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

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Abstract

Crinkleroot (*Cardamine* spp.) is an herbaceous understory plant in the Brassicaceae family. Plants in this family are known for high levels of glucosinolates, which are important anti-herbivore compounds that have garnered medicinal interest. Unlike many forest floor species, *Cardamine* grows both under rhododendron (*Rhododendron maximum* L.) thickets and on the open forest floor. The aim of this study was to investigate whether rhododendron affected soils and foliar characteristics of *Cardamine* leaves. The study was conducted in a rich cove forest in Pisgah National Forest near Barnardsville, NC. Twenty patches of *Cardamine* were located growing under rhododendron and twenty in the open forest, a 2-m radius circular plot was placed in each patch, and 25 basal leaves were harvested. The color of the abaxial (lower) surface of each leaf (purple or green) was recorded along with the associated herbaceous species, woody seedlings and saplings, and diameter at breast height (DBH) of the nearest overstory and understory species. One soil sample was taken from each plot and air-dried. 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* growing under rhododendron displayed significantly higher nitrogen and sulfur, lower biomass, and were more likely to have green abaxial surfaces compared to *Cardamine* growing in the open forest. Soils under rhododendron had significantly lower nitrogen and humic matter. Understory and overstory DBH did not vary significantly between areas. The most common herbaceous and woody species co-occurring with *Cardamine* were Christmas fern (*Polystichum acrostichoides* (Michx.) Schott) and tulip poplar (*Liriodendron tulipifera* L.) in both areas. These results indicate that rhododendron does impact soil and the foliar characteristics of this wintergreen perennial. Further studies investigating survival of *Cardamine* when transferred from an open forested area to underneath rhododendron may offer insight on how *Cardamine* is able to tolerate varying overstory environments.

Key Words: *Cardamine*, *Rhododendron*, glucosinolates

1. Introduction

Cardamine is a genus of approximately 200 species[1]. The plant of focus in this study is crinkleroot, which includes *Cardamine diphylla* (Michx.) A. Wood and *C. angustata* O.E. Schulz, two very similar species native to mature deciduous forests in North America. Both are wintergreen perennials that emerge in the fall and senesce in late spring[2, 3]. The two species can be distinguished by rhizome morphology and stem leaves, which are produced in spring. The stem leaves of *C. angustata* are narrower than the basal leaves, whereas those of *C. diphylla* are proportional to the basal leaves[1]. During the winter, both species have basal leaves that are very similar. Both species senesce in the spring as deciduous trees begin to break bud, and remain dormant throughout the summer[2, 3]. *Cardamine diphylla* and *C. angustata* are found in shady habitats in deciduous forests[4, 3], and have been described as widely scattered[4] and uncommon, but occurring in colonies when found[3].

Cardamine diphylla fills several unique ecological roles. It is considered an indicator of sugar maple (*Acer saccharum* Marshall) health in Vermont and New Hampshire[3], and several species of insects are known to feed on its leaves, including the flea beetle *Phyllotreta bipustulata* E. Smith and several caterpillars (*Pieris virginiensis* W. H. Edwards and *Pieris napi oleracea* Harris). Insect larvae that feed on *C. diphylla* have been shown to develop faster than larvae feeding on other Brassicaceae species, though the reason for this remains unknown[2]. *Cardamine angustata* is less prominent in scientific literature, so less is known about its ecological significance.

Brassicaceae species share the ability to produce glucosinolates, which are secondary metabolites composed of sulfur- and nitrogen-containing compounds that help protect plants against environmental stress and deter herbivory. Glucosinolates have been investigated in *C. diphylla,* while information on glucosinolates specific to *C. angustata* is lacking. 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]. Concentrations of glucosinolates are fairly consistent in the rhizome, excluding a 25% increase approximately two weeks before leaf emergence[2]. Glucosinolates have many potential human health benefits and medicinal properties. *Cardamine diphylla* contains a glucosinolate unique to its species[5], one that increases resistance to bacterial and fungal pathogens[6], some with cancer-fighting properties[7], and others that are promising for disease resistance, anti-inflammation, and cancer prevention[8, 3].

Cardamine leaves display multiple color morphotypes. The top (adaxial) surface can be green with a purple lower (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]. Other forest understory species with varying color morphotypes include cranefly orchid (*Tipularia discolor* (Pursh) Nutt.) and Galax (*Galax urceolata* (Poir.) Brummitt). 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 has been rejected by multiple studies[9]. Additionally, it has been shown that red- and purple-colored leaves contain less nitrogen than green leaves[9].

Forest understory plants require specific light regimes and usually depend on sunflecks or seasonal variation in light. Deciduous trees lose their leaves in fall, which allows light to penetrate to the forest floor, benefitting wintergreens such as *Cardamine*. Evergreen trees and shrubs prevent sunlight from reaching the forest floor throughout the year. rhododendron (*Rhododendron maximum* L.) is a tall shrub that prefers cove forests and north facing slopes, and covers 0.5 million ha in the southern Appalachians[10, 11]. rhododendron often takes advantage of canopy gaps in the overstory, and its abundance may be attributed in part to the decline of the American chestnut (*Castanea dentata* (Marshall) Borkh.)[12]. Rhododendron expands aggressively through branch layering and root sprouting[13], reducing light availability by 80%, sunfleck duration and frequency by 96%, and water availability by 20%[14]. Survivorship of tree seedlings such as *Quercus rubra* L., *Tsuga canadensis* (L.) Carrière, and *Prunus serotina* Ehrh. was shown to be significantly lower under *R. maximum*[13, 14]; however, few studies have examined the effects of rhododendron on herbaceous species.

The aim of this study is to investigate the effects of rhododendron on soils and foliar characteristics of *Cardamine*. Abaxial leaf color, nitrogen, and sulfur content will be compared between plants growing under rhododendron and on the open forest floor. Additionally, soil total nitrogen, pH, humic matter, and cation exchange capacity will be compared. Finally, habitat observations between areas will be compared including associated herbaceous species, woody seedlings and saplings, and diameter at breast height (DBH) of the nearest overstory and understory species.

2. Methods

The study was conducted in the vicinity of Corner Rock Creek and Staire Creek in the Coleman Boundary (Big Ivy) area of Pisgah National Forest near Barnardsville, NC (elevation 823-915 m). Samples were collected between December 2019 and January 2020. Since only basal leaves are evident in winter and distinguishing plants by their rhizome is destructive, our samples may have contained *Cardamine diphylla* and/or *C. angustata*.

Cardamine usually grows in diffuse patches, and 25 leaves were needed to produce sufficient dried material for leaf tissue analysis (1 g). We determined that a circular plot with a radius of 2 m was an appropriate size to harvest 25 leaves. Twenty plots were established in open forested areas, and 20 plots were established under rhododendron. Plot selection was accomplished by searching the study area for patches containing at least 25 leaves. When a patch was encountered, the quadrat was situated.

Leaves were harvested by cutting aboveground leaf tissue at the base of the petiole. The largest leaves within the quadrat were harvested first until 25 leaves were collected. Leaves that were unusually large or small, showed signs of herbivory, or had unusual growth forms were omitted. The color of the abaxial side of each leaf (either purple or green) was recorded. Leaves were placed in plastic bags, kept cool in the field, then dried in a forced hot air oven at

80 °C, and weighed. In each plot, associated herbaceous species, woody seedlings, and woody saplings were identified, along with the species and diameter at breast height (DBH) of the nearest overstory and understory species.

One soil sample was collected within each plot, consisting of 15, 2.5 cm x 10 cm soil cores, taken with a soil probe. Samples were homogenized and air-dried. Dry soil and leaf samples were sent to Waters Agricultural Lab in Warsaw, NC. Soils were analyzed for total nitrogen, pH, humic matter, and cation exchange capacity. Leaves were analyzed for nitrate nitrogen and sulfur. Foliar and soil variables were compared for plots under rhododendron and in the open forest using t-tests (alpha = 0.05).

3. Results

The color of the abaxial leaf surface varied significantly ($P < 0.001$) between areas. *Cardamine* in open plots had a mean of 73.2% purple leaves, while plants under rhododendron areas had an average of 21.6% purple leaves (Table 1). Leaf biomass also differed significantly between areas ($P = 0.039$); leaves in open plots were ~20% heavier than those underneath rhododendron. Both leaf nitrogen ($P < 0.001$) and leaf sulfur ($P < 0.001$) were significantly higher under rhododendron than in open areas.

Soil characteristics also varied with area. Soil nitrogen ($P = 0.036$) and humic matter ($P = 0.017$) were significantly higher in open plots. Soil pH and cation exchange capacity were similar in each area. The DBH of overstory and understory plants did not differ between areas ($P > 0.05$) (Table 2). In rhododendron plots, *Rhododendron maximum* was the closest understory plant, while in open forest plots, a variety of species were closest. *Cardamine* occurred closest to Tulip Poplar (*Liriodendron tulipifera* L.) in both areas. The most common co-occurring herbaceous species in both areas was Christmas Fern (*Polystichum acrostichoides* (Michx.) Schott).

Table 1. Foliar and soil characteristics for *Cardamine* growing under rhododendron and in the open forest.

Leaf Characteristics:	**Open Plots**	**Rhododendron Plots**	**P Value**
Purple undersides (%)	73.2	21.6	<0.001
Dry weight (g)	2.25	1.87	0.039
Nitrate Nitrogen (%)	3.52	4.3	<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

Table 2. Characteristics of overstory and understory plants for plots under rhododendron and in the open forest.

	Open Plots	**Rhododendron Plots**	**P Value**
DBH of nearest overstory tree (cm)	54.7	60.6	0.461
DBH of nearest understory tree (cm)	7.0	4.8	0.094
Nearest overstory tree	**Open Plot**	**Rhododendron Plot**	
Liriodendron tulipifera L. (%)	60.0	45.0	
Betula lenta L. (%)	10.0	15.0	
Acer saccharum Marshall (%)	10.0	-	
Fagus grandifolia Ehrh. (%)	5.0	15.0	
Quercus alba L. (%)	5.0	10.0	
Other species	10.0	15.0	
Nearest understory woody plant			
Fagus grandifolia Ehrh. (%)	25.0	-	
Carpinus caroliniana Walter. (%)	20.0	15.0	
Betula lenta L. (%)	10.0	-	
Rhododendron maximum L. (%)	5.0	80.0	
Other species	40.0	5.0	
Associated woody seedlings			
Smilax spp. (%)	11.2	16.1	
Fagus grandifolia Ehrh. (%)	10.1	3.2	
Fraxinus americana L. (%)	7.9	6.5	
Tsuga canadensis (L.) Carrière (%)	5.6	3.2	
Aesculus flava Sol. (%)	5.6	3.2	
Carpinus caroliniana Walter. (%)	4.5	3.2	
Acer rubrum L. (%)	4.5	3.2	
Euonymus americanus L. (%)	3.4	6.5	
Other species	47.2	54.9	
Associated herbaceous species			
Mitchella repens L. (%)	18.8	9.8	
Polystichum acrostichoides (Michx.) Schott (%)	17.2	26.8	
Carex spp. (%)	14.1	4.9	
Tipularia discolor (Pursh) Nutt. (%)	10.9	7.3	
Sedum spp. (%)	7.8	4.9	
Goodyera spp. (%)	3.1	4.9	
Hepatica spp. (%)	1.6	14.6	
Mitella spp. (%)	-	17.1	
Other species	26.5	26.8	

4. Discussion

The results of this study indicate that *Cardamine* foliar characteristics are influenced by the presence of rhododendron. Basal leaves growing under rhododendron had higher nitrogen and sulfur, lower biomass, and were more likely to have green abaxial surfaces. Basal leaves in the open forest had lower nitrogen and sulfur, higher biomass, and were more likely to have purple abaxial surfaces. The lower mass of leaves growing under rhododendron may be due to environmental stressors such as reduced sunlight and water[14].

Color morphotypes may also be associated with differences in sunlight. Green morphotypes (predominantly under rhododendron) contained more nitrogen than purple morphotypes (predominantly in the open forest). These results are consistent with the findings of Kytridis et al. (2008)[9]. Higher accumulation of anthocyanins in leaves growing in open plots may be due to higher sun exposure, as anthocyanins are thought to prevent photoinhibition. However, this has been rejected by others, so the reason for this difference requires more investigation[9].

The differences in foliar nitrogen and sulfur content between the two areas suggest differences in glucosinolate content, as nitrogen and sulfur are constituents of glucosinolates. Higher levels of nitrogen and sulfur in *Cardamine* growing under rhododendron suggest that these leaves may contain more glucosinolates than leaves growing in the open forest. It has been shown that the green morphotype of *Cardamine* has a higher glucosinolate content than the purple morphotype[9]. The reason for this requires further investigation. In both areas, *Cardamine* co-occurred with similar species: Tulip Poplar in the overstory, and Christmas Fern in the herbaceous layer. All species co-occurring with *Cardamine* are characteristic of mesic deciduous forests.

The results of this study raise the question of how long *Cardamine* has existed in areas now occupied by rhododendron, or whether *Cardamine* has been able to exploit the resource-limited niche provided by the expansion of rhododendron. The increase in abundance of rhododendron in the southern Appalachians is relatively recent[15], and it is unknown how long *Cardamine* colonies have occupied those areas. Further study could be done to evaluate *Cardamine* survivorship under rhododendron and in the open forest.

Significant differences in soil properties between habitat types also occurred. Soils in the open forest contained more nitrogen and more humic matter than soils under rhododendron. Higher soil nitrogen in open areas may be due to the higher levels of organic matter added to soils through deciduous leaf litter. Additionally, canopy leaf litter plays a role in supplying the humus layer[16], so lower humus in soils under rhododendron may be due to the lack of leaf litter produced by evergreen species.

In conclusion, *Cardamine* has been shown to display differing foliar characteristics under rhododendron and in the open forest. Plants growing under rhododendron had lower leaf biomass, higher sulfur and nitrogen content, and a higher frequency of green abaxial leaf surfaces. The higher nitrogen and sulfur content under rhododendron may be a sign of increased glucosinolate content. Further investigation of the trends between glucosinolate content, leaf morphotype, and nitrogen and sulfur content may offer valuable information on the ways the forest environment impacts both physical and chemical characteristics of *Cardamine* species. This is particularly valuable when investigating the medicinal properties that glucosinolates in *Cardamine* may contain.

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