RED ABAXIAL COLORING REDUCES HERBIVORY IN *COLUMNEA CONSANGUINEA*

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Abstract. Many plants have leaves with red coloration caused by anthocyanins. Anthocyanins may perform multiple functions for the plant but few of the hypothesized benefits have been rigorously tested. We tested the hypothesis that leaf herbivory on *Columnea consanguinea* is reduced by the presence of red abaxial spots. We compared herbivory on leaves with and without red spots while taking into account several potential covariates. Herbivory was significantly reduced on leaves with red spots and this benefit increased with spot size. The additional variation in herbivory was explained by branch volume, a measure of plant age and size, and not by any other potential covariate. A feeding trial confirmed that katydids avoid consuming leaf tissue with anthocyanins. We do not reject other hypothesized benefits of anthocyanins but suggest that anthocyanins may provide plants with multiple benefits.

Key words: abaxial, anthocyanin, Blood of Christ, columnin, herbivore deterrence, leaf coloration, 3-desoxyanthocyanin.

INTRODUCTION

Many plants have leaves with red pigmentation caused by localized accumulation of anthocyanins. Red pigmentation is taxonomically and ecologically widespread, occurring in all orders of plants and in most ecozones of the world (Lee & Gould 2002, Gould 2004). The presence of red pigmentation in leaves has puzzled evolutionary ecologists because there are clear costs but few verified benefits (Coley & Aide 1989, Gould 2004).

There may be a twofold cost of red pigmentation in leaves. First, manufacturing anthocyanins involves a significant investment of metabolic energy by plants (Gould 2004). Second, high levels of anthocyanins may reduce the photosynthetic efficiency of the leaf by interfering with photon capture (Gould & Lister 2006, Karageorgou & Manetas 2006). Several hypotheses have been put forward to explain this paradox, all of which propose benefits to the plants of foliar anthocyanin production. These hypotheses are of two broad types: physiological and ecological.

Anthocyanins in leaves may provide physiological benefits by reducing photoinhibition and the subsequent overproduction of free radicals (Gould 2004).

Red leaf tissue can filter out yellow-green light, preventing oversaturation of phytochemical response (Gould 2004). The benefit of reducing photoinhibition has been hypothesized for foliar anthocyanins exposed to high light intensities. Anthocyanins can also directly scavenge reactive oxygen produced by activated cholorphyll (Schaefer & Wilkinson 2004). Another potential physiological benefit of anthocyanins is the protection of defense compounds such as thiarubrines from photodegradation (Page & Towers 2002).

Anthocyanins in leaves have also been suggested as having ecological benefits such as attracting pollinators or reducing herbivory. It has been speculated that the red color in leaves caused by anthocyanins may attract avian pollinators (Morley 1974). Insect herbivores are also reported to preferentially avoid eating red pigmented leaves (Gould 2004, Gould & Lister 2006). This aversion of herbivores to red pigmented leaves may be caused by toxic effects of anthocyanins; however, anthocyanins have only been reported to be toxic to certain insect species, such as the tobacco budworm (Hedin *et al.* 1983). Leaves may also appear red even with low levels of anthocyanins if chlorophyll is particularly low (green chlorophyll masks the red color of anthocyanins). Understory tropical plants delay greening of young

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developing leaves as a strategy to reduce their nutritional value to herbivores (Kursar & Coley 1991). In such plants, chlorophyll increases only once the leaf is fully expanded and increased fiber content reduces palatability to herbivores (Coley 1983, Kursar & Coley 1991). Red leaf coloration may also make leaves appear less attractive to herbivores that are blind to the red part of the spectrum (Dominy et al. 2002). Almost all invertebrate herbivores lack a red receptor, making them insensitive to red light (Dominy et al. 2002, Schaefer & Wilkinson 2004). As a result, to these herbivores leaves with red anthocyanins appear dark in color, perhaps making them seem older and lower in quality than they actually are (Stone 1979, Lucas et al. 1998). Finally, it has been proposed that anthocyanins in leaves can reduce herbivory because of antifungal properties. Leaf-cutting ants, which feed leaf pulp to a cultivated fungus, have been shown to avoid collecting leaves or other food with high levels of anthocyanins (Coley & Aide 1989). However, a direct effect of anthocyanins on the ant-associated fungus has not been shown.

While red leaf pigmentation in temperate regions is associated with autumn senescence, in the tropics it is common year round in both understory herbs and canopy trees (Dominy et al. 2002). In the understory of tropical forests, anthocyanins are frequently on the underside of leaves, where they might increase light capture in low light environments by reflecting light transmitted through the leaf (backscattering) back to the chloroplasts for photosynthesis (Lee et al. 1979). Although there is some evidence that red abaxial coloration on leaves can increase absorbance at 700 nm (Lee & Graham 1986), the backscattering mechanism is not supported by current experimental evidence (Gould et al. 1995, Neill & Gould 1999, Hughes & Smith 2007, Hughes et al. 2008). Leaf anthocyanins may also be particularly beneficial to plants in terms of reducing photoinhibition during tropical sun flecks (Gould et al. 1995), although we note that sun flecks may only occasionally reach the undersides of leaves. If anthocyanins aid in herbivore deterrence, this may be particularly important in the tropics; some analyses suggest that herbivory rates are higher in the tropics than in temperate regions, especially on understory plants (Coley & Barone 1996).

In this study we examine the ecological effects of red pigmentation on the leaves of Columnea consanguinea Hanst., a common understory herb of Central American rainforests. This species has red leaf coloring in the form of discrete spots on the underside of leaves (Morley 1973, Gargiullo et al. 2008), the inspiration for the plant's common name, "Blood of Christ". The red pigment responsible for the spots on C. consanguinea leaves is 3-desoxyanthocyanin, also called columnin (Morley 1974). It has been proposed, but never demonstrated, that one purpose of this red abaxial coloration is to attract hummingbird pollinators to the flowers, which hang below the foliage (Morley 1974). The conspicuous red spot can be seen from below, the direction from which hummingbirds generally approach the flowers (Morley 1974); however, leaves contain red spots even on plants that have not yet developed flowers. In this study we address the possibility that the red spot serves additional functions that are secondary to attracting pollinators, such as reducing herbivory on leaves.

We test the hypothesis that leaf herbivory on C. consanguinea is reduced by the presence and size of red abaxial spots. This is a particularly useful species for this question, as leaves both with and without red spots are often found on the same branch (Fig. 1), allowing for a natural control. We quantified herbivory in over 300 leaves of C. consanguinea in a Costa Rican rainforest and examined if red spots explained more variation in herbivory than other ecological factors such as habitat type, branch height, the presence of galls or epiphytes, or measures of leaf, branch, or tree size. We also conducted a small preference experiment to examine if katydids prefer green over red leaf tissue.

METHODS

Study Site. Estación Biológica Pitilla (10°59'N, 85°26'W, elevation 659 m a.s.l.) is located in northwestern Costa Rica in the Área de Conservación Guanacaste, on the northeastern slope of Volcán Orosí (Allen 2001). The station is surrounded by pasture land as well as 10000 ha of primary forest and regenerating secondary forest that is between one and 80 years old (Lindblad 2001). Annual rainfall is *ca*. 3000 mm (unpublished data, Área de Conservación Guanacaste).

Herbivory. Herbivory (% of leaf area consumed) was compared between C. consanguinea leaves with red abaxial coloring and leaves without coloring to determine if the former is subject to less herbivory damage. Thirty plants were surveyed between 10
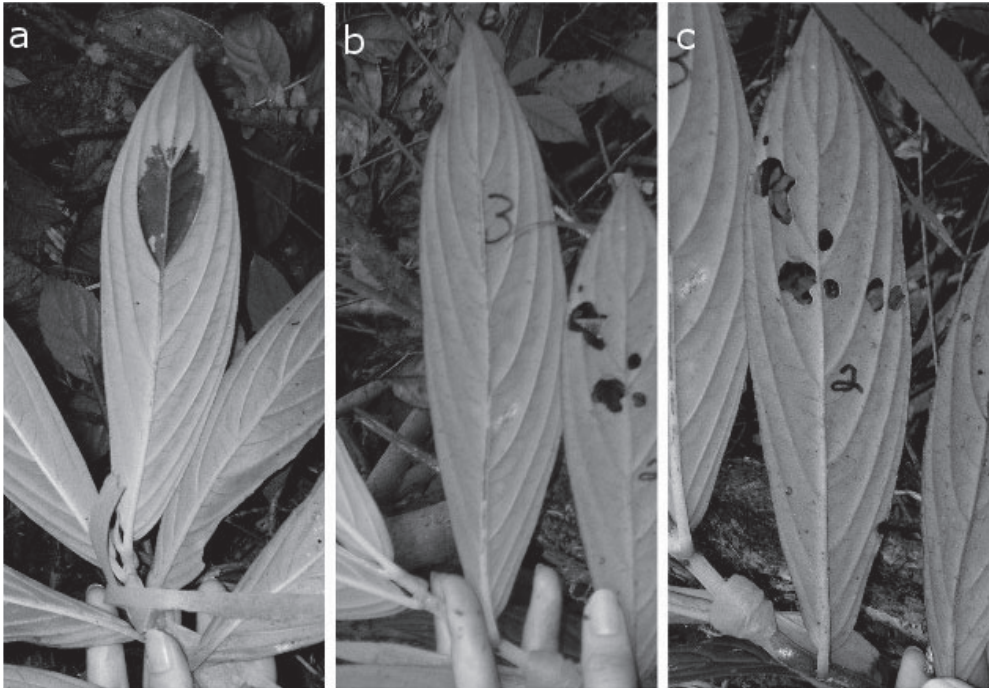
FIG. 1. Leaves of *Columnea consanguinea* with (a) a red abaxial spot, (b) no spot, and (c) loss of leaf tissue due to herbivory. In (c) the number "2" is approximately 1 cm high.

May 2007 and 12 May 2007. We excluded plants with less than three leaves either with or without a red spot. If multiple stems appeared to originate from one rootstock, those stems rooted together were considered one individual plant.

Herbivore damage was estimated visually as percent of leaf area consumed, and several additional leaf- and tree-level explanatory variables suspected to influence the degree of herbivory were also recorded. Leaf-level variables included leaf area (approximated by leaf length x width), branch volume (assumed cylindrical), red spot presence/absence, red spot size (small: 0.1 to 1 cm^2, medium: 1 to 3 cm^2, and large: greater than 3 cm^2), gall presence/absence, and average aboveground height of leaves for each branch. Tree-level variables were epiphyte presence/absence and habitat.

Habitats were classified into three categories, forest, dry marsh, and stream. Forest habitat was shaded, secondary (20-30 years old) forest with dense understory growth. The dry marsh was fern dominated with sparse surrounding understory

growth. The stream habitat was within 5 m of the banks of a pasture stream where vegetation was noticeably different from the other two habitats.

We assessed the effects of tree-, branch- and leaf-level variables on herbivory using a linear mixed model fitted with maximum likelihood techniques in the statistical program R (www.r-project.org). Tree and branch were considered random factors, with branch nested within tree. We log-transformed the percent herbivory (+0.1%) values to ensure that residuals were normally distributed and uncorrelated with fitted values. We used a backwards deletion procedure to reduce the full model. Comparisons of two models were assessed with log likelihood (L) tests, and effects within a model assessed with F tests.

Preference Experiment. To determine if herbivores prefer leaves without a red spot, feeding trials were carried out on 13 May 2007 using an unidentified species of katydid. Katydids were chosen because they are generalist herbivores. *Columnea consanguinea* leaves, one with and one without a large red spot,

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were collected from each of 11 plants in the vicinity of the station. For each plant the red spot was carefully cut from a leaf with a spot and an identically-sized green disc was cut from a leaf without a spot. One katydid and the pair of leaf discs were placed in a plastic cylindrical container (diameter = 13.8 cm, depth = 5.4 cm) for 24 hours. Leaf discs were placed with underside surfaces facing up to ensure the red spot was accessible to the katydid. Leaf discs were photographed before and after the feeding trial, and herbivory damage (percent of leaf area) was computed using Adobe Photoshop CS. The effect of the red spot on herbivory was analyzed with a linear mixed model, with katydid individual as a random factor.

RESULTS

Most of the variance in herbivory occurred between leaves on a branch (86.3%) rather than branches on a tree (13.5%) or between trees (<1%). We defined a factor "red spot" as having four levels, with the red spot either absent, or varying in size from small to medium to large. In univariate tests of explanatory variables, variation in leaf herbivory was significantly related to the red spot factor ($F_{3,144}$ = 3.42, p < 0.0001) and, marginally, leaf area ($F_{1,302}$ = 3.55, p = 0.06), but not to habitat type, branch height, branch volume, or the presence of leaf galls or epiphytes (all p > 0.05). In multivariate tests, the red spot factor continued to be significant, even after inclusion of all other explanatory variables (L = 59.2, p < 0.0001). However, leaf area was not significant in models with all other variables included (L = 1.56, p = 0.21), or even in a model with just the red spot factor (L = 3.03, p = 0.08), suggesting that its univariate correlation with herbivory was simply due to covariance with the red spot factor; large leaves tend to have red spots more often, and of greater size, than smaller leaves ($F_{3,348}$ = 30.4, p < 0.0001). The full model was reduced by backwards deletion, and the final model included only the red spot factor ($F_{3,299}$ = 21.2, p < 0.0001) and branch volume ($F_{1,299}$ = 6.40, p = 0.012). Herbivory increased with branch volume, but only after the presence and size of red spots were accounted for.

We then examined the red spot effect in more detail. The presence of a red spot resulted in lower herbivory ($F_{1,301}$ = 54.7, p < 0.0001, Fig. 2). Among
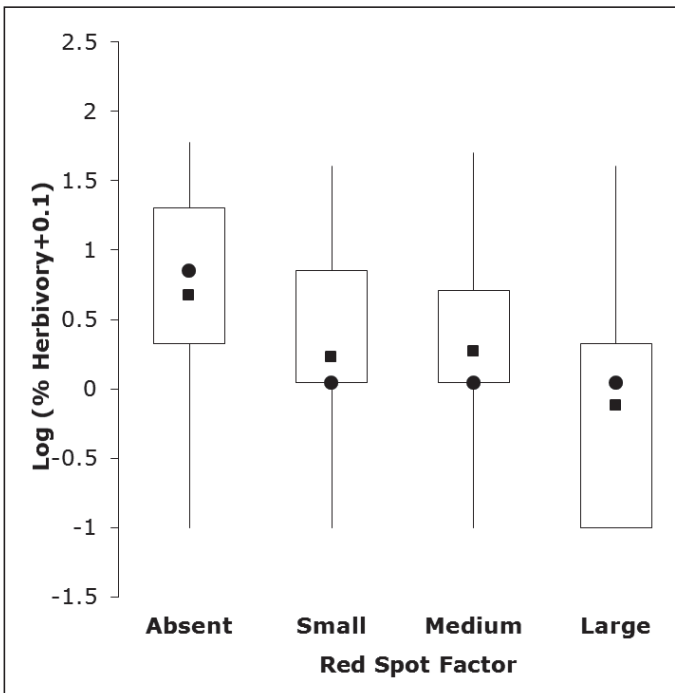

FIG. 2. The percentage of leaf tissue lost to herbivory (log-transformed) for four types of leaves, differentiated by the presence and size of the red spot on leaf undersides. The distribution of data is shown using box-whisker plots where the box bounds the interquartile range (middle 50% of data) and whiskers show the range of data that exceeds the interquartile box by < 1.5 x interquartile range. The median is indicated with a solid circle and the mean is indicated with a solid square.

leaves with red spots, the percent of the leaf consumed also decreased as the size of the red spot increased ($F_{3,144}$ = 3.42, p = 0.019, Fig. 2). Since leaves with large red spots are also larger in size, it is possible that the absolute amount of leaf consumed does not differ with leaf size although the proportion does. We therefore calculated absolute amounts of herbivory, and it too decreased with the presence ($F_{1,301}$ = 25.1, p < 0.0001) and size of red spots ($F_{3,144}$ = 3.61, p = 0.015).

Katydids tended to prefer to consume leaves without red spots (L = 3.86, p = 0.0495), with four individuals strongly preferring leaves without spots, two individuals consuming little of either, and one individual preferring the leaf with the red spot.

DISCUSSION

Although the primary benefit of the red spots on the underside of *Columnea consanguinea* leaves is often assumed to be the attraction of avian pollinators (Morley 1974), the results of this study suggest that the red spots may provide a secondary benefit to the plant by reducing leaf herbivory. Our survey found that herbivory was less on leaves with red spots compared with leaves without red spots, and this benefit to the plant increased with spot size. Correlative effects should always be viewed cautiously, because of the potential for confounding by covariates. However, even after accounting for numerous potential covariates (leaf area, galls, branch height and volume, epiphytes, and habitat), we still found red spots to significantly reduce herbivory. In fact, red spot size and presence accounted for the majority of variation in herbivory, with only branch volume (a measure of plant age and size) significantly explaining any additional variance. Furthermore, in a small preference experiment, katydids preferred to consume leaves without red spots. Although the power of this experiment is limited by small sample size, it is noteworthy that its results corroborate our survey. We would also like to note that although katydids are generalist herbivores, we do not have direct evidence for these species being important herbivores on *C. consanguinea*. As such, the results of this experiment should be interpreted as preliminary.

There are a number of possible reasons why herbivory was reduced on *C. consanguinea* leaves with red spots. In general terms, insects could be responding to toxic properties of the leaf, to visual cues, or to underlying nutritional differences. We address each class of hypothesis in turn.

Anthocyanins are unlikely to have direct toxic effects on herbivores as few cases of toxicity to invertebrates have been reported (Lev-Yadun & Gould 2009); however, antimicrobial and antifungal properties have been suggested by some authors (Lev-Yadun & Gould 2009). For example, Coley and Aide (1989) report that leaf-cutter ants avoid resources to which anthocyanins have been added, perhaps because of toxicity to the fungal symbionts of the ants. This would not explain why herbivores without fungal symbionts also avoid leaves with red coloration, such as katydids (this study) or temperate herbivores (Karageorgou & Manetas 2006).

Although anthocyanins are not known to be widely toxic to insect herbivores, they may still indirectly aid in chemical defenses against herbivory, perhaps by protecting antiherbivore compounds from photodegradation. For example, thiarubrines are a class of antiherbivore compound which can degrade in the presence of visible and ultraviolet light (Page *et al.* 1999). Anthocyanins shield the thiarubrines from photodegradation by absorbing in the same part of the visible spectrum (Page & Towers 2002). To date, there has been no research on potential antiherbivore compounds within *C. consanguinea* species, so it is not clear if a similar mechanism may operate in this species.

Red spots on *C. consanguinea* leaves could also potentially serve as an aposematic defense against herbivores, that is, as a reliable visual signal of toxicity. Although aposematic defenses may be strong deterrents to vertebrate consumers, their applicability to insect herbivory (e.g. Hamilton & Brown 2001) has been questioned as most insects lack a red receptor (Dominy *et al.* 2002, Schaefer & Wilkinson 2004) or show no color preference (Schaefer & Rolshausen 2007). Instead, insects may simply perceive red-colored leaves as dark (Dominy *et al.* 2002, Karageorgou & Manetas 2006). This has given rise to a second visual hypothesis, that insects avoid red-colored leaves because they appear to be aged, senescent, or dead (Stone 1979). It is possible that red spots on *C. consanguinea* leaves make them look diseased or senescent. Finally, green-colored herbivores may avoid red leaves because they are more visible to their predators against this background (Lev-Yadun *et al.* 2004). This could also apply to *C. consanguinea* leaves, but we note that red spots reduced herbivory by 70%, much more than the proportion of leaf area occupied by even the largest red spots.

As our study is observational, we cannot discount the possibility that some unmeasured factor covaries with the presence of red spots and is ultimately driving herbivory patterns. For example, leaves with red spots could be of lower nutritional quality, or be older and hence more defended (although we attempted to account for plant age by using plant size variables as covariates). Future studies could examine such explanations by comparing plant chemistry in leaves with and without red coloration, or by artificially coloring leaves to separate visual and chemical effects (Schaefer & Rolshausen 2007).

Regardless of the underlying mechanism, our study suggests that red abaxial coloration can provide plants with an important benefit: deterrence of herbivores. Gould and Lister (2006) critique the herbivore deterrence hypothesis as being largely untested. This study is one of the few to provide a direct test of the herbivore deterrence hypothesis. Our study also contributes to a limited number of feeding trials performed using red and acyanic leaves with non-aphid herbivores.

Finally, although our study supports the herbivore deterrence hypothesis, it does not discount other potential benefits of leaf anthocyanins, such as reducing photoinhibition and the production of free radicals (Gould 2004), protecting from oxidative stress (Schaefer & Wilkinson 2004), increasing light capture (Lee & Graham 1979), and attracting pollinators (Gould & Lister 2006).

For *C. consanguinea*, the most commonly cited (though untested) benefit of abaxial anthocyanins is the attraction of hummingbirds. It is possible that pollinator attraction is the primary benefit to *C. consanguinea* of red coloration, and herbivore deterrence a secondary benefit. We suggest that, in general, multiple benefits of anthocyanins in leaves are possible, and that each potential benefit needs to be carefully researched in order to arrive at a full explanation of the phenomena of red coloration.

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REFERENCES

Allen, W. 2001. Green phoenix: Restoring the tropical forests of Guanacaste, Costa Rica. Oxford University Press, New York.

Coley, P.D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecological Monographs 53(2): 209–233.

Coley, P.D., & T.M. Aide. 1989. Red coloration of tropical young leaves: a possible antifungal defence? Journal of Tropical Ecology 5: 293–300.

Coley, P.D., & J.A. Barone. 1996. Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27: 305–335.

Dominy, N.J., Lucas, P.W., Ramsden, L.W., Riba-Hernandez, P., Stoner, K.E., & I.M. Turner. 2002. Why are young leaves red? Oikos 98(1): 163–176.

Gargiullo, M.B., Magnuson, B., & L. Kimball. 2008. A field guide to the plants of Costa Rica. Oxford University Press, Inc., New York.

Gould, K.S., Kuhn, D.N., Lee, D.W., & S.F. Oberbauer. 1995. Why leaves are sometimes red. Nature 378(6554): 241–242.

Gould, K.S. 2004. Nature's Swiss Army knife: The diverse protective roles of anthocyanins in leaves. Journal of Biomedicine and Biotechnology 5: 314–320.

Gould, K.S., & C. Lister. 2006. Flavonoid Functions in Plants. Pp. 397–441 *in* Andersen, Ø. M., & K.R. Markham (eds.). Flavonoids: Chemistry, Biochemistry and Applications. Taylor and Francis Group, Boca Raton, Florida U.S.A.

Hamilton, W.D., & S. P. Brown. 2001. Autumn tree colours as a handicap signal. Proceedings of the Royal Society of London Series B - Biological Sciences 268(1475): 1489–1493.

Hedin, P.A., Jenkins, J.N., Collum, D.H., White, W.H., & W.L. Parrott. 1983. Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis virescens* F. American Chemical Society Symposium Series 208: 347–365.

Hughes, N.M., & W.K. Smith. 2007. Attenuation of incident light in *Galax urceolata* (Diapensiaceae): concerted influence of adaxial and abaxial anthocyanic layers on photoprotection. American Journal of Botany 94(5): 784–790.

Hughes, N.M., Vogelmann T.C., & W.K. Smith. 2008. Optical effects of abaxial anthocyanin on absorption of red wavelengths by understorey species: revisiting the back-scatter hypothesis. Journal of Experimental Botany 59(12): 3435–3442.

Karageorgou, P., & Y. Manetas. 2006. The importance of being red when young: Anthocyanins and the protection of young leaves in *Quercus coccifera* from insect herbivory and excess light. Tree Physiology 26: 613–621.

Kursar, T.A., & P.D. Coley. 1991. Nitrogen content and expansion rate of young leaves of rain forest species: Implications for herbivory. Biotropica 23 (2): 141–150.

Lee, D.W., Lowry, J.B. & B.C Stone. 1979. Abaxial anthocyanin layer in leaves of tropical rain forest plants: enhancer of light capture in deep shade. Biotropica 11 (1): 70–77.

Lee, D.W., & R. Graham. 1986. Leaf optical properties of rainforest sun and extreme shade plants. American Journal of Botany 73 (8): 1100–1108.

Lee, D.W., & K.S. Gould. 2002. Why leaves turn red. American Scientist 90 (6): 524–531.

Lev-Yadun, S., Dafni, A., Flaishman, M.A., Inbar, M., Izhaki, I., Katzir, G., & G. Ne'eman. 2004. Plant coloration undermines herbivorous insect camouflage. Bioessays 26 (10): 1126–1130.

Lev-Yadun, S., & K.S. Gould. 2009. Role of Anthocyanins in Plant Defence. Pp. 21–48 in Gould, K., Davies, K., & C. Winefield (eds.). Anthocyanins: Biosynthesis, Functions, and Applications. Springer, New York.

Lindblad, I. 2001. Diversity of poroid and some corticoid wood-inhabiting fungi along the rainfall gradient in tropical forests, Costa Rica. Journal of Tropical Ecology 17: 353–369.

Lucas, P.W., Darvell, B.W., Lee, P.K.D., Yuen, T.D.B., & M.F. Choong. 1998. Colour cues for leaf food selection for long-tailed macaques (Macaca fascicularis) with a new suggestion for the evolution of trichromatic colour vision. Folia Primatologica 69 (3): 139–152.

Morley, B. 1973. Materials for a treatment of Columnea in Panama. Annals of the Missouri Botanical Garden 60 (2): 449–460.

Morley, B.D. 1974. Notes on Some Critical Characters in Columnea Classification. Annals of the Missouri Botanical Garden 61 (2): 514–525.

Neill, S., & K.S. Gould. 1999. Optical properties of leaves in relation to anthocyanin concentration and distribution. Canadian Journal of Botany 77: 1777-1782.

Page, J.E., Block, E., & G.H.N. Towers. 1999. Visible-light photochemistry and phototoxicity of thiarubrines. Photochemistry and Photobiology 70 (2): 159–165.

Page, J.E., & G.H.N. Towers. 2002. Anthocyanins protect light-sensitive thiarubrine phototoxins. Planta 215: 478–484.

Schaefer, H.M., & Rolshausen, G. 2007. Aphids do not attend to leaf colour as visual signal, but to the handicap of reproductive investment. Biology Letters 3: 1–4.

Schaefer, H.M., & D.M. Wilkinson. 2004. Red leaves, insects and coevolution: a red herring? Trends in Ecology and Evolution 19 (12): 616–618.

Stone, B.C. 1979. Protective coloration of young leaves in certain Malaysian palms. Biotropica 11 (2): 126.