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A test of the bicolored fruit display hypothesis: Berry removal with artificial fruit flags[1]

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CRAMER, JENNIFER M., MARIA L. CLOUD, NATHAN C. MUCHHALA, ANASTASIA E. WARE, BRENT H. SMITH (Department of Biology, Earlham College, Richmond, IN 47374), AND G. BRUCE WILLIAMSON (Department of Biological Sciences, Life Sciences, Louisiana State University, Baton Rouge, LA 70803). A Test of the Bicolored Fruit Display Hypothesis: Berry Removal with Artificial Fruit Flags. J. Torrey Bot. Soc. 130:30–33. 2003.—The fruit flag (Stiles 1982) and the bicolored fruit display (Willson and Thompson 1982) hypotheses state that plants with fruit displays that provide contrast within the infructescence, the plant, or their surrounding environment, serve to attract birds, thereby increasing fruit consumption and seed dispersal. We simulated fruit flags by attaching infructescenses from pokeweed (*Phytolacca americana*) to wooden stakes wrapped with different colors of flagging tape—fluorescent pink, fluorescent green, and black. Pokeweed is a plant hypothesized to utilize fruit flags and has stems that turn from green to bright pink as berries along infructescences ripen to purple-black. In two habitats, removal rates from brightly colored stakes (pink and green) were significantly higher than those from black stakes, indicating that a bicolored fruit display increased fruit removal by birds. There were no consistent differences in removal from pink and green stakes suggesting that the particular color of the flag may not be as important as the contrast between the flag and the fruit.

Key words: fruit flags, bicolored fruit display, *Phytolacca*, seed dispersal, mutualism.

It has been hypothesized that plants produce visual signals, other than ripe fruits, to create vivid displays that may attract dispersal agents such as birds (Kerner 1895 apud Willson and Hoppes 1986; Thompson and Willson 1979; Stiles 1980, 1982; Willson and Thompson 1982; McDonnell et al. 1984; Webb 1985; Facelli 1993; Fuentes 1995). Stiles defined fruit flags "as contrastingly colored plant parts that are temporally or spatially associated with fruits, but are not quantitative indicators of the amount of ripe fruit available" (Stiles 1982, p. 500). Specifically, he identified "pre-ripening fruit flags" as unripe fruit or other associated plant parts that produce small-scale contrast within the plant. Somewhat simultaneously, Willson and Thompson (1982) hypothesized that "bicolored fruit displays" may increase attractiveness to locally breeding birds by providing contrast between ripe fruit and either pre-ripe fruit or other brightly colored structures. The concept that multiple colors on the plant may increase its attractiveness to seed dispersers, specifically birds, is central to both of these hypotheses. Here, we present the results of a field test of the "pre-ripening fruit flag/bicolored fruit display" hypotheses by varying the color of artificial flags while maintaining a single, natural fruit type. We hypothesized that more conspicuous flag colors, contrasting with ripe fruits, would be associated with higher rates of fruit removal.

Methods. The study was conducted in a mosaic of secondary habitats immediately southwest of Earlham College, Richmond, Indiana, USA. Artificial fruit flags were constructed of 2 cm × 2 cm × 150 cm wooden garden stakes completely wrapped with colored vinyl flagging. These flags were modeled after the plant *Phytolacca americana* L. (pokeweed) whose stems turn from green to bright pink in early September, coincident with the ripening of its purple-black berries (Willson and Melampy 1983; McDonnell et al. 1984). Four color treatments were employed. Fluorescent pink ("pink glo", Forestry Suppliers, Inc.) was chosen to approximate the natural color of *P. americana* fruiting stalks. Fluorescent green ("lime glo") flagging was chosen as a highly conspicuous color in tone (color quality) and brightness, like fluorescent pink, but one that was dissimilar to the nat-

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ural pink of the *P. americana* infructescences. Black was chosen as an inconspicuous color as it blended well into the background and did not contrast with the color of the *P. americana* fruits. Stakes without vinyl flagging were used in the fourth treatment as a natural color and to test for possible differences caused by the presence of flagging of any color.

A natural *P. americana* infructescence, containing 20–30 ripe fruits, was stapled to the top of each stake above a single large nail (4 mm diameter, 7 cm in length) which served as an avian perch. All infructescences had been collected in the study area in early October and were refrigerated to maintain fruit quality. The number of fruits on each stake was counted daily for 7 days (from October 6 through October 12, 1997) to monitor berry removal and the ground surrounding each stake was surveyed for fallen berries. Shriveled fruit and fruit of poor quality were removed daily and infructescences were replaced or supplemented when berry numbers fell below 20. No fruiting *P. americana* individuals were found in immediate proximity to the experimental infructescences.

Stakes were placed in four habitats: two types of secondary forests (Black Cherry Forest and Woodland) and two types of forest edges (Pasture and Corn Field). Ten stakes of each color were placed in each habitat (40 stakes × 4 habitats, 160 total) ordered randomly by color at 7.5 m intervals, along forest trails in Black Cherry Forest and Woodland habitats and parallel to and 1 m away from the forest edge in Pasture and Corn Field habitats. These habitats were not replicated, nor were there *a priori* predictions of removal rates among them; rather they were chosen to test avian responses to the fruit flags across an array of available habitats. Edges and forests often vary in disperser activity (Willson and Crome 1989; Gorchov et al. 1993).

Because the number of fruits per stake per day varied from 20 to 30, the proportion of fruits removed from each stake per day was used as the dependent variable. Preliminary analysis indicated no apparent need for repeated measures analysis: day-to-day fruit removal was independent of habitat (i.e., no habitat × day interaction), independent of color (i.e., no color × day interaction), and independent of color by habitat (i.e., no habitat × color × day interaction). Therefore, daily proportional removal of fruits per stake became the basic level of replication for statistical analyses. Proportions were arc-sin transformed in order to normalize variances for

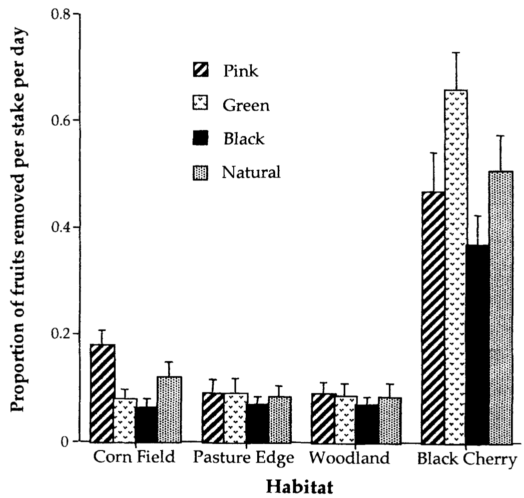

Fig. 1. Mean proportion (± S.E.) of *Phytolacca americana* berries removed per stake per day from stakes of four different colors in four different habitats.

statistical analysis. Contrasts used to test *a priori* hypotheses, correlations analyses, and ANOVAs were performed using Statistica.

Results. First, we tested whether the vinyl flagging used to wrap the colored stakes had an effect on fruit removal by comparing flagging of all colors (pink, green and black) to stakes without flagging or artificial color and found no apparent effect of the flagging material (contrast df = 1, t = −0.27, P = 0.787).

A priori hypotheses predicted that brightly colored flags represented by fluorescent stakes (pink and green) would have higher fruit removal rates than inconspicuous flags (black). Contrasts across all habitats showed that fluorescent green and pink stakes had fruit removal rates 71% and 77%, respectively, higher than black stakes (Fig 1., df = 1, t = −3.34, P = 0.0009). Removal rates from fluorescent pink stakes were not significantly different from those of fluorescent green stakes (contrast df = 1, t = 0.84, P = 0.40). A 2-way ANOVA demonstrated that color and habitat had significant effects on fruit removal rates, but a significant interaction term indicated that these color effects varied by habitat (Table 1), therefore *a priori* hypotheses were examined in each habitat independently (Table 2).

Removal rates for pink stakes were significantly higher than those for green stakes in the Corn Field (contrast df = 1, t = −3.09, P = 0.002) but they were significantly lower than removal from green stakes in the Black Cherry

Table 1. Two-way ANOVA of the effects of habitat and fruit flag color on daily removal proportions of *Phytolacca americana* berries. Means are shown in Fig. 1.

	d.f.	MS	F	P
Habitat	3	11.7814	125.96	0.0006
Color	3	0.37290	3.99	0.0077
Habitat*Color	9	0.27687	2.96	0.0018
Residual	1064			

Forest (contrast df = 1, t = 2.12, P = 0.035). Therefore, bicolored conspicuousness was important to fruit removal regardless of whether the artificial fruit flag resembled the natural infructescence color (pink) or not.

If conspicuous coloring were to have an effect on fruit removal, we hypothesized that it would be more evident at sites where overall dispersal rates were higher. Stakes with no artificial coloring (without flagging material) were used as an independent measure of fruit removal rates in each of the four habitats. Differences in fruit removal rates between fluorescent colored stakes and black stakes (pink minus black and green minus black) was used to measure fruit removed as a result of conspicuous coloring in each of the four habitats. We tested for a correlation between localized levels of fruit removal and the effect of color on fruit removal at each site and found a positive relationship that was significant for green minus black (R^2 =0.99, P = 0.002, df = 3), but not for pink minus black (R^2 = 0.59, P = 0.12, df = 3).

Discussion. These results confirm that conspicuous, contrasting coloration of the stakes is correlated with higher fruit removal and more so as overall dispersal activity increased. From our visual perspective, black stakes blended with shadows and provided the least amount of contrast with the fruits and with the background vegetation. Being less conspicuous, the black stakes attracted the attention of fewer birds as is evident by their lower rates of fruit removal.

Previous tests of the "preripening fruit flag/bicolored fruit display" hypotheses have yielded mixed results. Facelli (1993) found higher removal rates of the red berries of *Rhus glabra* from infructescences outfitted with red plastic flags relative to controls without flags, but contrastingly colored yellow flags showed no effect. Comparing black versus black-and-red fruit displays, Morden-Moore and Willson (1982) found that fruit removal at the bicolored displays was lower (7%) in one experiment, higher (8, 20, and 51%) in three experiments, and not different in three experiments—all with *Prunus serotina*. Willson and Melampy (1983) found higher dispersal from bicolored fruit displays for *P. serotina* and *Phytolacca americana* in a paired experimental design, but no difference for *Phytolacca americana* in a randomized design. Paired designs are useful due to extreme spatial and temporal heterogeneity in removal rates, but they also may be less realistic if birds perceive and visit any pair as a single entity (Morden-Moore and Willson 1982). Willson and Hoppes (1986) attached artificial red or green foliage sprays to fruiting branches of *Cornus*, *Vitis*, and *Parthenocissus* plants and found no differences in fruit removal rates based on color. However, because artificial flags were added to the branches of large individuals already in fruit, the location of plants and fruit availability may have been known to dispersers or the entire tree may have served as a flag, resulting in increased fruit removal regardless of the presence of artificial fruit flags on individual branches.

Table 2. Contrasts demonstrating differences between daily removal proportions of *Phytolacca americana* berries from fluorescently colored stakes (green and pink) and black stakes.

Habitat	t	P
Black Cherry Forest	−2.48	0.014
Corn Field	−2.40	0.017
Woodland	0.71	0.48
Pasture	−0.81	0.42

Our results are limited in scope, but they do support the fruit flag hypothesis using a randomized design and they demonstrate greater effects of flags where removal rates are higher, concurring with the results of Willson and Melampy (1983). However, the fluorescent colors chosen for our study are seldom seen in nature and may have produced artificially high differences (71% and 77%) with black flagging.

Stiles (1982) hypothesized that pre-ripening fruit flags serve to attract resident dispersers from immediate surroundings and prevent fruits from rotting on the plant while foliar fruit flags, large signals created by the majority of a plant's leaves turning color prior to other deciduous plants, perform as long-distance signals to alert migrating frugivores to fruit availability. Although the origin of the dispersers, residents versus migrants, was not known in our study, the results do support the preripening fruit flag/bicolored fruit display hypotheses by showing experimentally that with a consistent fruit type, there is less fruit removal from stakes with a dull color than with conspicuous colors. Further, the actual hue (shade) of a color may not be as important as the fact that it is conspicuous in tone or brightness (i.e., fluorescent).

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