Drew University College of Liberal Arts

Surviving or thriving: carbon trade-offs between growth, defense, and floral reproduction in *Rubus allegheniensis* and *R. phoenicolasius* in Northcentral New Jersey, USA

A Thesis in Environmental Science

By:

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Abstract

Little is known about the reproductive limits of semi-long-lived perennials, especially how their other two primary life history functions (growth and defense) may constrain reproductive investment. Understanding these constraints can help researchers produce cultivating best practices for perennials like *Rubus* that produce nutritious fruits. Here, we sought to elucidate the responses of carbon-demanding traits related to the aforementioned life history functions in *Rubus allegheniensis* (blackberry) and *R. phoenicolasius* (Japanese wineberry) to variable herbivory rates. We also discerned if trade-offs are present between these traits in these species. We observed traits related to physical defense (prickle intensity, leaf toughness, and stem basal diameter), growth (cane length and average leaf size), and floral reproduction (flower number, number of ripe fruits, fruit set, and fruit dry:wet mass ratio) as well as environmental characteristics related to carbon availability (rates of herbivory and light availability). We then used multiple regression models to characterize the relationships between these variables for both species. We found several potential induced defenses in these species, most in wineberry. Seven of the 29 trade-off models we ran returned significant results indicative of potential trade-offs between life history functions. Our results should spur further research into the defensive strategies utilized by these species because inducible defenses may result in trade-offs reducing yields in cultivation and also be a mechanism that increases the invasive potential of *Rubus*. In addition, our research suggests ways wineberry may be invading the study region.

Acknowledgements

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Introduction

Overview

We conducted observational research on two *Rubus* species, Allegheny blackberry (*R. allegheniensis*) and Japanese wineberry (*R. phoenicolasius*), during the summer of 2019, followed by sample and data analysis in the lab during the following academic year. Our research had two goals. First, we sought to characterize how herbivory affects investment toward defense, growth, and reproduction in semi-long-lived perennials. Second, we sought to discern if such plants exhibit trade-offs between these life history traits when carbon (an essential and finite resource required for all life history functions) is limited, as would be predicted by life history theory. If such trade-offs occur, we sought to additionally identify which trade-offs these were and to speculate on why some trade-offs occur and not others. This study is the beginning of a new line of research at Drew University led by Dr. Alex Bajcz concerning the reproductive ecology of the *Rubus* genus in northern New Jersey. As part of this research, we collected data on carbon investment towards growth, reproduction, and defense for these species. We also collected environmental data including leaf-level herbivory and light availability. This research addresses the following questions:

1. Are the defenses implemented by these species entirely constitutive, or could they be at least partially induced by leaf-level herbivory?
2. Do defensive and reproductive functions trade off within these species?
3. Do defensive and growth functions trade off within these species?
4. Do growth and reproductive functions trade off within these species?

5. Do any trade-offs exist *within* any of these life history functions (e.g., do reproductive traits ever trade off with one another)?
6. Do these two species experience similar trade-offs?

Below, I provide a brief review of our focal genus, of herbivory, of life history functions, and of trade-offs, before expanding upon the details of our experiment and our hypotheses and predictions.

Focal genus and its selection

We chose to study life history functions and trade-offs using species within the genus *Rubus* (a clade in the rose family) in part because they invest in several traits that clearly link to particular life history functions. Virtually all *Rubus* produce biennial canes—upright stems possessing vasculature, leaves, and woody stems and branches (Graham & Brennan 2018). The overall length of the cane and average size of leaves are traits indicative of carbon investment to growth because they influence carbon and space acquisition. Canes grow vegetatively and establish physical defenses in their first year of growth and then reproduce sexually in their second and final year. Stems, petioles, and even leaves often feature extensive physical defenses including prickles, hairs, and sometimes glandular trichomes (Björkman & Anderson 1990, Graham & Brennan 2018)—the size and density of which are traits indicative of carbon investment to defense. The second-year, reproductive canes produce fruits and seeds via open pollination. *Rubus* produce aggregates of drupelets, the whole of which is treated as the “fruit” relevant to dispersal and other ecological processes. The number of flowers and fruits produced, as well as the proportional dry mass of the fruits, are traits indicative of carbon investment towards reproduction. *Rubus* have perennial root and rhizome systems that typically live twelve or more years, and they are known to store extra nutrients within the rootstock for use in future seasons (Graham & Brennan 2018).

Additionally, given that *Rubus* contains hundreds of species all thought to produce edible fruits (a major component of a healthy human diet), it is particularly valuable to better understand this genus’ reproductive behaviors. *Rubus*, as a genus, is under-researched given burgeoning interest in berry crops for their potential health benefits. For example, Beattie et al. (2005) note that many berries have compounds with both antioxidant and anticancer properties. These compounds may also bolster the immune system, neurological functioning, and urinary tract health (Beattie et al. 2005). Nile and Park (2014) also found that red raspberry (*R. idaeus*) and black raspberry (*R. occidentalis*) contain high concentrations of vitamins B and C, zinc, iron, and folate, which are key nutrients that, when deficient, lead to significant health issues (Tontisirin et al. 2002, Müller & Krawinkel 2005, Tulchinsky 2010). All of these potential benefits provide added incentive to better understand the factors that stress these plants and place limits on their reproductive capacity, especially with respect to fruit composition and output.

Herbivory

Herbivory is the damage sustained by plants when fed on by animals. This damage can affect roots, stems, leaves, or reproductive structures. The three dominant categories of terrestrial herbivores are arthropods (e.g., insects), mollusks (e.g., snails and slugs), and mammals (e.g., deer). For our study, we examined leaf-level herbivory, presumably by insects. The negative and far-reaching impacts of herbivory on plant growth, survival, reproduction, and competitive ability are well-documented (Herms & Mattson 1992).

Species that are expected to experience high rates of herbivory, like *Rubus*, are valuable to study because the effects of herbivory may be more pronounced in them. While researching the percent of leaf herbivory across vascular plants, Turcotte et al. (2014) found that, on average, rosids experience more herbivory (8.2%) than other clades (overall average across all vascular plants = 5.3%). The authors also found that, within rosids, woody species experience 64% more herbivory on average than their non-woody counterparts. This indicates *Rubus* species (woody rosids) have the potential to experience higher-than-average herbivory rates. This raises questions about how these species have adapted to cope with higher herbivory rates and how this increased need for defense is balanced against their other life history functions. *Rubus* could therefore be a model for investigating how trade-offs between life history functions operate because they are among the perennial species in which they would be expected to be most pronounced.

Life and life history functions

Being alive is taxing. To remain alive and succeed, an organism must: maintain homeostasis, process energy and matter, respond to stimuli, reproduce, and more. We assume species across the tree of life have evolved to survive in their typical environments by satisfying all of the aforementioned requirements effectively and efficiently. In brief, they have evolved to *succeed*. But what does it mean, biologically, to succeed, as both an individual and as a species?

Success is often conceptualized in terms of Darwinian fitness—being able to survive long enough to pass on one's genes and to do so at a higher rate than intraspecific rivals (Pough 1989, Demetrius & Ziehe 2007, Lailvaux & Husak 2014). To succeed in these tasks often requires balancing several so-called life history functions, each associated with a suite of traits that contribute to their respective function or functions. These functions are growth, maintenance (and/or defense), reproduction, and storage (although this last one is sometimes considered optional or, alternatively, as part of maintenance).

The life history function of growth concerns the building of structures needed to acquire resources and outcompete rivals for resource access (e.g., generating leaves and roots on an ongoing basis), as well as to achieve the functional size and shape of the reproductive adult, but it also enables the three other life history functions. Defense is a separate function from growth; it often requires structures beyond those required for physical support or resource foraging. However, the two functions also overlap in that many defensive features are integrated with the physiology of growth-related structures (e.g. such as impregnating stems with wood). Storage can be considered a sub-function of growth, defense, or both because stored nutrients and water can enable an individual to survive through adverse conditions or forage during periods of resource scarcity. Reproduction is also reliant on growth because all reproductive structures need branches (vegetative tissues) to bear them.

Investment in defense is essential to survive to reproductive maturity in an ecological context where antagonistic relationships (e.g., herbivory and competition) and/or detrimental abiotic processes (e.g., extreme weather events) are common, and plants possess three general defensive strategies: little to no defense, a focus on inducible defenses, and a focus on constitutive defenses. Plants producing few or no defenses tolerate or re-grow quickly in response to herbivory or damage (Strauss & Agrawal 1999). Other plants may produce defensive compounds and structures but only in response to herbivory or other damage (inducible defense; Karban & Baldwin 1997). Still other plants produce defensive compounds and structures more or less continuously, even without a stimulus (constitutive defense; Karban & Baldwin 1997). Ito and Sakai (2009) used a model to assess the environmental conditions that select for each of these strategies. They found that if the biomass lost to herbivory was small and the probability of herbivory was low, a lack of defense would be optimal because plants would likely be able to tolerate or replace the small amounts of tissue generally lost. If damage caused by herbivory or abiotic forces was more intense and the probability of loss was high, then constitutive defenses would instead be ideal because being defended all the time would be prudent. However, if the probability of herbivory or abiotic stress was low but the amount of biomass lost during such adverse events was moderate, induced defenses could instead be optimal. Of course, plants can implement a combination of strategies as well (e.g., physical defenses may be constitutive but chemical defenses may be inducible).

Rubus are known to have several constitutive defenses: they will naturally produce structures like prickles, hairs, and trichomes even absent any herbivory or physical harm. This is expected because, as previously mentioned, *Rubus* are likely to experience higher-than-average herbivory rates. Gibson *et al.* (1993) found during simulated browsing experiments that prickles were not induced in *R. vestitus*, supporting this claim. However, Bazely *et al.* (1991) found an apparent induced response in *R. fruticosus*; prickle density, measured as prickles per internode (the length of stem in between branch points), significantly increased during simulated browsing experiments (control = 15 prickles/internode, leaf removal = 18 prickles/internode; $p < 0.05$). These studies suggest *Rubus* may employ a hybrid approach between constitutive and inducible physical defenses. Invasive *Rubus*, such as *R. phoenicolasius,* may experience less herbivory than native congeners, which led us to question whether this species in particular could be predisposed to some inducibility in their physical defenses.

Lastly, reproduction is the ultimate life history function because it is the process whereby Darwinian fitness is most directly achieved through the transfer of genes to the next generation. Plant reproductive strategies range widely, from entirely sexual to entirely asexual. *Rubus* can reproduce sexually via flowers and pollen. However, this genus can also reproduce asexually via vegetative (or clonal) reproduction, self-fertilization, and apomixis (Graham & Brennan 2018). In this study, we restrict our focus to only reproduction conducted via flowers, fruits, and seeds, which is likely a mix of sexual and asexual in these species. Given that this life history function is so vital to the success of both an individual and to a species in evolutionary terms, it is unsurprising this function is energy-intensive. For sexual reproduction in angiosperms to proceed, many accessory structures (e.g., peduncles and pedicels) need to be grown; compounds (nectars and floral volatiles) need to be produced; and the principle reproductive structures such as pollen, ova, seeds, and the fruit must be supported and protected (Obeso 2002). The significant energetic costs associated with the floral reproduction led us to wonder how *Rubus* plants manage these costs while still satisfying other life history functions.

Trade-offs

For this research, we focused on interrelationships between growth, defense, and floral reproduction. Each function is vital to the success of an individual and to the longer-term success of a species. As such, species are assumed to have evolved life history strategies to optimize relative investment to these functions (Obeso 2002). Because these functions are resource-demanding and energy-intensive, it is common to observe trade-offs between—and even within—them (Obeso 2002). A trade-off is defined as a negative relationship between two traits or functions—as one increases in extent or value, the other can, or must out of necessity, decrease (Mole 1994). Trade-offs can be observed at three scales: evolutionary, genetic, and physiological (Mole 1994). Evolutionary trade-offs are seen in overall life history patterns. For example, the difference between K- and r-selected species reflects a trade-off, with K-selected species prioritizing survivorship (growth plus defense) and r-selected species prioritizing reproductive capacity. Genetic trade-offs occur when the relative costs of expressing any trait A over any trait B change and thus incentivize or stimulate a switch in gene expression (e.g., allelochemical A is more nitrogen-rich than allelochemical B and thus more energetically costly to produce, so expression of B is favored in nitrogen-poor conditions). There is, however, as yet no easy way to assess the costs and benefits associated with the differential expression of any two non-lethal genes (Mole 1994). Most studies of trade-offs, including ours, instead have focused on physiological or phenotypic trade-offs. These occur when a negative relationship between physiological traits (themselves functions of both life history strategies and genotypes) can be identified. The cause of a physiological trade-off can be a genetic trade-off, an evolutionary trade-off, or both, but physiological trade-offs can also be the result of the interaction between genes and the environment (e.g., phenotypic plasticity and/or epigenetics), or physiological limitations (Mole 1994). It is known that, at some level, physiological trade-offs are inevitable because, in every system, resources are ultimately limited and so every organism must "decide" to which life history functions and their associated traits resources should be allocated. Evolution, we assume, adapts organisms to make these "decisions" wisely more often than not based on the environments they have historically occupied.

Most trade-off studies in plants have been performed on annuals because these species must successfully grow and reproduce in just one growing season and are thus easier to work with. As such, any tension between these two life history functions should be more pronounced, making observation of trade-offs easier. Perennials potentially have multiple seasons over which to reproduce, so they are under decreased pressure to ensure that any one season is a reproductive success. As such, these plants may be able to lessen or even avoid trade-offs between reproduction and other life history functions by spreading their reproductive investment out over time. Still, such trade-offs likely exist because they are ultimately unavoidable, especially when resources are scarce; they are perhaps just harder to observe and fewer people have attempted to look for them.

We chose to examine phenotypic trade-offs in *Rubus* because they are semi-long lived perennials about whose trade-offs little is currently known. Granted, there have been some studies to date on trade-offs within this genus. Gibson *et al.* (1993) found that cane growth rate was maintained at the expense of prickle production in *R. vestitus* at high levels of simulated browsing, indicating a potential trade-off between resource allocation to growth and defense for this species. Björkman and Anderson (1990) found trade-offs within defensive traits in *R. bogotensis*; they identified a significant negative relationship between leaf toughness and trichome density on the petiole ($p < 0.03$) for individuals with trichomes and between prickle length and leaf toughness ($p < 0.05$) for individuals without trichomes. When looking at reproductive effort (defined in their study as carbon invested to reproduction that must be diverted from vegetative growth), McDowell and Turner (2002) found that reproduction had increased physiological (growth-related) costs in the native *R. ursinus* but relatively no growth effect on growth for the invasive *R. discolor* in the Pacific Northwestern United States. These studies provide evidence that trade-offs do exist within this genus. Each study focuses on only one or two trait pairings in one or two *Rubus* species, however, preventing a fuller characterization of these species' (and thus the genus') reproductive ecology and behavior.

Experimental Questions and Hypotheses

We sought to document what impacts herbivory will have on defense, growth, and reproduction in two *Rubus* species due to their predisposition to high herbivory rates. We assumed both growth and reproduction would be negatively impacted by herbivory because it would both decrease resource availability and increase the need for defensive investment (Herms & Mattson 1992). We know that *Rubus* have many constitutive physical defenses. However, we predicted that:

1. Some physical defensive traits, namely prickles, might be partially induced by herbivory.

Additionally, we sought to document if and how defense, growth, and reproduction trade off in *Rubus* species when carbon is in tension, which should be frequent given that it is a frequently limiting resource (Bloom et al. 1985, Coley et al. 1985, Reekie & Bazzaz 1987). We chose to observe carbon-demanding traits related to our life history functions of interest because carbon's availability can be reliably estimated using field data. We made the following predictions:

2. Increased investment of carbon to defensive traits will result in a decrease in carbon allocation to reproduction.
3. Growth-related and defensive traits will experience carbon-allocation trade-offs, with the declining traits being those that develop second.

4. Carbon allocation to growth-related traits that occurs concurrently with reproduction (e.g., average leaf size) will coincide with decreased carbon allocation to reproductive traits.
5. Trade-offs may occur within life history functions, primarily between defensive traits as found by Björkman and Anderson (1990).
6. The native blackberry and invasive wineberry will both demonstrate trade-offs, but these will differ between the species (McDowell & Turner 2002).

Methods

Study system and species

For this research, we established 52 study plots across ten properties, most of which were county parks, in and around Madison, New Jersey, USA (Figure 1). 49 of the study plots were considered in this study because the other three plots only contained a third species for which we gathered insufficient data. The study region is northern temperate forest, with a majority of plots located close to hiking trails. Field data were collected from May 27th to August 7th, 2019.

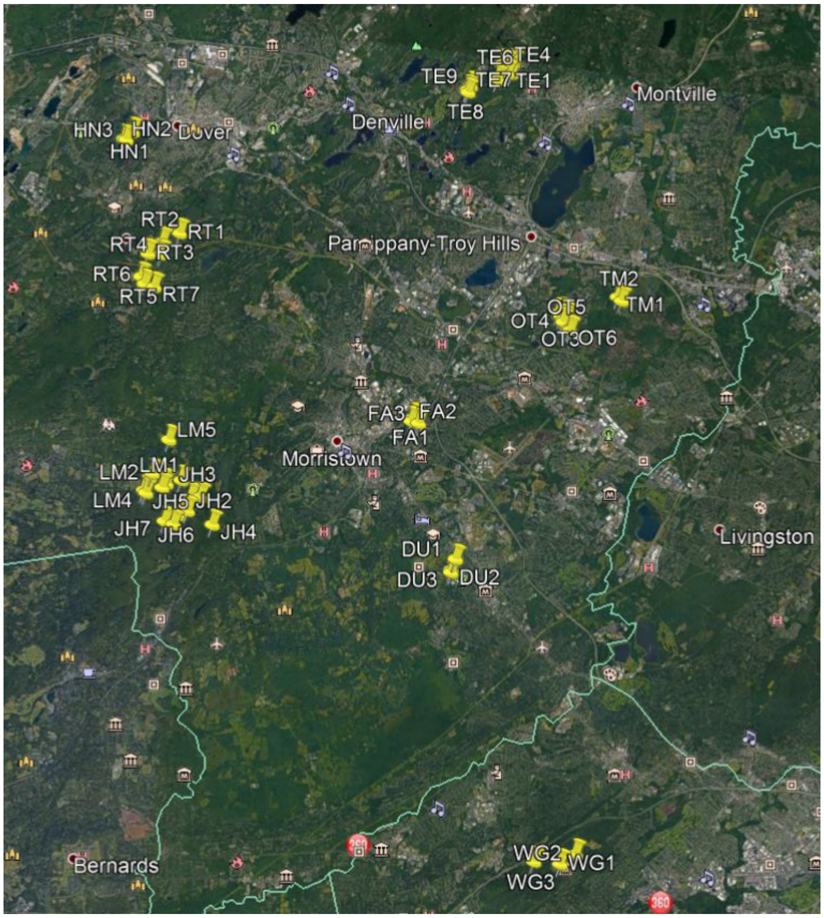


Figure 1. Satellite image with pins marking the locations of our 52 study plots in north-central New Jersey. The ten properties were Drew University (DU), Watchung Reservation (WG), Frelinghuysen Arboretum (FA), Old Troy County Park (OT), Troy Meadows Wetlands (TM), Tourne County Park (TE), Hedden County Park (HN), Randolph Trails at James Andrews Memorial County Park (RT), Lewis Morris Park (LM), and Jockey Hollow at Morristown National Historical Park (JH).

Our study species, blackberry (*Rubus allegheniensis)* and wineberry (*R. phoenicolasius),* are woody shrubs with similar life histories and physiologies, even though recent research suggests they are only distantly related within the genus (Carter *et al.* 2019). Blackberry are characterized by ribbed stems with a red-purple color and palmately compound leaves with three to five relatively long and narrow leaflets (Figure

2). They also have stout prickles on their stems, pedicels, and leaves. Wineberry are characterized by smooth stems covered in small red hairs and palmately compound leaves with three relatively wide and rounded leaflets, with the center one often distant from the other two (Figure 3). Wineberry have prickles, hairs, and glandular trichomes present on their stems, pedicels, and leaves.

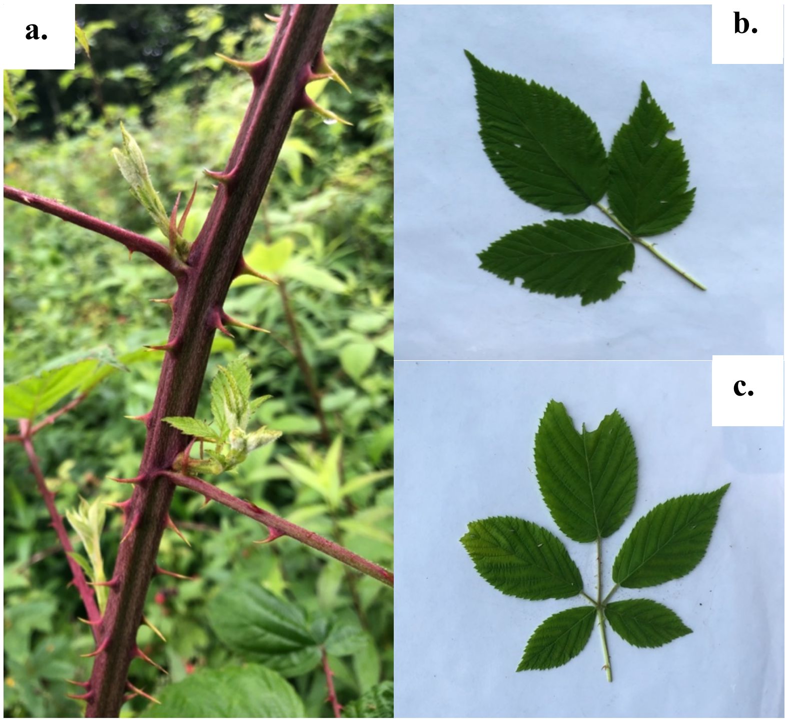


Figure 2. A picture of a blackberry stem (a) and two exemplary blackberry leaves (b and c). The stem shows the characteristic red-purple color and prickles that are present across the entire length. The two leaf morphs, a three-leaflet (b) and a five-leaflet (c) morph, can occur on the same cane.

Figure 3. A picture of an exemplary wineberry leaf and stem (bottom of image). The stem has the characteristic red color and short prickles and hairs present along its entire length. This leaf shape (a three-leaflet, palmately compound leaf with a larger central leaflet that is further away from the others) is typical for this species.

We predicted that individuals that invested more carbon to physical defenses would have decreased reproductive success and growth, and that individuals that invested more in growth would also experience a decrease in reproductive success due to an inability to invest limited carbon heavily into both functions concurrently. We also predicted that individuals receiving increased levels of herbivory would exhibit decreased investment towards both reproduction and growth but increased defensive investment (if possible). We also assumed that lower carbon availability in a plant's environment would exaggerate any trade-offs between defensive, reproductive, and growth-related traits, accounting for this possibility in our analyses.

To evaluate these predictions, we collected data on defensive, reproductive, and growth-related traits for *Rubus* floricanes (second-year, reproductive canes). We characterized investment into physical defenses by measuring prickle length and density, leaf toughness, and stem basal diameter. We believe stem basal diameter is a defensive character because a thicker stem may deter passage through a stand by large mammals as well as prevent stem breakage during contact or extreme weather events, especially for long canes. We characterized carbon investment to reproduction and subsequent reproductive success by quantifying flower number, fruit number, fruit set (the ratio of ripe fruits produced to flowers produced), and the fruit dry:wet mass ratio (also called fruit proportional dry mass). We characterized investment towards growth by measuring the length and average leaf area of each floricane. We examined multiple characters of defense, reproduction, and growth to assess for any potential trade-offs *within* these life history functions; focusing on only one trait for each function when such within-function trade-offs exist can lead to the mischaracterization of resource investment to the function as well as of the number and severity of trade-offs present (Mole 1994, Koricheva 2002, Barton & Koricheva 2010).

We also approximated carbon availability at each plot by quantifying maximum light availability and percent canopy cover. Plants acquire carbon via photosynthesis, and the rate of photosynthesis is proportional to the amount of light available. Limited access to carbon may increase tension between growth, defense, and reproduction given that all three functions are at least intermittently carbon-demanding (Koricheva 2002, Obeso 2002, Sletvold and Ågren 2015). We also approximated herbivory rates at each plot because greater herbivory would be predicted to stimulate defensive investment while simultaneously decreasing carbon acquisition, increasing the likelihood of trade-offs between defense and other functions that require carbon (Bazely et al. 1991).

Plot selection and setup

We required each study plot to have at least forty floricanes from at least one of the following *Rubus* species: wineberry*,* blackberry*,* and black raspberry (*R. occidentalis*). We labeled the species with the highest number of floricanes the dominant species at each plot. If one or both other species were present and had at least 20 floricanes, we labelled them as a subdominant species.

Once plots were selected, we prepared each for study by choosing twelve canes of the dominant species randomly (by tossing an object blindly and choosing the closest cane) or systematically (selecting every nth cane encountered on a random path through the stand). If subdominant species were present, we selected six canes of each using the same methods. We marked chosen canes for further study with color-coded pipe cleaners, which are easy to spot but cause little disruption to the plant when tied loosely.

Characterizing the environment

Two of our main objectives were to characterize herbivory levels and the availability of carbon at each plot. We took two measurements of light availability for each plot. We obtained an estimate of maximum light availability at each plot by measuring photosynthetically active radiation (PAR) at each plot center using a LI-COR LI-250A light meter. We took these measurements between June 11th and July 30th, 2019 during mid-day (10:30AM to 3:30PM) whenever possible and on up to three different days for each plot. Cloud cover conditions were also noted. Then, we selected the maximum value of our multiple readings for each plot. We also calculated the percent canopy cover for each plot using the mobile app CanopyApp (University of New Hampshire, version 1.0.3) between June 11th and June 22nd, 2019. Briefly, this involved taking a picture of the canopy above each plot center on a clear, sunny day. The app's software isolates and then calculates the percentage of pixels in the picture that were of structures intercepting light that would otherwise reach the forest floor (Figure 4). We then converted the percent canopy cover data to percent light transmittance (100% - percent canopy cover = percent light transmittance). We assumed the relative light transmittance at each plot in the early summer would be comparable to that of the rest of the growing season (i.e., a plot receiving more light during the early summer would also receive more light during the rest of the growing season).

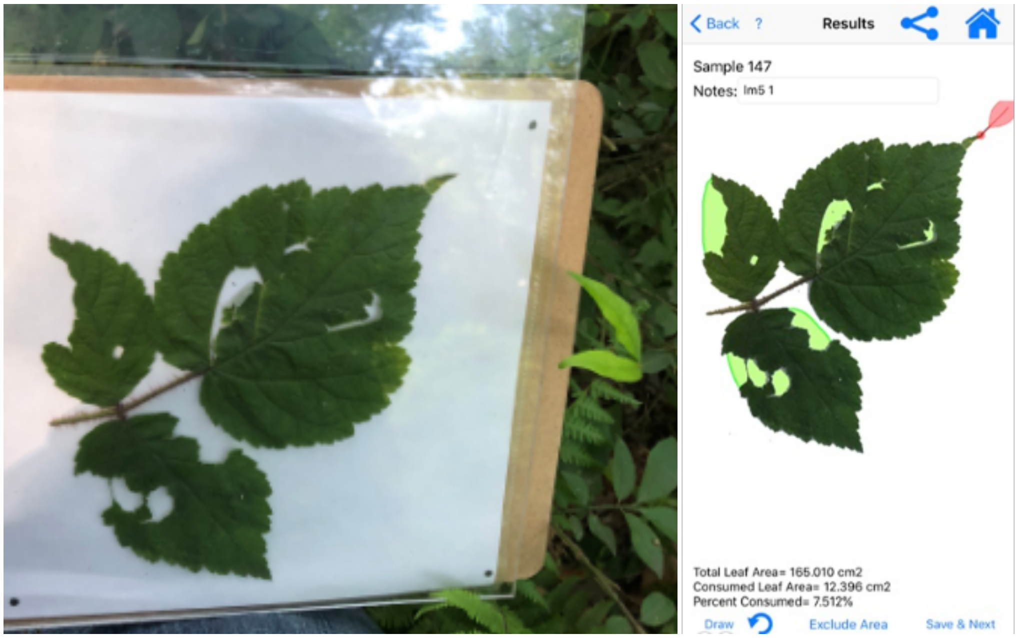
Measuring light in a meaningful way can often be difficult. We took both light measurements and canopy transmittance data to ensure we had consistently and meaningfully characterized the light conditions at each plot and, by extension, the carbon availability there. After we collected our data, we ran a correlation of these two proxies for carbon availability to assess whether these measurements were providing similar carbon availability information and found a moderately strong, positive relationship (r =

0.50). Because the correlation between these measurements was moderately strong, we decided that use of one measurement (percent light transmittance) would be sufficient for our analyses.

Figure 4. An image of the canopy taken above plot center (left) and the same image post-analysis in the mobile app CanopyApp (right). The app's software identifies and calculates the percentage of pixels in the picture that contain structures intercepting light, shown in pink.

To approximate rates of leaf-level herbivory by insects at each plot, we calculated the average percent leaf area loss (Lowman 1984, Williams & Sahli 2016, Sand-Jensen *et al*. 1994) via the mobile app Leaf Area (Adam Campbell, version 1.1.0). Briefly, four large black dots were drawn on a piece of white paper to form a 10cm x 10cm square. The leaves measured were those growing furthest from the ground on unmarked canes growing immediately next to marked canes. We harvested and measured six leaves (one per cane) of the dominant species and three leaves of any subdominant species at each plot. We placed each harvested leaf within the square and used a piece of plexiglass to hold it in place as well as to flatten it. A photo was taken, and the app's software was used to increase the contrast in the photo until only the leaf and any holes were visible

(Figure 5). We then used a drawing tool in the app to fill in missing leaf edges to get a more accurate reading. The app then calculated the total leaf area, the total area missing, and the percent leaf loss.



Sample 147
Notes: Im5 1

Total Leaf Area= 165.010 cm2
Consumed Leaf Area= 12.396 cm2
Percent Consumed= 7.512%

Figure 5. An image of a *R. phoenicolasius* leaf (left) and the same image post-analysis in the mobile app Leaf Area (right). The leaf is placed between plexiglass and a white sheet of paper with a 10x10cm square marked by four black dots on the corners. All non-white pixels are counted as leaf material; thus, the petiole is also included in the calculation of total leaf area. The lime-green areas (right) on the leaf are areas the app recognized as lost leaf area. The green lines along the edges were drawn with the app's drawing tool to fill in the missing leaf edge to improve estimation of leaf area and percent leaf loss.

Physical defenses

To characterize carbon allocation to physical defense, we quantified prickle length, prickle density, leaf toughness, and stem basal diameter. At the end of the field season, we cut marked canes between the root collar and the fourth visible branch node and brought each segment back to the lab. We also harvested three leaves from each cane (one healthy leaf each from the base, middle, and tip of the cane), if available. Both stem segments and leaves were placed in a -20°C freezer until later analysis.

We measured prickle density by first identifying the three growth nodes closest to the bottom of each cane segment. The number of prickles plus prickle scars between the first and second visible nodes were counted, and the internodal length was measured in centimeters using a ruler. These steps were repeated between the second and third node. We averaged these data to yield the mean prickle density (prickles per cm) for each cane. To obtain prickle lengths, five equally dispersed intact prickles (or less depending on the amount of prickles available) were selected from each cane segment and measured in millimeters using a ruler. These lengths were then averaged to produce an average prickle length for each cane. We then calculated prickle intensity (measured in mm*prickles/cm) by multiplying the prickle length and prickle density values from each plot; this metric was used as a measure of total carbon investment to prickles in our analyses.

We measured leaf toughness using a method similar to those of Lowman (1984) and Feeny (1970). We constructed an apparatus out of wood blocks and metal dowels (Figure 6). We pressed the center leaflet from a randomly selected leaf from each cane between the two halves of the apparatus, taking care not to place the midvein of the leaflet over the central hole. We then placed the metal plunger inside the top of the central hole, resting it on the surface of the leaflet. We placed an empty beaker on top of the plunger and slowly filled it with sand until the dowel attached to the plunger broke through the leaf. Three beakers (50mL, 100L, 250mL) were used to ensure that both very weak and very tough leaves could be measured because the larger beakers could alone break weaker leaves. The combined mass of the beaker, sand, and tape roll (used to increase stability of the beaker on the plunger) used was then recorded as a measure of leaf toughness. The mass of the plunger was assumed to be constant throughout the experiment and was not included. The values were thus measured in grams of sand + apparatuses needed to puncture the leaf, which will hereon be referred to as g of material.

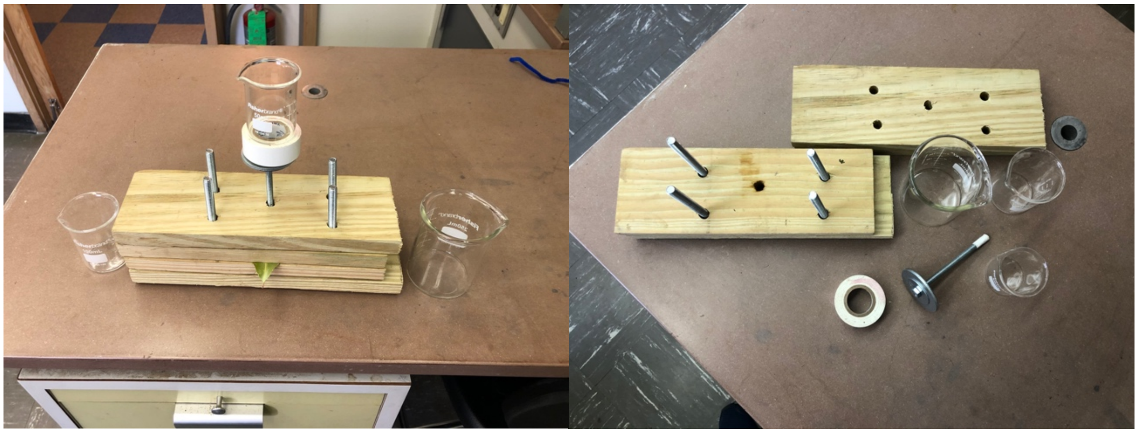


Figure 6. The leaf penetrometer constructed for this study in use (left) and disassembled (right). The penetrometer consists of four pieces of 4”x1” wooden planks with five drilled holes in a quincunx pattern. The four outer holes are used to hold the planks firmly together with metal bolts, while the center hole and a 3/8” metal dowel are used for puncturing the leaves. A roll of tape was used to help the beakers sit flat atop the plunger. Three different beakers (50mL, 100mL, 250mL) were used to account for very weak and very tough leaves. Leaf toughness was measured in grams of sand + apparatuses needed to puncture the leaf (or total g of material)

Stem basal diameter was also considered a measure of carbon allocation to defense. We used digital calipers (Precision Measuring Digital Caliper 150mm) to measure the diameter of the base of each cane segment in centimeters at every plot immediately following cane harvest.

Reproduction

We measured carbon allocation to reproduction by collecting the following data for each cane and plot: flower number, ripe fruit number, fruit set, and fruit dry:wet mass ratio. We counted all flowers on all marked canes in early June 2019. We then collected ripe fruit during the fruiting period in three rounds for all species, each round taking place one week apart from late June to early August 2019. In each round, we collected in plastic sandwich bags all visibly ripe fruits from our marked canes. We also separately collected all ripe fruits from the unmarked canes in the plot. A fruit was considered ripe based on subjective coloration; we considered blackberry fruits ripe if a majority of drupelets were black as opposed to red and wineberry fruits ripe if a majority of drupelets were a deep red as opposed to light orange. We brought all fruits to the lab and stored them in a -20°C freezer for later analysis.

Reproductive allocation metrics were determined as follows. We estimated fruit set by dividing the number of fruits gathered across all rounds for each cane by the number of flowers counted on each cane. To estimate the fruit dry:wet mass ratio (or the fruit proportional dry mass), we took a 4 g sub-sample of ripe fruits collected from each plot and species, weighed these, and then placed them in a 70°C drying oven for 48 hours. We then reweighed the dried fruits and divided the dry mass by the fresh mass. Higher values for this measure indicate a relatively greater investment to the carbon-containing portion of fruits (seeds, pulp, rind, sugars, etc.) irrespective of fruit size and number (Stapanian 1982).

Growth

We considered cane length and average leaf size as measures of carbon allocation to growth for our study species. At the end of the field season, we cut marked floricanes and measured the full length of each in centimeters using a measuring tape. If the cane was tip-rooted, we cut the tip as close to the ground as possible. If the cane was branched, we measured only the longest branch, so our values somewhat underestimate total cane length. We calculated average leaf size by taking the total leaf area measurements provided by the Leaf Area app as previously described and averaging them for each cane.

Statistical Analyses

We stored our data in Microsoft Excel and Google Sheets and then explored and analyzed them using RStudio (version 1.1.463). We used multiple linear regression models to assess the effects of herbivory on carbon investment toward growth-related, reproductive, and defensive traits. All data were averaged to plot for each species. The basic model structure was as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_3$$

where Y is a measure of carbon allocation (to defense, growth, or reproduction), X_1 is the average percent leaf loss (as a measure of the rate of leaf-level herbivory and the primary term of interest), X_2 is canopy light transmittance (as a proxy for carbon availability), and X_3 is the species (coded blackberry = 0 and wineberry = 1). The β values are regression coefficients estimated by the model. We included an interaction term between percent leaf loss and species (X_1X_3) because we assumed that the two species might respond differently to herbivory, in part because blackberry is native and wineberry is invasive to the study area. A significant β_4 value would indicate how much the β_1 value (the slope between herbivory and Y) would change for the second species (wineberry) relative to the first (blackberry). Canopy light transmittance (X_3) was included because we assumed plants receiving more light would have higher Y values because of greater overall carbon availability to invest. In other words, with greater carbon availability, plants may have enough carbon to offset any negative impacts herbivory may otherwise have had on growth, defense, or reproduction. This term corrects for any such effect.

The Y variables used in this set of models were flower number, number of ripe fruits, fruit set, fruit dry:wet mass ratio, leaf toughness, prickle intensity, and average leaf size. Percent leaf loss was the only X_1 variable used.

We also used multiple linear regression models to search for negative relationships between growth-related, defensive, and reproductive traits as potential evidence of physiological trade-offs. All data were averaged to plot for each species. The basic structure for these models was as follows:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_1X_4$$

where Y is a measure of carbon allocation (to defense, growth, or reproduction), X_1 is a measure of carbon allocation that occurs prior to or concomitant with that for measure Y (the primary term of interest), X_2 is cane length (as a measure of early investment towards growth), X_3 is canopy light transmittance (as a measure of carbon availability), and X_4 is species (coded as above). The β values are regression coefficients estimated by the model. The interaction term between species and the measure of carbon allocation

(X_1X_4) was included for the same reason as above. A significant β_5 would indicate how much the β_1 value would be expected to change for the second species (wineberry) relative to the first (blackberry). Canopy light transmittance was included in these models for the same reason as above. Similarly, cane lengths were included in these models because we assumed plants with longer canes will have higher Y values because of greater capacity for carbon uptake due to increased numbers of leaves as well as more physical locations at which to construct new structures. In other words, with greater cane lengths, plants may be able to obscure or reduce any trade-offs through their increased ability to forage for carbon via photosynthesis. In these models, evidence of a trade-off would be a negative β_1 regression coefficient, possibly in conjunction with a significant β_5 if the two species display (or don’t display) very different trade-offs (Figure 7).

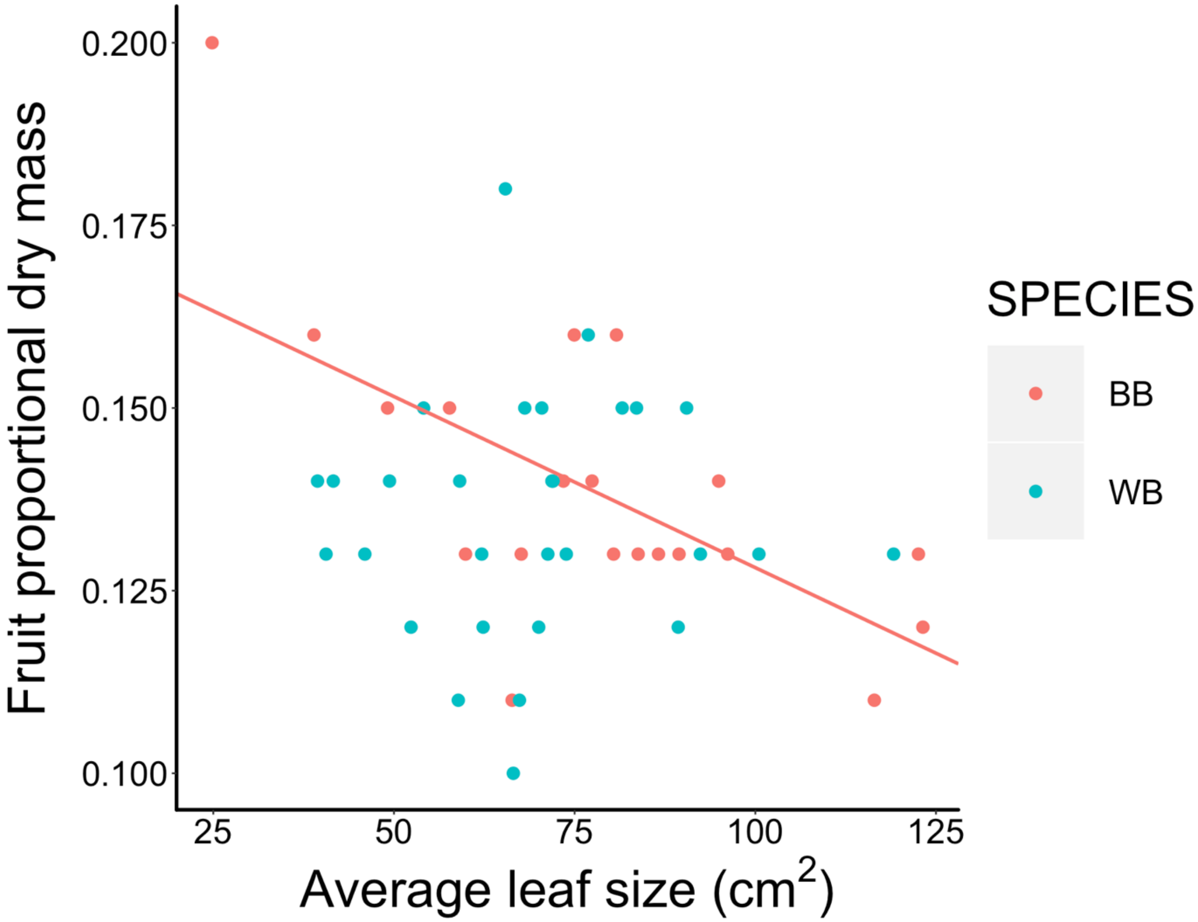


Figure 7. Linear relationship between two exemplary *Rubus* life history functions: reproduction (represented by fruit proportional dry mass; y-axis) and growth (represented by average leaf size; x-axis). The relationship shown is indicative of a physiological trade-off for blackberry (BB; red) only; as the value of one function increases, the other significantly declines. The solid red line is the best-fit line for blackberry from a multiple linear regression that accounted for differing light environments and cane lengths (see Methods). The regression coefficient for the slope of the line was statistically significant ($p = 0.015$). The multiple linear regression model did not return a statistically significant regression coefficient for wineberry (WB; blue).

The traits we considered as Y variables for these models were flower number, number of ripe fruits, fruit set, fruit dry:wet mass ratio, leaf toughness, prickle intensity, and average leaf size. The traits considered as X_1 variables were average leaf size, cane length, stem basal diameter, leaf toughness, and prickle intensity. Several Y/X_1 combinations were not considered because they were either the same trait (e.g., average leaf size versus itself), would have been a repeat test (e.g., both average leaf size and leaf toughness were possible X_1 and Y variables), or did not make sense temporally (e.g., the production of second-year leaves occurs one whole growing season after carbon investment to (most) prickles, thus average leaf size would not be expected to affect prickle intensity). When cane length was used as the X_1 variable, it was not also included as X_2, dropping the latter term out of the model.

We initially ran all models with the interaction term included. However, when the models returned an insignificant beta value for this term (i.e., there was no significant difference in the trade-off relationships between the two species), the interaction term was removed and the models re-ran. We also included weights in all models; double the weight was given to plots containing twelve canes of a given species than to plots containing just six canes. For all models, we checked to ensure the residuals were approximately normally distributed. When this assumption was violated (often as a result of Poisson-distributed count data for X_1 and/or Y), the model was re-run as a Poisson regression instead. Results were considered significant at a 95% confidence level ($p < 0.05$) and marginally significant at a 90-95% confidence level ($0.05 < p < 0.10$).

Results

Variability between species

The plasticity of investment toward reproductive, defensive, and growth-related traits differed between *R. allegheniensis* (Table 1) and *R. phoenicolasius* (Table 2). When comparing inter-quartile ranges (IQR), blackberry displayed a higher range of values compared to wineberry for leaf toughness (55.6g material vs. 37.9g material)*,* flower number (64.0 flowers vs. 24.7 flowers), stem basal diameter (2.44mm vs. 1.70mm), prickle intensity (3.7mm*prickles/cm vs. 2.67mm*prickles/cm), and average leaf size (27.23cm^2 vs. 20.92cm^2). Wineberry displayed a higher range of values than blackberry for fruit number (6.2 fruits vs. 3.2 fruits) and fruit set (0.19 vs. 0.08). Both species had similar levels of variability for fruit dry:wet mass ratio (0.02) and cane length (50cm).

The range of environments inhabited by plots also differed between the species. Canopy light transmittance was more variable across blackberry plots and less variable across wineberry plots (IQR = 35.09% transmittance vs. 18.28%), as was percent leaf loss (2.491% leaf area loss vs. 1.797%). On average, blackberry (Table 1) received more canopy light transmittance (45.72% vs. 32.44%) and more percent leaf loss (3.078% vs. 1.86%) than wineberry (Table 2).

It appears blackberry and wineberry may differ in their average expression of certain reproductive, defensive, and growth-related traits as well. Blackberry (Table 1) appears to have greater average flower numbers (48.3 flowers vs. 43.5 flowers), stem basal diameters (6.10mm vs. 5.65mm), leaf toughnesses (203.1g material vs. 184.7g material), and average leaf sizes (76.34cm^2 vs. 67.54cm^2) than wineberry (Table 2). On average, it appears wineberry has a greater ripe fruit production (6.6 fruits vs. 3.5 fruits), fruit set (0.17 vs. 0.062), prickle intensity (6.0mm*prickles/cm vs. 4.0mm*prickles/cm), and cane length (140cm vs. 130cm). These species have similar average fruit dry:wet mass ratios (0.14).

Table 1. Reproductive, defensive, and growth-related trait metadata for *Rubus allegheniensis*, as well as average canopy light transmittance and percent leaf loss metadata from study plots of this species in which it was at least subdominant (N = 22).

Trait	**Min**	**1st Q**	**Median**	**Mean**	**3rd Q**	**Max**
Flower number	0.00	6.71	23.6	48.3	70.7	151
Fruit number	0.0	0.083	0.92	3.5	3.3	26
Fruit set	0.0	0.0068	0.025	0.062	0.089	0.35
Fruit dry:wet mass ratio	0.11	0.13	0.13	0.14	0.15	0.20
Stem basal diameter (mm)	2.57	4.83	5.96	6.10	7.27	10.3
Prickle length (mm)	0.97	1.6	2.0	2.2	2.9	3.7
Prickle density (prickles per cm of stem)	0.64	1.1	1.8	1.8	2.4	3.0
*Prickle intensity (mm*prickles/cm)*	0.88	2.2	3.4	4.0	5.9	9.2
Leaf toughness (g material)	116.1	174.8	198.6	203.1	230.4	307.0
Cane length (cm)	49	100	130	130	150	230
Average leaf size (cm^2)	24.83	61.51	76.99	76.34	88.74	123.2
Canopy light transmittance (% by area)	12.77	23.16	37.55	45.72	58.25	100.0
Leaf loss (% by area)	0.0943	1.06	2.06	3.08	3.55	11.3

Table 2. Reproductive, defensive, and growth-related trait metadata for *Rubus phoenicolasius*, as well as average canopy light transmittance and percent leaf loss metadata from study plots of this species in which it was at least subdominant (N = 36).

Trait	Min	1st Q	Median	Mean	3rd Q	Max
Flower number	10.3	25.6	35.9	43.5	50.3	118
Fruit number	0.50	2.0	3.6	6.6	8.2	40.
Fruit set	0.016	0.065	0.12	0.17	0.25	0.50
Fruit dry:wet mass ratio	0.10	0.13	0.13	0.14	0.15	0.18
Stem basal diameter (mm)	3.28	4.87	5.49	5.65	6.57	8.12
Prickle length (mm)	1.7	2.4	2.7	2.7	2.9	3.9
Prickle density (prickles per cm of stem)	0.82	1.9	2.1	2.2	2.5	4.0
*Prickle intensity (mm*prickles/cm)*	2.4	4.3	5.7	6.0	6.9	15
Leaf toughness (g material)	145.8	160.6	172.3	184.7	198.5	315.1
Cane length (cm)	84	110	140	140	160	220
Average leaf size ($cm^{2)}$	39.42	53.68	66.29	67.54	74.60	119.1
Canopy light transmittance (% by area)	12.11	22.21	27.62	32.44	40.49	95.05
Leaf loss (% by area)	$1.00\text{x}10^{-3}$	0.142	0.829	1.86	1.94	13.9

Effects of herbivory

The data showed two instances where reproductive traits could be expected to significantly decrease and one instance where a defensive trait could be expected to significantly increase in response to increasing levels of leaf-level herbivory for both wineberry and blackberry, based on our regression model results. Ripe fruit number is expected to change in blackberry by a factor of 0.85 (or decrease by 15%) for every 1% increase in percent leaf loss ($\beta_{\text{MAIN EFFECT}}$ = -0.16; Table 3). In wineberry, ripe fruit number is instead predicted to change by a factor of 0.95 (or decrease by 5%; $\beta_{\text{INTERACTION TERM}} = 0.11$). Ripe fruit number in wineberry is also expected to be 2.36 times greater than in blackberry under identical circumstances ($\beta_{\text{SPECIES}} = 0.86$; Table 3). Fruit set is predicted to significantly decrease in both species by 0.011 for every 1% increase in leaf loss ($\beta_{\text{MAIN EFFECT}} = -0.011$; Table 3). Fruit set for wineberry is expected to be greater than for blackberry by 0.10 under identical circumstances ($\beta_{\text{SPECIES}} = 0.10$; Table 3). To put this into perspective, fruit set ranged from 0 to 0.35 in blackberry (Table 1) and from 0.016 to 0.50 in wineberry (Table 2), while leaf loss ranged from approximately 0.10% to 11.3% in blackberry (Table 1) and from 0.001% to 13.9% in wineberry (Table 2). This means that fruit set in plots with the most herbivory would be expected to be 0.12 (~34% of the maximum value) lower in blackberry and 0.15 (30% of the maximum value) lower in wineberry on average than in plots with the least amount of herbivory.

Prickle intensity is predicted to increase by 0.149mm*prickles/cm for every 1% increase in percent leaf loss for both species, albeit this result was only marginally significant ($\beta_{\text{MAIN EFFECT}} = 0.149$, $p = 0.091$; Table 3). Prickle intensity in wineberry is predicted to be significantly greater than in blackberry by 2.66mm*prickles/cm under identical circumstances ($\beta_{\text{SPECIES}} = 2.66$; Table 3). Prickle intensity ranged from 0.88 to 9.2mm*prickles/cm in blackberry (Table 1) and from 2.4 to 15mm*prickles/cm in wineberry (Table 2), meaning that the maximum expected increases are approximately 1.64mm*prickles/cm in blackberry (~18% of the maximum value) and

1.94mm*prickles/cm in wineberry (~13% of the maximum value) across the range of herbivory we observed here.

Our data indicated three traits in blackberry only—one reproductive, one growth-related, and one defensive— that are predicted to decrease in response to increasing rates of leaf-level herbivory, based on our models. For every 1% increase in leaf loss, flower number is expected to change by a factor of 0.948 (or decrease by 5%; $\beta_{\text{MAIN EFFECT}}$ = -0.0536; Table 3), average leaf size is predicted to change by a factor of 0.969 (or decrease by 3%; $\beta_{\text{MAIN EFFECT}}$ = -0.0313; Table 3), and leaf toughness is expected to be lower by a factor of 0.978 (or decrease by 2%; $\beta_{\text{MAIN EFFECT}}$ = -0.0227; Table 3; Figure 8).

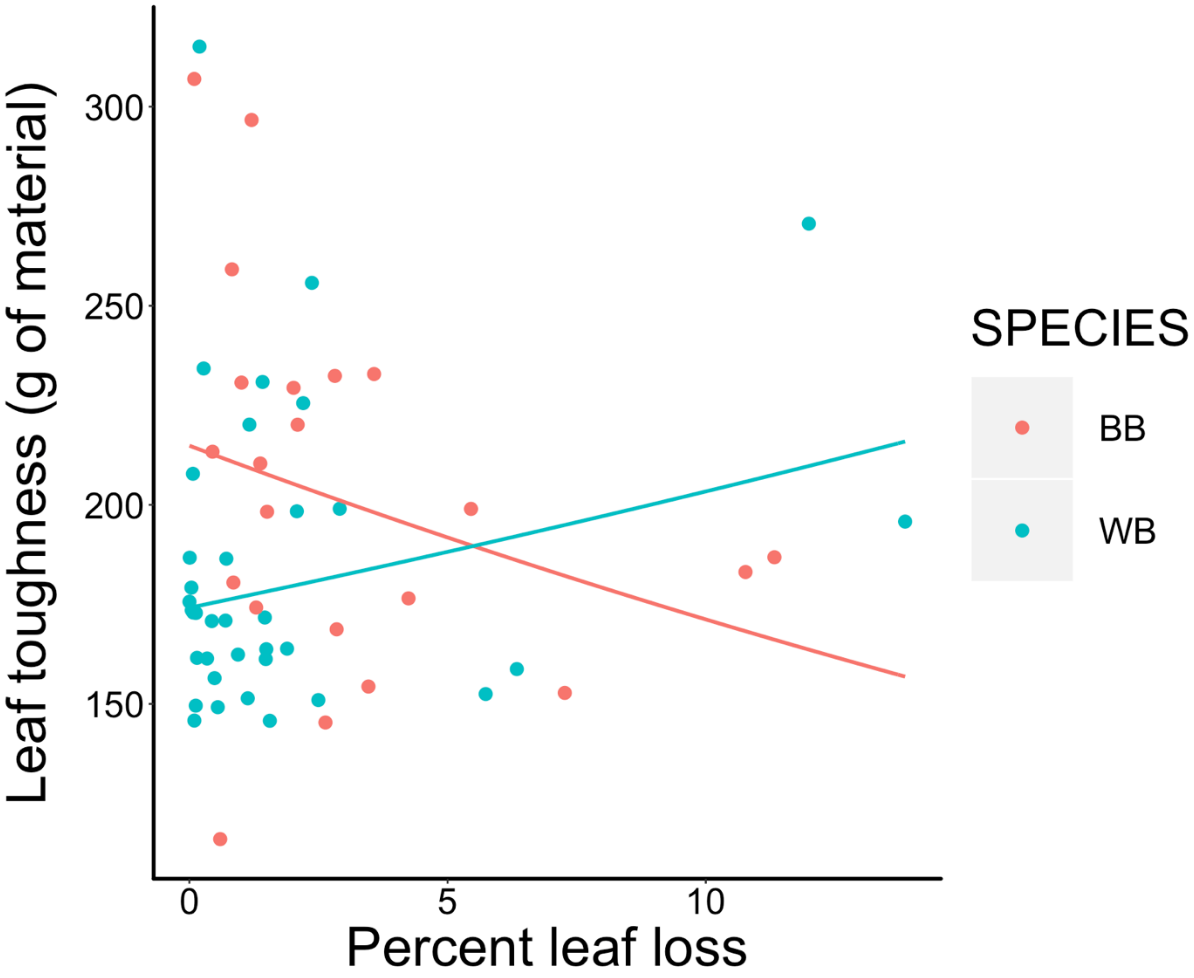


Figure 8. Non-linear relationship between a defensive trait (leaf toughness; y-axis) and leaf-level herbivory (percent leaf loss; x-axis) in blackberry (BB; red) and wineberry (WB; blue). This relationship is negative for blackberry and positive for wineberry, as indicated by their respective best-fit lines. The best fit lines are from a multiple Poisson regression that accounted for differing light environments. The regression coefficients for the lines were significant (both *p* values < 0.001).

These same three traits also showed significant relationships with percent leaf loss in wineberry, according to our models, but these relationships were instead *positive*. For every 1% increase in leaf loss, flower number is expected to change by a factor of 1.031 (or increase by 3%; $\beta_{\text{INTERACTION TERM}} = 0.0839$; Table 3), average leaf size by a factor of

1.035 (or increase by 4%; $\beta_{\text{INTERACTION TERM}} = 0.0662$; Table 3), and leaf toughness by a factor of 1.016 (or increasing by 2%; $\beta_{\text{INTERACTION TERM}} = 0.0382$; Table 3; Figure 8).

The last model we ran to assess leaf-level herbivory's effects on proportional fruit dry mass did not return a significant *p* value for either species ($p = 0.700$). All *p* values for these models can be found in Table 3.

Table 3. Multiple linear regression model results used to find evidence for the effects of leaf-level herbivory on reproductive, defensive, and growth-related traits in *R. allegheniensis* (blackberry) and *R. phoenicolasius* (wineberry), using combined data from plots containing one or both species. The main effect reported for each model indicates the degree to which the dependent variable changes in response to percent leaf loss. A species term was included to assess for any differences between species in the average value of the dependent variable. If the "Species – Wineberry" term was statistically significant, the beta value indicates how different the average value of the Y variable would be for wineberry versus for blackberry by adjusting the y-intercept of the resulting best-fit line. Models were run under the assumption that the two species could exhibit different responses to herbivory. If the interaction term was significant, the beta value indicates how the slope of the relationship between the dependent variable and leaf loss would differ for wineberry versus for blackberry. When the interaction was not significant, the model was simplified by removing the interaction term. Probability values were deemed significant (bold) or marginally significant (italics) at $p < 0.05$ and $0.05 < p < 0.1$, respectively.

Dependent variable	**Independent variable**[1]		**Regression coefficient (β)**	**Test statistic (t)**	***p* value**
Flower number					
	Percent leaf loss*	Main effect	-0.0536	-5.932	**0.000**
		Species – Wineberry	-0.0433	-0.981	0.327
		Interaction term	0.0839	8.207	**0.000**
Fruit number					
	Percent leaf loss*	Main effect	-0.16	-3.549	**0.000**
		Species – Wineberry	0.86	5.459	**0.000**
		Interaction term	0.11	2.405	**0.016**
Fruit set					
	Percent leaf loss	Main effect	-0.011	-2.275	**0.027**
		Species – Wineberry	0.10	3.058	**0.003**
Fruit dry:wet mass ratio					
	Percent leaf loss	Main effect	$-2.9\text{x}10^{-4}$	-0.387	0.700
		Species – Wineberry	$-1.8\text{x}10^{-3}$	-0.337	0.737
Average leaf size					
	Percent leaf loss*	Main effect	-0.0313	-4.931	**0.000**
		Species – Wineberry	-0.329	-9.946	**0.000**
		Interaction term	0.0662	8.865	**0.000**
Leaf toughness					
	Percent leaf loss*	Main effect	-0.0227	-5.940	**0.000**
		Species – Wineberry	-0.211	-10.401	**0.000**
		Interaction term	0.0382	8.150	**0.000**
Prickle intensity					

Percent leaf loss	Main effect	0.149	1.723	*0.091*
	Species – Wineberry	2.66	4.433	**0.000**

[1] an * indicates a Poisson regression rather than a linear regression (see Methods).

Trade-offs

We detected evidence of three potential trade-offs occurring in both *Rubus* species, one between reproduction and growth, one between reproduction and defense, and one between growth and defense. According to our regression model results, fruit number is predicted to change by a factor of 0.980 (or decrease by 2%) for every 1cm^2 increase in average leaf size for both species ($\beta_{\text{MAIN EFFECT}}$ = -0.020; Table 4; Figure 9). However, the number of ripe fruits is expected to be 2.16 times larger in wineberry than in blackberry under identical circumstances (β_{SPECIES} = 0.77; Table 4). For every 1mm*prickle/cm increase in prickle intensity, ripe fruit number is expected to significantly change by a factor of 0.905 (or decrease by 9%) in blackberry ($\beta_{\text{MAIN EFFECT}}$ = -0.10; Table 4) and by 0.967 (or decrease by 3%) in wineberry ($\beta_{\text{INTERACTION TERM}}$ = 0.066; Table 4). Because the interaction term was only marginally significant (p = 0.075), we re-ran the model with the species codings reversed and revealed a marginally significant main-effect p value (p = 0.053) for wineberry ($\beta_{\text{MAIN EFFECT WB}}$ = -0.038), indicating that the trade-off is less steep for this species. Ripe fruit number values were predicted to be 2.12 times greater for wineberry than for blackberry under identical circumstances (β_{SPECIES} = 0.75). The last potential trade-off in both species indicated by our results was between average leaf size and prickle intensity (Figure 10). Average leaf size is predicted to change by a factor of 0.946 (or decrease by 5%) in blackberry ($\beta_{\text{MAIN EFFECT}}$ = -0.055; Table 4) and by a factor of 0.975 (or decrease by 2%) in wineberry for every 1mm*prickle/cm increase in prickle intensity ($\beta_{\text{INTERACTION TERM}}$ = 0.030

respectively; Table 4). Average leaf size is predicted to be 0.779 times smaller for wineberry than for blackberry under identical circumstances ($\beta_{SPECIES} = -0.25$; Table 4).

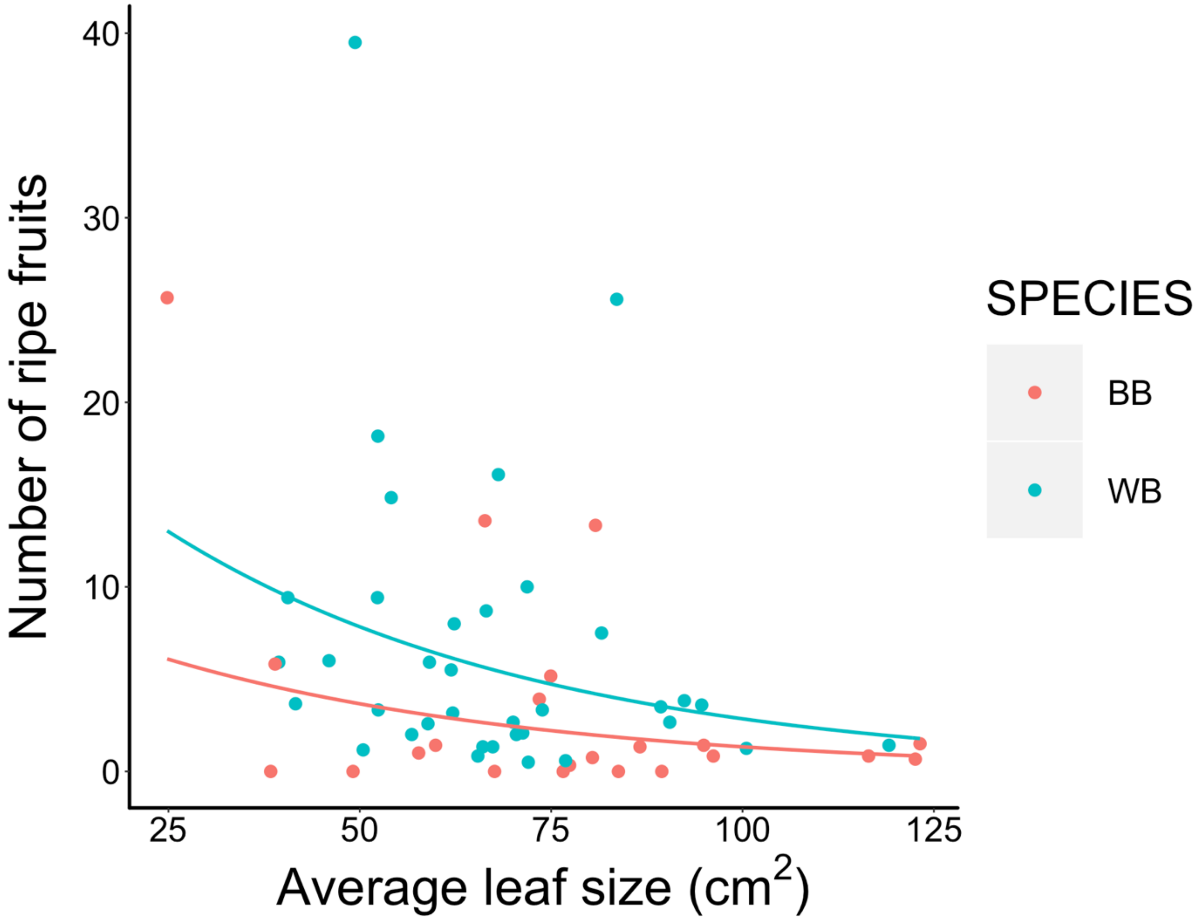


Figure 9. Non-linear relationship between a reproductive trait (number of ripe fruits produced; y-axis) and a growth trait (average leaf size; x-axis) in blackberry (BB; red) and wineberry (WB; blue). This relationship is negative for both blackberry and wineberry, as shown by their respective best-fit lines, and is consistent with a physiological trade-off for both species. The species experience similar curvature in the relationship ($\beta_{MAIN\ EFECT} = -0.020$; Table 4), but wineberry produces, on average, more ripe fruits than blackberry ($\beta_{SPECIES} = 0.77$; Table 4). The best fit lines are from a multiple Poisson regression that accounted for differing light environments and cane lengths. The regression coefficients for the lines and also the difference between the species were significant (all p values < 0.001).

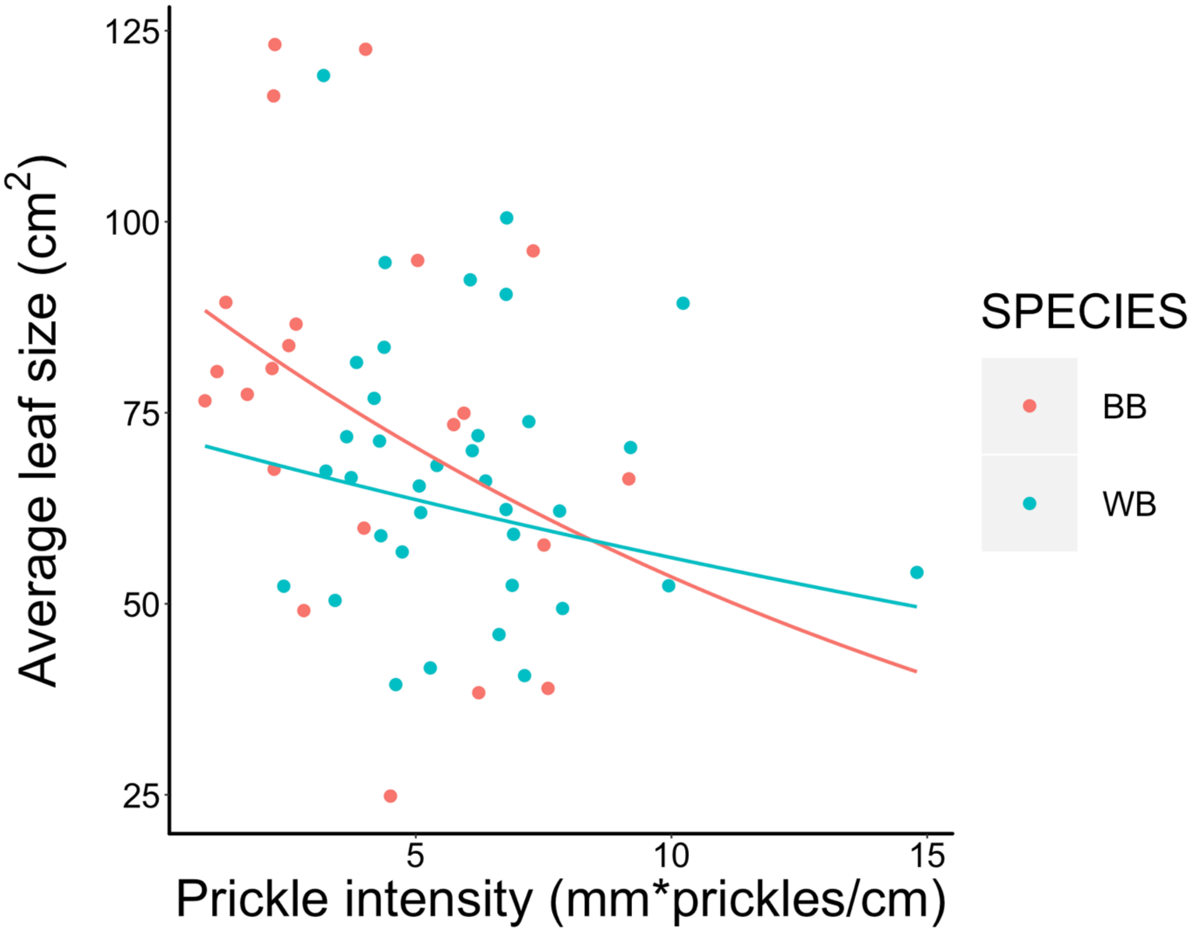


Figure 10. Non-linear relationship between a growth trait (average leaf size; y-axis) and a defensive trait (prickle intensity; x-axis) in blackberry (BB; red) and wineberry (BB; blue). This relationship is negative for both blackberry and wineberry, as shown by their respective best-fit lines, and is consistent with a physiological trade-off for both species. The species experience different curvatures in the relationship ($\beta_{\text{INTERACTION TERM}} = 0.030$; Table 4), and wineberry produces, on average, smaller leaves than blackberry ($\beta_{\text{SPECIES}} = -0.25$; Table 4). The best fit lines are from a multiple Poisson regression that accounted for differing light environments and cane lengths. The regression coefficients for the lines and also the difference between the species were significant (all *p* values < 0.01).

Our regression models also indicated evidence of three potential trade-offs that only occur within blackberry. Flower number is expected to decrease by a factor of 0.997 (or by 0.3%) for each unit increase in leaf toughness ($\beta_{\text{MAIN EFFECT}} = -0.00254$; Table 4;

Figure 11). To put this into perspective, the minimum value of leaf toughness in blackberry was 116.1g of material needed to puncture a leaf and the maximum was 307g (Table 1). This means, for the plots we observed, that the plot with the toughest leaves would be expected to produce 57.3% fewer flowers than the plot with the least-tough leaves. The next potential trade-off identified by our models was between flower number and prickle intensity. An average individual would be expected to have 4.8 fewer flowers for every 1mm*prickles/cm increase in prickle intensity, although this result was only marginally significant ($\beta_{\text{MAIN EFFECT}} = -4.8$; $p = 0.079$; Table 4). With a range of 0.88 to 9.2mm*prickles/cm for prickle intensity for blackberry (Table 1), the plot with the highest prickle intensity would be expected to produce 40 fewer flowers per stem (~26% of the maximum value) than the plot with the lowest prickle intensity. The last potential trade-off we found evidence for in blackberry was between fruit proportional dry mass and average leaf size. The fruit proportional dry mass is expected to decrease by 0.00047 for every 1cm^2 increase in average leaf size ($\beta_{\text{MAIN EFFECT}} = -4.7\text{x}10^{-4}$; Table 4). The fruit proportional dry mass ranged from 0.11 to 0.20 (11% to 20% dry content by mass) and average leaf size values ranged from 24.83cm^2 to 123.2cm^2 in blackberry (Table 1). This means, for our plots, that the plot with the largest leaves would be expected to produce fruit with a proportional dry mass that is 0.046 units (23% of the maximum value) lower than the plot with the smallest leaves.

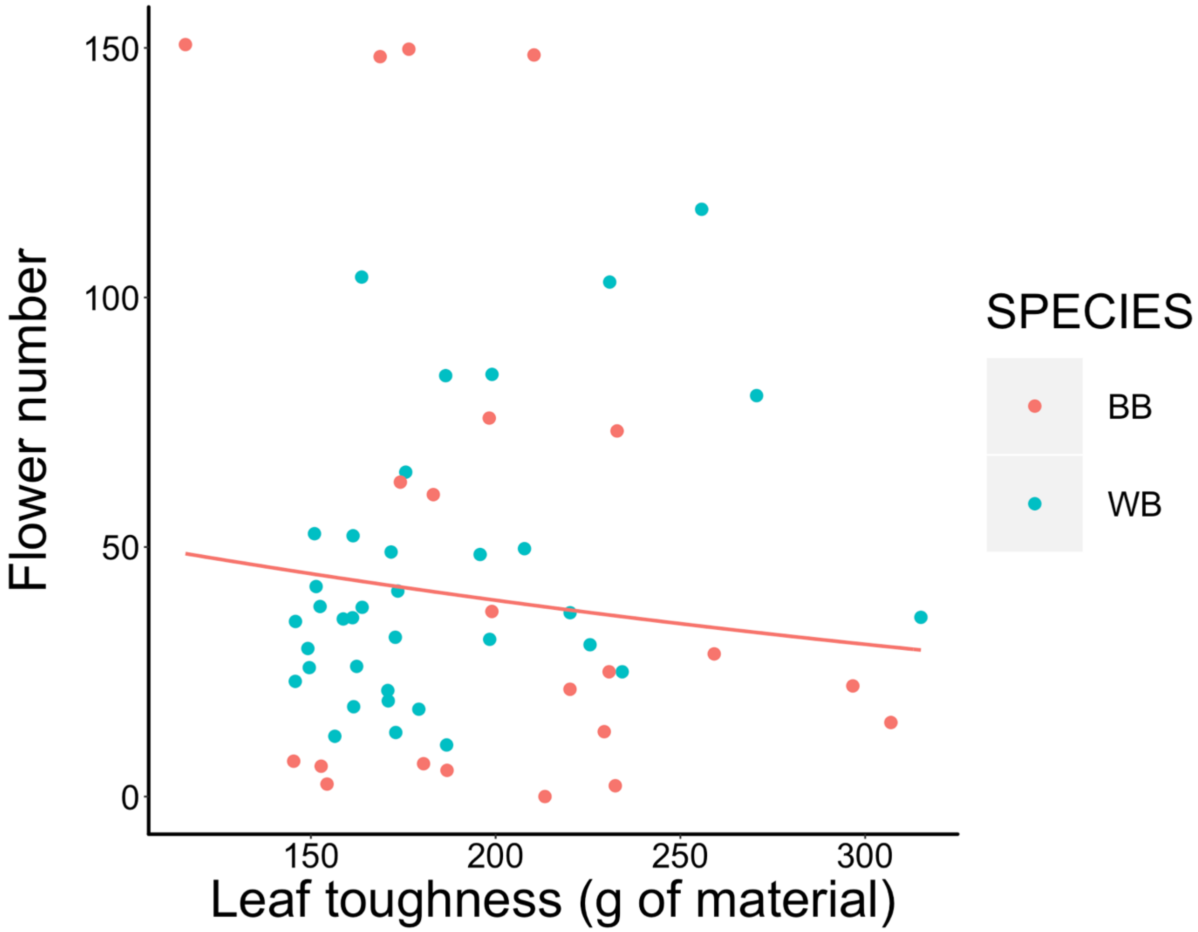


Figure 11. Non-linear relationship between a reproductive trait (flower number; y-axis) and a defensive trait (leaf toughness; x-axis) in blackberry (BB; red). This relationship is negative for this species, as indicated by the solid red best-fit line, and is consistent with a physiological trade-off ($\beta_{\text{MAIN EFFECT}} = -2.54\text{x}10^{-3}$; Table 4). The best fit line is from a multiple Poisson regression that accounted for differing light environments and cane lengths. The regression coefficients for the line was significant ($p < 0.001$). The model did not return a statistically significant regression coefficient for wineberry (WB; blue).

Only one potential trade-off was identified in wineberry only. Fruit set would be predicted to decrease by 0.071 for every 1mm increase in stem basal diameter according to the results of our regression model ($\beta_{\text{INTERACTION TERM}} = -0.039$; Table 4; Figure 12). Despite the interaction term being only marginally significant ($p = 0.077$; Table 4), rerunning the model with the species codings reversed revealed a significant main-effect *p* value ($p = 0.007$) for wineberry ($\beta_{\text{MAIN EFFECT WB}} = -0.071$). Stem basal diameter for this species ranged from 3.28mm to 8.12mm (Table 2), meaning that, for our plots, the plot with the thickest stems would be expected to have a fruit set that is 0.34 units (~69% of the maximum value) lower than the plot with the thinnest stems. In fact, plots with average basal diameters a little over 8mm were predicted to produce no fruits at all (fruit sets < 0; Figure 12).

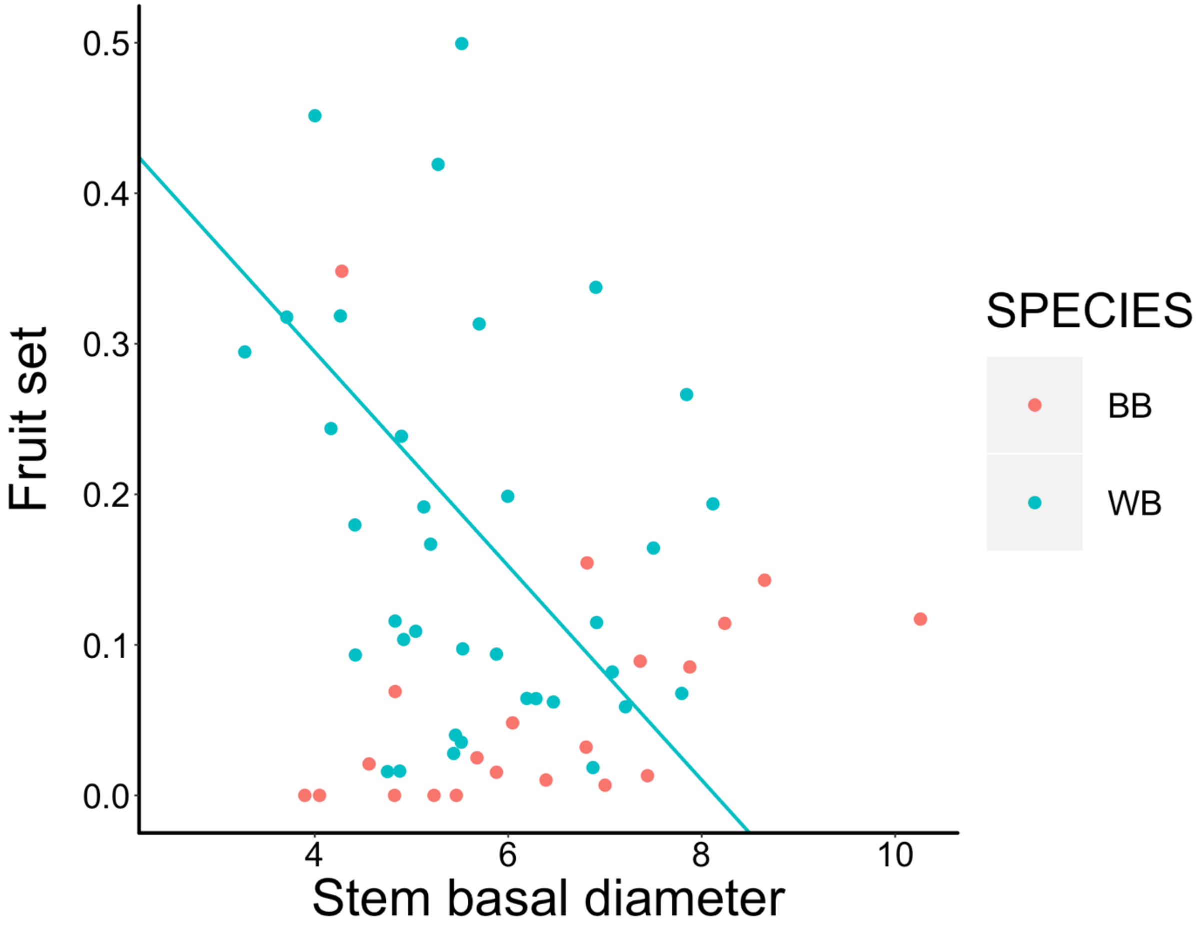


Figure 12. Negative linear relationship between a reproductive trait (fruit set; y-axis) and a defensive trait (stem basal diameter; x-axis) in wineberry (WB; blue). The relationship is negative, as shown by the solid blue best-fit line, and is consistent with a physiological trade-off for this species ($\beta_{\text{INTERACTION TERM}} = -0.039$; Table 4) The best-fit line is from a

multiple linear regression that accounted for differing light environments and cane lengths. The regression coefficient for the slope of the line was significant ($p < 0.01$). The model did not return a statistically significant regression coefficient for blackberry (BB; red).

Ten of our regression models returned significant results indicating a positive relationship between variables for both species. Three additional models indicated significant positive relationships between carbon-related variables for wineberry and significant negative relationships for blackberry. Lastly, two models indicated a significant positive relationship between variables for one species (one for wineberry and one for blackberry) and no relationship for the other (all p values > 0.1). These positive or non-significant relationships are not indicative of trade-offs; as such, they will not be discussed here but can be found in Table 4. All other regression models (N = 10) we ran to look for evidence of trade-offs did not return a significant p value for the independent variable of interest for either species (all p values all > 0.1). All p values for these analyses can be found in Table 4.

Table 4. Multiple linear regression model results used to look for evidence of trade-offs between reproductive, defensive, and growth-related traits in *R. allegheniensis* (blackberry) and *R. phoenicolasius* (wineberry), using combined data from plots containing one or both species. The main effect reported for each model indicates the degree to which the dependent variable changes in response to the independent variable. A species term was included to assess for any differences between species in the average value of the dependent variable. If the "Species – Wineberry" term was statistically significant, the beta value indicates how different the average value of the Y variable would be for wineberry versus for blackberry by adjusting the y-intercept of the resulting best-fit line. Models were run under the assumption that the two species could exhibit different relationships between the independent and dependent variables. If the interaction term was significant, the beta value indicates how the slope of the relationship between the dependent variable and independent variable would differ for wineberry versus for blackberry. When the interaction was not significant, the model was simplified by removing the interaction term and then re-ean. Probability values were deemed significant (bold) or marginally significant (italics) at $p < 0.05$ and $0.05 < p < 0.1$, respectively.

Dependent variable	**Independent variable[1]**		**Regression coefficient (β)**	**Test statistic (t)**	***p* value**
Flower number					
	Average leaf size*	Main effect	4.92x10^{-3}	4.480	**0.000**
		Species – Wineberry	0.480	4.026	**0.000**
		Interaction term	-5.92x10^{-3}	-3.927	**0.000**
	Cane length*	Main effect	0.012	31.142	**0.000**
		Species – Wineberry	0.0067	0.201	0.841
	Basal diameter*	Main effect	0.212	10.021	**0.000**
		Species – Wineberry	0.138	3.866	**0.000**
	Leaf toughness*	Main effect	-2.54x10^{-3}	-3.872	**0.000**
		Species – Wineberry	-1.06	-6.521	**0.000**
		Interaction term	5.43x10^{-3}	6.752	**0.000**
	Prickle intensity	Main effect	-4.8	-1.791	*0.079*
		Species – Wineberry	-28	-1.739	*0.088*
		Interaction term	5.8	1.892	*0.064*
Fruit number					
	Average leaf size*	Main effect	-0.020	-7.862	**0.000**
		Species – Wineberry	0.77	6.314	**0.000**
	Cane length*	Main effect	0.010	5.597	**0.000**
		Species – Wineberry	0.19	0.507	0.612

		Interaction term	0.0051	2.275	**0.023**
	Basal diameter*	Main effect	0.18	2.349	**0.019**
		Species – Wineberry	2.2	4.846	**0.000**
		Interaction term	-0.16	-2.674	**0.008**
	Leaf toughness*	Main effect	$-3.8\text{x}10^{-3}$	-1.466	0.143
		Species – Wineberry	-1.1	-1.986	**0.047**
		Interaction term	0.011	3.765	**0.000**
	Prickle intensity*	Main effect	-0.10	-2.838	**0.005**
		Species – Wineberry	0.75	3.233	**0.001**
		Interaction term	0.066	1.779	*0.075*
Fruit set					
	Average leaf size*	Main effect	-0.016	-1.015	0.310
		Species – Wineberry	0.99	1.206	0.228
	Cane length*	Main effect	$-1.9\text{x}10^{-3}$	-0.226	0.821
		Species – Wineberry	1.1	1.468	0.142
	Basal diameter	Main effect	-0.032	-1.281	0.206
		Species – Wineberry	0.32	2.446	**0.018**
		Interaction term	-0.039	-1.803	*0.077*
	Leaf toughness	Main effect	$1.4\text{x}10^{-5}$	0.035	0.972
		Species – Wineberry	0.12	3.287	**0.002**
	Prickle intensity	Main effect	$-3.0\text{x}10^{-4}$	-0.036	0.971
		Species – Wineberry	0.12	3.039	**0.004**
Fruit dry:wet mass ratio					
	Average leaf size	Main effect	$-4.7\text{x}10^{-4}$	-2.549	**0.015**
		Species – Wineberry	-0.042	-2.093	**0.042**
		Interaction term	$5.0\text{x}10^{-4}$	2.011	*0.051*
	Cane length	Main effect	$-5.2\text{x}10^{-5}$	-0.664	0.510
		Species – Wineberry	$-7.7\text{x}10^{-4}$	-0.138	0.891
	Basal diameter	Main effect	$2.5\text{x}10^{-3}$	0.693	0.492
		Species – Wineberry	$5.1\text{x}10^{-4}$	0.086	0.931
	Leaf toughness	Main effect	$-9.8\text{x}10^{-5}$	-1.665	0.103

		Species – Wineberry	$-2.6\mathrm{x}10^{-3}$	-0.469	0.641
	Prickle intensity	Main effect	$-1.6\mathrm{x}10^{-4}$	-0.118	0.907
		Species – Wineberry	$-3.9\mathrm{x}10^{-4}$	-0.060	0.952
Average leaf size					
	Cane length*	Main effect	$2.6\mathrm{x}10^{-3}$	7.425	**0.000**
		Species – Wineberry	-0.19	-7.522	**0.000**
	Basal diameter*	Main effect	$9.00\mathrm{x}10^{-3}$	0.525	0.599
		Species – Wineberry	-0.186	-6.823	**0.000**
	Prickle intensity*	Main effect	-0.055	-5.978	**0.000**
		Species – Wineberry	-0.25	-4.386	**0.000**
		Interaction term	0.030	2.705	**0.007**
Leaf toughness					
	Average leaf size*	Main effect	$2.46\mathrm{x}10^{-3}$	4.428	**0.000**
		Species – Wineberry	$4.99\mathrm{x}10^{-2}$	0.849	0.396
		Interaction term	$-1.83\mathrm{x}10^{-3}$	-2.452	**0.014**
	Cane length*	Main effect	$-1.6\mathrm{x}10^{-4}$	-0.556	0.578
		Species – Wineberry	-0.24	-4.488	**0.000**
		Interaction term	$1.0\mathrm{x}10^{-3}$	2.595	**0.009**
	Basal diameter*	Main effect	0.0454	4.034	**0.000**
		Species – Wineberry	-0.202	-3.384	**0.000**
		Interaction term	0.0212	2.140	**0.032**
	Prickle intensity*	Main effect	0.022	5.995	**0.000**
		Species – Wineberry	-0.16	-8.934	**0.000**
Prickle intensity					
	Cane length	Main effect	0.021	2.705	**0.009**
		Species – Wineberry	2.1	3.619	**0.000**
	Basal diameter	Main effect	0.485	1.258	0.214
		Species – Wineberry	2.38	3.849	**0.000**

[1] an * indicates a Poisson regression rather than a linear regression (see Methods).

Discussion

Our research goals were to assess how traits associated with carbon allocation to defense, reproduction, and growth in *Rubus* species would correlate with leaf-level herbivory. We also wanted to discern if traits associated with these three life history functions would trade off with one another as theory would predict, and if so, specifically which—and how many—of these trade-offs would exist. Our data suggest the potential for several induced defense responses in these species, as well as the potential presence of several trade-offs between reproduction and the other two life history functions for both species. Additionally, our data suggest potential strategies wineberry may be using to invade and outcompete its native congeners in New Jersey.

Variability between species

The variability data we obtained for our two study species suggest a potential explanation for how wineberry is successful as an invasive species in Northern New Jersey. On average, wineberry plots received less light (and thus had less carbon available, assuming equal photosynthetic capacities) than blackberry plots did. Despite this, wineberry plots were, on average, able to produce more ripe fruits, do so more efficiently (as measured by fruit set), and produce longer canes than blackberry. The more efficient reproduction in wineberry could be due to the species receiving less average leaf-level herbivory and, as a consequence, being less incentivized to invest carbon to tougher leaves (and other defenses) compared to blackberry.

Effects of herbivory

It is common for leaf-level herbivory to negatively affect reproductive output by eliminating photosynthetic tissues and reducing rates of carbon acquisition (Herms and Mattson 1992). The decreases we observed in both fruit number and fruit set for both species in plots with higher rates of herbivory are thus not surprising. Our observation that the native species, blackberry, experienced a more dramatic decrease in fruit number with increasing rates of herbivory than the invasive wineberry did is consistent with the results of McDowell and Turner (2002), who found that the invasive *R. discolor* in the Northwestern United States had greater reproductive success than the native *R. ursinus.* In their study, *R. ursinus* produced, on average, many fewer fruit (23.5 ± 4.4 vs. 720.3 ± 123.9; $p < 0.001$), with less combined fruit and flower biomass relative to plant size (0.22 ± 0.03 g g^{-1} vs. 0.36 ± 0.02 g g^{-1}; $p = 0.01$), than did *R. discolor*. They contended these differences were due to *R. discolor* being able to invest more resources to reproduction without consequently diverting those resources as heavily from vegetative functions (like growth and defense) as *R. ursinus* had to. If our species are behaving similarly, this could explain why blackberry's reproductive output is predicted to decrease more severely when the needs for increased growth (to regrow lost vegetative tissue) and defense (to prevent the loss of more vegetative tissue) are present. The predicted decrease of flower number as herbivory increases in blackberry but not wineberry also supports the notion that blackberry's reproductive output is reliant on resources that would have otherwise been allocated to growth or defense but that this pressure may be less for wineberry.

Observing higher prickle intensity values for both blackberry and wineberry when leaf-level herbivory rates were higher, though only a marginally significant trend ($p = 0.091$), was more unexpected. These results suggest that carbon allocation to prickles may have a degree of inducibility and can be increased when an individual is subjected to regular leaf-level herbivory. The possibility of prickle inducibility in *Rubus* is supported by the results of Bazely et al. (1991). In that study, *R. fruticosus* displayed increased prickle density (prickles per internode) in response to simulated browsing ($p < 0.05$). However, a study by Gibson et al. (1993) on *R. vestitus* found that simulated browsing did not have a significant impact on prickle density (prickles per internode; $p > 0.05$). These conflicting results suggest that the extent to which prickles can be produced "on demand" may differ across the genus and that data on the response of prickle production to leaf-level herbivory is needed from more species before any conclusions can be reached.

It is interesting to note that prickles are primarily used to limit browsing by larger mammals and not by insects, so the fact that it may nevertheless increase in response to leaf-level herbivory is curious. We did not measure herbivory by large mammals at our plots, but it has been observed previously that these species (likely ungulates) are frequent browsers of *Rubus* (Bazely *et al.* 1991; Gibson *et al.* 1993). It is possible that this response to more frequent herbivory, if it indeed occurs, is a response to leaf tissue damage of any biotic kind and is primarily geared towards deterring larger herbivores and the induction is maladaptive or neutral when insect herbivory is high. We did not quantify resource investment to hairs and trichomes, defensive structures geared more towards discouraging insect herbivory, so it is possible we could have missed a similar and more appropriately scaled induced response involving these structures to the herbivory we were observing.

It is also possible that higher rates of herbivory in more well-defended plots (in this instance, plots with more overall carbon allocated to prickles) could actually be a result of the increased defenses. Prickles are primarily a defense against larger herbivores (small and large mammals). If these herbivores are no longer visiting plots that are well-defended against them, it is possible that insects will seek refuge in these plots more frequently and/or in higher numbers due to the decreased disturbance and predation and competition pressure. Another possibility is that insect herbivores may use defense level as a proxy for nutrition such that a well-defended plant could be more nutritious than a less-defended one. Because the prickles are not a defense that deters insect herbivores specifically, insects will still be able to eat these more well-defended plants. This possibility, that by better defending themselves individuals may be increasing their likelihood of suffering herbivory by either signaling their nutritional value to insect herbivores or providing them refuge, should be explored to provide clarity on this relationship.

The fact that a positive relationship between leaf toughness and leaf-level herbivory is predicted by our models in wineberry but not blackberry yields a possibility: that leaf toughness may be an induced defense (at least partially) for wineberry but not blackberry. This suggests that the invasive wineberry may be able to successfully invest resources to reproduction without diverting them from vegetative functions (in this case defensive traits such as leaf toughness), similar to *R. discolor* (McDowell & Turner

2002). If this is the case, then wineberry would be able to more freely invest resources to both reproduction *and* defense, as opposed to being forced to choose how resources should be allocated between the two functions.

The potential inducibility of both increased leaf toughness and prickle intensity in wineberry suggests that this species may initially invest fewer resources to defense and only invest more when a demonstrated need arises (or, alternatively, when prior experience indicates a likely need). Williams and Sahli (2016) found that in their study sites in Pennsylvania, wineberry received half the amount of leaf-level herbivory than its native congener, which for their study was black raspberry (*R. occidentalis*; $p < 0.001$). Our data corroborate this, showing that the wineberry in our plots received, on average, 40% less leaf-level herbivory than the native blackberry (Tables 1 and 2). This is not surprising, as wineberry is invasive and thus many insects here may not yet be adapted to feed on it. These growing conditions being experienced by wineberry here in New Jersey—moderate amounts of leaf area loss plus infrequent herbivory—are thought to be optimal for the strategy of relying primarily on induced rather than constitutive defenses (Ito & Sakai 2002). In short, wineberry may be capable of having a lower leaf toughness and prickle intensity initially but then increasing this investment to protect itself when stimulated to. This more economical allocation of carbon to defense may explain how wineberry is able to better minimize carbon trade-offs between defense and other life history traits than blackberry and thus outcompete its native congeners here in New Jersey.

Trade-offs

Of the 29 trade-off models we ran, seven (the null expectation being 1.5) returned significant results indicative of physiological trade-offs involving carbon between life history functions for at least one of our two study species. Two were detected between reproductive and growth-related traits, four between reproductive and defensive traits, and one between defensive and growth-related traits. Of these trade-offs, three occurred in both species, three occurred only in blackberry, and one occurred only in wineberry. This suggests that, overall, these species have likely evolved to reduce the number of carbon trade-offs they exhibit as much as possible by developing optimized allocation strategies that rarely result in two essential functions having a strong demand for the same limited carbon at the same time. However, it also demonstrates that these trade-offs cannot be entirely eliminated because some concurrent investment is unavoidable and resources are always finite.

The trade-offs between growth and either reproduction or defense all included average leaf size as the growth trait while our other measure of growth, cane length, was not involved in any significant trade-offs. This may be explained, as noted by McDowell and Turner (2002), by the fact that reproduction is more likely to experience tension with concurrent rather than prior growth in perennial plants. The leaves we measured were all produced during the same year as reproductive structures, so this growth-related trait could reasonably be expected to trade off with reproductive traits more directly than cane growth, investment to which occurred a full year earlier. That said, first-year growth could, to some extent, limit the amount of carbon in storage that would be available for reproduction in the second year. We did not measure first-year average leaf size nor cane growth rates, so it is possible these growth traits could have had such an effect on stored carbon, but we did not see evidence of this directly.

The apparent trade-off between average leaf size and prickle intensity could potentially be due to increased relative allocation of carbon to prickles in the developing first-year cane, limiting the carbon allocated to storage that would later be available to invest into leaves at the start of the second year. It is possible that wineberry and blackberry could both be reducing the investment of carbon to prickle production during the first growing season to increase their stored carbon and thus maintain a higher rate of leaf growth in the second growing season. This is only possible if previously stored carbon is an essential source of carbon for second-year leaves and that this trade-off between carbon allocation to prickle production and storage exists. Because we did not observe or quantify how stored carbon is utilized by our study species, we cannot say for certain which traits would rely, to any extent, on stored carbon versus newly acquired carbon.

An additional growth-defense trade-off between cane growth rate and prickle production (prickles per cm and prickles per internode) has been documented in *R. vestitus* (Gibson et al. 1993), but we were unable to confirm or deny the possibility of this trade-off in our study species. Because we were unable to measure the first-year cane growth rates of our marked canes, we are unable to say whether or not this trait could also have potentially traded off with prickle production in wineberry and blackberry or have influenced our observed trade-off between second-year vegetative growth and prickle production. We did measure cane length, which can be used as a proxy for cane growth rate, and this did not return any significant results indicative of any trade-off relationships.

The potential trade-offs between reproduction and defense suggested by our models support our predictions that increased carbon investment to defense (whether by "choice" or by "necessity") can negatively impact carbon-demanding reproductive characters produced at the same time or soon thereafter. Prickle intensity trading off with flower number and ripe fruit production in blackberry indicates that these reproductive measures could potentially rely, to some extent, on previously stored carbon, given that prickles are produced in the year prior to reproduction. That leaf toughness also trades off with flower number in blackberry indicates that these two functions may be competing for carbon during the reproductive season within this species as well and that there's a need to defend photosynthetic structures even at the expense of reproductive output. These findings are consistent with the aforementioned results of McDowell and Turner (2002) that a native *Rubus* species may be more likely to experience tension between resource allocation to vegetative structures (in this case, prickles and leaves) and to reproduction (in this case, the number of flowers and ripe fruits produced).

It is interesting to note that blackberry is predicted to experience six trade-offs, five of which could curtail reproduction, while wineberry is only predicted to experience four, only three of which could negatively impact reproduction. This could help explain why wineberry, on average, has relatively higher reproductive success (in terms of number of ripe fruits and also fruit set) compared to blackberry, perhaps providing insight into how wineberry succeeds as an invasive species in New Jersey, possibly at the expense of its native congeners.

Future directions

We would recommend that future studies analyze life history function-related traits that we were unable to quantify in our research to more fully and accurately characterize these species' reproductive ecology. For example, Björkman and Anderson (1990) found that a *Rubus* species (*R. bogotensis*) without trichomes tended to have tougher leaves, as previously described, suggesting that these two characters may play a similar role and would not be simultaneously invested in. Our results are consistent with such a relationship; wineberry possesses trichomes and has, on average, lower leaf toughness values than blackberry, which lacks trichomes. However, because we did not quantify the trichomes on wineberry within our plots and compare these data to those of leaf toughness, we cannot confirm the presence of this relationship in this species. Knowing whether such a relationship exists is important because it would help to shed light on wineberry's full defensive strategy against insect herbivores specifically, which may aid this species' ability to outcompete native congeners and resist natural population regulation in regions where insects are the dominant herbivores. We would also recommend observing the dynamics between hairs and trichomes and rates of leaf-level herbivory; we predict that these traits may potentially have a degree of inducibility in wineberry under such herbivory as seen in leaf toughness and prickle intensity. One further recommendation would be to measure first-year cane growth rates and assess whether or not this growth-related trait trades off with prickle intensity in these species, a possibility indicated by Gibson et al. (1993). This work would help clarify the reproductive behavior of these species and ultimately provide guidance on which traits should be observed during subsequent manipulation and/or simulation experiments.

We also recommend experiments be conducted to simulate variable levels of herbivory as well as reproductive effort (via flower removal and hand pollination) to confirm the presence or absence of the effects of herbivory as well as trade-offs indicated by our study. By exaggerating herbivory in a controlled environment, the impacts it has will be made more evident and reduce the possibility of confounding variables while helping to test the possible cause-effect relationships indicated by our results. Similarly, exaggerating the amount of reproductive effort a plant must support will increase the tension put on that individual's resource supply, thus increasing the chances of observing trade-offs between reproductive traits and those associated with defense and growth (Sletvold & Ågren 2015). The clearer understanding of these species' reproductive ecology that could be gleaned from these experiments would allow researchers to recommend best practices for cultivating these species for their reproductive products (e.g., establishing guidelines for when treatment for insect herbivory is warranted because it is likely to significantly reduce yield).

Lastly, we would recommend that individual rootstocks be studied across multiple years to assess if impacts of herbivory or trade-offs are simply being distributed across time, making them harder to discern in any given year. A project of this type does not appear to have been carried out for this genus to date. This would allow researchers to observe if the ecological conditions facing an individual one year affect its behavior in subsequent years, as might be expected for conditions such as herbivory and carbon availability. We recommend observing first- and second-year cane pairs from the same individual across at least two growing seasons to determine if the behavior of the second-year cane also affects the first-year cane's reproductive behaviors and outputs the following season. Similarly, herbivory can be observed on a second-year cane to see if there is an impact on the allocation of resources to defense in future canes, indicating defense induction over longer time scales.

Conclusion

We began this research driven by six questions:

1. Are the defenses implemented by these species entirely constitutive, or could they be at least partially induced by leaf-level herbivory?
2. Do defensive and reproductive functions trade off within these species?
3. Do defensive and growth functions trade off within these species?
4. Do growth and reproductive functions trade off within these species?
5. Do any trade-offs exist *within* any of these life history functions (e.g., do reproductive traits ever trade off with one another)?
6. Do these two species experience similar trade-offs?

Our results supported five of our six predictions. We observed evidence that prickle intensity and leaf toughness may be at least partially inducible in one or both study species. Additionally, our data suggest that carbon allocation to defensive traits (leaf toughness, prickle intensity, and stem basal diameter) could potentially lead to decreased investment to reproductive outputs (flower number and fruit number) as well as reproductive success (fruit set). Our models also revealed a potential trade-off between average leaf size (a growth-related trait) and prickle intensity (a defensive trait). The potential trade-offs between growth-related (average leaf size) and reproductive (ripe fruit number and proportional fruit dry mass) traits support our prediction that increased concurrent investment of carbon to one life history function will frequently result in a decreased investment to the other, putting potential limits on how successfully these species can reproduce under field-typical conditions. Our models did not indicate any trade-offs between traits within a single life history function, contrary to our prediction, although we also did not consider a large number of these particular trade-offs. Lastly, we observed that blackberry and wineberry may both experience trade-offs but these are not always similar in type or intensity between the two species.

The primary goal of this research was to better understand the reproductive ecology and limitations of the *Rubus* genus. By studying blackberry and wineberry, we were able to discover several potential trade-offs between life history functions and were also able to identify potentially inducible defenses. We also identified several mechanisms by which wineberry may be outcompeting its native congeners in New Jersey and thus succeeding as an invasive (namely through the inducibility of prickles and leaf toughness as well as more efficient reproduction). This research, having proposed several responses to herbivory and trade-offs between life history functions, can provide a foundation for future studies concerning the reproductive ecology of these species in particular or this genus more generally.

Research contributions

Brady Thexton (BJT) designed the experiment, carried out laboratory analyses on stem and leaf samples, and wrote the manuscript with guidance from Dr. Alex Bajcz (AWB); BJT, AWB, and Colleen Dabrowski carried out the fieldwork; BJT, Morgan Zielenski, and Tess Needham carried out laboratory analysis on fruit samples.

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