Genetic Variation in Adventitious Rooting, Seed Germination, and Berry Phenolic Content of Black Elderberry (*Sambucus canadensis*) in New Brunswick

by

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Abstract

Black elderberry (*Sambucus canadensis*), a North American shrub valued for its ecological and medicinal properties, remains underexplored compared to its European counterpart, *Sambucus nigra*. This study investigates genetic variation in seed germination, adventitious rooting, and phenolic content (chlorogenic acid and rutin) among wild *S. canadensis* populations in New Brunswick, Canada. Ten populations from diverse biogeographic zones were sampled. Germination success varied significantly (59%–78%), with coastal populations germinating faster. Phenolic concentrations ranged widely (chlorogenic acid: 487–1825 ng; rutin: 884–2404 ng), showing strong correlation ($\beta = 0.735$, $p < 0.001$). Root development showed limited site variability and no correlation with plant size. Results highlight substantial genetic and phenotypic diversity, underscoring the species' potential for ecological restoration, sustainable agriculture, and bioactive compound production. This research informs population selection for adaptability and enhanced bioactive compound production.

Dedication

To my family, whose unwavering support, patience, and encouragement have been the foundation of this journey. Thank you for believing in me, standing by me through every challenge, and inspiring me to reach for my goals. I am deeply grateful for your love and understanding, which have given me the strength and motivation to persevere. This work would not have been possible without you.

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1. Introduction

This study explores the genetic and physiological traits of black elderberry (*Sambucus canadensis*) from wild populations in New Brunswick, focusing on phenolic content in berries, seed germination from open-pollinated sources, and root development in softwood cuttings. These traits were chosen to gain insights into the adaptability, ecological roles, and potential applications of *S. canadensis* in sustainable agriculture and restoration. Through fieldwork and laboratory analyses, we collected berries, seeds, and cuttings from various *S. canadensis* populations. Phenolic content was quantified, seed germination evaluated, and clonal propagation potential examined using advanced analytical methods.

S. canadensis is a deciduous, fruit-bearing shrub native to North America, belonging to the Adoxaceae family (Lee and Finn, 2007). It is widespread across New Brunswick and is recognized by its compound leaves, which typically feature 5–9 leaflets, and clusters of small, fragrant white or cream-colored flowers that bloom from late June to early July. The dark purple to black berries ripens from mid-August to early September (Charlebois et al., 2010).

Elderberries were used traditionally by various cultures including Ancient Romans, Native Americans, and North American settlers, for their practical and medicinal applications (Charlebois et al., 2010; Osbaldeston and Wood, 2000; Mabey, 1996; Grieve, 1931). Traditionally, elderberries were used for crafting materials, natural dyes, remedies, and artisanal products (Stang, 1990; Moerman, 1998; Thompson, 1969; Durand et al., 1981). Known for their rich nutrient profile, elderberries contain vitamins, minerals, and bioactive compounds such as phenolic acids and flavonoids, contributing to

their antioxidant and anti-inflammatory properties (USDA, 2008; Schmitzer et al., 2012). These traditional uses have spurred contemporary interest in *S. canadensis*, with recent research increasingly focused on understanding its health benefits and applications.

The potential health benefits of *S. canadensis* has spurred recent research into the nutritional composition and medicinal properties of the berries as well as techniques for plant cultivation (Mathieu et al., 2015). This species holds value not only for its historical and cultural significance but also for its potential in sustainable agriculture, natural health products, and ecological restoration (Shelef et al., 2017). *S. canadensis* is thus positioned to contribute significantly to both sustainable health practices and ecosystem management in North America and beyond (Lee and Finn, 2007; Charlebois et al., 2010). However, despite its potential, *S. canadensis* has not been studied as extensively as its European counterpart, *Sambucus nigra.*

Significant variability in phytochemical content has been highlighted in different *S. canadensis* genotypes (Lee and Finn, 2007; Özgen et al., 2010). Both genetics and environment can affect the variation in total phenolic content, anthocyanin levels, and antioxidant capacity. Environmental conditions, such as climate, soil type, and agricultural practices, impact the concentrations of bioactive compounds in *S. canadensis* tissues (Thomas et al., 2008; Finn et al., 2008). This variability presents breeding opportunities to select and develop cultivars with enhanced levels of beneficial compounds (Finn et al., 2008; Özgen et al., 2010).

Quantifying genetic variation within and among wild populations of *S. canadensis* in New Brunswick fills this research gap, laying the groundwork to explore the plant's adaptability, biochemical potential, and ecological roles. Assessing traits such as phenolic

content, seed germination patterns, and root development across populations provides valuable insights into the plant's genetic diversity and its potential utility for commercial cultivation and medicinal use (Lee and Finn, 2007; Mathieu et al., 2015). Understanding this genetic variation is crucial for improving *S. canadensis* cultivars tailored to North American conditions and optimizing the production of bioactive compounds that contribute to its health-promoting properties (Shelef et al., 2017).

Integrating Pigliucci's (2001) concept of "Phenotypic Plasticity" offers a framework for understanding how *S. canadensis* responds to environmental variability. Phenotypic plasticity—the ability of a single genotype to produce different phenotypes in response to environmental conditions—is a crucial factor in plant adaptation and survival. In elderberries, phenotypic plasticity may explain observed variability in phytochemical content and other traits across environments. Recognizing phenotypic plasticity's role is essential for optimizing breeding programs and cultivation practices, as it allows for selecting genotypes with favorable plastic responses that maintain high bioactive compound levels despite environmental variability.

Root development, germination success, and phenolic content are interconnected traits that provide insights into the adaptability and ecological fitness of *S. canadensis*. Early root development, crucial for establishing softwood cuttings, contributes to plant resilience by enhancing nutrient uptake, water absorption, and stability—qualities essential for survival in fluctuating environments. Root development impacts soil stabilization and ecosystem function, making it relevant for restoration projects in erosion-prone areas.

Seed germination success is critical for the recruitment and spread of *S. canadensis* populations. Genetic factors influencing seed dormancy and germination rates can determine how well populations adapt to site-specific environmental pressures. As environmental changes increasingly affect seed viability, understanding the genetic basis of germination can inform effective restoration practices, especially in variable climates. Identifying populations with high seed viability and reproductive potential could enhance restoration strategies by supporting rapid establishment and resilience in restored areas.

Phenolic compounds, especially chlorogenic acid and rutin, are secondary metabolites in *S. canadensis* berries that significantly contribute to plant defense and stress resilience. These compounds help protect against herbivory, pathogens, and environmental stresses like drought and UV exposure. High phenolic content enhances elderberries' ecological role in supporting biodiversity and increases their value as bioactive resources in natural health products. While variability in phenolic concentrations across populations offers opportunities for selecting genotypes with optimal bioactive profiles, further research is needed to determine whether the genotypes with the highest commercial potential also align with those that provide the greatest ecological benefits. Exploring this relationship could maximize both ecological and commercial outcomes.

The genetic regulation of these three traits—root development, seed germination, and phenolic content—reflects complex adaptive strategies supporting *S. canadensis*'s success across diverse habitats. Each trait's expression can be influenced by genetic factors, environmental conditions, or their interaction; a concept captured by phenotypic plasticity. Understanding how these traits manifest across environments reveals the

adaptive capacity of *S. canadensis*, guiding efforts to develop resilient, ecologically valuable populations suitable for both commercial and conservation applications.

By examining phenotypic plasticity of these traits in wild populations, this study aims to expand knowledge of *S. canadensis*'s adaptive mechanisms. This understanding supports the goal of fostering a sustainable resource base, ensuring that *S. canadensis* populations thrive and contribute to ecosystem health, biodiversity, and natural product markets in the face of environmental change.

Studies on the genetic diversity, physiological traits, and medicinal properties of *S. canadensis* remain limited (Charlebois et al., 2010). This is notable given the global interest in natural health products and the therapeutic applications of elderberries, rich in essential amino acids, vitamins, and phenolic compounds critical to human health (USDA, 2008; Schmitzer et al., 2012). Addressing this gap, the present study quantifies genetic variation within and among wild populations of *S. canadensis* in New Brunswick.

2. Methods

2.1. Field Sampling

The objective of the site selection process was to ensure genetic diversity and minimize population overlap across the study area. *S. canadensis* is a shrub commonly found in full sun, particularly along forest edges, stream banks, wetlands, and open fields, where it coexists with species like birch (*Betula sp.),* eastern hemlock (*Tsuga canadensis*), balsam fir (*Abies balsamea*), and tamarack (*Larix laricina*). These habitat types are characteristic of *S. canadensis* populations throughout New Brunswick. To locate viable populations for study, we utilized data from the Atlantic Canada Conservation Data Centre (ACCDC) and iNaturalist. Following initial identification from these records, we conducted field visits to verify the presence of *S. canadensis* plants and assess their suitability for sampling.

Over 30 sites were initially surveyed, with criteria designed to maximize genetic diversity and minimize the risk of sampling genetically similar populations. At each site, plants were spaced at least three meters apart to reduce the likelihood of selecting plants from the same clone that originated from root suckering or layering. This distance aligns with observations of clonal spread in other woody species, where root suckers typically remain within a few meters of the parent plant (e.g., Cain et al., 1998; Jones et al., 2000). We also ensured that populations were independent by maintaining a minimum distance of 50 kilometers between sites. This spacing accounted for the natural range of pollen and seed dispersal, as *S. canadensis* is primarily wind-pollinated, with seed dispersal occurring via birds, mammals, and insects that are attracted by the plant's cyanogenic berry compounds (Vander Wall and Moore, 2016).

Sampling was stratified across three distinct biogeographic zones in New Brunswick: inland (e.g., Sites 1, 2, 3, 8), Bay of Fundy coastal (e.g., Sites 5, 9, 10), and Gulf of St. Lawrence coastal (e.g., Sites 4, 6, 7). This stratification was carried out using ArcGIS, considering ecological factors such as climate, soil types, plant communities, geology, water systems, and elevation variability. The stratified approach ensured that the sites selected were representative of the diversity in *S. canadensis* habitats, contributing to a broader understanding of the species' environmental adaptability (Figure 1).

Figure 1. Distribution and habitat of *S. canadensis* in New Brunswick. Map showing sampled populations of *S. canadensis* across central New Brunswick, Bay of Fundy coastal, and Gulf of St. Lawrence coastal zones. ACCDC and iNaturalist data confirmed species presence, with sampling points spaced a minimum of 50 km apart to minimize genetic overlap. Plants at each site were spaced at least three meters apart, with ten plants sampled per site across six populations, capturing ecological variation in soil, climate, and topography.

To capture genetic variation within and across sites, preliminary surveys and initial rooting trials conducted in the first year guided the sampling design. Based on these findings, we established a sampling scheme targeting a minimum of six plants per site, with ten cuttings collected from each plant, resulting in a total of 600 cuttings across ten populations. This approach was intended to ensure adequate representation of the genetic diversity at each site and across the broader study area (Lee & Finn, 2007; Mathieu et al., 2015). For germination experiments, seeds were collected from the same plants used for softwood cuttings, ensuring consistent representation across all experiments. Phenolic analyses were conducted on berries collected from these plants, allowing comparisons of genetic and trait variability across softwood cuttings, germination, and phenolic content.

Sample sizes were determined according to expected population sizes, survival rates, and observed rooting success from initial trials. To address concerns about disentangling genetic and maternal effects, seeds and cuttings were propagated under controlled conditions to minimize environmental variability. Statistical models included random effects for maternal plant identity to account for maternal influences on traits like seed germination success. For each plant, detailed observations were recorded, including plant size, health status, habitat type, and leaf characteristics. Each plant was tagged sequentially to facilitate tracking over the growing season, and GPS coordinates were documented using a Garmin GPSMAP® 65, which provides an accuracy of up to ±3 meters under optimal conditions. This ensured precise mapping and supports future monitoring of populations.

2.2 Softwood Cuttings

Softwood cuttings were collected between late June and July to capture the period from flower bud emergence to early fruit set, a timing shown to be optimal for rooting success due to the presence of active, healthy tissue. Studies have demonstrated that collecting softwood cuttings during late spring to early summer maximizes rooting potential, as the tissues are in a metabolically active state that supports successful root formation (Hartmann et al., 2010; Dirr & Heuser, 2006; Howard, 1994; Loach, 1988). At each field site, observations on plant size, health status, habitat type, and leaf characteristics were recorded to ensure that cuttings were obtained from healthy plants in typical habitat conditions. Each plant was tagged to facilitate later identification and berry collection throughout the season.

All cuttings were collected within a two-week period between July 2 and July 16, 2023. Cutting tools and containers were sterilized with alcohol between samples. Hardwood branches were selected based on the presence of at least two shoots measuring a minimum of 10 cm in length, with one to two leaves each. Cuttings were placed into plastic bags containing damp paper towels and the bags placed into coolers with ice packs for transport to the processing site.

Upon arrival at the processing site, softwood cuttings were cut from hardwood branches and trimmed to a standard length of 15–25 cm and prepared with two to four nodes and a maximum diameter of 1 cm to ensure consistency across samples (Figure 2). To promote rooting, a revised soaking protocol from a 2021 trial was used: the cuttings were placed in water-filled glass jars and soaked for two to three weeks, eliminating the need for rooting hormones

In July 2023, the cuttings were transplanted into a controlled environment in central New Brunswick. To simulate natural shading, a 30% shade cloth was applied overhead, and watering was carried out each morning to maintain soil moisture, with additional watering in the evenings on days when temperatures exceeded 27°C. Plants were arranged using a randomized block design with ten blocks, each containing six rows of ten cuttings. Sixty random cuttings were selected for ten blocks to account for potential environmental factors such as light intensity, air circulation, and water distribution (Figure 4).

All plants from all study sites were compiled into an Excel sheet with unique ID codes in the format "S[Site number]-[Plant number]_[Replicate number]." Random numbers were assigned to these ID codes to facilitate unbiased allocation. The plants were then randomly distributed into ten blocks, each containing six rows of ten cuttings, ensuring equal representation across the blocks. The randomized block design enabled each of the ten blocks to account for localized environmental variability, such as slight differences in microclimate within the experimental area. This design ensured that any observed differences in growth or trait expression among cuttings were due to the inherent variability in the study population rather than environmental inconsistencies.

Diameter and height of each cutting were recorded weekly from June to October, with additional observations on leaf count, browning, pest damage, new shoots, and overall vigour. In late October, as the cuttings approached dormancy, root volume was measured using a water displacement method. Growing media was washed from the roots of the cuttings and the roots submerged in a 500 mL beaker containing 200 mL of water (Figure 3). The displacement caused by the roots was recorded to quantify root volume.

Figure 2. Collection and preparation of *S. canadensis* softwood cuttings. Stages of softwood cutting preparation from initial harvest to pre-planting processing, showing selected vegetative shoots from woody stems (left and middle) and measured, processed for uniformity (right).

Figure 3. Root production for elderberry cuttings following two-week soak in water.

Figure 4. *S. canadensis* cuttings from ten populations. Cuttings arranged in ten blocks, each containing six rows of ten cuttings, within a controlled propagation environment in Rusagonis, NB, from June to October 2023.

2.3. Seed Germination

Berries were harvested from during August 26 to September 9, 2023, during peak ripeness, indicated by their glossy black-purple color. The berries were processed using a blender and sieve method. Berry processing was conducted using a standard kitchen

blender (Model: Ninja® Foodi™ Cold & Hot Blender), with water added to fully submerge the berries. The mixture was pulsed on a low setting approximately 20-30 times, allowing for intermittent settling between pulses, over a total duration of two to four minutes. This technique effectively separated the pulp from the seeds while minimizing seed damage. Seed viability was subsequently assessed using the flotation method described by Justice and Bass (1978). For this process, the container was filled with one liter of tap water, allowing viable seeds to sink while non-viable seeds floated to the surface. After a 30-minute flotation period, floating material was removed, and the flotation step was repeated for an additional 30 minutes to ensure thorough separation and isolation of viable seeds. Afterward, the seeds were dried to a target humidity of 10% and stored in labeled paper bags by plant and population. The bags were then placed in an indoor greenhouse structure with a dehumidifier in my house, ensuring consistent, cool, and dry conditions until the germination trials began.

Ten random samples of 100 seeds from each collection were weighed to calculate average seed weight per population calculated. Embryo maturity was assessed by bisecting random seeds to check for developed embryos, ensuring that only fully developed seeds were selected for the germination trials. Seeds deemed non-viable using the flotation method and cut test were not subjected to germination trials, as confirming their lack of viability was outside the scope of this study.

Seeds received chemical scarification to overcome the impermeability of their seed coats, which is often a barrier to germination. Following USDA Natural Resources Conservation Service protocols (2023), the seeds were immersed in 90% sulfuric acid for 10 minutes, after which they were thoroughly rinsed and air-dried to remove any residual

acid. This scarification process is critical to enhance germination rates. The seeds were then placed on moistened Vera-Pak Germination Paper (Seedburo Equipment Company) within closed germination trays on slotted racks. Each piece of germination paper was moistened with 125 mL of dissolved oxygen (DO) water, and an additional 125 mL of DO water was added to the bottom of each tray to maintain consistent humidity. The trays were securely sealed to maintain a controlled environment conducive to germination.

For each plant, we assessed germination using four replicates of 25 seeds, totaling 100 seeds per plant. With six plants sampled per site across ten different sites, the study included a total of 600 seeds per site, and 6,000 seeds overall. To ensure unbiased seed distribution, Python programming was used to randomize seed placement across the 60 germination trays, allowing for rigorous experimental controls on plant and site distribution.

Prior to germination, the trays underwent a 60-day cold stratification period at 4°C in a walk-in cold storage unit to simulate overwintering conditions. Germination was carried out in a growth chamber (Conviron CMP5090) set at 18°C with 65% relative humidity, providing a 12-hour photoperiod with fluorescent lighting from 7:00 AM to 7:00 PM, while nighttime conditions were maintained at 15°C with no light.

Temperature, humidity, and any signs of mold or desiccation were monitored every other day throughout the germination trial. Germination progress was tracked using a vigour scale from 0 to 5 (Table 1, Figure 5). The number of seed within each class was assessed during each assessment. Germination day was recorded as the number of days from the start of the trial (Day 0: February 14, 2024) to the first emergence of the

radicle for each seed. Observations continued daily until no further germination was observed, and the mean germination day was calculated by averaging the germination days for all seeds that successfully germinated within each replicate.

Table 1. Germination vigour classes used to assess *S. canadensis* seed germination over 48 days. Stages of seed germination ranging from no development (0) to full leaf formation (5), with each vigour class indicating specific growth milestones.

Vigour Class	Description
0	No vigour
1	Radicle emergence
2	Cotyledon emergence
3	Epicotyl present
4	First leaf
5	Two leaves

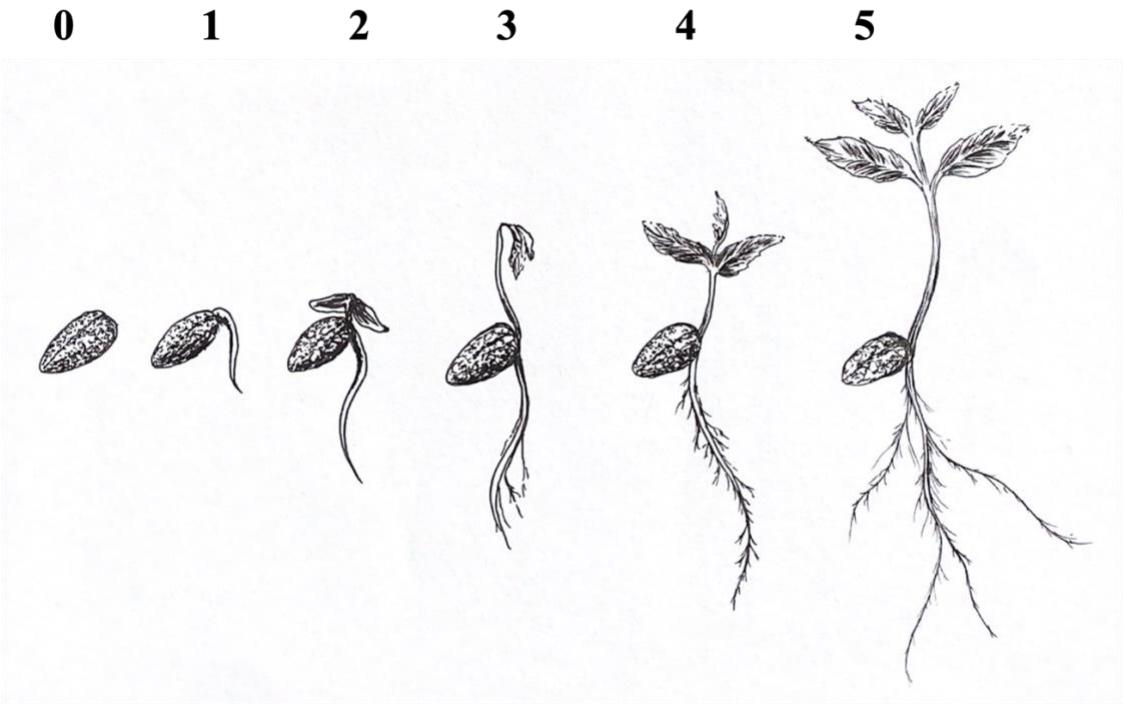

Figure 5. *S. canadensis* seed vigour classes. Illustration by Lena Beckley (2024) From left to right: 0 (no growth) to 5 (two leaves), with stages including radicle emergence (1), cotyledon emergence (2), epicotyl presence (3), cotyledon fully expanded (4), and first leaves (5).

2.4. HPLC-MS/MS: Analysis of Chlorogenic and Rutin Concentration in Berries

On April 15, 2024, berry samples were randomly selected from six plants at each of ten populations. From each plant, 220 grams of berries were measured, yielding a total of 60 samples across the ten sites. Each 220-gram sample was divided into two replicates for analysis, resulting in 120 total replicates. On the day of analysis, samples were thawed to room temperature and homogenized in liquid chromatography-mass spectrometry (LC-MS) grade methanol (OmniSolv; CAS-No: 67-56-1) at a 1:2 (w:v) ratio. Homogenization was performed using a Brinkmann PT 10/35 homogenizer (110 Volts, 6 Amps, 60 Hz; Brinkmann Instruments Co., Switzerland) until minimal solid pulp remained.

Following homogenization, we centrifuged a 1 mL aliquot for 10 minutes at 15,000 RPM (Model: 5425, Eppendorf). The resulting supernatant was then transferred to sanitized 1 mL microtubes and stored at 4°C until further analysis.

Sample Filtration and Injection:

Prior to injection, a 1 mL syringe fitted with a 0.2 μm membrane filter (VWR North America; Cat. No. 28145-491) was rinsed twice with LC-MS grade methanol. A 100 μL sample of the supernatant was then mixed with 100 μL of LC-MS grade methanol, filtered, and transferred into a 2 mL autosampler vial, which was capped (VWR North America; Cat. No. 46610-724A).

HPLC-MS/MS Analysis:

Rutin and chlorogenic acid levels were quantified at the University of New Brunswick's Department of Chemistry, using an Ultivo Triple Quadrupole LC-MS/MS

system (Agilent, CA, USA). The system featured a Poroshell 120 EC-C18 column (100 x 4.6 mm, particle size: 2.7 μm; Agilent) and a photodiode array detector covering a 190-640 nm range. Rutin and chlorogenic acid standards (prepared from 97% purity powders, CAS No: 472-61-7; Sigma Aldrich) were injected at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 μg/μL.

Mobile Phase and Elution Gradient:

The mobile phase consisted of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B). A linear elution gradient was applied, starting at a 1:3 ratio (A:B) from 0-5.6 minutes, shifting to 1:99 from 5.6-6 minutes, and returning to 1:3 from 6.01-8 minutes. The flow rate remained constant at 0.6 mL/min throughout the run.

2.5. Statistics

All statistical analyses were conducted in R (version 4.2.0). Data were checked for normality and homoscedasticity, with transformations applied as needed to meet assumptions of the statistical tests. 'Site' and 'Plant' were treated as categorical variables, with 'Plant' nested within 'Site' to address the hierarchical structure of the data. Environmental variables, including proximity to water bodies, slope, and aspect, were standardized to ensure comparability across sites.

To evaluate variation in root development, germination success, and phenolic concentrations (chlorogenic acid and rutin) in *S. canadensis* across sites, we performed Pearson correlation analyses and linear regression models. Pearson correlation coefficients were used to identify relationships between germination, root displacement

volume, and phenolic concentrations. Linear regression models assessed whether germination percentage predicted root volume displacement and whether chlorogenic acid concentrations predicted rutin concentration, based on their roles in seedling development and phenolic biosynthetic pathways (Lambers et al., 2008; Cheynier et al., 2013). Additional regressions investigated the effects of chlorogenic acid and rutin on germination success, as these compounds are linked to seed viability and stress response (Bailly et al., 2004; Lattanzio et al., 2006). Significance was evaluated at $p < 0.05$.

2.5.1. Softwood Cuttings

Root development was assessed by measuring root displacement volume in milliliters. To evaluate the influence of plant height and diameter on root displacement volume, a linear mixed-effects model (LMM) was employed using the lme4 and lmerTest packages. Random effects for 'Site' and 'Plant' accounted for variability across sites and individual plants. The model specification was as follows:

$$\text{RootDisplacement\_Volume.ml}_{ijk} = \beta_0 + \beta_1 \,\text{Height}_{ijk} + \beta_2 \text{Diameter}_{ijk} + u_i + v_{ij} + \varepsilon_{ijk}$$

In this equation, β_0 is the intercept; β_1 and β_2 are fixed-effect coefficients; $u_i$is the random effect of the *i*-th site; v_{ij} is the random effect of the *j*-th plant within the *i*-th site; and ε_{ijk} is the residual error. We used the lme4 package to fit the model and assessed the significance of fixed effects using Satterthwaite's approximation for degrees of freedom with the lmerTest package.

2.5.2. Germination

Seed germination percentage was calculated as the proportion of seeds that germinated (vigour rankings 1 through 5) over the total number of seeds tested. A generalized linear mixed model (GLMM) with a binomial distribution was fitted using the glmer function and lme4 package. Fixed effects included 'SeedWeight' and 'Row' (position in the growth chamber), while random effects included 'Site,' 'Plant,' and 'Tray' to account for hierarchical and spatial variability. Row was treated as a fixed effect to account for potential systematic variations within the growth chamber, such as light or temperature gradients, while tray was modeled as a random effect to capture unstructured noise between trays. The model specification was as follows:

$$\text{logit}(P_{ijk}) = \beta_0 + \beta_1 \text{SeedWeight.mg}_{ijk} + \beta_2 \text{Row}_{ijk} + u_i + v_{ij} + \omega_k$$

In this equation, P_{ijk} is the probability of germination; β_0, β_1, and β_2 are fixed-effect coefficients; u_i is the random effect of the *i*-th site; v_{ij} is the random effect of the *j*-th plant within the *i*-th site; and ω_k is the random effect of the *k*-th tray. Diagnostics for model fit and overdispersion were conducted using residual plots and other metrics provided by the performance package.

Row position was specifically tested for its effect on germination success to ensure that spatial positioning within the growth chamber did not influence results. The analysis indicated no significant differences in germination success among rows (Chamber_RowMR: \beta = -0.039, p = 0.591; Chamber_RowTR: \beta = -0.079, p = 0.283).

Additional GLMM analysis incorporated 'Mould.Percent', 'chlorogenic acid (Conc_Chloro.ng), rutin concentration (Conc_Rutin.ng), and seed weight as fixed effects,

with site as a random effect. Germination was modeled as a continuous variable using the glmer function from the lme4 package.

2.5.3. HPLC-MS/MS Analysis: Chlorogenic Acid and Rutin Concentrations

Differences in concentrations of chlorogenic acid and rutin were analyzed using, separate linear mixed-effects models fitted for each compound with the lme4 and lmerTest packages. Fixed effects included 'SeedWeight' and environmental variables, while 'Site' and 'Plant' were treated as random effects. The models for chlorogenic acid and rutin were as follows:

Chlorogenic acid:

$$\text{Conc\_Chloro.ng}_{ij} = \beta_0 + \beta_1 \text{SeedWeight.mg}_{ij} + \beta_2 \text{EnvironmentalVariables}_{ij} + u_i + v_{ij} + \varepsilon_{ij}$$

Rutin:

$$\text{Conc\_Rutin.ng}_{ij} = \beta_0 + \beta_1 \text{SeedWeight.mg}_{ij} + \beta_2 \text{EnvironmentalVariables}_{ij} + u_i + v_{ij} + \varepsilon_{ij}$$

In these equations, β_0, β_1, and β_2 are fixed-effect coefficients; 'EnvironmentalVariables' included standardized distance to water bodies, slope, and aspect; u_i is the random effect of the *i*-th site; v_{ij} is the random effect of the *j*-th plant within the *i*-th site; and ε_{ij} is the residual error.

2.5.4 Environmental Variable Analysis

Environmental variables were analyzed to assess site-specific factors influencing *S. canadensis* populations, focusing on hydrological, topographic, and soil characteristics. High-resolution spatial data from the Service New Brunswick Data Catalogue (2024) served as the foundation for these analyses, including water features, wetlands, and a 10 m resolution Digital Elevation Model (DEM). Environmental metrics were derived using ArcGIS Pro (version 3.4), with additional data preparation and visualization performed in R (version 4.2.0) using ggplot2.

Proximity to water bodies, wetlands, and watercourses was calculated using tools in ArcGIS Pro. The Euclidean Distance tool was applied to polygonal features (e.g., water bodies and wetlands), measuring the straight-line distance from each sampling site to the nearest feature, with all measurements derived from the 10 m resolution DEM. For linear features such as watercourses, the Near Tool was used to determine the shortest distance between sampling sites and the nearest segment of the watercourse. These variables were included to assess hydrological influences, as water availability can significantly affect plant growth and survival.

Moisture availability at each site was estimated using the Topographic Wetness Index (TWI). TWI combines two components: the upslope area contributing to surface runoff and the local slope at a given location. Higher TWI values indicate areas with greater potential for soil saturation and moisture retention. To derive TWI, the 10 m resolution DEM was used to calculate flow accumulation (representing the upslope contributing area) and slope, which were then combined using the formula:

$$\text{TWI} = \ln\left(\frac{\text{Flow Accumulation}}{\tan(\text{Slope})}\right)$$

Additional site characteristics included slope and aspect, both derived from the DEM. Slope represents the steepness of the terrain, influencing factors like soil stability and drainage, while aspect indicates the direction a slope faces, affecting exposure to sunlight and microclimatic conditions.

Soil data were integrated as a raster dataset containing categorical information about soil types across the study area. GPS coordinate points for sampling sites were mapped to corresponding raster cells, with each site assigned the soil type of the cell it intersected. This approach allowed the integration of localized soil characteristics into the environmental analysis. Soil types were categorized using unique soil codes that encapsulate key attributes such as texture, drainage, and fertility. These original soil type classifications, which inherently account for the combination of these attributes, were used in the analysis without deriving new soil types.

All environmental variables were standardized using z-score standardization, a common method in statistical analysis that involves subtracting the mean and dividing by the standard deviation across all sites. This process ensured that variables were on a consistent scale, facilitating comparability across sites and compatibility with subsequent multivariate analyses such as Principal Component Analysis (PCA) and regression models. By deriving and standardizing these variables, this study established a robust framework for assessing environmental influences on *S. canadensis* populations.

2.5.5. Principal Component Analysis and Multivariate Analysis

Principal Component Analysis (PCA) was conducted using the psych and ggbiplot packages to simplify data and summarize relationships among multiple traits, including germination percentage, root displacement volume, seed weight, and phenolic

concentrations (chlorogenic acid and rutin). PCA reduces dimensionality by transforming the original set of variables into a smaller number of uncorrelated components, capturing most of the variation present in the data. This approach identifies underlying patterns while minimizing redundancy among highly correlated variables. Prior to PCA, the degree of correlation among traits was assessed using variance inflation factor (VIF) scores and Pearson correlation coefficients. This preliminary analysis confirmed that multicollinearity was within acceptable thresholds, justifying the inclusion of all traits in the PCA.

All traits were standardized to ensure equal weighting across variables with different measurement units. Standardization involved subtracting the mean and dividing by the standard deviation for each variable, transforming them into a common scale with a mean of 0 and a standard deviation of 1.

PCA was performed on the correlation matrix to account for differences in measurement scales. The number of retained components was determined using scree plots and the Kaiser criterion, selecting components with eigenvalues greater than 1. Variable loadings on each principal component were examined to interpret trait contributions to observed patterns.

Results from the PCA informed hierarchical clustering to group sites and plants based on trait and environmental similarities. These clusters were analyzed using t-tests with Bonferroni corrections and Analysis of Variance (ANOVA) with Tukey's HSD post-hoc tests to compare trait means across groupings. Hierarchical clustering for grouping traits was conducted using the cluster, dendextend, and ggplot packages.

2.5.6. Mixed-Effects Models

Linear mixed-effects models (LMMs) were implemented using the lme4 package to fit the LMMs and lmerTest package to calculate p-values for fixed effects, facilitating hypothesis testing within the mixed-effects framework. These packages allowed for the evaluation of the effects of environmental variables on seed germination, root development, and phenolic concentrations. Fixed effects included distance to roads (Dist_Roads.m) and soil type (Soil_code), while random intercepts were assigned to 'Site' to account for location-specific variation. Germination percentage, root displacement volume, and chlorogenic acid concentration served as dependent variables. Model diagnostics, including assessments for fit, rank deficiencies, and boundary conditions, ensured robustness and reliability.

2.5.7. Within-Site and Across-Site Variation Analysis

To investigate genetic and microenvironmental differences within sites, nested linear mixed models (LMMs) were used, focusing on germination percentage, root displacement volume, and phenolic concentrations (chlorogenic acid and rutin). In these models, 'Plant' was nested within 'Site' to partition variance associated with these traits and assess the influence of site-specific factors. This approach aimed to capture genetic variation within sites while acknowledging that environmental heterogeneity could limit the ability to detect clear genetic signals.

Across-site variation was evaluated by integrating environmental and site-level effects on the same traits. A linear mixed-effects model was applied with 'Site' as a random effect to account for spatial variability among locations. Fixed effects included root displacement volume, chlorogenic acid concentration, rutin concentration, distance

to roads, distance to wetlands, and soil type. This dual-scale modeling approach allowed for the identification of overarching trends in trait-environment interactions across populations while capturing localized site-specific patterns.

We recognize that site-level environmental factors could confound the detection of genetic differences, particularly for traits like germination percentage, which are strongly influenced by maternal and environmental effects. Controlled propagation conditions were used to reduce environmental variability during experiments, but the potential for residual maternal effects was acknowledged. Despite these limitations, the nested LMM approach provided a framework for exploring variation within and among sites while accounting for environmental influences.

3. Results

Results are presented across three distinct biogeographic zones in New Brunswick, representing inland sites (1, 2, 3, 8), Bay of Fundy coastal sites (5, 9, 10), and Gulf of St. Lawrence coastal sites (4, 6, 7), to highlight spatial variability in the rooting of softwood cuttings, seed germination, and berry phenolics.

3.1. Softwood Cuttings

Root displacement volume (RDV), a measurement of root development, showed minor variation across the study sites, with means ranging from 2.54 mL at Site 10 to 3.65 mL at Site 1 (Table 2). Tukey's HSD tests identified Site 1 as having significantly higher RDV than Site 10 ($p < 0.05$). However, most sites did not exhibit statistically significant differences (Figure 6). Mean plant height ranged from 18.60 cm (Site 8) to 20.87 cm (Site 9), while mean stem diameter was consistently clustered between 0.98 cm (Sites 8 and 9) and 1.02 cm (Sites 7 and 10).

Table 2. Mean root displacement volume (mL), height (cm), and diameter (cm) across study sites, with Tukey's HSD groupings ($\alpha = 0.05$).

Site	Mean Root Displacement (mL)	Mean Height (cm)	Mean Diameter (cm)
1	3.65 [a]	19.60 [c]	1.01 [d]
5	3.24 [ab]	19.85 [c]	1.01 [d]
4	3.22 [ab]	19.92 [c]	1.01 [d]
2	3.20 [ab]	19.06 [c]	1.01 [d]
3	3.12 [ab]	20.02 [c]	1.00 [d]
8	2.98 [ab]	18.60 [c]	0.98 [d]
9	2.98 [ab]	20.87 [c]	0.98 [d]
6	2.80 [ab]	20.19 [c]	1.00 [d]
7	2.70 [ab]	20.31 [c]	1.02 [d]
10	2.54 [b]	19.29 [c]	1.02 [d]

Root displacement volume demonstrated higher within-site variability at Sites 6 and 10, with ranges extending from 0.5 to 7.5 mL and 0.3 to 6.9 mL, respectively. In contrast, plant height and stem diameter exhibited narrower ranges, suggesting less variability. For example, plant height at Site 9 spanned 17–25 cm, while stem diameter at Site 10 ranged from 0.8–1.2 cm (Figure 6). ANOVA results confirmed the lack of significant differences across sites for RDV ($F = 1.65$, $p = 0.10$), plant height ($F = 1.14$, $p = 0.34$), and stem diameter ($F = 1.08$, $p = 0.38$) (Table 3).

Table 3. ANOVA results for the effects of site on plant height, stem diameter, and root displacement volume.

Trait	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Height (cm)	Sites	9	233.69	25.97	1.14	0.34
Diameter (cm)	Sites	9	0.10	0.01	1.08	0.38
Root Displacement (mL)	Sites	9	54.17	6.02	1.65	0.10

[1] *Degrees of freedom (Df), Sum of Squares (Sum Sq), Mean Squares (Mean Sq), F-value (F Value), and p-value (Pr(>F)).*

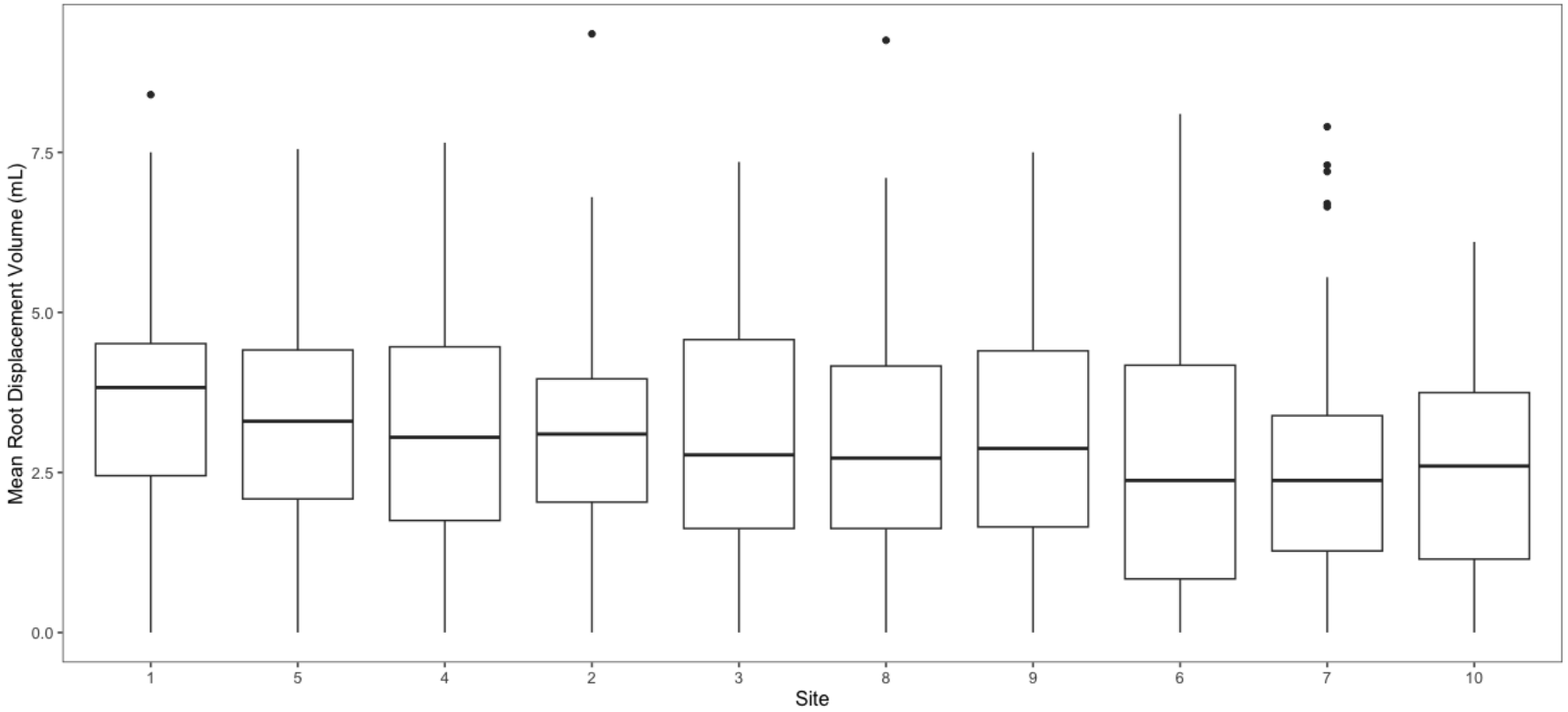

Figure 6. Distribution of root displacement volume across study sites (*n = 60 per site*). Boxplot of root displacement volume measurements for *S. canadensis* cuttings collected from 6 plants per site, with 10 cuttings per plant, across ten study sites. The figure shows median values, interquartile ranges, and variability across sites.

Root displacement volume (RDV) and plant height showed a wide distribution of data points without a consistent pattern across all sites (Figure 7). Similarly, the scatter plot of RDV and stem diameter displayed dispersed points with no apparent clustering or alignment (Figure 8). The scatter plot of plant height and stem diameter also revealed a broad and scattered distribution of points across sites (Figure 9).

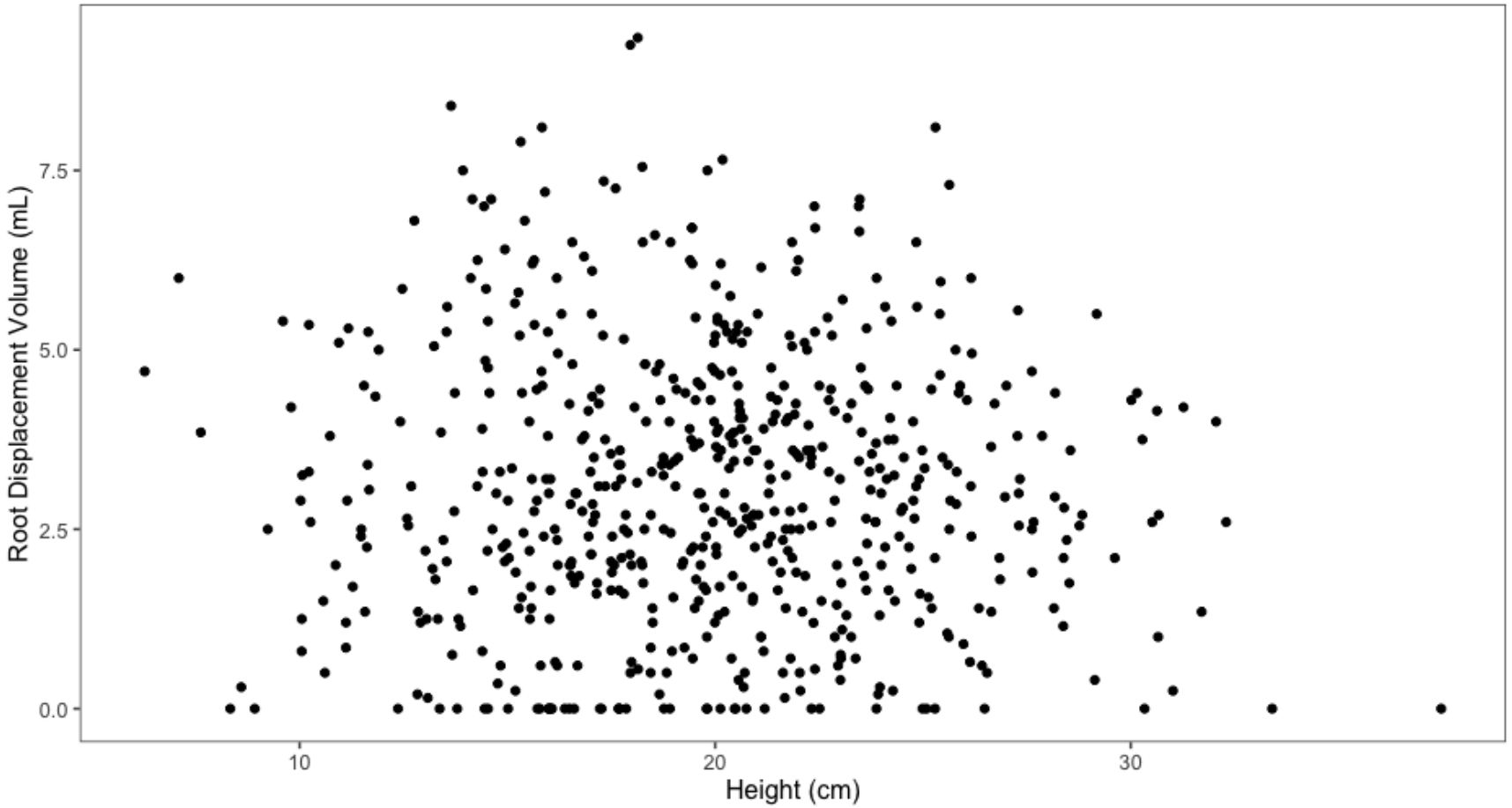

Figure 7. Relationship between plant height and root displacement volume across all study sites.

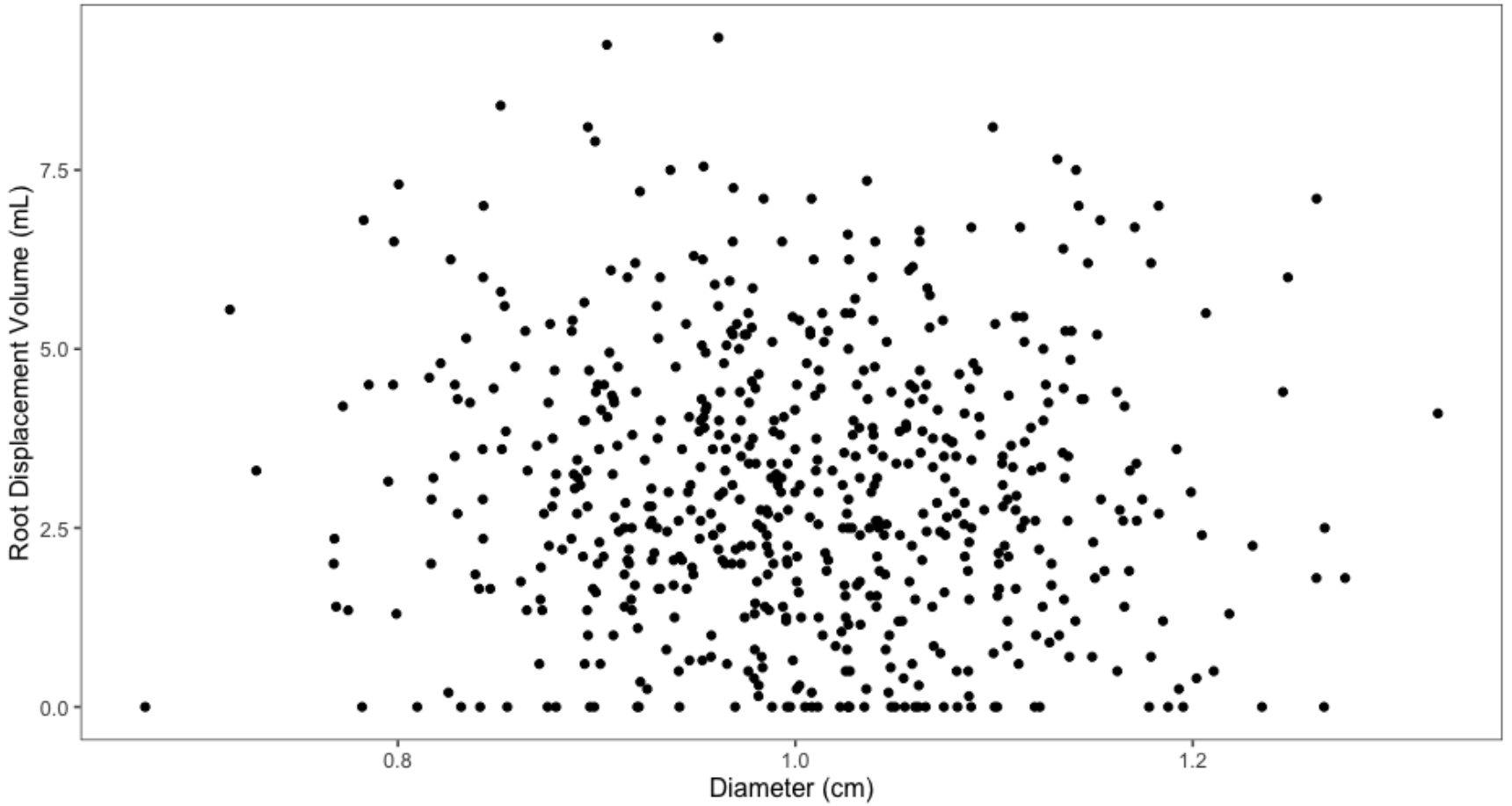

Figure 8. Relationship between stem diameter and root displacement volume across all study sites.

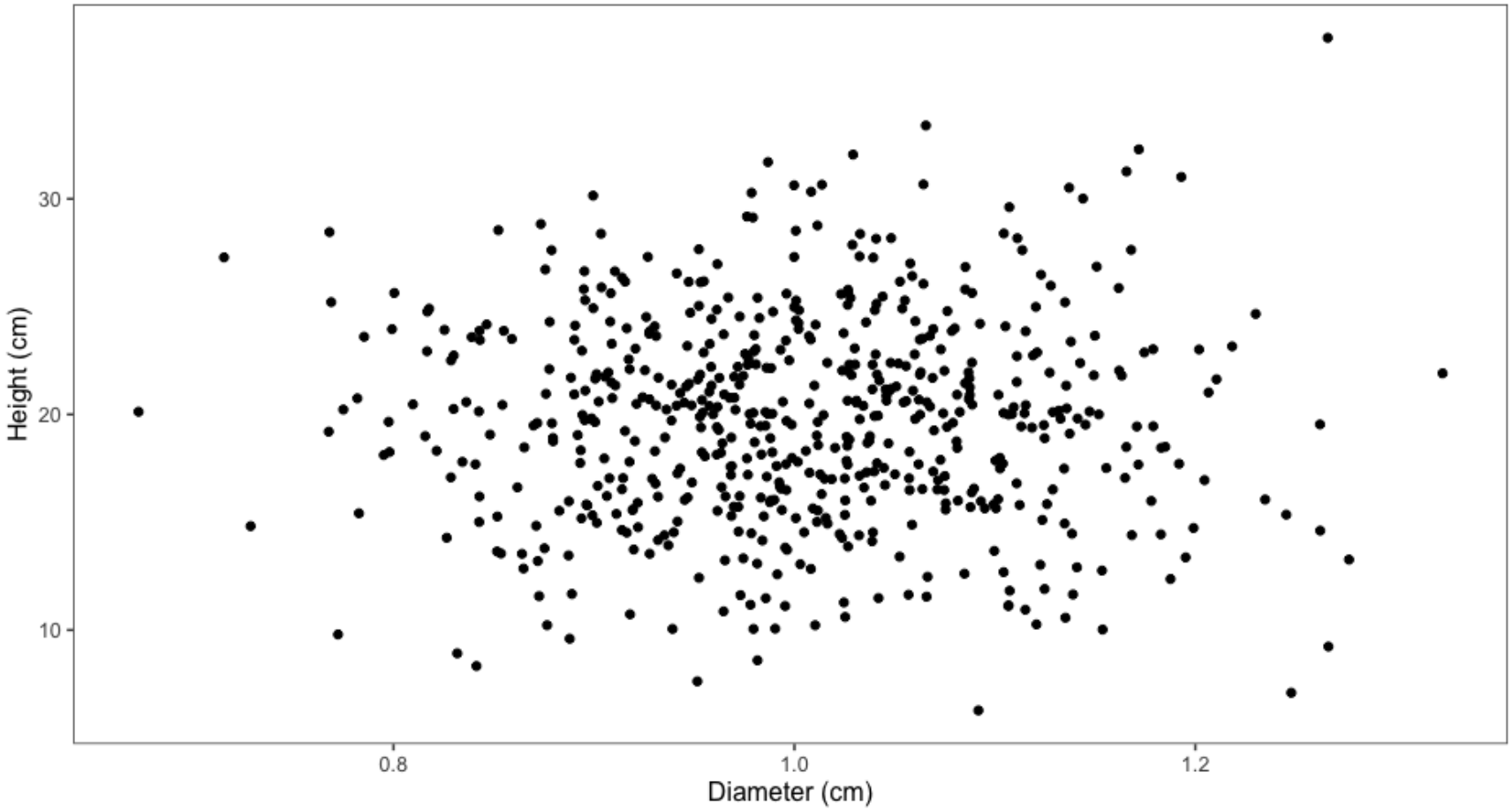

Figure 9. Relationship between plant height and stem diameter across all study sites.

The PCA was conducted to reduce the dimensionality of the dataset and summarize the variation across environmental variables and plant traits. Nine principal components (PCs) were identified, collectively explaining 76.2% of the total variance in the dataset (Figure 10). The first five PCs accounted for the majority of the variation (76.2%), with PCs 6 through 9 contributing finer-scale variation.

PC1 explained 28% of the total variation, driven by environmental variables, including negative contributions from distance to roads (Dist_Roads.m: -0.445) and positive contributions from distances to waterline (Dist_WL.m: 0.544), watercourse (Dist_WC.m: 0.493), and water body (Dist_WB.m: 0.364). PC2 accounted for 14.5% of the variation, influenced by aspect (Aspect: -0.561), distance to roads (Dist_Roads.m: 0.426), and distance to watercourses (Dist_WC.m: 0.415).

Plant-specific traits were the primary contributors to PCs 3, 4, and 5, which together explained 33.9% of the variation. PC3 (11.8%) included contributions from root displacement volume (RootDisplacement_Volume.ml: 0.520), height (Height.cm: -0.708), and diameter (Diameter.cm: -0.352). PC4 (11.3%) included diameter (Diameter.cm: 0.695), height (Height.cm: -0.503), and root displacement volume (RootDisplacement_Volume.ml: -0.368). PC5 (10.8%) included root displacement volume (RootDisplacement_Volume.ml: -0.671), diameter (Diameter.cm: -0.552), and slope (-0.329).

Environmental variables dominated PCs 1, 2, 6, and 7, while plant traits contributed most strongly to PCs 3, 4, and 5. These PCs collectively describe the patterns of variance among traits and environmental gradients across study sites.

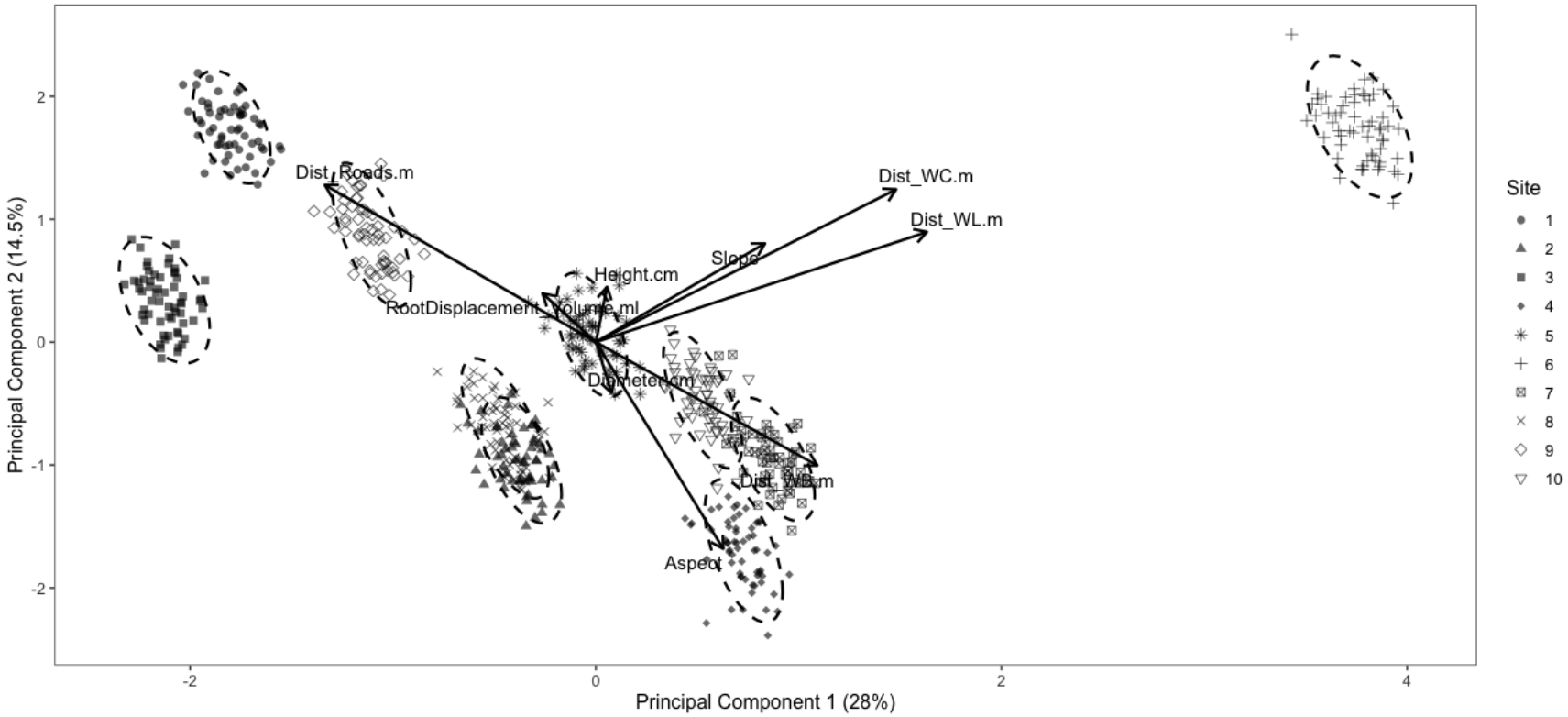

Figure 10. Principal component analysis (PCA) biplot of root development traits and environmental variables. The biplot includes environmental variables (e.g., proximity to water bodies, soil type), showing distinct clustering for Sites 1 and 10. Root displacement volume contributed most strongly to PC1, while environmental gradients influenced variation along PC2. Ellipses represent 95% confidence intervals for site clustering.

3.2 Germination

Total germination percentages varied across sites, with notable differences in variability and mean values (Table 4). Site 7 exhibited the highest mean germination percentage (77.70%) and a standard deviation of 21.09%, indicating relatively consistent germination success among sampled plants. In contrast, Site 4 had the lowest mean germination percentage (59.40%) and the highest variability, with a standard deviation of 36.48%. Similarly, Site 6 recorded a low mean germination percentage (63.29%) with a high variability (34.90%).

Sites 5 and 8 displayed moderate variability, with standard deviations of 32.50% and 26.46%, respectively, and comparable mean germination percentages of 67.57% and 66.40%. These sites demonstrated greater variability among plants within the site compared to Sites 1 and 2, which had lower standard deviations of 25.42% and 24.74%, respectively. Interestingly, Site 10 displayed slightly lower variability (25.41%) and achieved a mean germination percentage of 72.96%.

The mean germination day was relatively consistent across most sites, ranging from 30 to 32 days. However, Sites 9 and 10 showed significantly earlier germination, with mean germination days of 18 and 19, respectively, suggesting faster germination in these populations.

Minimum germination percentages across sites were consistently low, reflecting the inclusion of plants with near-zero germination success. In contrast, the maximum germination percentages across sites remained high (96–99%), indicating that some plants at each site achieved nearly full germination. This pattern highlights substantial

within-site variability in germination success, particularly at Sites 4, 6, and 9, where higher standard deviations were observed.

Table 4. Summary of germination rates and mean germination days across study sites, including significant differences ($p < 0.05$) based on Tukey's HSD test.

Site	Std Dev	Mean Germination (%)	Min Germination (%)	Max Germination (%)	Mean Germination (Day)
7	21.09	77.70 [a]	0	99.16	30
9	20.23	74.37 [ab]	0	99.37	18
1	25.42	73.44 [abc]	0	99.37	32
2	24.74	73.16 [abc]	0	98.36	30
10	25.41	72.96 [abc]	0	99.72	19
3	29.49	71.18 [bc]	0	97.95	30
5	32.50	67.57 [cd]	0	96.20	31
8	26.46	66.40 [cd]	0	96.48	31
6	34.90	63.29 [de]	0	99.09	32
4	36.48	59.40 [e]	0	96.40	27

Cumulative germination counts increased across all sites, with sites reaching varying plateaus by the end of the trial (Figures 11 and 12). Sites 7, 9, and 1 exhibited higher cumulative germination percentages, plateauing at 77.70%, 74.37%, and 73.44%, respectively. Sites 5 and 8 showed slightly lower cumulative germination percentages, plateauing at 67.57% and 66.40%, respectively. Germination speed also varied, with Sites 7 and 9 achieving 77.70% and 74.37% germination by 30 days, whereas Sites 5 and 8 required over 40 days to approach their plateaus.

The total germinants observed in Vigour Classes 3 and 4 (Figures 11 and 12) varied across sites, with notable differences in patterns of increase and final totals by day 44. Sites 1 and 7 exhibited the highest germination totals for both vigour classes, with Vigour Class 4 reaching approximately 140 germinants and Vigour Class 3 achieving around 130 germinants. These sites displayed the steepest rates of increase between days 25–40, followed by a plateau toward day 44.

Sites 9 and 10 showed moderate germination totals, with Vigour Class 4 reaching approximately 120 germinants and Vigour Class 3 reaching around 110 germinants. The rates of increase for these sites were steady and continuous, with no sharp plateaus by day 44. Similarly, Site 6 demonstrated moderate germination totals of approximately 110 and 100 germinants for Vigour Classes 4 and 3, respectively, and exhibited consistent increases through the observation period.

In contrast, Sites 4 and 8 had the lowest germination totals, with Vigour Class 4 reaching approximately 70 and 90 germinants, respectively, and Vigour Class 3 achieving approximately 60 and 80 germinants. These sites displayed flatter slopes, indicating slower rates of increase throughout the observation period. Site 3 also

demonstrated lower germination totals, with Vigour Class 4 reaching approximately 100 germinants and Vigour Class 3 achieving around 90 germinants, accompanied by slower rates of increase after day 35.

Across all sites, Vigour Class 4 consistently showed higher totals and slightly faster rates of increase compared to Vigour Class 3. The rates of germinant accumulation were steepest for Sites 1, 7, and 9, while Sites 4 and 8 showed markedly slower rates of increase and lower final totals by day 44. These patterns highlight variability in germination dynamics across sites and vigour classes.

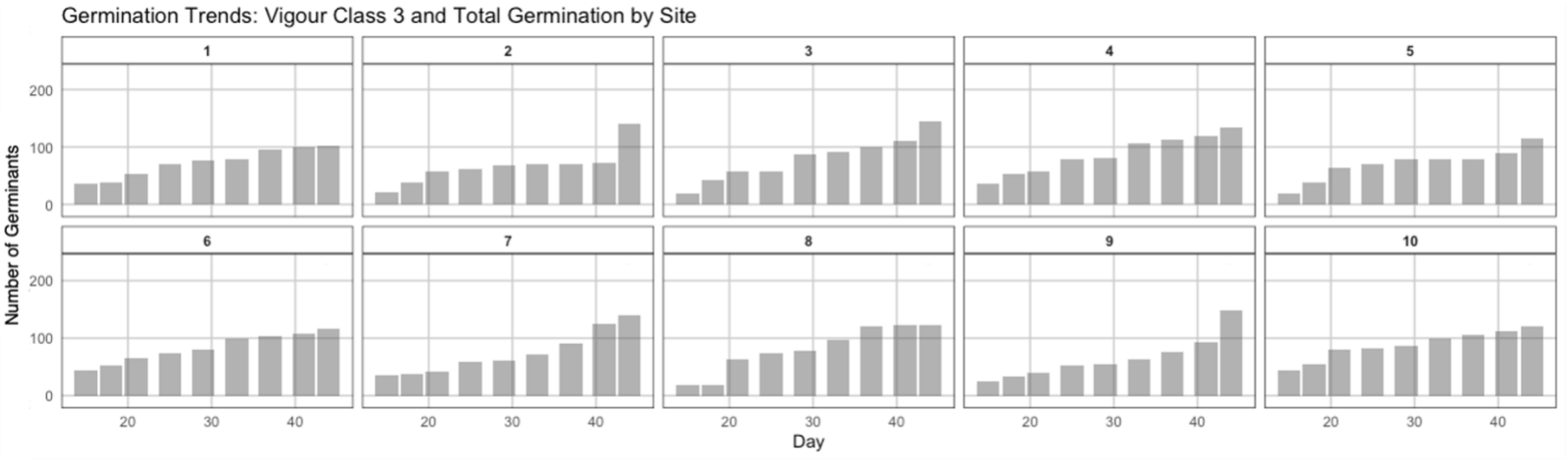

Figure 11 Total number of vigour 3 ranked germinants over time by site.

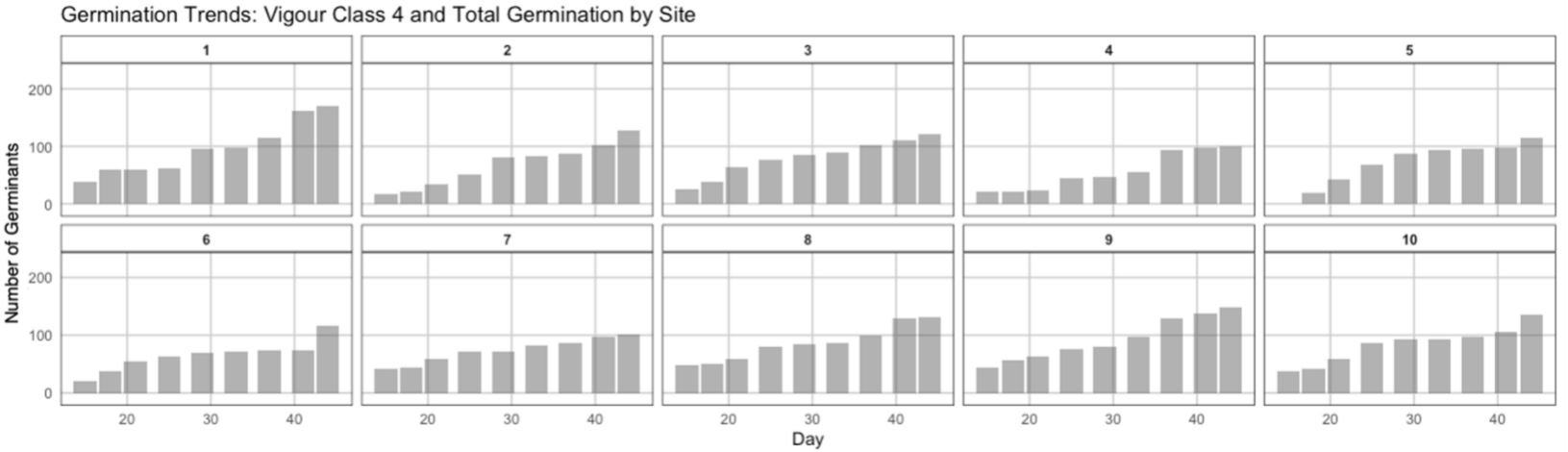

Figure 12. Total number of vigour 4 ranked germinants over time by site.

Seed weight varied significantly among sites ($F_{9,45} = 8.86$, $p < 0.001$), while no significant variation was observed among plants within sites ($F_{5,45} = 0.45$, $p = 0.808$). Seed weight showed no significant effect on germination success ($\beta = -0.0082$, $p > 0.05$). Confidence intervals for the estimated effects of seed weight and row position both overlapped with 0, indicating no significant influence of these predictors on germination success (Figure 13).

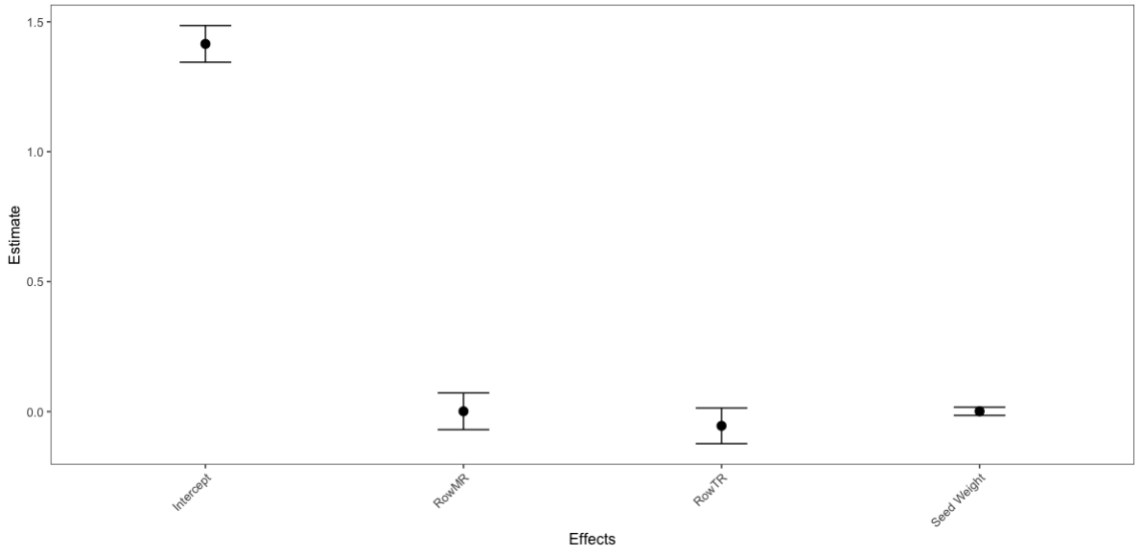

Figure 13. Effects of row position and seed weight on germination rates. Error bar plots visualize estimated effects. F or row positions, germination rates were measured from 20 trays per row, each containing 100 seeds, resulting in 2,000 seeds assessed per row (BR, n = 2000 seeds; TR, N = 2000 seeds; TR, n = 2000). For seed weight, the effects are based on ten random samples of 100 seeds each, representing population-level averages. Error bars represent 95% confidence intervals.

The inclusion of Vigour Ranks 3 and 4 in the analysis aimed to explore potential differences in post-germination outcomes among seeds. However, these ranks did not significantly influence the analysis results. The GLMM revealed no significant effect of Vigour Rank on germination success (Estimate = 0.001, Std. Error = 0.008, z-value = 0.148, p = 0.882), and the row effects remained non-significant (Table 5).

Table 5. Summary of fixed effects from generalized linear mixed model on germination success, including chamber row and vigour rank

Effect	Estimate	SE	z-value	p-value
Intercept	1.415	0.036	39.578	<2e-16
Chamber Row MR	0.001	0.036	0.014	0.989
Chamber Row TR	-0.055	0.035	-1.585	0.113
Vigour Rank	0.001	0.008	0.148	0.882

Principal component analysis (PCA) of germination traits, plant vigour, and environmental variables provided insights into the underlying patterns of variability across sites (Figure 14). A total of eight principal components explained 76.2% of the total variation, with the first two PCs accounting for 47.42%. PC1, contributing 31.23% of the variation, was driven primarily by environmental variables, such as distances to waterline (-0.546), watercourse (-0.496), and water body (-0.366), which were negatively associated with proximity to these features. Distance to roads (0.448) positively influenced PC1, while topographic variables like slope (-0.270) and aspect (-0.215) had minor contributions. Germination percentage contributed minimally (0.014).

PC2, explaining 16.19% of the variance, emphasized spatial orientation, with aspect (-0.556) and distance to roads (0.445) playing dominant roles. Slope (0.299) also

contributed positively. Like PC1, germination traits, such as germination percentage (-0.016), had negligible contributions to this axis.

PC3, accounting for 12.84% of the variance, highlighted germination percentage (0.834) as the most significant contributor, contrasting with plant vigour represented by Vigour Rank (-0.352). This component isolated the relationship between germination and plant vigour, with minor contributions from environmental and topographic variables. Figure 14 demonstrates site clustering along PC1 and PC2. Site 1 (purple cluster) is positioned in the top right quadrant, while Site 10 (yellow cluster) is located closer to the center-left, reflecting differences in environmental and germination traits.

PC4 (12.53% of the variance) was dominated by plant vigour (-0.898), while PC5 (10.78%) reflected a mix of environmental and germination traits, driven by slope (0.571), distance to water bodies (0.459), and germination percentage (0.349). PCs 6 through 8 captured finer-scale variations in topography and environmental distances, with negligible contributions from germination traits.

Environmental distances and topographic variables were the primary drivers of variability, dominating PCs 1, 2, 5, 6, 7, and 8. Germination percentage had a significant influence on PC3, while plant vigour dominated PC4. Combined, the first two PCs explained nearly half of the total variation, reflecting the dominance of environmental variables. Germination and vigour-related traits contributed to subsequent PCs but were less impactful overall.

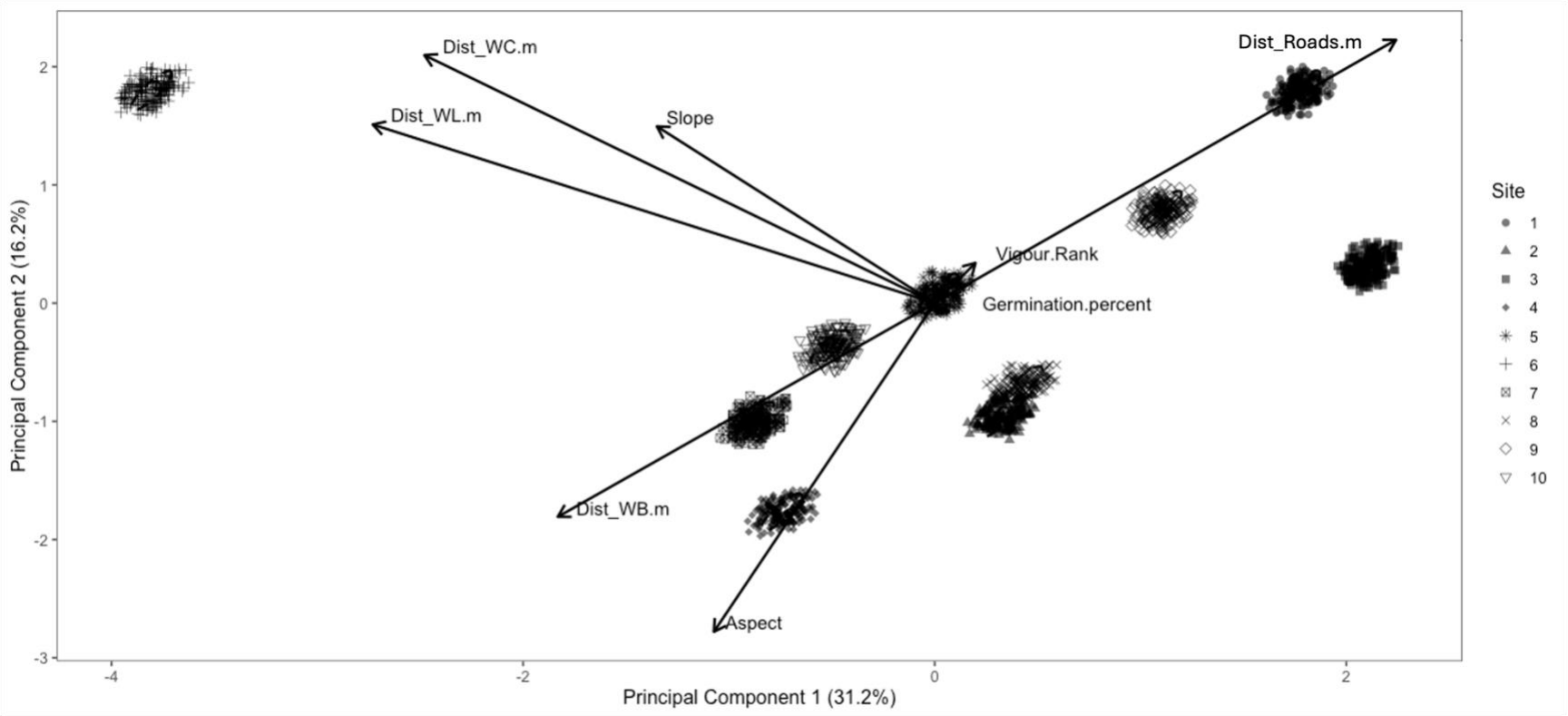

Figure 14. PCA biplot of principal components for *S. canadensis* germination rates and environmental variable analysis. The biplot illustrates site clustering and relationships among germination traits (e.g., germination percentage, seed weight) and environmental variables (e.g., proximity to water bodies, slope, aspect) along Principal Components 1 (31.8%) and 2 (16.0%). Vectors represent the contributions of traits and environmental variables to the principal components, while ellipses depict 95% confidence intervals for each site. The site gradient (color intensity) reflects site-specific contributions to the principal components.

3.3. HPLC MS-MS: Chlorogenic Acid and Rutin Concentration in Berries

Chlorogenic acid and rutin concentrations varied significantly across sites (Table 6). The highest mean chlorogenic acid concentration was recorded at Site 4 (1825 ng), while the lowest concentration was observed at Site 8 (487 ng). Rutin concentrations were also highest at Site 5 (2404 ng) and lowest at Site 6 (884 ng). Within-site variability was noted, with Site 5 displaying the greatest heterogeneity in rutin concentrations, as indicated by a broad interquartile range. Conversely, Site 9 exhibited the smallest variability for both compounds (Figures 15 and 16).

Significant within-site variation in chlorogenic acid concentrations was detected at Site 3 ($F = 25.19$, $p < 0.05$), Site 4 ($F = 18.55$, $p = 0.02$), and Site 5 ($F = 20.24$, $p < 0.05$). A near-significant trend was observed at Site 6 ($F = 4.27$, $p = 0.05$) (Table 7). For rutin concentrations, significant within-site differences were identified at Site 3 ($F = 23.24$, $p < 0.05$), Site 4 ($F = 9.53$, $p = 0.05$), and Site 5 ($F = 13.72$, $p < 0.05$), with a marginally significant trend at Site 10 ($F = 4.96$, $p = 0.08$).

Across sites, chlorogenic acid concentrations ranged from 487 ng at Site 8 to 1825 ng at Site 4, with moderate variability reflected in standard errors. Rutin concentrations displayed a broader range, from 884 ng at Site 6 to 2404 ng at Site 5. Despite these variations, ANOVA results indicated significant within-site differences at several sites for both chlorogenic acid and rutin concentrations.

Table 6. Mean phenolic concentrations of chlorogenic acid and rutin (ng) in *S. canadensis* berries from ten sites presented in descending order of chlorogenic acid levels.

Site	Chlorogenic Acid (ng)	Rutin (ng)
4	1825	1863
7	1500	2198
5	1280	2404
2	648	1127
3	634	1579
10	611	1506
9	582	1303
6	576	884
1	570	1050
8	487	1584

Table 7. ANOVA results for phenolic concentrations within sites.

Site	F-value (Chlorogenic Acid)	p-value (Chlorogenic Acid)	F-value (Rutin)	p-value (Rutin)
1	7.61	0.02	1.78	0.37
2	0.22	0.94	2.50	0.17
3	25.19	<0.05	23.24	<0.05
4	18.55	0.02	9.53	0.05
5	20.24	<0.05	13.72	<0.05
6	4.27	0.05	6.31	0.02
7	3.38	0.09	0.75	0.62
8	0.89	0.50	1.03	0.46
9	1.67	0.33	0.19	0.83
10	1.40	0.37	4.96	0.08

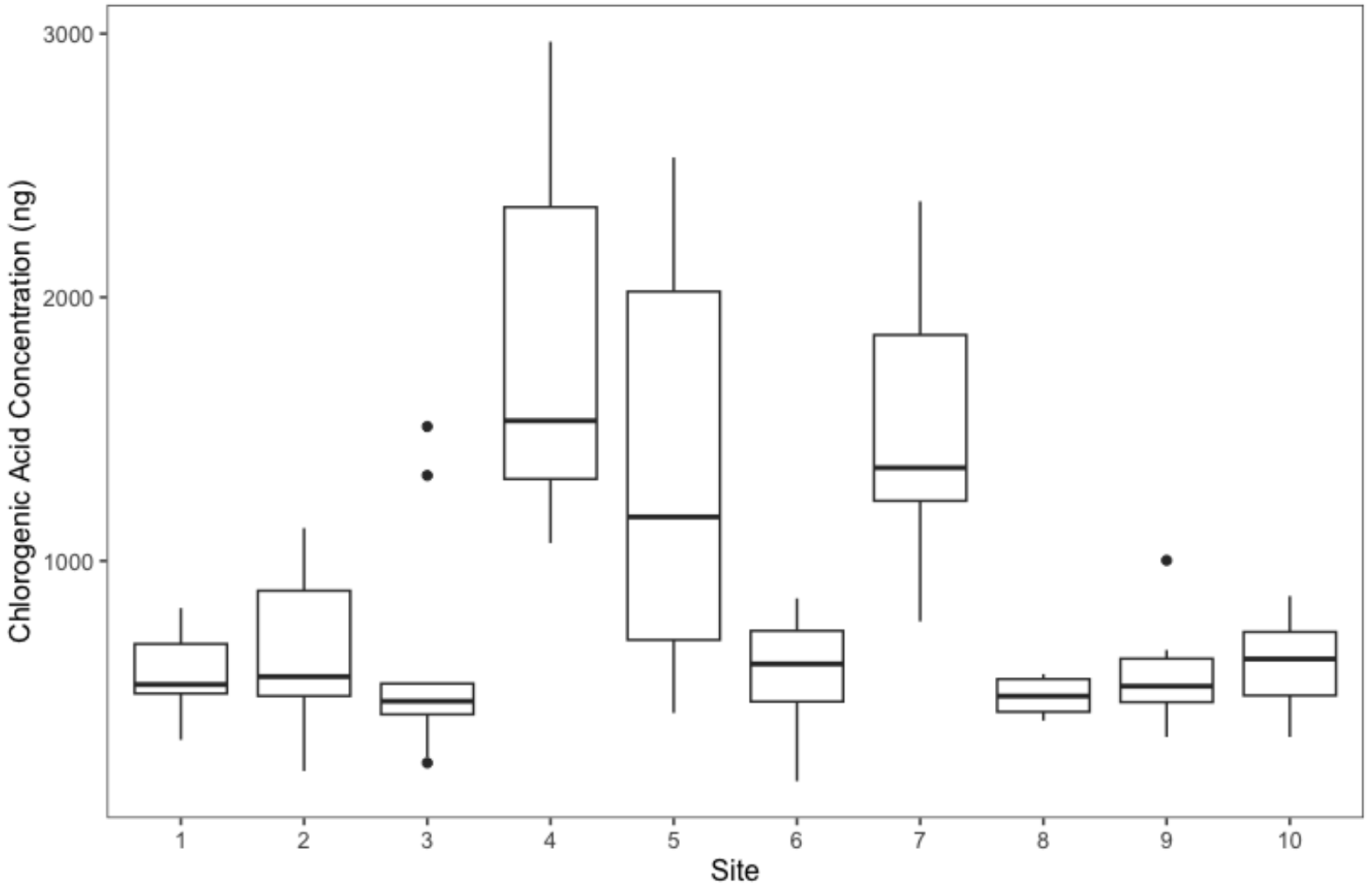

Figure 15. Site-specific variation in chlorogenic acid concentration in *S. canadensis* berries. Boxplots depict site-specific variability with interquartile ranges, median values, and outliers.

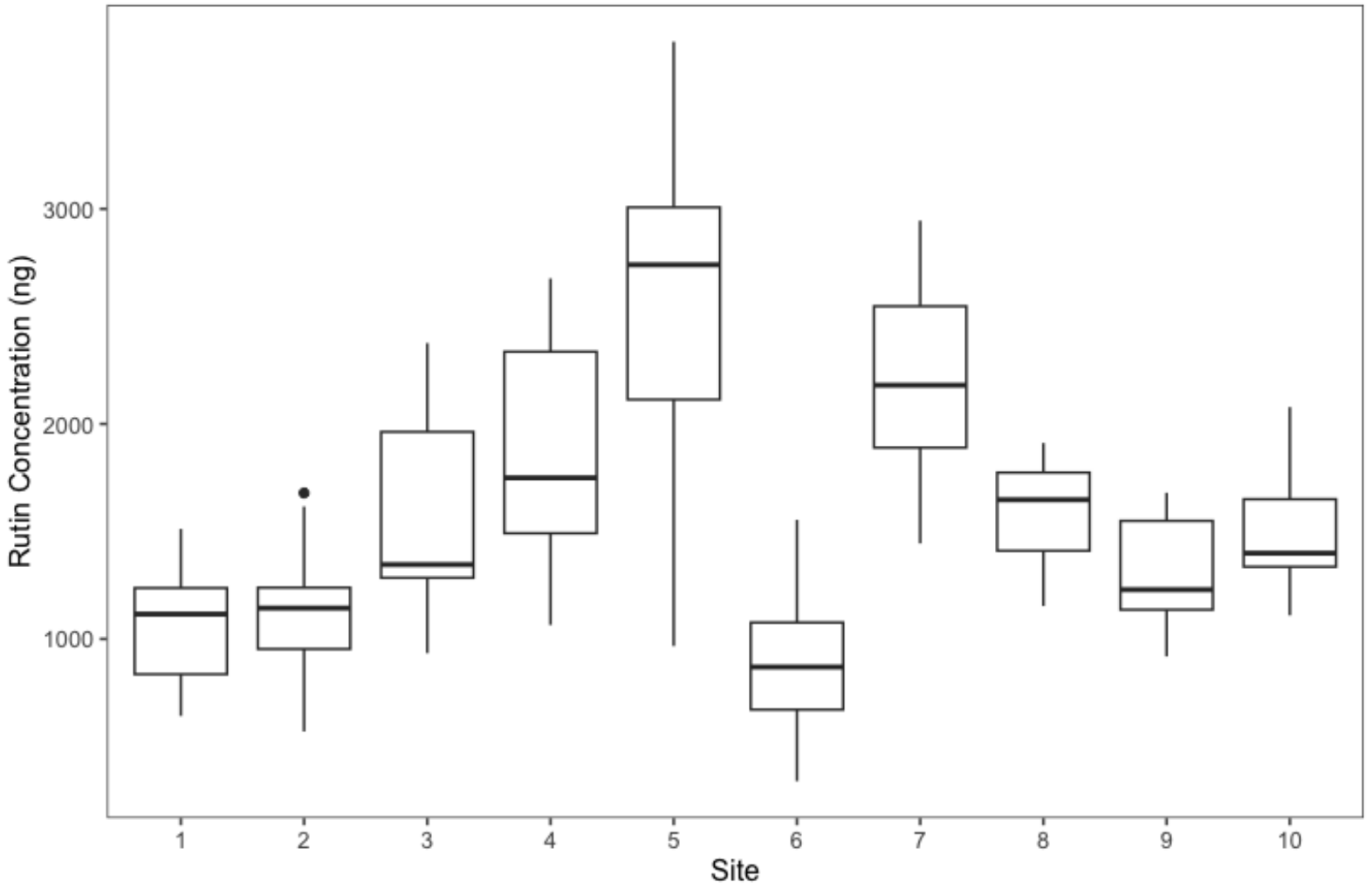

Figure 16. Site-specific variation in rutin concentration in *S. canadensis* berries. Boxplots display interquartile ranges, medians, and outliers for rutin concentrations across sites.

A significant positive relationship between chlorogenic acid and rutin concentrations was identified ($R^2 = 0.518$, $p < 0.001$) (Figure 17). The regression line demonstrated that increases in chlorogenic acid concentrations were consistently associated with higher rutin concentrations across all sites. Chlorogenic acid concentrations tended to be higher than rutin concentrations across most sites, but both compounds showed a pattern of co-variation.

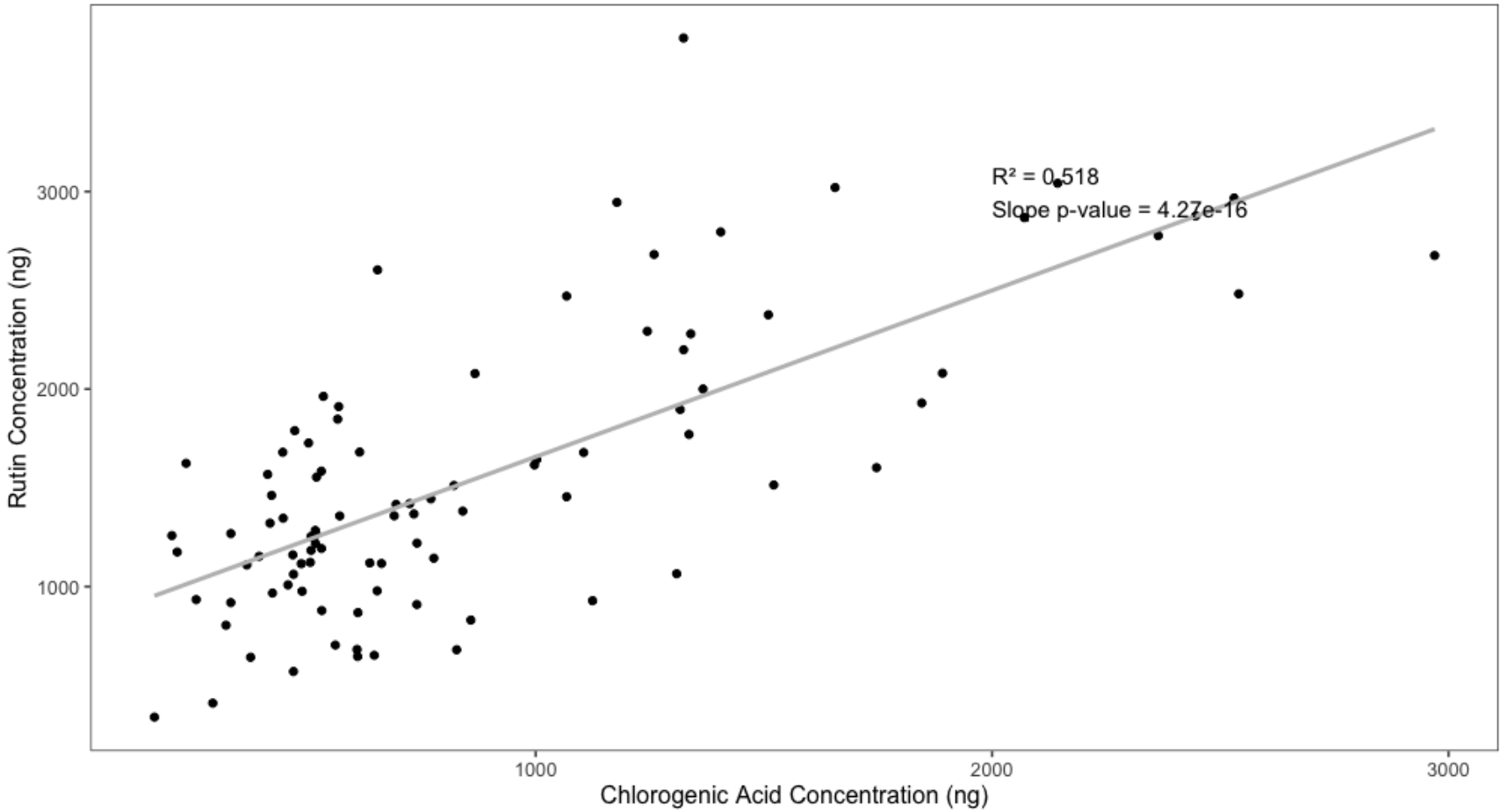

Figure 17. Linear relationship between chlorogenic acid and rutin concentrations in *S. canadensis* berries across ten sites. The regression line ($R^2 = 0.518$, $p < 0.001$) highlights a strong positive correlation.

When assessing environmental factors, proximity to water features, roads, slope, and aspect did not reveal significant direct associations with chlorogenic acid or rutin concentrations based on ANOVA results (Table 8). However, subtle site-specific differences were captured through multivariate analyses.

Table 8. ANOVA results for phenolic concentration variation across sites.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sites	9	150.8	16.756	3.20	0.001
Residuals	238	1250.6	5.250		

Note: Degrees of freedom (Df), Sum of Squares (Sum Sq), Mean Squares (Mean Sq), F-value (F Value), and p-value (Pr(>F)).

The PCA revealed that variation in phenolic concentrations and environmental variables across sites was predominantly explained by the first two principal components (PCs), which together accounted for 62.14% of the total variance (Figure 18). PC1, explaining 32.91% of the variation, was primarily driven by positive contributions from chlorogenic acid concentration (loading = 0.88) and environmental distances such as distance to waterline (2.55), watercourse (2.32), and water body (2.13). In contrast, distance to roads (-2.39) showed a strong negative contribution. These results suggest that PC1 reflects a gradient influenced by proximity to water features and phenolic content.

PC2 accounted for 29.23% of the variation, contrasting phenolic concentrations with select environmental variables. Chlorogenic acid (-2.78) and rutin (-2.83) concentrations contributed negatively, while distances to waterlines (1.43) and

watercourses (1.67) contributed positively. This component highlights the relationship between phenolic concentrations and environmental gradients at specific sites.

Subsequent components captured more localized variability. PC3 (14.83% of variation) emphasized topographic features, such as slope (2.47) and aspect (-3.80), while PC4 (9.49%) highlighted contrasting contributions from slope (-4.06) and environmental distances. Later PCs, such as PC5 and PC6, underscored the relative importance of phenolic compounds versus environmental factors.

Phenolic compounds, particularly chlorogenic acid and rutin, were key contributors to PCs 1, 2, 5, 6, and 7, indicating their dominant role in explaining site-level variation. Environmental variables such as distances to water bodies, slope, and aspect primarily influenced PCs 3, 4, and 8. The biplot (Figure 20) illustrates clustering of sites based on phenolic profiles and environmental gradients, with ellipses representing 95% confidence intervals for each site.

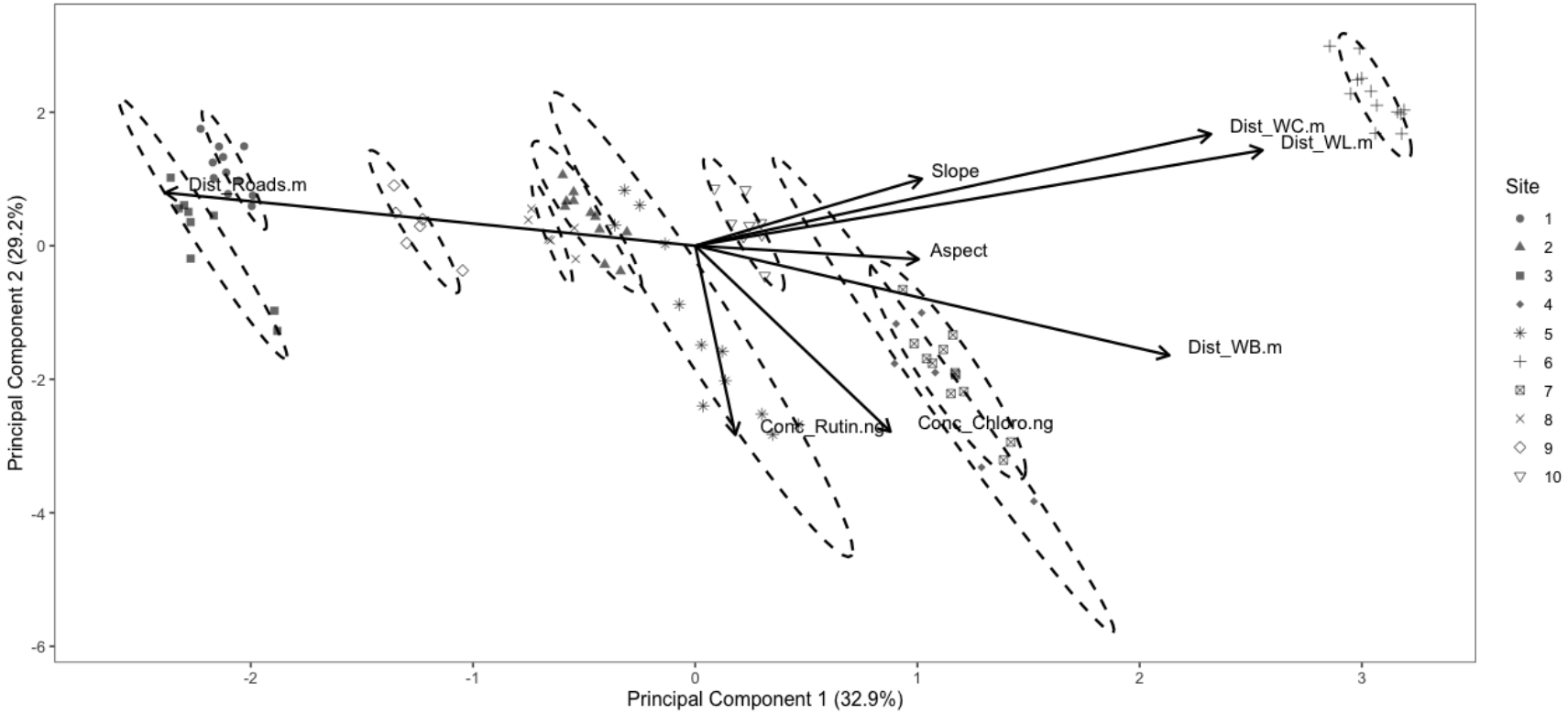

Figure 18. PCA biplot of phenolic concentrations and environmental variables. Clustering highlights site-specific phenolic profiles. Vectors indicate variable contributions to PC1 and PC2.

3.4. Overall Comparison of Traits

The evaluation of combined traits across sites revealed distinct patterns of variation in germination, phenolic concentrations, and environmental factors. Generalized linear mixed models (GLMMs) were employed to assess the effects of chamber row positions and phenolic concentrations on germination success, accounting for tray-level variability. The first model, which excluded phenolic concentrations, indicated no significant effects of chamber row positions (middle row: $\beta = 0.208$, $p = 0.476$; top row: $\beta = 0.097$, $p = 0.740$) on germination success (Table 9). The marginal R^2 of 0.001 showed that fixed effects contributed minimally to explaining variance, while the high conditional R^2 (0.980) highlighted the dominant role of tray-level variability.

The second model included phenolic concentrations (chlorogenic acid and rutin), with chlorogenic acid showing a significant negative association with germination success ($\beta = -0.000409$, $p = 0.046$), while rutin and chamber row positions remained non-significant. Incorporating scaled phenolic concentrations in the refined model yielded similar results, with scaled chlorogenic acid retaining its significant negative effect on germination success ($\beta = -0.248$, $p = 0.046$). Across all models, tray-level variability remained the dominant source of variance (conditional $R^2 > 0.979$).

Table 9. Summary of generalized linear mixed model (GLMM) results for germination success across sites.

Model	Fixed Effects	β Estimate	p-value	Marginal R^2	Conditional R^2
Model 1 (no phenolics)	**Chamber Row (middle)**	0.21	0.48	< 0.05	0.98
	Chamber Row (top)	0.10	0.74		
Model 2 (with phenolics)	**Chamber Row (middle)**	0.20	0.49	< 0.05	0.98
	Chamber Row (top)	0.08	0.75		
	Chlorogenic Acid	< -0.05	0.05		
	Rutin	< 0.05	0.68		
Model 3 (with scaled phenolics)	**Chamber Row (middle)**	0.19	0.49	< 0.05	0.98
	Chamber Row (top)	0.08	0.76		
	Scaled Chlorogenic Acid	-0.25	0.05		
	Scaled Rutin	0.05	0.69		

Mean germination percentage varied across sites but showed no significant associations with phenolic concentrations or environmental distances to water features (Table 10). Chlorogenic acid concentration displayed a weak negative association (β = -0.0102, p = 0.216), while rutin concentration showed a weak positive association (β = 0.0090, p = 0.195). Root displacement volume had negligible influence on germination percentage (β = -0.0027, p = 0.556). Chamber row positions also demonstrated minimal effects on germination success, with non-significant estimates for both middle (β = 0.0179, p = 0.123) and top rows (β = 0.0058, p = 0.591).

Among environmental variables, slope was negatively associated with germination percentage (β = -0.0117, p = 0.014), as shown in Table 10. No significant relationships were observed between germination percentage and distances to water bodies (β = -0.0012, p = 0.874), water lines (β = 0.000017, p = 0.634), or water courses (β = 0.000016, p = 0.543). Aspect (β = 0.0049, p = 0.352) and proximity to roads (β = 0.0092, p = 0.171) displayed no significant effects. Distance to Water Bodies positively correlated with Chlorogenic Acid (r = 0.628) and Rutin (r = 0.381). Distance to Roads had a weak negative correlation with Chlorogenic Acid (r = -0.290).

Statistical results showed that fixed effects accounted for a negligible proportion of the variance in germination percentage, with a low marginal R^2. Most variability was explained by residual variance, as reflected in the high conditional R^2. Random effects analysis revealed no variability at the site level (Variance = 0.0000) and minimal variability at the plant level (Variance = 0.0001). Residual variance was the dominant source of unexplained variability (Residual Variance = 0.0130).

Table 10. Results of combined GLMM for germination percentage including environmental variables

Effect	Estimate	SE	t-value	p-value
Fixed Effects				
(Intercept)	0.79	< 0.05	79.82	< 0.05
Chlorogenic Acid Concentration (ng)	< -0.05	< 0.05	-1.24	0.22
Rutin Concentration (ng)	< 0.05	< 0.05	1.30	0.20
Root Displacement Volume (mL)	< -0.05	< 0.05	-1.24	0.22
Chamber Row (middle)	< 0.05	< 0.05	1.30	0.20
Chamber Row (top)	< 0.05	< 0.05	0.54	0.59
Distance to Water Body (m)	< -0.05	< 0.05	-0.16	0.87
Distance to Wetland (m)	< 0.05	< 0.05	0.48	0.63
Distance to Water Course (m)	< 0.05	< 0.05	0.61	0.54
Slope (m)	< -0.05	< 0.05	-2.45	< 0.05
Aspect (deg)	< 0.05	< 0.05	0.93	0.35
Distance to Roads (m)	< 0.05	< 0.05	1.37	0.17
Random Effects				
Site	0	0	-	-
Plant	< 0.05	< 0.05	-	-
Residual Variance	< 0.05	0.11	-	-

[4] *Standard error (SE)*

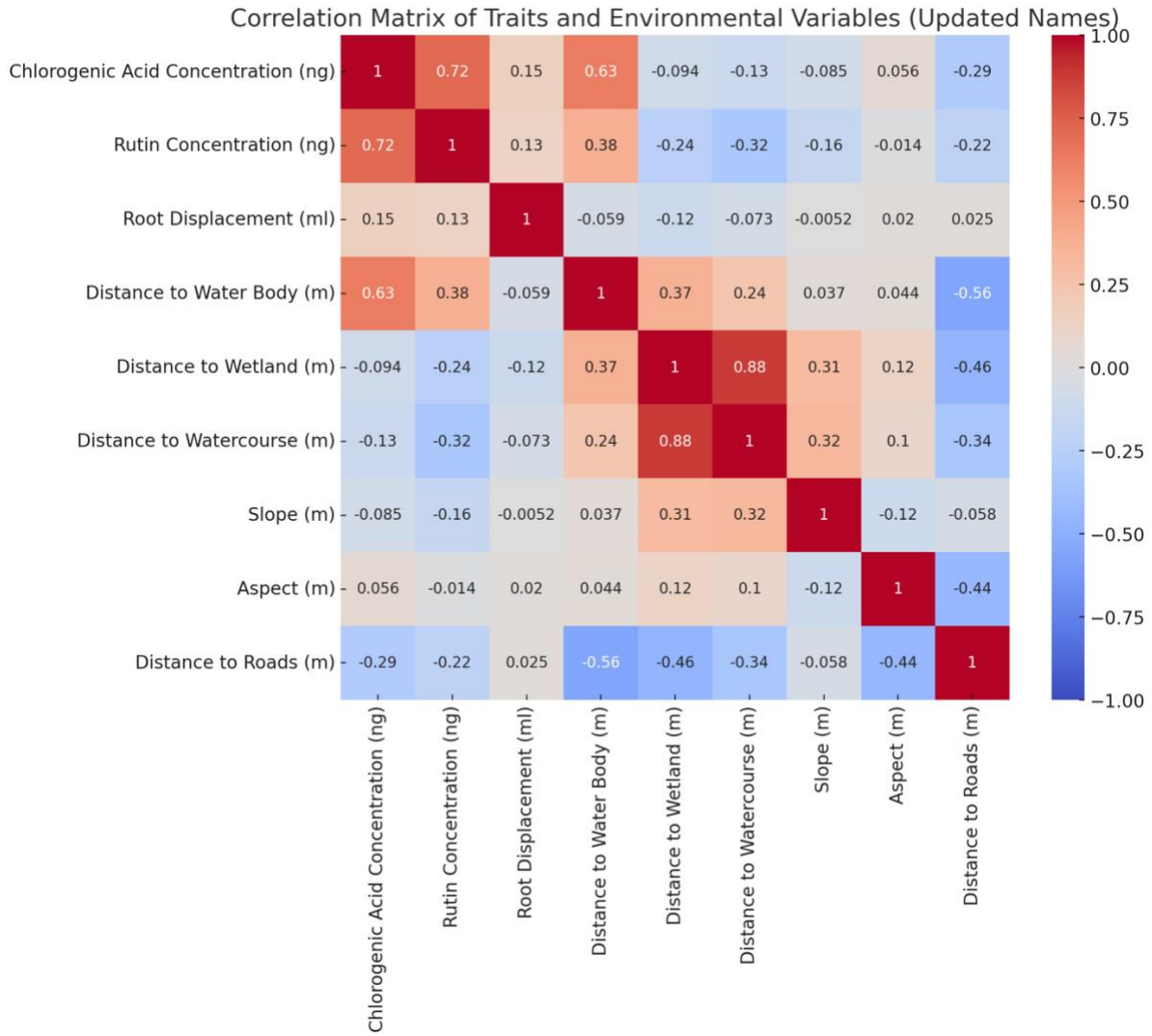

Figure 19. Heatmap of correlations between plant traits and environmental variables across study sites.

The principal component analysis (PCA) revealed the distinct contributions of environmental variables, phenolic concentrations, and plant performance traits to each principal component (PC). Together, the first eight PCs explained 61.2% of the total variation in the dataset, with PC1 and PC2 capturing the majority of the variance. PC1, which explained 22.28% of the variation, was primarily driven by environmental gradients, with positive contributions from the distance to roads and negative contributions from distances to water-related features, including waterlines, watercourses, and water bodies (Figure 20). Slope also contributed negatively to PC1, indicating that sites with closer proximity to water and steeper slopes tended to cluster together. These results suggest that PC1 reflects a gradient contrasting proximity to human infrastructure with natural water features and topography.

PC2 accounted for 18.82% of the variation and was dominated by phenolic traits and plant performance metrics. Positive contributions included chlorogenic acid and rutin concentrations, which were strongly correlated, and root displacement volume. Distances to waterline and water bodies contributed negatively, indicating a contrast between phenolic concentrations and water-related proximity. PC2 highlights biochemical and root development variability across sites, suggesting phenolic traits are a key driver of this component.

PC3, explaining 11.13% of the variation, incorporated plant performance and environmental orientation traits. Germination percentage and aspect contributed positively, while slope and vigour rank contributed negatively. This component reflects a balance between plant reproductive success and site-specific environmental conditions, including topographic steepness and plant vigour.

PC4, which accounted for 8.66% of the variation, focused on structural plant traits and their relationship with topographic variables. Positive contributions included aspect and root displacement volume, while height and stem diameter contributed negatively. This PC highlights a distinction between structural plant development and orientation-related site characteristics.

PC5 explained 7.93% of the variation, with stem diameter emerging as the dominant contributor. Vigour rank also contributed positively, suggesting this component primarily captures structural and performance variability among plants. Contributions from other variables, including phenolic concentrations and environmental distances, were minimal.

PC6 accounted for 7.66% of the variation and emphasized root development and germination traits. Root displacement volume and germination percentage were the strongest contributors, while minor contributions were observed from environmental distances, such as distance to roads. This component underscores the importance of plant performance traits in driving within-site variability.

PC7 explained 6.56% of the variation, representing a combination of environmental and structural influences. Positive contributions included slope and height, while negative contributions were observed for distances to water features, reflecting a mix of topographic and environmental variability.

PC8 accounted for 1.12% of the variation and captured subtle differences in environmental distances. Distance to watercourses contributed positively, while distance to waterlines and minor effects from phenolic concentrations contributed negatively.

This component reflects finer-scale variability in water-related site features and their association with biochemical traits.

Overall, environmental variables dominated the earlier components (PC1 and PC3), with phenolic concentrations and plant performance traits being the primary drivers of PC2 and subsequent components. Structural traits, including height and stem diameter, were more influential in PC4 and PC5, while later components (PC6 to PC8) captured finer-scale variability across root development, phenolic traits, and environmental distances. This analysis demonstrates how different traits and variables interact to shape site-level and within-site variability.

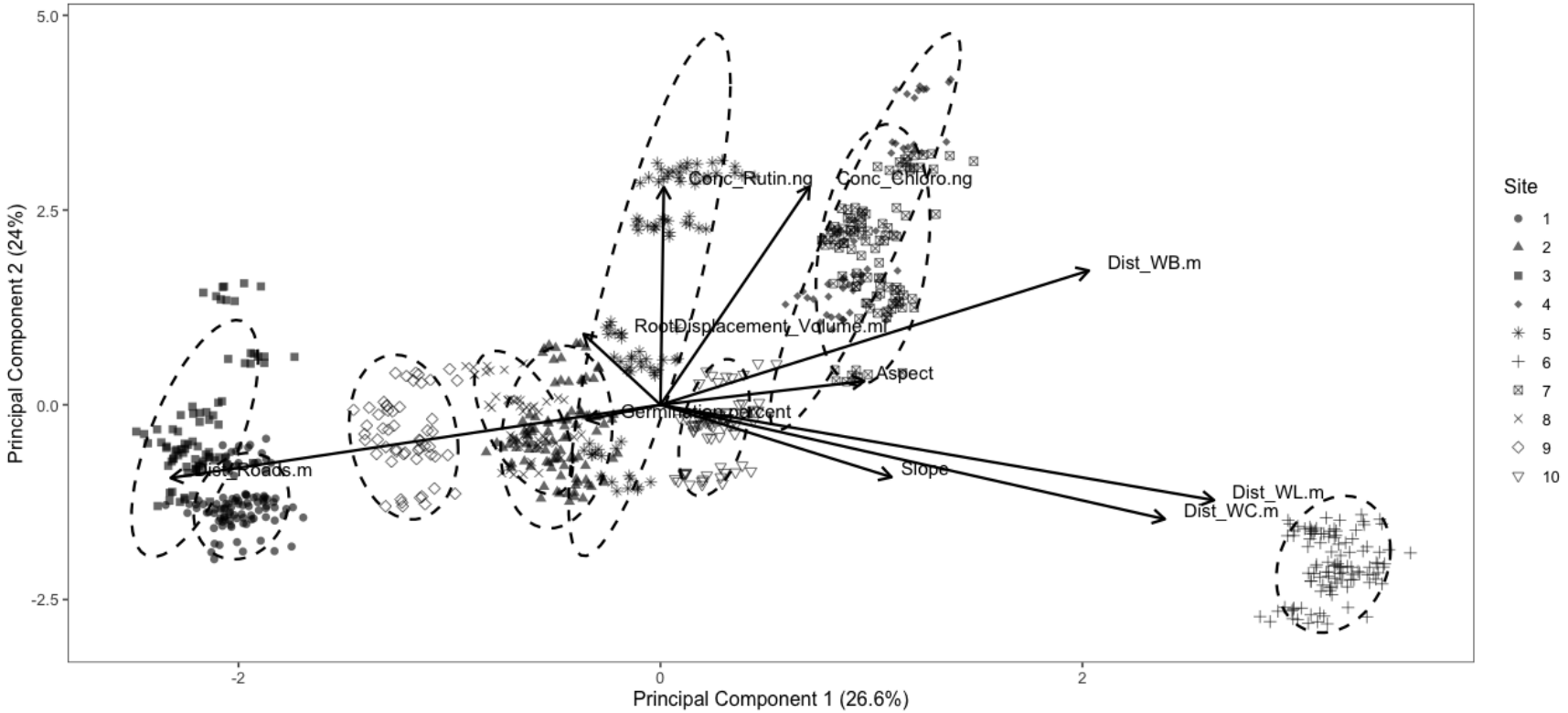

Figure 20. PCA biplot of combined traits and environmental variables for *S. canadensis* populations. Biplot illustrating the relationships between principal components and combined trait data, including root displacement volume, germination rates, berry phenolic concentrations (chlorogenic acid and rutin), and environmental variables (aspect, slope, distance to water bodies). Principal components explain 61.2% of the total variance, with PC1 dominated by phenolic traits and PC2 influenced by topographic factors. Vectors represent trait and variable contributions, with lengths indicating the strength of influence. Ellipses show site groupings based on shared traits and environmental conditions.

Hierarchical clustering of combined traits revealed four main clusters, highlighting ecological variation among sites (Figure 21). Cluster 1, consisting primarily of Inland sites, exhibited higher germination rates and moderate phenolic concentrations. Cluster 4, dominated by Bay of Fundy sites, had the lowest germination rates, steeper slopes, and the highest environmental distances. Cluster 2 (Gulf sites) and Cluster 3 (transitional sites) displayed intermediate levels of phenolic concentrations and germination traits (Figure 24).

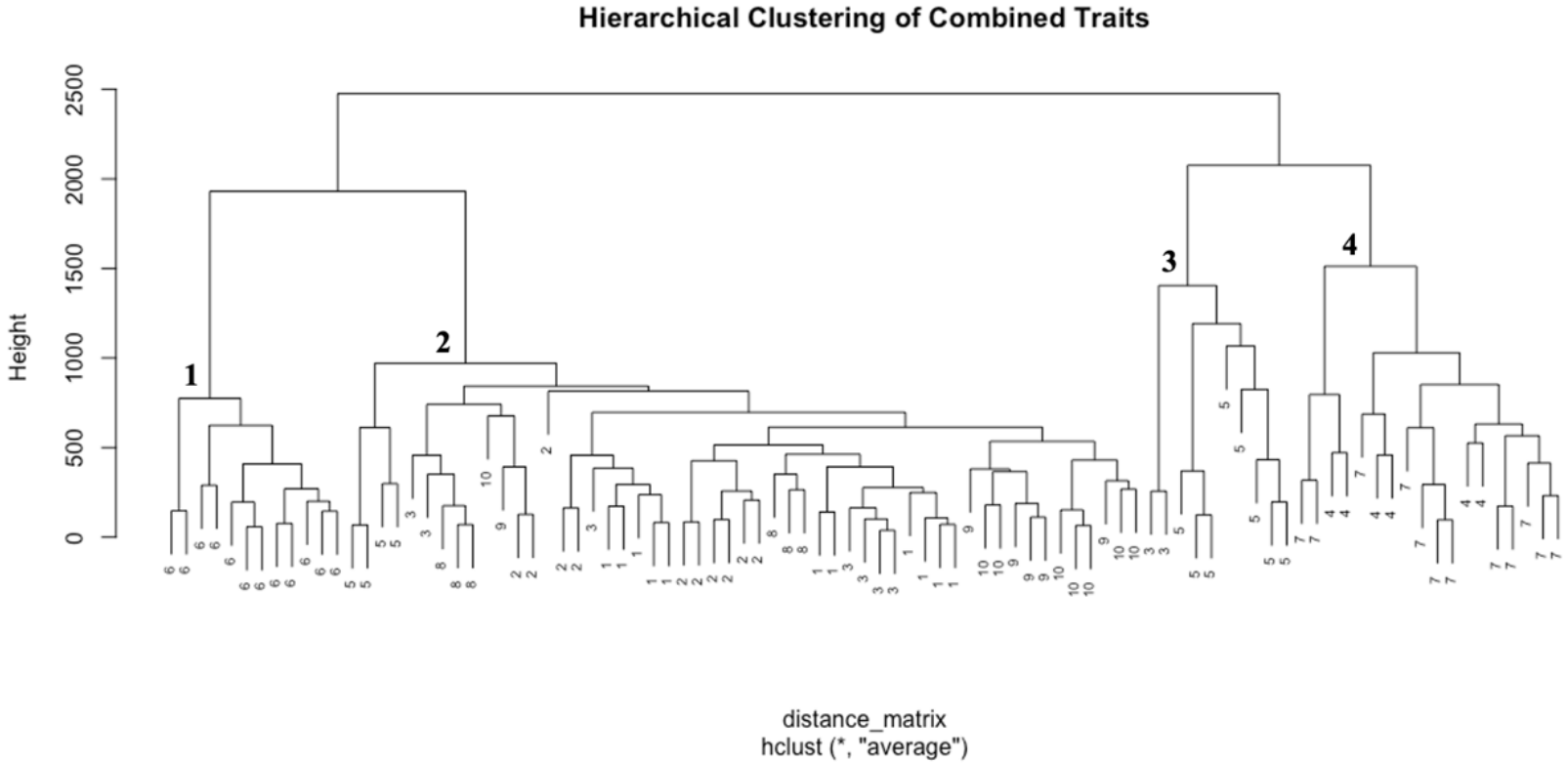

Figure 21. Hierarchical clustering dendrogram of combined traits in *S. canadensis* populations. Dendrogram representing relationships among populations based on combined trait data, including germination percentage, phenolic concentrations, and environmental variables. The vertical axis represents dissimilarity (Euclidean distance) between sites, with clustering performed using average linkage. Four main clusters are highlighted, reflecting distinct trait-environment associations: Cluster 1 (high germination rates, moderate phenolics, mostly Inland sites), Cluster 2 (moderate traits, Gulf of St. Lawrence sites), Cluster 3 (mixed traits, transitional sites), and Cluster 4 (low germination, steep slopes, Bay of Fundy sites).

Phenolic traits showed clear distinctions across clusters, with the highest concentrations of chlorogenic acid and rutin observed in Cluster 3. Cluster 1 had lower phenolic concentrations, while Cluster 4 exhibited the lowest germination rates and steep slopes (Figure 21). Environmental variables such as distance to roads, wetlands, and water bodies revealed gradients among clusters. For instance, Cluster 1 had moderate distances to water courses, while Cluster 4 showed the greatest distances.

Root displacement volume also varied among clusters, with higher values in Clusters 1 and 2 and lower values in Cluster 4. Similarly, Cluster 4 sites had the steepest slopes, which were associated with reduced germination success compared to gentler slopes in Cluster 1. Boxplots (Figure 22) illustrate these relationships, showing how combined traits and environmental gradients shaped site-level variation.

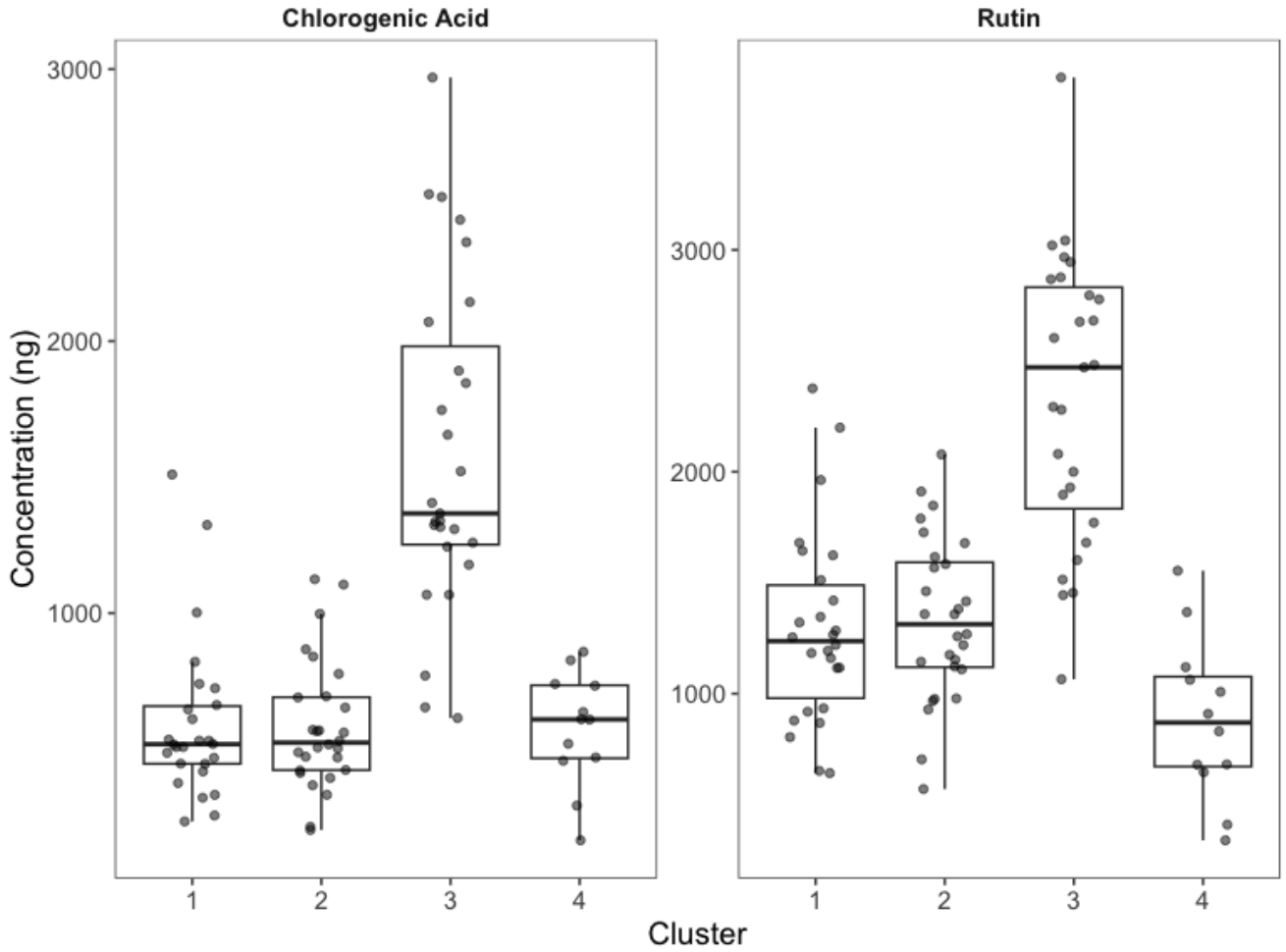

Figure 22. Phenolic concentrations (chlorogenic acid and rutin) by cluster grouping from PCA analysis of *S. canadensis* populations. Boxplot displaying phenolic concentrations (chlorogenic acid and rutin) across clusters identified from PCA of combined traits and environmental data.

Across all analyses, environmental variables such as slope, proximity to water bodies, and distances to roads emerged as critical contributors to site-level variability. Phenolic concentrations, particularly chlorogenic acid, were strongly correlated with each other and with site characteristics, while germination traits demonstrated clear site and cluster-specific trends. The PCA and clustering results (Figure 23) underscore the complex interplay of environmental and biological factors in shaping the traits of *S. canadensis* populations.

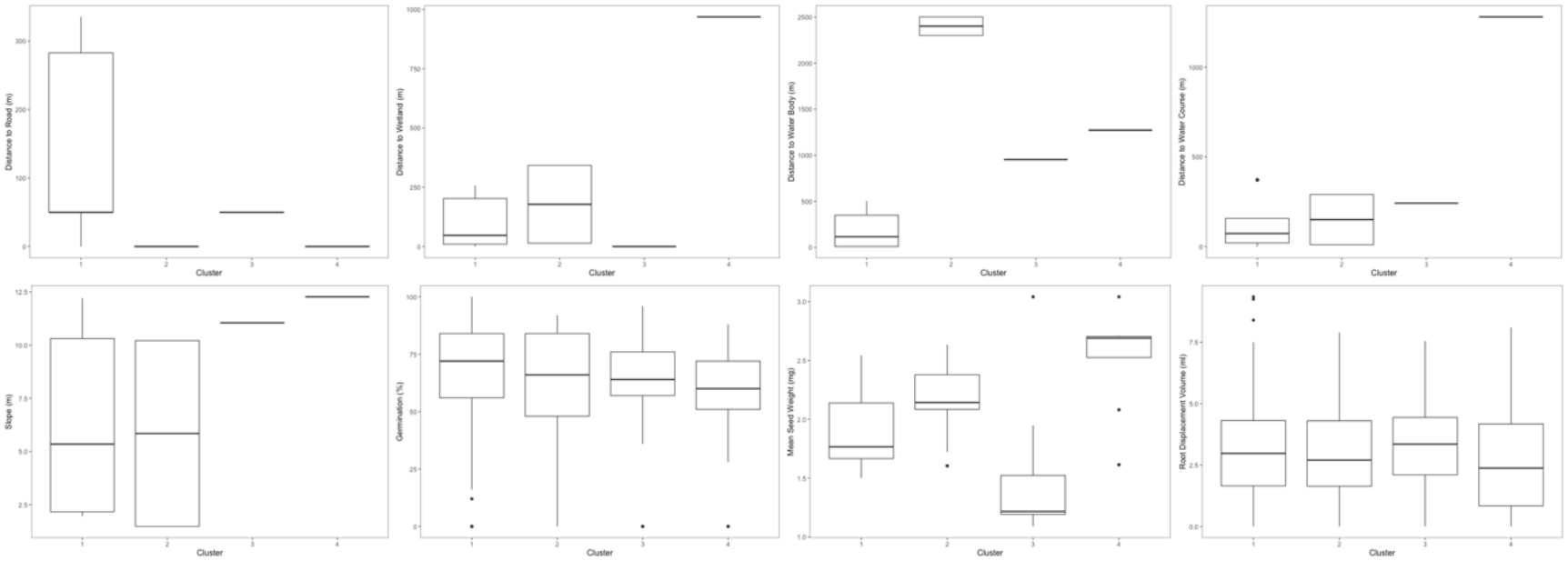

Figure 23. Environmental and trait variables across PCA-derived clusters for *S. canadensis* populations. Boxplots showing (top row, left to right): distance to road (m), distance to wetland (m), distance to water body (m), and distance to water course (m); (bottom row, left to right): slope (%), germination rate (%), mean seed weight (mg), and root displacement volume (mL). Data are grouped by primary and subcluster groupings identified through PCA of environmental and plant traits.

4. Discussion

This study explores the relationships between germination rates, root development, and phenolic content in *S. canadensis* populations, emphasizing the critical role of genetic diversity and phenotypic plasticity in adaptation to diverse environmental conditions. By examining trait variation within and among inland, Gulf of St. Lawrence, and Bay of Fundy populations, the findings provide insights into ecological adaptability and implications for restoration, agriculture, and natural health product development.

4.1. Softwood Cuttings

Root development, measured by root displacement volume, exhibited notable variation among and within sites, consistent with studies examining root system variability in response to environmental gradients (e.g., Brown et al., 2020; Wilson et al., 2018). Among inland populations, particularly at Site 1, the highest mean root displacement volumes were observed, coupled with relatively low within-site variability. These patterns may reflect adaptations to stable soil and moisture conditions typical of inland environments, which are less prone to hydrological disturbances compared to coastal zones. However, higher nutrient levels or faster growth rates could also play a role in driving greater root development at these sites. While the health of maternal plants was assessed, source differences in leaf surface area or stored reserves may have contributed to these observations. These factors should be considered in future studies.

Populations in the Gulf of St. Lawrence region displayed moderate root displacement volumes with greater variability within sites, potentially due to heterogeneous environmental pressures, such as moisture gradients, soil quality, and exposure to climatic variability, including wind and precipitation (Kozlowski, 1997).

Such variability may drive selective pressures for traits promoting root plasticity in response to local conditions. In contrast, Bay of Fundy populations exhibited significantly lower root displacement volumes, likely reflecting cumulative abiotic stressors, including steep slopes, reduced soil stability, nutrient limitations, and tidal dynamics. These findings align with studies highlighting environmental constraints on root initiation and elongation in areas with abiotic stressors, such as high erosion potential or salinity (Smith et al., 2019).

Root initiation and development are influenced by genetic, environmental, and physiological factors. Among these, soil composition and moisture availability are particularly significant, affecting aeration, water retention, and nutrient access (Grimaldi et al., 2016). Sandy soils in inland regions favor deeper root penetration due to their low waterlogging potential. In contrast, compacted or clayey soils, characteristic of coastal areas, restrict root elongation and development. Similarly, consistent moisture levels support uniform root growth, whereas fluctuating moisture conditions, as observed in Gulf populations, may contribute to increased variability in root traits.

Although environmental factors are critical in natural settings, all softwood cuttings in this study were treated uniformly. Soil type, moisture levels, and other environmental variables were controlled, ensuring observed differences in rooting traits were not influenced by these external factors. The variability in rooting traits observed across populations is therefore likely a reflection of genetic differences or local adaptations of the parent plants to their environments. However, stored reserves in maternal plants could also influence root development and should be explored further.

The distinct rooting traits across populations may indicate underlying genetic or epigenetic influences shaped by local environmental pressures. Parent plants in inland regions, where soils are sandy and moisture levels stable, may have evolved traits favoring deeper or more extensive root systems. Alternatively, greater nutrient availability and faster growth rates in these regions could enhance root development. Future studies should assess whether sites with greater root development also produce taller plants or exhibit differences in stored reserves and leaf surface area. Conversely, Gulf populations, which experience fluctuating moisture and denser soils, may exhibit greater variability in rooting traits. These adaptations highlight the role of local selection pressures in shaping rooting capacity and development.

Although this study did not directly evaluate the impact of soil and environmental variability on rooting traits, future research could compare cuttings grown in contrasting soil types and moisture conditions to better understand genetic and environmental influences. Genetic analyses of parent plants could help elucidate the genetic basis of rooting traits observed in different populations.

Maintaining a consistent treatment protocol in this study allowed for a controlled evaluation of rooting potential across populations. However, the role of environmental selection on parent plants remains an important area for future investigation, bridging experimental insights with natural adaptations in diverse contexts.

Environmental stresses significantly impact root development. Steep slopes in Bay of Fundy populations impose physical and hydrological constraints, while tidal dynamics and saltwater intrusion further limit nutrient absorption and root elongation (Huang & Glick, 2017). In addition to environmental influences, hormonal regulation

plays a central role. Auxins and cytokinins are critical for root initiation and elongation, with site-specific environmental cues or genetic differences potentially driving observed patterns in root development (Aloni et al., 2010). Microbial associations, such as symbiotic relationships with mycorrhizal fungi, enhance root growth and nutrient uptake, particularly in nutrient-poor soils (Smith & Read, 2008). Variations in microbial communities across sites may partly explain differences in root displacement volume observed in this study.

Genetic variation likely drives much of the observed variation in root traits, reflecting adaptations to local conditions. Adaptation to environmental heterogeneity influences root system architecture in many species (Pérez-Jaramillo et al., 2016). Clonal propagation limitations are particularly relevant to softwood cuttings, which rely on stored carbohydrates for root development. Differences in carbohydrate reserves and phenolic profiles among cuttings may also influence root initiation success (Leakey, 2004).

Collectively, these factors shape root development and highlight the complexity of plant responses to environmental conditions. Future studies that incorporate detailed soil analyses, moisture monitoring, and genetic characterization could further disentangle the contributions of these factors to root variability across *S. canadensis* populations.

The variation in root traits observed in this study has practical implications for restoration and agricultural practices. Cuttings/seedlings from the inland populations, those with rapid and strong root development, could be used for bank stabilization and/or in areas with nutrient-poor soils. Gulf populations, with their plasticity, may be better suited in areas with highly variable microsites. While salt tolerance was not assessed in

this study, the potential for Bay of Fundy populations to contribute tidal-resistant traits warrants further investigation. However, this application remains speculative and requires future research to confirm.

The lack of correlation between root displacement volume and above-ground traits, such as plant height and stem diameter, suggests independence in the regulatory mechanisms for root and shoot growth. This may align with resource partitioning strategies, optimizing specific functional traits in response to varying environmental pressures (Brouwer, 1962; Poorter & Nagel, 2000). However, this study did not measure changes in stem diameter growth over time, nor were the plants grown beyond the initial season. Extending the growth period or assessing stem diameter changes more comprehensively could provide greater insight into potential trade-offs or interactions between root and shoot development. While resource partitioning offers a valuable framework for understanding these results, further studies are needed to explore this hypothesis in the context of extended growth periods and environmental variability.

Gulf populations, characterized by moderate root development and high phenolic production, may allocate more resources to chemical defenses, enhancing resilience against biotic stressors such as herbivory or microbial infections. In contrast, Bay populations, growing in steep and erosion-prone terrains, appear to prioritize structural root adaptations that enhance anchorage and soil stability. These population-specific traits suggest that localized environmental pressures have shaped divergent strategies for resource allocation and growth.

The variation in root traits among Gulf, Bay, and Inland populations may reflect underlying genetic differences. Smaller, geographically isolated populations, such as

those in the Bay of Fundy region, often exhibit lower levels of genetic diversity due to restricted gene flow and higher levels of inbreeding. Such genetic bottlenecks could reduce inter-plant variation and constrain adaptive capacity, potentially explaining the limited root development observed in these populations. Conversely, Inland and Gulf populations, which are larger and less isolated, may exhibit greater genetic variation, enabling more pronounced phenotypic plasticity and adaptation to local conditions (Hoban et al., 2016; Pérez-Jaramillo et al., 2016).

Differences in site characteristics such as soil compaction, hydrologic conditions, and microclimate may have resulted in genetic selection for root initiation and development in the cuttings, but this was beyond the scope of this study. The link between genetic variation and root traits is critical for understanding how wild populations of *S. canadensis* adapt to diverse environments. Molecular studies focusing on population-level genetic diversity and gene expression profiles could provide insights into the genetic basis of root phenotypic plasticity. Such information would also help identify specific populations with superior root traits for use in restoration or breeding programs. For instance, sandy soils in Inland populations may favour genotypes capable of rapid root growth, while plants growing in the Bay population may be comprised of genotypes better adapted to growing in compacted or saline soils (cf. Grimaldi et al., 2016; Kozlowski, 1997).

Molecular and physiological factors also play a key role. The regulation of root growth through hormonal pathways, particularly auxins and cytokinins, likely varies across populations in response to environmental cues (Aloni et al., 2010). Investigating transcriptomic responses to environmental stressors could elucidate how *S. canadensis*

populations modulate root development in different habitats. Furthermore, the role of symbiotic relationships, such as mycorrhizal associations, in enhancing root nutrient uptake and growth under challenging conditions warrants further exploration (Smith & Read, 2008).

Phenotypic plasticity in root traits enables *S. canadensis* to optimize resource acquisition and resilience in diverse environments (Pigliucci, 2001). This adaptability is particularly valuable for ecological restoration efforts, where robust root systems are essential for stabilizing soils, managing hydrology, and promoting biodiversity recovery (Ballero et al., 2009). While this study observed significant variation in root development traits across populations, it did not directly assess the degree of plasticity. Measuring phenotypic plasticity requires experimental exposure of genotypes to controlled, contrasting environmental conditions to evaluate trait variability. Therefore, the observed variation in root traits among Inland and Gulf populations may reflect environmental influences, genetic differentiation, or a combination of both. Despite this limitation, the superior root development traits of Inland and Gulf populations suggest that they are promising candidates for targeted restoration strategies, particularly in erosion-prone or drought-affected areas.

As climate change accelerates soil erosion and alters water availability, the role of resilient plant species like *S. canadensis* becomes increasingly critical. Populations with superior root systems can mitigate these impacts by improving soil stabilization, enhancing water retention, and supporting ecosystem recovery. Leveraging genetic diversity and adaptive traits from Inland and Gulf populations could help develop restoration strategies that are both ecologically and economically sustainable. To enhance

our understanding of root trait variability and its applications, future studies should integrate comprehensive approaches across multiple disciplines. First, detailed soil and hydrological analyses are essential for measuring soil composition, moisture availability, and other abiotic factors that influence root development across populations. These analyses would help clarify the environmental drivers of root variability. Second, genomic and transcriptomic studies are needed to investigate the genetic basis of root phenotypic plasticity, which could reveal key adaptive loci and molecular pathways underpinning root development. Third, population-level genetic studies should be conducted to assess genetic diversity within and among populations, providing valuable insights into the evolutionary pressures shaping root traits. Finally, long-term monitoring of root and shoot traits under changing environmental conditions would elucidate the role of phenotypic plasticity in climate adaptation.

By integrating environmental, genetic, and molecular data, future research can better inform conservation and restoration strategies, ensuring the long-term viability of *S. canadensis* populations in the face of environmental change.

4.2. Germination

Seed germination varied significantly across sites, reflecting the influence of environmental and genetic factors on germination strategies. Inland populations achieved the highest germination success (e.g., 95% at Site 1). Teasing apart genetic and maternal effects remains a challenge, as maternal effects may reflect superior environmental conditions of the parent plants rather than purely genetic traits. Future studies that incorporate controlled environmental trials or molecular markers could help separate these influences. This may reflect inherent seed viability or genetic adaptations to the

stable soil types characteristic of inland environments, which may provide favorable germination conditions. Although environmental factors such as temperature or soil nutrients were not explicitly measured in this study, the consistent conditions in germination trials (e.g., standardized moisture levels, substrate type) highlight genetic differences or seed quality as key drivers of germination success. The observed variation in germination success between inland, Gulf, and Bay populations may therefore reflect site-specific adaptations or genetic diversity within populations (Shelef et al., 2017; Pérez-Jaramillo et al., 2016).

Bay and Gulf populations exhibited lower germination success, suggesting that seeds from these regions may face environmental challenges or possess less genetic robustness compared to inland populations. Smaller Gulf populations may have experienced reduced pollen availability, potentially influencing seed quality and subsequent germination success. Differences in soil type between regions could also influence germination outcomes. For example, inland soils tend to retain water while draining efficiently, potentially mimicked by the consistent moisture conditions of the germination trials, thereby supporting higher germination rates (Stang, 1990). Coastal populations may exhibit traits reflecting adaptations to fluctuating or stress-prone environments, such as dormancy or slower germination, but these traits may not have been fully expressed under uniform trial conditions.

Germination success varied significantly across populations, reflecting adaptations to local environmental conditions and site-specific pressures. Inland populations exhibited the highest germination rates, with some sites achieving over 85% success. However, this success could also reflect maternal environmental effects rather

than purely genetic traits. Controlled trials and seed source standardization could help elucidate the extent of these influences. These populations likely benefit from relatively stable environmental conditions, such as consistent moisture levels, well-drained soils, and minimal hydrological disturbances. The larger population sizes observed at inland sites may also contribute to higher genetic diversity, promoting adaptive capacity and robust germination traits.

In contrast, coastal populations demonstrated greater variability in germination success, consistent with the heterogeneous environmental conditions of the Gulf and Bay of Fundy regions. Gulf sites exhibited intermediate germination success, with rates averaging around 70%. This variability may be influenced by adaptations to fluctuating moisture levels and localized microclimates. Smaller population sizes at some Gulf sites could limit genetic diversity, contributing to uneven germination performance. Additionally, variability in soil composition, proximity to wetlands, and hydrological dynamics may affect seed hydration and germination timing (Stang, 1990).

Bay of Fundy populations, characterized by smaller population sizes and greater environmental challenges, exhibited the lowest germination rates, averaging 59% at Site 4. These populations also displayed the shortest germination windows, suggesting significant constraints on seed viability and germination potential. Environmental pressures such as steep slopes, tidal dynamics, saltwater intrusion, and nutrient limitations may restrict seed hydration, delay germination onset, and reduce germination success (Kozlowski, 1997; Huang & Glick, 2017). Additionally, smaller population sizes may reduce genetic variation and pollen availability, further limiting germination potential. Beyond environmental factors, genetic variation likely plays a critical role in

shaping germination strategies across populations. Inland populations may exhibit higher genetic diversity or retain alleles favorable for germination under stable conditions, while coastal populations, particularly those in the Bay of Fundy, may have experienced genetic bottlenecks due to smaller population sizes or geographic isolation. Reduced genetic variation within these populations could limit adaptive responses to environmental pressures, further exacerbating germination challenges (Pérez-Jaramillo et al., 2016).

The reference to phenotypic plasticity in germination success requires clarification. Germination success itself may not exhibit plasticity in the same way timing might. Instead, variability in germination success is more likely attributable to genetic or maternal environmental effects rather than plasticity per se. This plasticity, driven by environmental cues such as temperature and soil moisture, allows populations to maintain reproductive success across variable habitats (Pigliucci, 2001; Wilson et al., 2018).

The observed positive correlation between chlorogenic acid concentration and germination success suggests that biochemical traits influence germination under variable conditions. Phenolic compounds like chlorogenic acid are associated with stress tolerance, protecting seeds against abiotic stressors such as UV radiation and oxidative damage, as well as biotic pressures like herbivory. Gulf populations with elevated phenolic levels may benefit from enhanced resilience in their variable environments, whereas inland populations achieve high germination success through favorable environmental stability (Lattanzio et al., 2006; Sgrò et al., 2011).

Interestingly, seed weight was not a significant predictor of germination success in *S. canadensis*. This finding contrasts with expectations for many species, where larger seeds are associated with higher germination success in resource-limited environments

due to greater nutrient reserves (Thomas et al., 2008). The lack of correlation in this study may reflect *S. canadensis*'s adaptive strategies, which prioritize traits such as biochemical resilience or germination timing over seed size. Additionally, differences in seed collection timing and maturity may contribute to this lack of relationship. Seeds collected at varying maturity stages may display differences in dormancy status, nutrient reserves, or phenolic content, which could influence germination outcomes. Future studies should standardize seed collection protocols and assess maturity to better understand these dynamics.

The significant variation in seed weight among sites ($p < 0.001$) suggests that site-level factors, such as environmental conditions or localized genetic differences, may influence seed development. This could reflect site-specific adaptation or maternal effects related to resource availability during seed formation. The lack of significant variation among plants within sites ($p = 0.808$) indicates that seed weight is consistent within populations, which may suggest limited genetic or environmental variability at this scale. Despite this variation, the absence of a significant effect of seed weight on germination outcomes implies that other factors, such as seed viability, dormancy mechanisms, or biochemical traits, may play a more critical role in determining germination success.

Given that seed weight did not significantly impact germination, this suggests that resource allocation within the fruit (e.g., phenolic content or nutrient reserves) may be more critical than seed size alone. Nutritional content and fruit quality may also impact seed germination. Variability in fruit nutrient composition, influenced by environmental conditions or plant health, could affect seed nutrient reserves and metabolic activity during germination. Fruits grown in nutrient-poor or stress-prone environments, for

example, may produce seeds with lower carbohydrate or lipid reserves, reducing their ability to sustain early growth stages. These nutritional differences might partially explain the observed variation in germination success across populations.

Future studies should explore how fruit size, seed number per berry, and fruit nutrient quality interact to influence germination traits. Investigating these factors alongside biochemical markers, such as phenolic content, could clarify how maternal investment in fruit and seed development impacts germination and seedling establishment. Additionally, research examining seed weight in relation to field survival and seedling growth, rather than just initial germination, could provide insights into its broader ecological and agricultural relevance. For restoration and agricultural applications, prioritizing populations with traits linked to phenolic resilience and germination success over seed size alone may yield more reliable outcomes in stress-prone or degraded environments.

Expanding research across multiple growing seasons would provide a more comprehensive understanding of how environmental changes, such as temperature and precipitation shifts, influence germination patterns. Genetic analyses to identify loci associated with phenolic production and germination traits could further elucidate the mechanisms driving observed patterns and inform restoration strategies.

Although this study highlights distinct germination patterns across inland, Gulf, and Bay of Fundy populations, the restoration recommendations require further support. Inland populations with high germination success may serve as ideal seed sources for stable environments. The consistent germination success observed in inland populations

aligns with the stable environmental conditions, such as well-drained soils, mimicked in the controlled trials.

Gulf populations, with their elevated phenolic content, may exhibit greater resilience in stress-prone areas, while Bay of Fundy populations, despite their lower germination rates, could provide valuable traits for rapid germination in dynamic tidal ecosystems. These distinctions suggest inland populations could stabilize soils in erosion-prone areas, while Gulf populations may enhance restoration outcomes in environments with variable hydrology or abiotic stressors.

4.3. HPLC MS-MS: Chlorogenic and Rutin Concentration in Berries

Phenolic content, including chlorogenic acid and rutin concentrations, varied significantly among populations grouped by geographic region. Gulf of St. Lawrence populations (e.g., Site 8) exhibited the highest concentrations of both phenolic compounds, potentially reflecting an adaptive response to environmental pressures such as herbivory, fluctuating moisture availability, and UV stress. Elevated levels of chlorogenic acid and rutin have been linked to enhanced resistance to herbivore feeding and oxidative damage, serving as chemical deterrents while also protecting against abiotic stressors (Grace & Logan, 2000). However, this study did not directly assess herbivory pressure or other biotic stressors, and so the connection between phenolic content and herbivory remains speculative. Future research explicitly measuring herbivory rates alongside phenolic content would help clarify this relationship.

In contrast, Bay of Fundy populations exhibited lower phenolic concentrations, suggesting a trade-off between chemical defenses and structural adaptations. These populations may allocate more resources to structural compounds like lignin to withstand

the physical stresses of tidal dynamics, steep slopes, and saltwater intrusion. This trade-off aligns with resource prioritization models, but without direct measurements of lignin content or structural adaptations, further testing is needed to substantiate these hypotheses. This reflects a prioritization of resilience to abiotic stress over chemical defense mechanisms, particularly in environments where herbivory pressure may be lower or less frequent (Kozlowski, 1997). Such trade-offs are well-documented in other species, where plants in resource-limited or physically challenging environments emphasize structural traits over chemical deterrents (Smith & Jones, 2019).

Inland populations displayed moderate phenolic concentrations, reflecting a balanced strategy of growth and defense. The relatively stable environments in inland regions, with consistent moisture and soil conditions, may reduce the need for elevated chemical defenses, allowing for optimal growth and reproduction. This balanced allocation of resources may be advantageous in environments where herbivory is less intense, and abiotic pressures are moderate. These findings reflect observations in other plant species, but the study did not measure how this balance is achieved or whether phenolic levels correlate with reproductive success. These findings align with studies showing that plants in stable environments allocate resources to growth and reproduction while maintaining moderate levels of chemical defenses (Shelef et al., 2017).

The positive correlation between chlorogenic acid and rutin concentrations suggests a co-regulated biosynthetic pathway, which may enhance ecological fitness and stress tolerance. These phenolic compounds likely work synergistically to protect against environmental stressors, including herbivory and oxidative damage (Schmitzer et al., 2012). Elevated phenolic levels in Gulf populations suggest a heightened capacity to

tolerate the variable coastal conditions, whereas inland populations achieve high germination success through stable environmental conditions that reduce the need for heightened chemical defenses.

Although this study did not explicitly assess environmental drivers of phenolic variability, such as herbivory pressure, nutrient availability, or moisture stress, future research should address these factors. Further studies investigating how site-specific environmental parameters influence phenolic production would help clarify the role of these compounds in plant adaptation. Understanding the role of environmental stressors in driving phenolic production is crucial for interpreting the adaptive significance of these traits across different populations. Additionally, genomic and transcriptomic studies investigating the regulation of phenolic biosynthesis could reveal key molecular mechanisms underlying their production (Pérez-Jaramillo et al., 2016). Identifying genetic loci associated with high phenolic content would inform breeding programs aimed at enhancing stress tolerance and improving the resilience of crops or restoration species.

High-phenolic Gulf populations hold significant commercial potential for the nutraceutical industry, where chlorogenic acid and rutin are valued for their antioxidant properties and potential health benefits (Schmitzer et al., 2012). These populations could be prioritized for cultivating high-phenolic cultivars for use in antioxidant products. Inland populations, with their moderate and stable phenolic concentrations, provide a reliable source for consistent product quality in the natural health market. Meanwhile, Bay populations, despite their lower phenolic content, play an essential ecological role in

maintaining biodiversity and supporting pollinator networks, emphasizing the importance of conservation and restoration efforts in tidal and coastal ecosystems.

Future research should examine correlations between phenolic content and ecological traits, such as herbivory resistance or pollinator interactions, to verify the ecological roles of these compounds. Additionally, assessing the role of phenolic concentrations in plant survival under stress would strengthen their application in restoration and agriculture.

4.4. Overall Comparison of Traits

The integration of Principal Component Analysis (PCA) and hierarchical clustering analyses revealed significant patterns in trait-environment interactions across the three regions studied: inland, Gulf of St. Lawrence, and Bay of Fundy populations. Inland populations exhibited tight clusters characterized by high germination rates and consistent root displacement, suggesting adaptation to stable, homogeneous conditions. Environmental factors such as moisture availability and nutrient composition likely support higher seed viability in these regions. These populations capitalize on favorable and consistent growth conditions typical of inland environments, where water retention in soils and minimal temperature fluctuations enhance germination success (Shelef et al., 2017; Pérez-Jaramillo et al., 2016).

Gulf of St. Lawrence populations, in contrast, displayed more distinct clustering primarily influenced by phenolic concentrations and environmental gradients, such as proximity to water bodies and slope. The variability observed in germination success and phenolic content likely reflects the dynamic and heterogeneous environmental conditions in the Gulf region, which experiences frequent moisture fluctuations and exposure to

saltwater (Huang & Glick, 2017). Elevated phenolic concentrations in these populations may represent an adaptive response to stressors such as herbivory and fluctuating moisture levels. Higher phenolic content is often associated with increased stress tolerance (Lattanzio et al., 2006). However, this study did not assess phenotypic plasticity directly. A detailed study exposing genotypes to different environments would be required to determine the degree of plasticity in trait expression.

Bay of Fundy populations showed the greatest dispersion across the PCA axes, indicating adaptation to extreme and fluctuating tidal environments. These conditions impose significant constraints on seedling establishment due to steep slopes, tidal dynamics, and salinity stress (Kozlowski, 1997). The lower germination success and shorter germination windows observed in these populations suggest stronger selective pressure to rapidly complete life cycles under suboptimal germination conditions (Huang & Glick, 2017). The broader dispersion observed in these populations aligns with the idea that high phenotypic plasticity is essential to cope with dynamic and unpredictable environments (Pigliucci, 2001). However, the observed variation may also reflect reduced genetic diversity in smaller populations, limiting their adaptive capacity to these stressors.

Trait interactions varied significantly across regions, particularly with respect to seed weight and germination success. Inland populations exhibited a strong positive correlation between seed weight and germination success, indicating that larger seeds in these environments may have a higher likelihood of successful germination due to greater energy reserves and nutrient availability (Thomas et al., 2008). This correlation should be interpreted cautiously, as environmental effects on maternal plants may also influence

seed size and germination success. Further studies standardizing seed collection conditions are recommended.

Phenolic concentrations also varied significantly by region, with Gulf populations exhibiting the highest levels of chlorogenic acid and rutin. These higher concentrations likely reflect an adaptive strategy for coping with high-stress environments, where phenolic compounds protect against herbivory, UV radiation, and other biotic and abiotic stressors (Lattanzio et al., 2006; Sgrò et al., 2011). Bay populations, in contrast, showed the lowest concentrations of these compounds, potentially prioritizing structural adaptations to withstand harsh tidal conditions over chemical defenses (Kozlowski, 1997). Inland populations exhibited intermediate phenolic concentrations, balancing growth and defense strategies suitable for stable environments with minimal abiotic stressors.

Expanding the environmental dataset to include factors such as slope stability, herbivory intensity, and detailed hydrological conditions would enhance understanding of how these traits are shaped by environmental factors. Incorporating genetic analyses could also help pinpoint loci associated with these adaptive traits, providing valuable information for developing restoration strategies and targeted breeding programs to enhance trait resilience (Pérez-Jaramillo et al., 2016).

The observed variability in trait performance across populations underscores the critical importance of preserving genetic diversity within *S. canadensis* populations. Inland populations, with their high germination success and root development, are particularly valuable for stabilizing soils and improving water retention in restoration projects. Gulf populations, with their elevated phenolic content, may contribute

significantly to ecosystem resilience in stress-prone areas. Bay populations, despite their lower phenolic content, are vital for maintaining biodiversity and supporting ecosystem services in dynamic tidal environments. Integrating population genetics and phenotypic trait data into restoration planning will optimize efforts to ensure long-term ecological stability in the face of environmental change (Ballero et al., 2009; Wilson et al., 2018).

5. Conclusions

This study highlights the critical interplay between genetic diversity, phenotypic plasticity, and environmental factors in shaping the adaptive traits of *S. canadensis*. By examining trait variation across inland, Gulf of St. Lawrence, and Bay of Fundy regions, we identified distinct patterns of adaptation. Inland populations demonstrated higher germination rates and robust root systems, reflecting their suitability for stable environments. Conversely, Gulf and Bay populations exhibited unique adaptations to coastal stressors, including elevated phenolic concentrations and rapid germination strategies, underscoring their resilience in dynamic and challenging habitats.

These findings emphasize the importance of conserving genetic diversity within *S. canadensis* populations to support resilience against climate change and increasing environmental pressures. Populations with high phenolic content and superior germination traits offer significant potential for applications in ecological restoration, agriculture, and the nutraceutical industry. Leveraging these adaptive traits in targeted restoration efforts can enhance soil stabilization, water retention, and ecosystem recovery, particularly in erosion-prone or stress-affected areas.

Future research should prioritize elucidating the genetic and molecular mechanisms underlying trait variability and adaptation. Long-term studies across multiple seasons, integrating genetic analyses and environmental assessments, will refine our understanding of how *S. canadensis* populations respond to changing conditions. These insights will inform restoration strategies and breeding programs, fostering ecological and commercial sustainability in the face of accelerating environmental change.

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