ethanol for 72 hrs. Leaves specimens were later rinsed thoroughly in running tap water. Leaf segments were sectioned from identical regions of the leaf using a microtome (Leica Microsystems, Wetzlar, Germany), with unriped pawpaw as the sectioning medium. The resulting sections were stained with 1% (w/v) aqueous solution of Aniline chloride (Lillie, 1954) for about 5 minutes at room temperature. Excess stain was rinsed off with water and sections mounted in glycerine. To test for the presence of tannins, sections were stained in methylene blue (Thomas *et al.,* 1973).

Data Collection and Analysis

Observations were made under light microscope and photographs were taken with microscope photometer (Optiphot, Nikon, Tokyo). The midrib was examined and various cell types (parenchyma, collenchyma, sclerenchyma, xylem, phloem, trichomes and tannin) were identified and counted. Cell frequency was estimated as the number of cells per square millimetre ($mm^{2)}$.

RESULTS

Distribution of Cell Types in Resistant and Susceptible Lines

The results showed differences in the distribution of cell types in both lines (Table 2). Collenchyma and total xylem cells were equally distributed in resistant and susceptible lines. There was an average of 35 and 58 xylem and collenchyma cells per mm^2 respectively in susceptible and resistant lines (Table 2). More parenchyma cells per unit area were recorded in susceptible than resistant lines.

Total phloem cells were about two-fold more in susceptible lines than resistant lines. More sclerenchyma cells were also recorded in susceptible lines (73 per mm^2) than resistant lines (57 per mm^2) (Table 2). Trichomes were almost three-fold more in susceptible lines (115.5 per mm^2) than in resistant lines (40.0 per mm^2) (Table 2). Moreover the trichomes were about 6-fold longer in the susceptible lines (363.78µm) than resistant lines (56.56µm) (Figure 2). However, there were more tannin cells per unit area in the resistant than susceptible lines (Table 2) (Figure 3).

Table 1: *Milicia* spp. progenies and their seed sources in the trial plot at Mesewam nursery

Code name	Line	Seed source	Region	Forest type
AA8	Resistant	Abofour	Ashanti	Dry semi-deciduous
AA12	Resistant			
AA28	Susceptible			
AA35	Resistant			
AB10	Susceptible	Akotaa	Ashanti	Moist semi-deciduous
BK7	Susceptible	Kintampo	Brong Ahafo	Dry semi-deciduous
EM1	Resistant	Mpraeso	Eastern	Moist semi-deciduous
VH1	Susceptible	Ho	Volta	Dry semi-deciduous

Table 2: Distribution of various cells per unit area (mm^2) in resistant and susceptible progenies (values are means ± SE). Values for xylem and phloem represent total number of cells

Cell type	Relative abundance (mm^2)	
	Resistant	Susceptible
Collenchyma	37 ± 3	37 ± 3
Xylem (total)	37	37
Parenchyma	787.5 ± 84.7	1354 ± 127.02
Phloem (total)	768	1403
Sclerenchyma	57.75 ± 20.65	73 ± 27.64
Trichomes	40.0 ± 3	115.5 ± 14.56
Tannin localization cells	838.25 ± 113.73	332.5 ± 171.44

Tannin Cells in Progenies

In There was variation in abundance per square millimetre tannin cells in both resistant and susceptible lines, even amongst progenies from the same source (Table1 and Figure 1).

In general resistant lines had more tannin cells than susceptible lines (Figure1 and Figure 3). However AA28 a susceptible line had a fairly high amount of tannin cells (Figure 1).

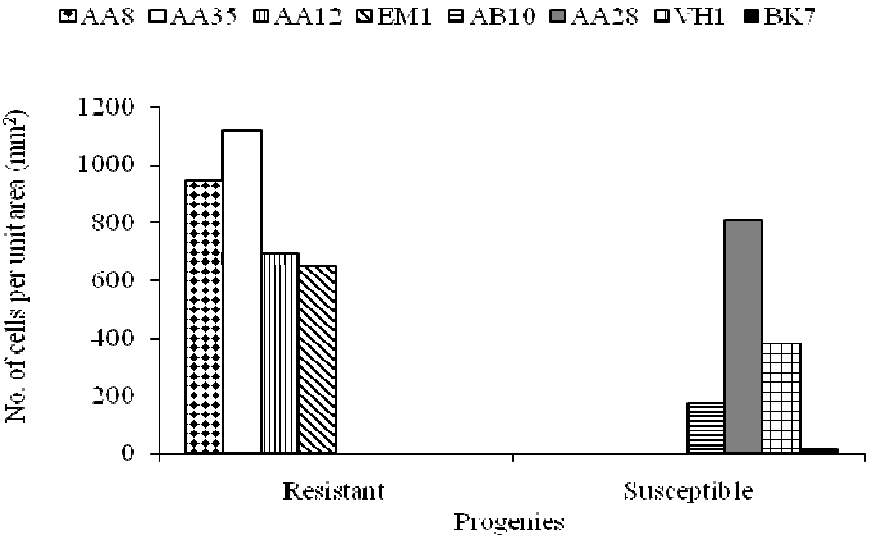
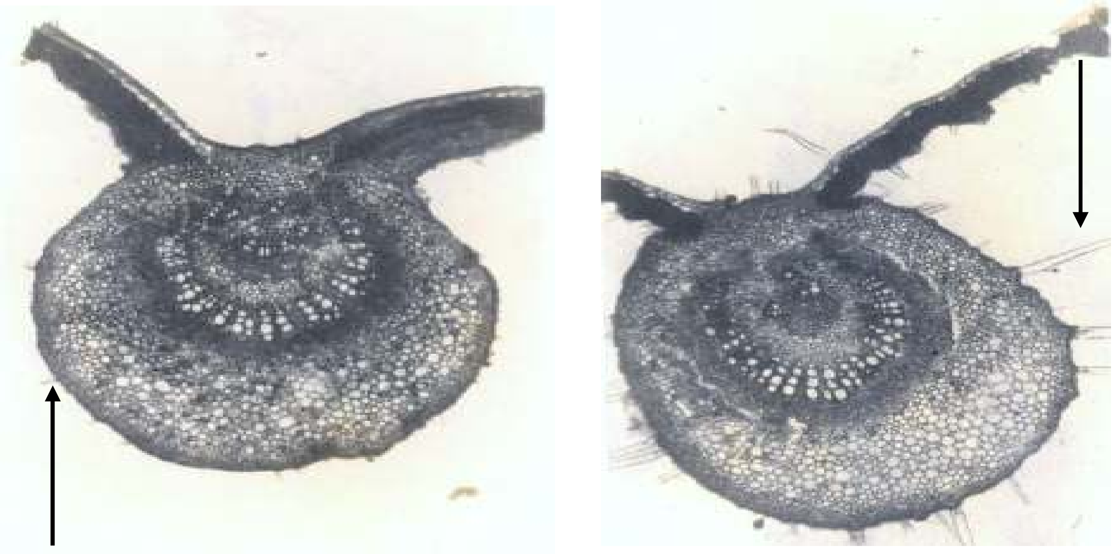
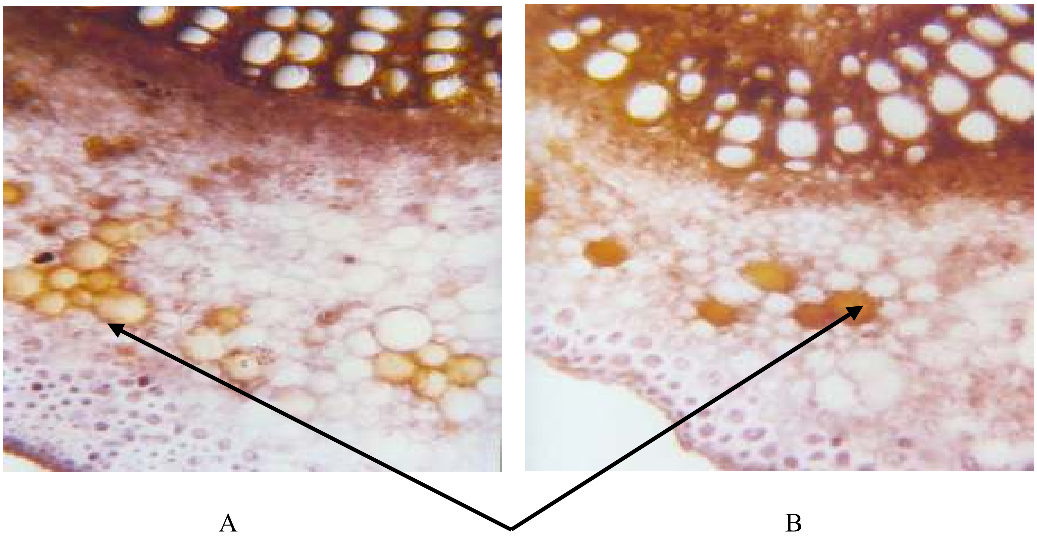


Figure 1: Distribution of tannin cells in Resistant and Susceptible progenies



A B

Figure 2: Transverse section of a leaf midrib of *Milicia* spp. showing A: resistant lines with few and shorter trichomes (black arrow), and B: susceptible lines with many, and longer trichomes



A B

Tannin cells

Figure 3: Transection of *Milicia* leaves midrib showing distribution of tannin cells in A: Resistant lines, and B: Susceptible lines

DISCUSSION

Morphological traits (eg. density of trichomes) and chemical characteristics (eg. distribution of secondary metabolites) of plants play pivotal roles in plant-insect relationships (Barbehenn *et al.,* 2005; Gutschick, 1999; Agrawal and Rutter, 1998; Agren and Schemske, 1993; Kozlowski, 1992). The results indicated that parenchyma cells were about two-fold more in susceptible than resistant lines (Table 2). Earlier studies by Cobbinah (1986) had shown that *P. lata* prefer young leaves for the formation of galls. Parenchymatous cells are known to divide to form gall tissue enclosing *Phytolyma* spp. (White, 1966). It is possible that the preference for susceptible lines is due to the abundance of these cells in the susceptible lines. More phloem cells were recorded in susceptible lines than in resistant lines (Table 2). A strong relationship has been demonstrated between galls and veins and this has been attributed to the fact that gall formers are phloem feeders (Cobbinah, 1986). Furthermore more phloem cells in the susceptible lines might probably explain why the veins in susceptible lines are quite larger than those in the resistant lines (Figure 2). Larger veins in the susceptible lines might serve as physical stimulants for selection and oviposition by *P. lata*. Moreover the presence of less phloem cells in the resistant lines (Table 2) may also be as result of the cost associated with the production of defence chemicals like tannin (Rudgers *et al.,* 2004; Kraus *et al.,* 2003; Kozlowski, 1992; Gulmon and Mooney, 1986). When tannins are present in leaves in high concentration they may inhibit photosynthesis (Kozlowski, 1992). Coley (1986) noted that opportunity costs are incurred as resources are allocated to defence rather than for other functions. Defence chemicals are mostly large molecules (Rudgers *et al.,* 2004), and their synthesis may involve indirect cost of inhibition of vegetation and reproduction growth because of diversion of carbon to defence (Gulman and Mooney, 1986). There is therefore considerable competition for photosynthate between the formation of defences and growth processes (Lorio and Sommers, 1986).

The results also showed preponderance of trichomes in the susceptible than resistant lines (Table 2 and Figure 2). This observation is in agreement with findings from Traw and Bergelson (2003), Traw and Dawson (2002), and Agrawal (2000). The higher number of trichomes in the susceptible lines may have been a result of induced responses from the *Milicia* plants due to attack by *P. lata* (Wittstock and Gershenzon, 2002). According to Traw and Bergelson (2003), many plant species respond to insect damage by increasing the density and/or number of trichomes on new leaves. Results from Agrawal (2000) showed a strong correlation between increase in number of trichomes per unit leaf area and induced resistance in *Lepidium virginicum.* Traw and Dawson (2002) also found increased leaf trichome densities per unit area in *Brassica nigra* leaves following attack by insect herbivores.

It is evident from this study that there is variation in tannin cell abundance per unit area in *Milicia* spp. (Figure 1). This can be expected since tannin concentration has been found to differ among genotypes and individuals of the same species (Ayres *et al*., 1997; Coley, 1986) which reflects genetic differences among individuals (Kraus *et al.,* 2003). The results also showed that in general resistant lines of *Milicia* spp. had more tannin cells than susceptible lines (Table 2 and Figure 3). This observation is in accordance with a number of studies that have demonstrated deterrent effects of tannins on feeding patterns and population dynamics of insects (Nagy *et al.,* 2004; Kozlowski, 1992; Coley, 1986; Feeny, 1970). Coley (1986) reported that individual *Cecropia peltata* seedlings with high tannin content had lower damage levels from armyworm, *Spodoptera latifscia*. Phenolics such as tannin may have negative impacts on insect herbivores as feeding deterrents, binding agents and generators of oxygen radicals (Appel, 1993; Kozlowski, 1992; Karowe, 1989). Nagy *et al.,* (2004) noted that tannins are important components of both constitutive and inducible defence system of most plants. Since *Milicia* spp. is frequently attacked by *P. lata*, accumulating high levels of tannins may be the optimal protection measure for the species (Wittstock and Gershenzon, 2002). This possibly could explain the relatively high resistance of genotypes AA8, AA12, AA35 and EM1 (Table 1 and Figure 1). Even though genotype AA28 was susceptible, it had a relatively high amount of tannin cells (Figure 1). This was unexpected, however Wagner and Evans (1985) demonstrated that secondary compounds can be induced to accumulate in foliage in response to leaf damage. Moreover differences in tannin composition in the resistant and susceptible lines could also explain the variation in susceptibility/resistance to *P. lata* (Barbehenn *et al.,* 2009). A more detailed chemical study will be required to explain further the discrepancy.

Implications for Management of *Phytolyma lata*

The question of exploring effective strategies to manage *P. lata* has always been of prime importance in plantation development in Ghana, and Africa in general. This implies that a wide range of integrated techniques and approaches need to be considered to deal with the challenge this insect pest poses. It is apparent from our study that the level of resistance in *Milicia* species may be influenced by cell characteristics of individual progenies. This observation offers a good opportunity to explore the technique of breeding naturally resistant progenies to *Phytolyma*.

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