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The Effect of Rhododendron on Soils and Foliar Characteristics of a Wintergreen Perennial Herb

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Abstract

Cardamine diphylla (Crinkleroot) is a common wintergreen found in scattered colonies across mesic forests of North America. It is known for high levels of glucosinolates, which are important anti-herbivore compounds characteristic of Brassicaceae. Crinkleroot displays varying color morphotypes of green and purple on the abaxial and adaxial sides of the basal leaves. Unlike many forest floor herbs, *C. diphylla* grows both under Rhododendron (*Rhododendron maximum* L.) thickets and on the open forest floor. The aim of this study was to investigate whether *Rhododendron* affects soils and foliar characteristics of *C. diphylla*. Our study was conducted in a rich cove forest in Pisgah National Forest near Barnardsville, NC. Twenty patches of *C. diphylla* growing under *Rhododendron* were compared with twenty patches in the open forest. The frequency of green or purple abaxial color on the basal leaves was recorded, and leaf and soil samples were collected for analysis. Foliar samples were analyzed for nitrogen and sulfur content, and soil samples were analyzed for total nitrogen, pH, humic matter, and cation exchange capacity. *Cardamine diphylla* growing under *Rhododendron* displayed significantly higher nitrogen and sulfur content, lower biomass, and were more likely to have green abaxial surfaces compared to *C. diphylla* growing in the open forest. Soils under *Rhododendron* had significantly lower nitrogen and humic matter. These results indicate that *Rhododendron* does impact soils and the foliar characteristics of this wintergreen perennial. Transplant studies would offer insight on how *C. diphylla* is able to tolerate varying overstory environments.

Key Words: *Cardamine diphylla*, *Rhododendron*, glucosinolates, leaf morphotypes

1. Introduction

Cardamine diphylla (Michx.) A. Wood (Crinkleroot) is a wintergreen herb that emerges in the fall, senesces in late spring, and is dormant throughout the summer [2, 3]. It occurs in scattered colonies throughout moist, shady habitats in deciduous forests[4, 3].

Cardamine diphylla leaves display multiple color morphotypes. The adaxial surface can be green with a purple abaxial surface, or both sides of the leaf can be completely green or completely purple. The purple color is due to accumulated anthocyanins, which are non-photosynthetic leaf pigments[9]. This trait is displayed in other forest understory herbaceous species including *Tipularia discolor* (Pursh) Nutt. and *Galax urceolata* (Poir.) Brummitt. Variation in color may also indicate nutritional differences in leaves; red and purple leaf morphotypes have been shown to contain less nitrogen than green leaf morphotypes[9]. Accumulation of anthocyanins has been proposed to protect against photoinhibition brought on by abiotic stressors such as cold temperatures, excess light, or nutrient deficiencies, though this theory is still being debated[9].

Brassicaceae species produce glucosinolates, which are secondary metabolites composed of sulfur- and nitrogen-containing compounds. Glucosinolates help protect plants against environmental stress, deter herbivory, and contain compounds with many potential human health benefits and medicinal properties[3, 5, 6, 7, 8]. Levels of glucosinolates in

Cardamine diphylla tend to be higher in shorter (and most likely younger) leaves compared to longer (and most likely older) leaves[2].

Unlike many other understory species, *C. diphylla* has been observed under the canopy of *Rhododendron maximum* L. Forest understory plants require specific light regimes and usually depend on sunflecks or seasonal variation in light. Like other evergreen shrubs, *Rhododendron* prevents sunlight from reaching the forest floor throughout the year. *Rhododendron* is prevalent in cove forests and covers 0.5 million ha in the southern Appalachians[10, 11]. It grows in canopy gaps and reduces light availability, sunfleck duration, and water availability[14]. Its recent increase in abundance may be attributed in part to the decline of the American chestnut, *Castanea dentata* (Marshall) Borkh.[12]. Survivorship of tree seedlings is reduced under *R. maximum*[13, 14], but few studies have examined the effects of *Rhododendron* on herbaceous species.

We investigated the effects of *Rhododendron* on soils and foliar characteristics of *C. diphylla*. Abaxial leaf color, nitrogen, and sulfur content were compared between plants growing under *Rhododendron* and on the open forest floor. Soil total nitrogen, pH, humic matter, and cation exchange capacity were also compared.

2. Methods

The study was conducted in the vicinity of Corner Rock Creek and Staire Creek in the Big Ivy area of Pisgah National Forest near Barnardsville, NC (elevation 823-915 m). Samples were collected between December 2019 and January 2020. This mesic cove forest is characterized by Tulip Poplar (*Liriodendron tulipifera* L.), Sweet Birch (*Betula lenta* L.), American Beech (*Fagus grandifolia* Ehrh.), and Rhododendron (*Rhododendron maximum* L.). Additional understory species include Christmas Fern (*Polystichum acrostichoides* (Michx.) Schott.) and Partridgeberry (*Mitchella repens* L.).

Twenty circular plots (radius 2 m) were established in open forested areas, and 20 plots were established under *Rhododendron*. Plots were located after searching the study area for patches containing at least 25 leaves, which was the number of leaves needed to produce sufficient dry material for foliar analyses (1 gram). When a patch was encountered, the quadrat was situated, and leaves were harvested by cutting at the base of the petiole.

The abaxial leaf color (purple or green) of each leaf was recorded, and leaves from each plot were placed in plastic bags and kept cool. Samples were dried in a forced hot air oven at 80 °C and weighed. Soils were sampled by taking 15, 2.5 cm x 10 cm soil cores from throughout each plot. Soil samples were homogenized, air-dried, and analyzed for total nitrogen, pH, humic matter, and cation exchange capacity. Leaves were analyzed for nitrate nitrogen and sulfur. All samples were analyzed at Waters Agricultural Lab in Warsaw, NC. Foliar and soil variables were compared for plots under *Rhododendron* vs. the open forest using t-tests (alpha = 0.05).

3. Results

The color of the abaxial leaf surfaces varied significantly between areas ($P < 0.001$). *Cardamine diphylla* in open plots had 73.2% purple leaves, while plants under *Rhododendron* had 21.6% purple leaves (Table 1). Leaf biomass also differed significantly between areas ($P = 0.039$), with leaves in open plots ~20% heavier than leaves underneath *Rhododendron.* Leaf nitrogen and leaf sulfur were both significantly higher under *Rhododendron* than in open plots ($P < 0.001$ in both analyses).

Soil characteristics also varied between understory habitats. Soil nitrogen ($P = 0.036$) and humic matter ($P = 0.017$) were significantly higher in open plots, but soil pH and soil cation exchange capacity did not differ between areas ($P > 0.05$).

Table 1. Foliar and soil characteristics for *C. diphylla* growing under *Rhododendron* and in the open forest.

Leaf Characteristics:	**Open Plots**	***Rhododendron* Plots**	**P Value**
Purple abaxial surface (%)	73.2	21.6	<0.001
Dry weight (g)	2.25	1.87	0.039
Nitrate nitrogen (%)	3.52	4.30	<0.001
Sulfur (%)	0.84	1.05	<0.001
Soil Properties:			
Total nitrogen (%)	0.37	0.29	0.036
pH	5.4	5.2	0.308
Humic matter (%)	0.53	0.38	0.017
Cation exchange capacity (meq)	10.5	9.9	0.359

4. Discussion

Results indicate that *C. diphylla* foliar characteristics are affected by the presence of *Rhododendron*. Basal leaves contained higher nitrogen and sulfur and had lower biomass under *Rhododendron* than in open areas. *Cardamine diphylla* under *Rhododendron* had a higher frequency of green abaxial leaf surfaces, while leaves in open plots had a higher frequency of purple abaxial surfaces. The reduced biomass of leaves growing under *Rhododendron* may be due to stressors such as reduced sunlight and water availability associated with co-occurring with *Rhododendron*[14]. Nitrogen and sulfur are constituents of glucosinolates[2], so higher levels of foliar nitrogen and sulfur in *C. diphylla* growing under *Rhododendron* suggest these leaves may contain more glucosinolates than leaves growing in the open forest.

The higher frequency of purple morphotypes occurring with lower foliar nitrogen in open plots is consistent with Kytridis et al. (2008) in which red phenotypes of *Cistus creticus* leaves contained lower nitrogen[9]. Anthocyanins in upper leaf tissue act to shade deeper leaf tissues from UV radiation, resulting in a sunscreen effect that adjusts the ratio of chlorophyll a and chlorophyll b[9]. The presence of anthocyanins results in lower photosynthetic rates[9], which may allow *C. diphylla* to adapt to high light conditions in open plots after deciduous leaf fall.

Soils in the open forest contained more humic matter than soils under *Rhododendron*, possibly due to the higher levels of organic matter added to these soils through deciduous leaf litter[16]. These results are consistent with Wurzburger and Hendrick (2007) in which soils under *Rhododendron* had a higher accumulation of organic matter and nitrogen in the O horizon than the A horizon compared to other Southern Appalachian hardwood soils[17]. *Rhododendron* leaf litter is high in polyphenols such as tannins, which make leaf litter resistant to decomposition and mineralization[17]. Lowering the accessible humic matter and inorganic nitrogen in the soil is thought to be competitively advantageous for *Rhododendron*[17], though our results show that *C. diphylla* is successful in *Rhododendron* soils. Although *Rhododendron* soils were lower in nitrogen, associated *C. diphylla* foliar samples were higher in nitrogen than samples in open plots. Lowered soil nitrogen under *Rhododendron* may benefit glucosinolate production in *C. diphylla* as other Brassicaceae species have displayed reduced glucosinolate production when growing in high nitrogen soils[18].

The significant differences in color, nutrient content, and biomass in *C. diphylla* leaves of varying habitats raises many questions regarding forest composition and the potentially opportunistic nature of *C. diphylla*. It is unknown whether the patches of *C. diphylla* predated the arrival of *Rhododendron*, or whether *C. diphylla* was able to exploit the resource-limited niche provided by the expansion of *Rhododendron*. The increased abundance of *Rhododendron* in the southern Appalachians is relatively recent[15], and it is unknown how long *C. diphylla* colonies have occupied those areas. Further investigation of *C. diphylla* survivorship under *Rhododendron* and in the open forest may offer answers to these questions.

In conclusion, *C. diphylla* displayed differing foliar characteristics under *Rhododendron* than in the open forest. Plants growing under *Rhododendron* had lower leaf biomass, higher sulfur and nitrogen, and a higher frequency of green abaxial leaf surfaces. The higher foliar nitrogen and sulfur content under *Rhododendron* may be a sign of increased glucosinolate content, and the high foliar nutrient content suggests that *C. diphylla* finds *Rhododendron* environments suitable. Transplant studies of *C. diphylla* would offer insight to its ability to grow in varying overstory environments. Further investigation of the trends between glucosinolate content, leaf morphotype, and occurrence of *Rhododendron* may offer valuable information on how changing forest environments impact both physical and chemical characteristics of *C. diphylla* and other wintergreens.

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