Establishment of southern highbush blueberry cultivars and suppression of Phytophthora root rot using cover crop and soil amendment treatments

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ABSTRACT

Phytophthora root rot is a major disease of blueberry that relies on cultural practices, including soil amendments, for disease management. We evaluated the effect of three cover crops on the establishment and growth of southern highbush blueberry (SHB) plants and on their ability to suppress Phytophthora root rot infection of plants grown in a field previously identified to be infested with *Phytophthora cinnamomi*. In the spring and fall for three years, six soil amendment treatments [three cover crops (*Legume*, *Grass*, and *Brassica*), aged ground pine bark (*Bark*), fungicide drench (*Ridomil*), and none (*Control*)] were applied and tilled into the soil six months later. After three years, all soil amendment treatment plots were tilled into the soil, and two-year-old plants of three SHB cultivars were transplanted into each treatment plot and

grown for three years. Three bioassays failed to detect the presence of *P. cinnamomi* in the field; therefore, we were unable to determine the effect of the cover crops on Phytophthora root rot. Plant growth measurements (leaf and flower development scores and plant vigor ratings) and final measurements (cane count, total stem length and weight, cane diameter, and root measurements) showed no differences between plants grown in the *Control* treatment and those grown in the three cover crop treatments (*Brassica, Grass, and Legume*); however, plants in the *Bark* treatment scored higher than those in the *Control* treatment in these parameters. Results support previous reports of the beneficial effect of organic soil amendments (e.g., peat moss, sawdust, bark) on blueberries.

INTRODUCTION

Phytophthora root rot, incited by *Phytophthora cinnamomi* Rands, is a major disease of blueberry (*Vaccinium* spp.) world-wide (Milholland and Oudemans 2017). Southern highbush blueberry (SHB) cultivars (*V. corymbosum* interspecific hybrids), in general, are more susceptible to *P. cinnamomi* than the rabbiteye blueberry cultivars (*V. virgatum* Aiton) commonly grown in the southeastern USA (Stafne et al. 2024). While some blueberry cultivars are less susceptible to root rots than others, resistant cultivars are not available (Smith 2002; Yeo et al. 2016). Chemical control options are limited and include mefenoxam (Ridomil Gold SL), oxathiapiprolin (Orondis Gold 200), oxathiapiprolin + mefenoxam (Orondis Gold), and phosphonates (potassium phosphite); however, these generally suppress root rot symptoms but do not eliminate the pathogen (Brannen et al. 2009; Milholland and Oudemans 2017; Oliver 2024; Sial et al. 2024). Management of Phytophthora root rot relies primarily on cultural

practices, e.g., clean planting stock, good site selection (acidic, well-drained, sandy-silt loam soils), planting on raised beds, use of drip rather than overhead irrigation, and soil amendments to improve drainage (Milholland and Oudemans 2017; Sial et al. 2024; Stafne et al. 2024; Ward 2023; Yeo et al. 2017). Cover crops are often recommended, especially for organic production, before planting to increase organic matter, improve soil structure, suppress pathogens and nematodes, and to provide habitat and nectar for beneficial insects (Carroll et al. 2013; Demchak and Kime 2017; Everts 2016; Garcia-Salazar et al. 2020; Himmelstein et al. 2016; Kader et al. 2017; Kuepper and Ames 2024; Panth et al. 2020).

Cover crops typically fall into three classes (grass, legume, and brassica) and may be planted in the fall and/or spring (Burdine 2019). Grass species are the main component in many cover crop systems due to their rapid growth and deep fibrous roots that hold soil particles together to reduce erosion and recycle nutrients from below the crop root zone (Burdine 2019; Carroll et al. 2013). Annual ryegrass (*Lolium multiflorum* Lam.), often used as a winter cover crop, breaks up soil compaction and allows better water infiltration, its fibrous root system helps prevent erosion, and it is a scavenger of leftover nutrients (Anonymous 2024a). Brown top millet (*Urochloa ramosa (L.) T. Q. Nguyen*), a warm-season grass, is used as a fast-growing cover crop for erosion control and for suppression of root-knot nematode populations in vegetable crops (McSorley et al. 1999). Legumes convert nitrogen gas from the atmosphere into plant-available nitrogen in the soil. Two legumes, hairy vetch (*Vicia villosa* Roth) and crimson clover (*Trifolium incarnatum* L.), grown as winter cover crops and tilled into the soil the following spring suppressed Fusarium wilt (*Fusarium oxysporum* f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hansen) of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)

(Himmelstein et al. 2016). Another legume, 'Iron and Clay' cowpea (*Vigna unguiculata* (L.) Walp.), grown as a summer cover crop, reduced population levels of root-knot nematode in tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) crops the following fall (McSorley et al. 1999). Brassica species have been reported to suppress weed germination, lower nematode populations, and suppress pathogens (Burdine 2019; Carroll et al. 2013). Mustard greens (*Brassica juncea* (L.) Czern.), a brassica cover crop, reduces weed germination via a natural herbicide (glucosinolate) (Anonymous 2024b), suppresses *Rhizoctonia* on potatoes (*Solanum tuberosum* (L.) through the release of sulfur containing chemicals with fungicidal and nematicidal properties (Everts 2016), and suppresses Pythium, Fusarium and Rhizoctonia root rots in soybeans (*Glycine max* (L.) Merr.) (Anonymous 2024b). Canola or rapeseed (*Brassica napus* (L.) is primarily grown for its oil but is also grown as a fast-growing drought-tolerant cover crop that reduces soil compaction, captures residual nitrogen, and helps improve water infiltration (Anonymous 2024c).

The primary objective of this study was to determine if cover crops would suppress Phytophthora root rot infection and reduce symptoms in blueberry plants grown in a field naturally infested with *P. cinnamomi*. The secondary objective was to determine the effect of soil amendment treatments on the establishment and growth of SHB blueberry plants. The six-year study was conducted in two three-year phases. During phase one, soil amendment treatments were applied twice yearly; and during phase two, the effect of these treatments on the establishment and growth of SHB blueberry plant growth and root rot disease were assessed.

MATERIALS AND METHODS

Study site. The study was conducted at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Thad Cochran Southern Horticultural Laboratory (TCSHL) in Poplarville, MS USA (lat. 30°85′36″N, long. 89°49′94′W, elevation 97 m, USDA hardiness zone 9a). Soil type was Ruston fine sandy loam with a 2% slope, a pH of 5.5, and a soil electrical conductivity (EC) of 0.65 mS/cm at the initiation of these studies. This research field had tested positive for *P. cinnamomi* in a previous study evaluating SHB cultivars (Smith and Miller-Butler 2017). At the end of that study, all blueberry plants were removed; the field was subsoiled, tilled, and seeded with annual winter ryegrass at a rate of 100.9 kg/ha (90 pounds/acre) in October 2015. The following spring (April 2016), the ryegrass was mowed and the clippings were tilled into the soil before applying soil amendment treatments.

Soil amendments study. There were six soil amendment treatments: 1) *Control* (Fallow), no cover crop or other treatment; 2) *Ridomil* fungicide drench, same as the control treatment except the fungicide, mefenoxam (Ridomil Gold SL, Syngenta Crop Protection, LLC, Greensboro, NC, USA) was applied as a drench at a rate of 4.9 L/ha (3.5 pint/ac) broadcast twice each year in the spring and again in the fall); 3) *Grass cover crop*, brown top millet, seeded at a rate of 33.6 kg/ha (30 lb/ac), was grown during the summer; and annual ryegrass, seeded at a rate of 100.9 kg/ha (90 lb/ac), was grown during the winter); 4) *Brassica cover crop*, mustard, seeded at a rate of 11.2 kg/ha (10 lb/ac), was grown during the summer; and 'Dwarf Essex' canola, seeded at a rate of 11.2 kg/ha (10 lb/ac), was grown during the winter; 5) *Legume cover crop*, 'Iron and Clay' cowpea, seeded at a rate of 95.3 kg/ha (85 lb/ac), was grown during the summer; and

crimson clover, seeded at a rate of 16.8 kg/ha (15 lb/ac), was grown during the winter; and 6) *Bark*, a 8 cm (3 inch) layer of aged, ground bark was placed on top of the bed at the same time that the summer and winter crops were seeded and was disked into the soil at the same time as the cover crops (Burdine 2020; Sheahan 2012, 2014; Young-Mathews 2012). All the cover crop treatments were seeded broadcast over tilled soil and hand raked into the soil. The first summer soil amendment treatments were applied in March 2016 and the final winter soil amendment treatments were applied each summer and winter for three years. Subsequently in this paper, soil amendment treatments will be referred to as "treatments". Phase one study statistical design was a randomized complete block with five replications. Treatments were randomly assigned to plots within each of the five rows (replications). Each treatment plot was 9 m long x 1.5 m wide (30 ft long x 5 ft wide) with a 3.6 m (12 ft) grass alley between rows. To enhance drainage, a 3 m wide (10 ft wide) shallow [~0.3 m (1 ft) deep] grass drainage ditch ran from east to west between treatment plots across the five rows.

Soil samples were taken from each treatment plot by collecting ten or more soil cores from the top 15 cm (6 inch) from random locations within each plot for a total amount of soil sample of at least 2 pounds (900 grams) per plot. Soil samples collected one month after the first summer crop was tilled into the soil and again six months later after the first winter crop was tilled into the soil were sent to the Mississippi State University Extension Service Soil Testing Laboratory (Mississippi State, MS USA) for soil analysis [pH, available phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn), and total soluble salts (TSS)]. Additional soil samples were collected from each treatment plot periodically (March 2018,

February 2019, September 2019, May 2021, October 2021, April 2022), air-dried, and stored in the dark at room temperature [22° C (72° F)] for later pH and electrical conductivity (EC) measurements and *Phytophthora* bioassays. Their EC and pH were measured in our laboratory using a handheld combo waterproof pH, EC/TDS and temperature tester (Hanna Instruments, Model H198130, Romania).

Blueberry plant growth and root rot disease assessment. Two-year-old plants of three root-rot susceptible SHB cultivars, 'Gumbo' (Stringer et al. 2018), 'Pearl' (Stringer et al. 2013), and 'Legacy' (USDA, ARS and New Jersey Agricultural Experiment Station, Rutgers University 1993), were obtained from a commercial blueberry nursery in #1 trade gallon pots [3.4 L (0.9 gal)] and were transplanted into the previously described treatment plots in March 2019. Plants were spaced 1.2 m (4 ft) apart with two plants of each of the three cultivars in each 7.3 m (24 ft) long treatment plot. Phase two statistical design was a randomized split-plot with treatments as whole plots and two-plant cultivar plots as subplots with five replication blocks. Plants were grown following standard commercial practices for SHB blueberries in the southeastern USA (Stafne et al. 2024). During the first year, the field was irrigated as needed via drip tape. However, in March 2020, the USDA/ARS TCSHL facility was shutdown per USDA/ARS Covid-19 protocols, and the authors were not permitted on site for the following two years. One technician was allowed to periodically collect soil samples and make visual plant ratings. No irrigation, fertilizers, or weed control were applied to the plants during this 24-month shutdown.

In March 2021, each plant was rated for leaf and flower development. Leaf development was visually rated on a scale of 0 = no leaves to 4 = leaves abundant. Flower

development was rated on a similar scale, 0 = no flowers to 4 = flowers abundant; however, no plant received a flower development rating of 4. The number of surviving plants, plant vigor, and crop load were determined May 2021. Crop load was a visual estimate of the quantity of berries on each plant and ranged from 0 = no berries to 5 = most fruit among the plants in the field. Plant vigor was visually rated on a scale ranging from 0 (plant dead) to 6 (most vigorous among the plants in the field). A late spring freeze in March 2022 killed all the flowers on all the plants in the study.

In July 2022, three years after the blueberry plants were planted, phase two of the study was terminated and final plant and root data were collected. The above-ground portion of each plant was harvested by cutting each cane 10 cm (4 inch) above ground level. Stem count, total stem length (cm), length of living stems (cm), stem fresh weight (g), diameter of the largest cane at the cut base (mm), and percentage living stem (number of living stems/total number of stems x 100) were collected. Roots of each plant were excavated and all soil and potting media dislodged by vigorous shaking; and total root fresh weight (g), root length (cm), and root ball diameter (cm) were measured. Each root ball was rated as "pot bound" (score = 1) if its roots did not extend beyond the diameter of the original pot in which it had been grown or as "not pot bound" (score = 0) if its roots extended into the field soil.

Phytophthora bioassays – Baiting Bioassay. A floating leaf bait technique (Ferguson and Jeffers 1999) was used to determine the presence of *Phytophthora* spp. in soil collected periodically from each treatment plot. Twenty-five grams of soil from each plot were placed in a 125-mL glass jar, 55 mL deionized water was added to cover the soil to a depth of 2 cm, and eight 3-5 mm pieces of *Camellia japonica* L. leaves were floated in each jar and incubated for 3 days at

room temperature in the light. Leaf pieces were then removed, blotted dry, and placed under the surface of a Phytophthora specific agar, PARPHV8 (Ferguson and Jeffers 1999; Smith 2012; Smith et al. 2017). After 3 and 5 days, isolates displaying typical *P. cinnamomi* morphology were tested using a Phytophthora genus only alkaline phosphatase label ELISA test (Phytophthora PathoScreen® Kit, Catalog #PSA 92600; Agdia, Elkhart, IN USA).

Blueberry seedling assay. Seed were collected from SHB cultivar 'Biloxi' open pollinated berries harvested from plants grown in a high tunnel with two other SHB cultivars ('Star' and 'Legacy'), dried, and stored in a refrigerator. Seed were sowed onto moist, ground sphagnum moss in small insert trays [13x20 cm (5x7 inches)] that were placed in a [28x53 cm (11x21 inch)] greenhouse flat. An incubation chamber was created by placing the greenhouse flat into a water tight flat and covering it with a clear propagation dome. Seeds were germinated in the laboratory with a 12 hr dark/12 hr light cycle and temperature 26°C (78°F) and misted with water each day. When the seedlings were ~5 cm (2 inch) high, 7 seedlings/pot were transferred to 10 cm (4 inch) pots containing 600 ml (2.5 cups) soil from each treatment plot. Pots containing seedlings were maintained in a warm greenhouse and watered daily with tap water. Two studies were conducted. Assay 1: In June 2016, seedlings were transplanted into pots containing soil collected from each treatment plot at the beginning of the study (May 2016) and the seedlings were harvested in April 2017. Assay 2: In November 2016, seedlings were transplanted into soil collected from treatment plots after the first summer treatments were tilled into the soil (September 2016), and the seedlings were harvested in September 2017. The number of seedling, their total weight, and weight/seedling in each pot were recorded. Any root rot symptoms on each were recorded. Statistical design was a randomized complete block

with five replications. Treatments were the same as in the soil amendment study and were randomly assigned to pots grouped on the greenhouse bench by replication.

Phytophthora bioassays - Lupine seedling assay. Soil [925 ml (4 cup)/tray] collected from treatment plots at the end of the study (July 2022), was placed in insert trays [~13x20 cm (~ 5x7 inches)], and seeded with 18 lupine (Lupinus angustifolius L.) seed/tray in October 2022. In addition to soil from the treatment plots, three comparison soils were included; i) Pc positive, soil from a site with Phytophthora root rot symptomatic blueberry plants and a history of P. cinnamomi infestation, ii) Pc negative, soil from a site with no history of P. cinnamomi infestation and blueberry plants that did not have symptoms of Phytophthora root rot, and iii) Pc unknown, soil from a site with Phytophthora root rot symptomatic blueberry plants, but with no history of *P. cinnamomi* infestation. Insert trays containing seeds were placed humidity chambers constructed by placing a 1020 greenhouse flat [~28 cm w x 54 cm l x 6 cm d (10.9" w x 21.4" | x 2.4" d)] with drainage holes inside a similar flat with no drainage holes and covered with a clear humidity dome. Each humidity chamber contained six insert trays and was maintained in the laboratory at a temperature of 26°C (78°F) on a light cart with 24 hr light supplied by GroLux 20W T12 Wide Spectrum Fluorescent bulbs (Sylvania, Wilmington, MA USA) and watered three times/week with tap water. Lupine seedlings began to emerged 4 days after seeding, and the humidity domes were removed three weeks after seeding. Once a month, each insert tray was submerged in water for ~48 hours to simulate a flood event. The number of surviving seedlings was counted 4 and 100 days after seeding (Chee and Newhook 1965; Darvas 1979; Hoitink et al. 1977). Statistical design was a randomized complete block with five

replications. Treatments were the same as in the soil amendment study and were randomly assigned to trays grouped on the light cart by replications.

Statistical analysis

To estimate the effects of soil amendment treatment and its interaction with cultivar on the means of the different response variables, we fit linear mixed models (LMMs). Models included random intercepts corresponding to a split plot design: one for replicate block and one for main plot (i.e., soil amendment treatment nested within replicate), except for soil chemistry variables (Tables 1 & 2) which were not measured at the plot level and therefore only had random intercepts for replicate. Soil chemistry variables other than pH were log-transformed prior to fitting Gaussian LMMs, and response variables were back-transformed. Soil pH and electrical conductivity (Table 2) were measured at multiple time points, therefore we fit a generalized additive mixed model (GAMM) with a separate thin plate spline for each soil amendment treatment, and estimated means from that model for each treatment at each time point.

Probability of survival in September 2021 and June 2022 were separately modeled with binomial generalized linear mixed models (GLMMs) with logit link functions; to mitigate the issue of complete separation by treatment, regularizing Normal(0, 5) priors were put on the fixed effects. Categorical score variables (leaf development, flower development, crop load, fruit ripeness, and vigor ratings; Table 3) were modeled with cumulative logistic GLMMs with ordered multinomial response distribution and cumulative logit link functions. The cumulative models were fit using a Bayesian approach, with Normal(0, 5) priors on the fixed effects and

Gamma(1, 1) priors on the random effect standard deviations. Hamiltonian Monte Carlo was used to sample the posterior distributions, with 4 Markov chains each having 2000 discarded warmup iterations and 1000 retained sampling iterations.

Stem count (Table 4) was modeled with a Poisson GLMM with log link function.

Proportion of live stems (Table 4) was modeled with a zero-inflated Beta GLMM with logit link function, fixed effects on the zero-inflation parameter, and regularizing Normal(0,5) priors put on the fixed effects of the zero-inflation parameter because of complete separation. Total stem length, cane diameter, and stem weight (Table 4) were modeled with Gamma GLMMs, excluding five zeros from plants that did not survive from the model, thus those estimates are conditional on the plant having survived. Live stem length (Table 4) was modeled with a zero-inflated Gamma GLMM, again with fixed-effects on the zero-inflation parameter with Normal(0, 5) priors.

Root ball diameter, length of longest root, and root ball weight (Table 5) were log-transformed and fit with Gaussian LMMs; again, zeros for the five plants that did not survive were excluded. Probability of being pot bound (Table 5) was fit with a binomial GLMM with logit link function and Normal(0, 5) priors on the fixed effects. For the data from the blueberry seedling assays (Table 6), replicate nested within assay was fit with a random intercept, and soil amendment treatment, assay, and their interaction were treated as fixed effects. Because assay did not explain significant variance, we presented means averaged over assay. Seedling count was analyzed with a Poisson GLMM. Total seedling weight and weight per seedling were log-transformed and analyzed with Gaussian LMMs. For the lupine seedling assay data (Table 7), the only fixed effect was soil amendment treatment, and the only random effect was replicate.

Proportion of germinated seedlings at 4 and 100 days were fit with binomial GLMMs. Simulated residual plots were examined for all models to ensure that assumptions were met.

Analysis of variance was done using joint F-tests for models assuming a normal response distribution, and joint Chi-squared tests for all other models. In all cases, we compared means using the Tukey adjustment of p-values and the Sidak adjustment for the 95% confidence interval end points. Tukey-adjusted p-values < 0.05 were considered significant. Means were compared between soil amendment treatments averaged over cultivars, between cultivars averaged over soil amendment treatments, and between soil amendment treatments separately within each cultivar. For the Bayesian cumulative logistic models (Table 3), means for each treatment combination were estimated by taking the mean of the posterior expected values of the category probabilities weighted by the score values. Differences between those weighted means were assessed using the Bayesian maximum a posteriori p-value (pMAP), with pMAP < 0.05 considered significant (Makowski et al. 2019).

Statistical analysis was done using R software v4.4.1 (R Core Team 2024) and Stan software v2.35.0 (Stan Development Team 2024), including the R packages glmmTMB v1.1.10 (Brooks et al. 2017), emmeans v1.10.4 (Lenth 2024), mgcv v1.9-1 (Wood 2011), and brms v2.21.0 (Bürkner 2018). Data and code needed to reproduce the analysis in this manuscript are archived on Ag Data Commons (Smith et al. 2024).

RESULTS and DISCUSSION

Effect of treatments on pH and nutrient levels. Analysis of soil samples collected one month after the first summer treatments were tilled into the soil and six months later after the first winter treatments were tilled into the ground showed a significant main effect of treatment and sampling date for pH, TSS, and all extractable nutrients except Zn (Pr>F <0.0001) (Table 1). There were significant treatment*sampling date interactions (Pr>F < 0.02) for all extractable elements except P, K, and Zn (Table 1). In general, the three cover crops (Brassica, Grass, and Legume) had a greater impact on soil pH and nutrients compared to the Ridomil, Bark, and Control treatments. Examples include: soil pH at the 9/2016 sampling date was significantly higher (95% CI) in the *Ridomil, Bark*, and *Control* treatments (4.60, 4.66, and 4.78, respectively) than in Brassica and Legume treatments (4.3 and 4.24, respectively), while at the 4/2017 sampling date, pH was significantly higher (95% CI) in the Bark treatment (4.64) than in Brassica, Grass, and Legume treatments (3.94, 3.94 and 3.98, respectively). Mg was highest in the Bark and Ridomil treatments in 9/2016; and in 4/2017, it was highest in the Bark, Control, and Ridomil treatments. There were no differences among treatments in Ca levels in 9/2016, but in 4/2017, Ca was higher in the Bark and Control treatments than in the Legume treatment (Table 1). The *Brassica* and *Legume* cover crops effectively lowered soil pH in this trial below the acceptable range for blueberry production (pH 4.5 to 5.5 in well-drained soils). However, using cover crops may have an additional benefit for those growers looking to reduce soil pH. Correlations between P. cinnamomi levels and soil pH, fertility, and texture have been reported; e.g., Moreira and Martins (2005) found no restriction to P. cinnamomi activity in the soil pH range of 3.4 to 6.5; but they did isolate the pathogen more frequently (P = 0.04) in soils with pH

≥5.1; Scarlett et al. (2013) reported a positive association between high levels of nutrients, particularly nitrogen, and *P. cinnamomi*, but found that this association was species specific; and Shearer and Tippett (1989) observed that low fertility, especially low phosphorus levels, of Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest soils were more conducive for survival of *P. cinnamomi*.

Soil amendments study - Effect of treatments on pH and EC. Average pH and EC of soil collected once or twice yearly from treatment plots showed that the soil pH ranged from 4.0 to 5.7 and all were within the 4.5 to 5.5 acceptable level for blueberries (Stafne et al. 2024) (Table 2, Fig. 1); except, the 5.7 pH at the start of the study which was at just above the acceptable range. Soil pH from all treatments was reduced sharply after the first sample date, then gradually increased at each sample date for the duration of the experiment. EC levels ranged from 0.09 to 0.19 mS/cm and were within acceptable levels for blueberries for all sampling dates except the first (May 2016) and last sampling dates (April 2022) (Table 2, Fig. 1). EC levels for all treatments dropped dramatically after the first sample date and continued to drop until April 2022 when they increased sharply. The increase in EC levels in April 2022 likely was due to the return of regular fertilizer applications to blueberry sub-plots following the return of the staff to the research facility after the Covid protocols were dropped in March 2022.

Blueberry plant growth and root rot disease assessment. There were no significant differences due to treatment in the probability of live plants in September 2021 or in June 2022 (P = 0.91 and 0.44 respectively) or in the treatment by cultivar interaction at either rating date data (not shown). There was a significant treatment by cultivar interaction at the June 2022 rating date with 'Gumbo' having a higher probability of live plants (98.9%, 95% CI = [83.6%, 99.9%]) than

Pearl (80%, 95% CI = [48%, 94.5%]). Leaf development ratings (March 2021) and vigor ratings (June 2022) of plants in the Bark treatment were greater than those of plants in all other treatments (Table 3). Among the three cultivars, leaf development ratings (March 2021) of 'Gumbo' and 'Legacy' plants were more advanced than those of 'Pearl' plants. Vigor ratings in September 2021 were higher for 'Legacy' plants than for 'Gumbo' plants, but at the conclusion of the study, 'Gumbo' plants received higher rating than 'Legacy' or 'Pearl' plants (Table 3). The interactions between the treatments and cultivars are shown on Table 3. The Bark soil amendment treatment had a beneficial effect on leaf development and plant vigor. Application of bark (especially as a mulch) has the known benefits of reduced weed competition, enhanced soil moisture retention, and maintaining cooler soil temperatures in hot growing conditions (Kader et al. 2017). Both 'Gumbo' and 'Pearl' were bred and released from USDA-ARS in Poplarville, MS, while 'Legacy' was bred in New Jersey. All are considered southern highbush blueberries, and even though there were some differences among them in ratings, the ratings are not consistent enough to determine which cultivar is superior under these growing conditions.

Final plant measurements. Three years after blueberry plants were transplanted into treatment plots, plants harvested from the *Bark* treatments had more canes per plant (3.4 canes/plant) compared to plants harvested from the *Brassica, Legume,* and *Ridomil* treatments (2.1, 2.1 and 2.0 canes/plant, respectively), and among the cultivars, 'Gumbo' plants had more canes/plant (2.9 canes/plant) than 'Legacy' and Pearl (2.0 and 2.1 canes/plant, respectively). In addition, cane diameter of plants harvested from the *Bark* treatments was greater (41.9 mm) than the cane diameter of plants from the other five treatments (18.8 mm average).

Interactions between cultivars and treatments for total stem length, live stem length, live stem weight, cane diameter, and percentage of live stems are shown in Table 4. The root system of each plant was excavated; and root ball diameter, length of the longest root, and root ball weight were measured. Among the treatments, plants from the *Bark* treatment had the longest roots and the largest and heaviest root balls (Table 5). Among the cultivars, 'Gumbo' plants had longer roots and larger and heavier root balls than 'Pearl' plants.

These results indicate that blueberry plants benefit from amending the soil with bark. While bark is frequently used in blueberry production, the cost of purchasing and applying bark has increased substantially in recent years leading growers to look for more cost-effective alternatives. This is understandable, but this study again demonstrated that bark provides a great benefit when establishing blueberry plants. 'Gumbo' outperformed 'Pearl' and 'Legacy' in most measured parameters. Upon its release, 'Gumbo' was shown to outperform 'Pearl' and some other southern highbush cultivars (Stringer et al. 2018). Even though there were no differences in final plant data among treatments, other than that due to the *Bark* treatment, this does not necessarily mean the treatments did not affect root rot disease incidence. In ornamental field grown nursery production, Panth et al. (2020) reported lower root rot disease severity and recovery of soilborne fungi and oomycetes from soils with cover crops compared to the non-cover cropped soil; however, they reported no differences in plant fresh weight and root weight between the two treatments.

Phytophthora bioassays, Baiting Bioassay. The camellia leaf assay did not yield any isolates that displayed typical *P. cinnamomi* morphology, nor did any of the blueberry plants show

typical above-ground disease symptoms, i.e., discoloration of the leaves (yellowing or reddening), defoliation, or stunted growth or below ground symptoms, i.e., root necrosis or a reduction in the number of roots (Milholland 1995). Even though *P. cinnamomi* had routinely been isolated from the test field using the camellia leaf assay during a five-year study conducted in the same field immediately preceding the current study, we concluded that the pathogen was no longer present in the soil. Therefore, we could not test our major objective of determining if cover crops would suppress Phytophthora root rot infection and reduce symptoms in young blueberry plants grown in a field infested with *P. cinnamomi*. But we could test our secondary objective of determining the effect of the cover crop treatments on the establishment and growth of SHB blueberry plants.

Blueberry seedling assay. Blueberry seedlings were transplanted and grown for five months in soil collected from each treatment plot. There were no significant differences (α = 0.05) in main effects in the number of surviving seedlings, seedling weight, or weight/seedlings between the soil from the two assays or among the soil from the six treatments, nor were there significant assay by treatment interactions (Table 6). On average, 68% of the seven seedlings transplanted into each treatment soil survived. Surviving seedlings did not display typical Phytophthora root rot symptoms, i.e., leaves yellow with burned margins, no new foliage, or root decay. Addition of a flood event to the protocol might have enhanced the probability of detecting the pathogen if it were present. Many reports have noted a correlation between Phytophthora root rot severity and flood events; e.g., wet soil conditions stimulated germination of *Phytophthora* spores (Sterne et al. 1977); poor growth of blueberry plantings on poorly drained soils was associated with Phytophthora root rot (Sterne 1982); flooding increased disease severity

(MacDonald and Duniway 1978); and incidence was higher with weekly and biweekly flooding than with monthly or no flooding (de Silva et al. 1999).

Lupine seedling assay. After the failure of the blueberry seedling assay to indicate Phytophthora in the soils from the treatment plots, treatment soils were tested using a lupine seedling assay which has been utilized for many years to detect *Phytophthora* in soils (Allardyce et al. 2012; Chee and Newhook 1965; Darvas 1979; Gunning and Cahill 2009; Hoitink et al. 1977). Several flood events and three comparison soils were included in the lupine assay. There were significant differences in lupine seedling counts 4 and 100 days after seeding among the soils from the six treatment plots and three comparison sites (Table 7). Higher seedling counts 4 and 100 days after seeding soils from the Pc unknown and Pc negative comparison soils suggested fewer lupine seedlings due to a lower population of *Phytophthora* zoospores in these soils compared to soils from the treatment plots. There were significantly fewer seedlings in the soils from the Brassica and Grass treatments than in the soils from the Pc negative and Pc unknown treatments (Table 7). Roots of surviving seedlings in Brassica treatment displayed typical symptoms of Phytophthora root rot, i.e., sharply defined brown lesions above the root tip (Chee and Newhook, 1965) indicating the presence of *Phytophthora* zoospores in soil from the Brassica treatment. The lupine assay did not indicate significant differences ($\alpha = 0.05$) among the six treatment soils which agrees with the results of the blueberry seedling assay.

CONCLUSIONS

There were no significant differences between the *Control* treatment and the three cover crop treatments (*Brassica, Grass, and Legume*) in leaf and flower development scores, percentage

living plants, crop load, or plant vigor, signifying that the cover crop treatments did not influence plant growth, flower development, or fruit production. Leaf development ratings and final vigor ratings of plants in the Bark treatment were greater than those of plants in all other treatments. Likewise, after three years growth, final measurements of plants from the Bark treatment were higher than measurements from plants in all other treatments. These results support previous reports of the beneficial effect of organic soil amendments (e.g., peat moss, sawdust, and bark) on blueberries and the recommendations to add these amendments to soils before planting blueberries (Ames and Espinoza 2023; Bouska et al. 2018; Foulk et al. 2021; Himelrick 2008; Krewer and Ruter 2012; Nesbitt et al. 2013; Pavlis and Sciarappa 2005). Specific examples include: Cline (2013) recommended bringing soil organic matter to 3% or greater by amending soil with 3% or greater from naturally-occurring humic matter or by soil amendments added prior to planting blueberries in North Carolina; Demchak and Kime (2017) stated that blueberries produce best in soils with a high organic matter content and recommended blueberry growers increase the organic matter content in their soils by adding rotted sawdust, peat moss, or other composted organic matter and/or by plowing in cover crops. Demchak and Kime (2017) also recommended adding organic matter in the planting hole, especially for clay soils. Kuepper and Ames (2024) further stated that because blueberries have a shallow root system, they may benefit from the soil disease suppressive qualities of an organic mulch. Improved soil moisture may have been a contributing factor to the growth of plants in the Bark treatment, but it is not easy to narrow it down to this one component. Addition of pine bark has several benefits beyond soil moisture, including weed suppression, addition of organic matter, and reduction in soil temperature during summer months. Even though our results did

not indicate any Phytophthora root rot suppression due to the three living cover crops, cover crops do play an important role in the years prior to planting blueberries through improvement of soil organic matter, breaking up compaction layers, erosion control, and suppression or elimination of weeds (Carroll et al. 2013).

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The author(s) declare no conflict of interest.

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TABLES

- 2 **Table 1**. Interaction of soil amendment treatment by soil sample date of soil samples collected one month after the first summer soil
- 3 amendment treatments (September 2016) and one month after the first winter soil amendment treatments (April 2017) were tilled
- 4 into the soil. Each sample was analyzed for pH; available phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn);
- 5 and total soluble salts (TSS).

Soil amendment treatment ^a							
Soil sample							
date	рН	P (ppa b)	K (ppa)	Mg (ppa)	Zn (ppa)	Ca (ppa)	TSS (mmhos/cm)
September 2016	Estimate [95% CI] c,d	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]
Control	4.60 [4.42, 4.78] ab	125 [81.3, 193] c	192 [153, 242] c	162 [127, 206] ab	6.8 [5.6, 8.2] a	906 [667, 1230] a	0.1 [0.084, 0.12] c
Bark	4.66 [4.48, 4.84] a	126 [81.6, 194] bc	249 [197, 313] bc	185 [145, 235] a	7.8 [6.4, 9.5] a	1070 [791, 1460] a	0.1 [0.084, 0.12] c
Brassica	4.30 [4.12, 4.48] c	245 [159, 377] a	267 [212, 336] abc	127 [100, 162] b	7.73 [6.4, 9.4] a	830 [612, 1130] a	0.17 [0.15, 0.21] b
Grass	4.34 [4.16, 4.52] bc	206 [134, 318] ab	380 [302, 478] a	162 [128, 206] ab	6.97 [5.7, 8.5] a	874 [644, 1190] a	0.25 [0.21, 0.29] a
Legume	4.24 [4.06, 4.42] c	271 [176, 417] a	344 [273, 433] ab	169 [133, 214] ab	7.9 [6.6, 9.7] a	1060 [780, 1440] a	0.28 [0.23, 0.33] a
Ridomil	4.78 [4.6, 4.96] a	134 [87.2, 207] bc	201 [159, 253] c	182 [143, 231] a	6.7 [5.5, 8.1] a	977 [720, 1330] a	0.1 [0.084, 0.12] c
April 2017							
Control	4.26 [4.08, 4.44] b	117 [75.8, 180] b	143 [114, 180] bc	127 [100, 162] ab	6.11 [5.1, 7.5] a	769 [566, 1040] ab	0.2 [0.17, 0.24] a
Bark	4.64 [4.46, 4.82] a	85.6 [55.6, 132] b	175 [139, 221] bc	166 [131, 211] a	7.2 [5.9, 8.8] a	953 [702, 1290] a	0.1 [0.08, 0.12] b

Brassica	3.94 [3.76, 4.12] c	238 [155, 367] a	185 [147, 233] bc	80 [62.9, 102] c	6.1 [5.0, 7.5] a	596 [439, 809] bc	0.2 [0.17, 0.24] a
Grass	3.94 [3.76, 4.12] c	201 [130, 310] a	272 [216, 343] a	97.4 [76.6, 124] bc	6.4 [5.3, 7.8] a	652 [480, 885] bc	0.24 [0.19, 0.29] a
Legume	3.98 [3.8, 4.16] c	224 [146, 346] a	196 [156, 247] ab	84.1 [66.1, 107] c	5.7 [4.7, 6.9] a	536 [395, 728] c	0.22 [0.18, 0.26] a
Ridomil	4.26 [4.08, 4.44] b	115 [74.4, 177] b	133 [106, 168] c	125 [98, 158] ab	5.9 [4.9, 7.2] a	748 [551, 1020] abc	0.20 [0.17, 0.24] a

- ^a Control (Fallow) no treatment; Bark (Ground pine bark) [summer and winter 8 cm (3 inch) layer of aged, ground pine bark seeded 6 and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer - mustard (Brassica juncea (L.) Czern.) 7 seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (B. napus L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; Grass 8 cover crop [summer - brown top millet (Urochloa ramosa (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter - annual 9 10 ryegrass (Lolium multiflorum Lam.) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; Legume cover crop [summer - 'Iron and Clay' cowpea (Vigna unguiculata (L.) Walp.) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter - crimson clover (Trifolium incarnatum L.) seeded at a 11 rate of 16.8 kg/ha (15 lb/ac)]; Ridomil (mefenoxam) [summer and winter - fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac) 12 broadcast]. 13
- b ppa = pounds per acre; TSS = total soluble salts.
- 15 ° 95% CI = 95% confidence intervals are displayed in brackets after the mean and have been adjusted with the Sidak multiple 16 comparison adjustment.
- d p-values on which the multiple comparison letters are based have been adjusted with the Tukey adjustment.

Table 2. Interaction of soil sample dates and soil amendment treatments on estimated marginal means of pH and electrical conductivity (EC). Separate Tukey adjustments are made within each time point. EC levels were determined by 1:1 volume laboratory extract analysis.

Soil Amendment	pH	EC (mS/cm)		
Treatment ^a	Estimate [95% CI] ^{b, c}	Estimate [95% CI]		
5/1/2016				
Control	5.35 [5.00, 5.7] a	0.516 [0.328, 0.811] ab		
Bark	5.52 [5.17, 5.87] a	0.436 [0.277, 0.685] b		
Brassica	5.31 [4.96, 5.66] a	0.828 [0.527, 1.30] ab		
Grass	5.42 [5.06, 5.77] a	0.887 [0.565, 1.39] a		
Legume	5.32 [4.97, 5.67] a	0.805 [0.512, 1.26] ab		
Ridomil	5.73 [5.37, 6.08] a	0.591 [0.376, 0.929] ab		
9/1/2017				
Control	4.37 [4.05, 4.69] ab	0.134 [0.0861, 0.207] b		
Bark	4.62 [4.32, 4.92] a	0.041 [0.0265, 0.0639] c		
Brassica	4.17 [3.84, 4.5] ab	0.192 [0.124, 0.298] ab		
Grass	4.03 [3.70, 4.37] b	0.285 [0.184, 0.441] a		
Legume	4.32 [4.01, 4.64] ab	0.218 [0.14, 0.337] ab		
Ridomil	4.37 [4.04, 4.71] ab	0.142 [0.0913, 0.22] b		
2/1/2019				
Control	4.44 [4.19, 4.69] a	0.175 [0.126, 0.241] a		

Bark	4.23 [3.99, 4.47] a	0.163 [0.118, 0.226] a
Brassica	4.24 [3.99, 4.49] a	0.195 [0.141, 0.27] a
Grass	4.27 [4.02, 4.52] a	0.190 [0.137, 0.263] a
Legume	4.23 [3.98, 4.48] a	0.199 [0.144, 0.275] a
Ridomil	4.40 [4.14, 4.65] a	0.176 [0.127, 0.243] a
9/1/2019		
Control	4.59 [4.33, 4.85] a	0.134 [0.0955, 0.189] a
Bark	4.26 [4.01, 4.51] a	0.187 [0.133, 0.263] a
Brassica	4.38 [4.12, 4.65] a	0.141 [0.100, 0.198] a
Grass	4.49 [4.23, 4.75] a	0.122 [0.087, 0.172] a
Legume	4.33 [4.08, 4.59] a	0.138 [0.0981, 0.194] a
Ridomil	4.56 [4.30, 4.82] a	0.132 [0.0941, 0.186] a
5/1/2021		
Control	4.72 [4.45, 4.99] a	0.066 [0.046, 0.096] a
Bark	4.53 [4.28, 4.79] a	0.084 [0.058, 0.123] a
Brassica	4.51 [4.23, 4.78] a	0.072 [0.050, 0.105] a
Grass	4.59 [4.30, 4.87] a	0.071 [0.049, 0.102] a
Legume	4.52 [4.25, 4.78] a	0.070 [0.046, 0.102] a
Ridomil	4.71 [4.43, 4.99] a	0.063 [0.044, 0.092] a
10/1/2021		
Control	4.72 [4.51, 4.93] a	0.14 [0.107, 0.185] a
Bark	4.57 [4.36, 4.78] a	0.18 [0.137, 0.237] a

Brassica	4.55 [4.34, 4.76] a	0.155 [0.118, 0.205] a
Grass	4.59 [4.37, 4.8] a	0.148 [0.113, 0.195] a
Legume	4.53 [4.32, 4.74] a	0.150 [0.114, 0.197] a
Ridomil	4.72 [4.51, 4.94] a	0.136 [0.103, 0.178] a
4/1/2022		
Control	4.72 [4.40, 5.04] a	0.561 [0.366, 0.860] a
Bark	4.60 [4.29, 4.9] a	0.793 [0.516, 1.22] a
Brassica	4.63 [4.30, 4.95] a	0.624 [0.407, 0.957] a
Grass	4.61 [4.28, 4.94] a	0.553 [0.361, 0.847] a
Legume	4.54 [4.22, 4.86] a	0.587 [0.383, 0.900] a
Ridomil	4.76 [4.43, 5.08] a	0.554 [0.361, 0.849] a

layer of aged, ground pine bark seeded and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer - mustard (*Brassica juncea (L.) Czern.*) seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (*B. napus* L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; <u>Grass</u> cover crop [summer - brown top millet (Urochloa ramosa (L.) T.Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter - annual ryegrass (*Lolium multiflorum*) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; <u>Legume</u> cover crop [summer - 'Iron and Clay' cowpea (*Vigna unguiculata (L.) Walp.*) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter - crimson clover (*Trifolium incarnatum* L.) seeded at a rate of 16.8 kg/ha (15 lb/ac)]; <u>Ridomil</u> (mefenoxam)

[summer and winter - fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac) broadcast].

^a Control (Fallow) no treatment; Bark (Ground pine bark) [summer and winter - 8 cm (3 inch)

- 31 b 95% CI = 95% confidence intervals are displayed in brackets after the mean and have been
- 32 adjusted with the Sidak multiple comparison adjustment.
- 33 c p-values on which the multiple comparison letters are based have been adjusted with the
- 34 Tukey adjustment.

Table 3. Interaction of soil amendment treatments and cultivars on blueberry leaf and flower development (March 2021); crop load
 and fruit ripeness (May 2021); and plant vigor ratings (September 2021 and June 2022).

Cultivar x Soil	Leaf development ^a	Flower development ^b	Crop load ^c	Fruit ripeness ^d	Vigor rating e 9/21	Vigor rating 6/22
Amendment Treatment f	Estimate [95% CI] ^{g, h}	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]
Gumbo						
Control	1.4 [0.596, 2.22] b	1.7 [0.734, 2.85] a	1.7 [0.758, 2.85] a	1.3 [0.574, 2.01] a	1.8 [0.636, 3] ab	2.3 [1.73, 2.94] b
Bark	2.9 [2.03, 3.56] a	2.0 [0.73, 3.4] a	2.0 [0.791, 3.49] a	1.1 [0.289, 1.98] a	1.7 [0.394, 2.97] ab	4.6 [4.26, 4.87] a
Brassica	1.8 [0.847, 2.72] ab	1.8 [0.758, 2.97] a	1.7 [0.76, 2.97] a	1.5 [0.767, 2.24] a	2.0 [0.603, 3.25] ab	2.8 [2.11, 3.4] b
Grass	1.7 [0.785, 2.58] ab	1.5 [0.586, 2.7] a	1.7 [0.54, 2.79] a	1.1 [0.426, 1.9] a	1.3 [0.196, 2.67] ab	2.4 [1.77, 3.08] b
Legume	1.6 [0.761, 2.48] b	1.4 [0.492, 2.77] a	1.4 [0.462, 2.82] a	1.0 [0.34, 1.85] a	1.2 [0.16, 2.53] b	2.5 [1.84, 3.2] b
Ridomil	1.5 [0.702, 2.44] b	1.5 [0.487, 2.9] a	1.5 [0.459, 2.97] a	1.4 [0.517, 2.19] a	3.5 [2.19, 3.97] a	2.9 [2.2, 3.59] b
Legacy						
Control	1.9 [0.913, 2.87] b	0.8 [0.155, 2.02] a	0.8 [0.172, 1.99] a	1.1 [0.296, 2.07] a	2.0 [0.624, 3.13] a	1.8 [1.15, 2.45] b
Bark	4.0 [3.75, 4] a	0.6 [0.0617, 2.29] a	0.6 [0.0596, 2.27] a	1.8 [0.512, 3.2] a	1.7 [0.389, 2.99] a	4.1 [3.49, 4.45] a
Brassica	1.2 [0.375, 2.24] bc	1.9 [0.783, 3.27] a	1.9 [0.713, 3.38] a	0.9 [0.275, 1.7] a	2.6 [1.11, 3.68] a	1.2 [0.653, 1.81] b
Grass	0.3 [0.0517, 1.12] c	2.7 [0.973, 4.26] a	2.6 [1.03, 4.26] a	1.8 [0.708, 2.8] a	2.3 [0.842, 3.48] a	0.9 [0.357, 1.53] b
Legume	1.5 [0.604, 2.53] bc	0.7 [0.114, 1.92] a	0.7 [0.117, 1.92] a	0.8 [0.158, 1.74] a	2.1 [0.756, 3.33] a	1.7 [1.08, 2.3] b
Ridomil	2.5 [1.47, 3.41] b	0.8 [0.155, 2.09] a	0.8 [0.151, 2.09] a	0.9 [0.196, 1.86] a	3.7 [2.42, 3.98] a	1.9 [1.28, 2.49] b
Pearl						
Control	0.2 [0.0161, 0.744] b	1.9 [0.828, 3.23] a	1.9 [0.812, 3.2] a	1.3 [0.536, 2.1] ac	2.5 [1.25, 3.58] a	0.7 [0.226, 1.28] b
Bark	2.6 [1.65, 3.28] a	1.7 [0.723, 2.98] a	1.8 [0.773, 3.01] a	1.7 [0.866, 2.45] ab	1.8 [0.385, 3.05] a	4.3 [3.83, 4.66] a
Brassica	0.1 [0.0047, 0.628] b	2.3 [0.91, 3.78] a	2.3 [0.943, 3.87] a	2.0 [1.25, 3.03] a	1.8 [0.415, 3.22] a	1.1 [0.576, 1.73] b

Grass	0.6 [0.154, 1.46] b	1.6 [0.615, 2.95] ab	1.6 [0.649, 3.01] ab	1.2 [0.493, 1.97] ac	2.3 [0.766, 3.44] a	1.7 [1.02, 2.34] b
Legume	0.7 [0.172, 1.7] b	0.2 [0.011, 0.97] b	0.2 [0.0108, 1] b	0.2 [0.011, 0.97] c	2.3 [0.919, 3.46] a	1.1 [0.462, 1.89] b
Ridomil	0.2 [0.0326, 0.92] b	0.7 [0.146, 1.77] ab	0.7 [0.126, 1.85] ab	0.5 [0.0847, 1.3] bc	3.1 [1.59, 3.9] a	0.7 [0.257, 1.33] b

- ^a Leaf development was visually rated on a scale of 0=No leaves to 4=Leaves abundant.
- 39 b Flower development was visually rated on a scale of 0=No flowers to 4=Leaves abundant.
- 40 ° Crop load was a visual estimate of the quantity of berries on each plant and ranged from 0 = no berries to 5 = most fruit among all
- the plants in the field.
- d Ripeness Stage: 0=no berries, 1=all green berries, 2=mix of stages with green berries most dominant, 3=mix of stages with all stages
- equally represented, 4=mix of stages with ready to harvest most dominant, 5= all berries ready to harvest.
- ^e Plant vigor was visually rated on a scale ranging from 0 (plant dead) to 6 (most vigorous among the plants in the field).
- 45 f Control (Fallow) no treatment; Bark (Ground pine bark) [summer and winter 8 cm (3 inch) layer of aged, ground pine bark seeded
- and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer mustard (Brassica juncea (L.) Czern.)
- seeded at a rate of 11.2 kg/ha (10 lb/ac), winter 'Dwarf Essex' canola (*B. napus* L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; *Grass*
- cover crop [summer brown top millet (Urochloa ramosa (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter annual
- ryegrass (Lolium multiflorum Lam.) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; Legume cover crop [summer 'Iron and Clay' cowpea
- 50 (Vigna unguiculata (L.) Walp.) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter crimson clover (Trifolium incarnatum L.) seeded at a

- rate of 16.8 kg/ha (15 lb/ac)]; *Ridomil* (mefenoxam) [summer and winter fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac)
- 52 broadcast].
- 53 g 95% CI = 95% credible intervals are displayed in brackets after the mean.
- ^h Bayesian maximum *a posteriori* p-values () were used to generate the multiple comparison letters.

Table 4. Interaction of soil amendment treatments and cultivars on total stem length, length of live stems, cane diameter at the base, total weight of stems, and percentage of live stems (number of live stems/total number of stems*100) at the conclusion of the study in July 2022.

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Cultivar x Soil	Total stem length (cm)	Live stem length (cm)	Cane diameter (mm)	Stem weight (g)	Live stems (%)
Amendment Treatment ^a	Estimate [95% CI] ^{b, c}	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]
Gumbo					
Control	187 [128, 275] b	74 [40.6, 134] b	21.5 [14.4, 31.9] b	71 [37.6, 135] b	47.0 [27.3, 67.7] c
Bark	377 [257, 553] a	366 [203, 660] a	51.8 [34.8, 77.1] a	528 [279, 1000] a	92.1 [82.5, 96.7] a
Brassica	188 [128, 276] b	147 [81.3, 264] b	23.3 [15.7, 34.6] b	115 [60.5, 220] b	82.1 [65.1, 91.8] ab
Grass	190 [129, 279] b	121 [64.6, 225] b	24.2 [16.3, 36] b	110 [57.7, 210] b	58.6 [36.3, 77.9] bc
Legume	193 [131, 286] b	187 [91.3, 383] ab	24.8 [16.5, 37.2] b	143 [74.9, 272] b	86.0 [67.8, 94.7] ab
Ridomil	213 [145, 314] ab	174 [96.3, 313] ab	27.4 [18.4, 40.8] b	127 [66.3, 242] b	84.1 [68.2, 92.9] ab
Legacy					
Control	169 [115, 249] a	93 [46.7, 183] ab	20.5 [13.8, 30.7] ab	86 [45.1, 165] b	53.3 [31.5, 73.9] b
Bark	200 [136, 294] a	199 [110, 359] a	32.6 [21.9, 48.5] a	289 [153, 548] a	92.0 [82.3, 96.6] a
Brassica	126 [85.7, 185] a	52 [26.7, 100] b	15.9 [10.7, 23.7] b	46 [24.3, 87.4] b	45.2 [24.0, 68.4] b
Grass	132 [89.7, 194] a	61 [28.2, 131] b	15.7 [10.5, 23.4] b	58 [30.3, 112] b	58.3 [31.4, 81.0] b

Legume	122 [82.9, 180] a	64 [32.6, 127] b	15.7 [10.5, 23.4] b	56 [29.3, 107] b	47.1 [25.4, 70.0] b
Ridomil	123 [83.5, 180] a	37 [20.1, 69.3] b	.0 [10.1, 22.2] b	51 [27.0, 96.9] b	40.1 [21.2, 62.5] b
Pearl					
Control	120 [79.9, 179] b	61 [23.9, 154] bc	14.3 [9.41, 21.7] bc	38 [19.4, 72.7] b	44.9 [17.3, 76.1] bc
Bark	336 [229, 493] a	311 [173, 561] a	43.4 [29.2, 64.6] a	436 [230, 827] a	87.9 [74.6, 94.7] a
Brassica	113 [75.7, 170] b	31 [15.2, 62.1] c	14.3 [9.47, 21.7] bc	39 [20, 75.2] b	32.0 [14.2, 57.2] c
Grass	165 [110, 247] b	75 [39.6, 141] bc	26.0 [16.9, 39.9] ab	86 [42.6, 172] b	50.5 [29.1, 71.6] bc
Legume	166 [113, 245] b	170 [74.3, 387] ab	17.8 [11.9, 26.5] bc	99 [50.5, 193] b	73.9 [44.9, 90.8] ab
Ridomil	100 [65.5, 153] b	38 [15.2, 92.6] c	13 [8.39, 20.1] c	41 [20.8, 82.2] b	38.6 [13.8, 71.2] bc

^a <u>Control</u> (Fallow) no treatment; <u>Bark</u> (Ground pine bark) [summer and winter - 8 cm (3 inch) layer of aged, ground pine bark seeded and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer - mustard (*Brassica juncea* (*L.*) *Czern.*) seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (*B. napus* L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; <u>Grass</u> cover crop [summer - brown top millet (Urochloa ramosa (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter - annual ryegrass (*Lolium multiflorum*) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; <u>Legume</u> cover crop [summer - 'Iron and Clay' cowpea (*Vigna unguiculata* (*L.*) *Walp.*) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter - crimson clover (*Trifolium incarnatum* L.) seeded at a rate of 16.8 kg/ha (15 lb/ac)]; <u>Ridomil</u> (mefenoxam) [summer and winter - fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac) broadcast].

- 66 b 95% CI = 95% confidence intervals are displayed in brackets after the mean and have been adjusted with the Sidak multiple
- 67 comparison adjustment.
- 68 ^c p-values on which the multiple comparison letters are based have been adjusted with the Tukey adjustment.

Table 5. Main effects of soil amendment treatments on length of the longest root, root ball diameter, root ball weight, and
 percentage pot bound root balls at the end of the study in July 2022.

oil Amendment	Root length (cm)	Root ball diameter (cm)	Root ball Weight (g)	Pot bound (%)
reatmenta	Estimate [95% CI] ^{b, c}	Estimate [95% CI]	Estimate [95% CI]	Estimate [95% CI]
Control	16.9 [13.8, 20.7] b	26.4 [21.8, 31.9] b	126 [93.7, 170] b	10.2 [16.8, 43.2] a
ark	47.9 [39.2, 58.5] a	85.7 [71.2, 103] a	504 [377, 674] a	0.3 [2.8e-04, 74.2] a
rassica	17.5 [14.3, 21.3] b	28 [23.3, 33.7] b	120 [90, 161] b	6.8 [0.42, 55.8] a
Grass	16.0 [13.0, 19.6] b	27 [22.4, 32.6] b	138 [103, 185] b	32.0 [9.81, 67.0] a
egume	19.0 [15.6, 23.3] b	32.6 [27.1, 39.4] b	136 [102, 183] b	10.8 [1.88, 43.1] a
idomil	15.6 [12.7, 19.1] b	26 [21.6, 31.4] b	126 [94.2, 170] b	22.0 [5.31, 58.6] a
Cultivar				
Gumbo	27.5 [23.9, 31.7] a	44.4 [38.9, 50.6] a	208 [168, 259] a	6.3 [1.05, 29.8] a
egacy	18.9 [16.4, 21.8] b	31.8 [27.8, 36.3] b	174 [140, 216] a	17.4 [3.46, 55.3] a
earl	15.7 [13.6, 18.2] c	27.1 [23.6, 31] c	118 [94.3, 147] b	4.17 [0.38, 32.9] a
earl	15.7 [13.6, 18.2] c	27.1 [23.6, 31] c	118 [94.3, 147] b	4.17 [0.38,

and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer - mustard (Brassica juncea (L.) Czern.)

seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (B. napus L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; Grass

- cover crop [summer brown top millet (*Urochloa ramosa* (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter annual
- 75 ryegrass (Lolium multiflorum Lam.) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; Legume cover crop [summer 'Iron and Clay' cowpea
- 76 (Vigna unguiculata (L.) Walp.) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter crimson clover (Trifolium incarnatum L.) seeded at a
- rate of 16.8 kg/ha (15 lb/ac)]; *Ridomil* (mefenoxam) [summer and winter fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac)
- 78 broadcast].
- 79 b 95% CI = 95% confidence intervals are displayed in brackets after the mean and have been adjusted with the Sidak multiple
- 80 comparison adjustment.
- 81 °p-values on which the multiple comparison letters are based have been adjusted with the Tukey adjustment.

Table 6. Effects of blueberry seedling assays 1 and 2 (means averaged over the two assays) on the number, weight, and weight/seedling of blueberry seedlings harvested 9 months after being transplanted into soil collected from soil amendment treatment plots: Assay 1 (blueberry seedlings transplanted into soil collected from plots at the beginning of the study) and Assay 2 (blueberry seedlings transplanted into soil collected from treatment plots fall 2016 after first summer treatments were tilled into the soil).

Soil Amendment	Seedling count	Seedling weight (g)	Weight/Seedling (g)	
Treatment ^a	Estimate [95% CI] ^{b, c}	Estimate [95% CI]	Estimate [95% CI]	
Control	5.1 [3.52, 7.36] a	9.5 [5.67, 16.0] a	1.9 [1.18, 3.02] a	
Bark	4.4 [2.96, 6.54] a	10.6 [6.30, 17.8] a	2.5 [1.55, 3.97] a	
Brassica	4.9 [3.36, 7.13] a	11.3 [6.70, 18.9] a	2.3 [1.46, 3.74] a	
Grass	4.9 [3.32, 7.09] a	10.9 [6.46, 18.3] a	2.3 [1.44, 3.69] a	
Legume	4.6 [3.10, 6.76] a	7.2 [4.26, 12.1] a	1.7 [1.06, 2.71] a	
Ridomil	4.5 [3.04, 6.66] a	9.6 [5.70, 16.1] a	2.2 [1.35, 3.47] a	

^a <u>Control</u> (Fallow) no treatment; <u>Bark</u> (Ground pine bark) [summer and winter - 8 cm (3 inch) layer of aged, ground pine bark seeded and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer - mustard (*Brassica juncea (L.) Czern.*) seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (*B. napus* L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; <u>Grass</u> cover crop [summer - brown top

- 90 millet (Urochloa ramosa (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter annual ryegrass (Lolium multiflorum) seeded at a
- rate of 100.9 kg/ha (90 lb/ac)]; Legume cover crop [summer 'Iron and Clay' cowpea (Vigna unguiculata (L.) Walp.) seeded at a rate of 95.3
- kg/ha (85 lb/ac), winter crimson clover (*Trifolium incarnatum* L.) seeded at a rate of 16.8 kg/ha (15 lb/ac)]; *Ridomil* (mefenoxam) [summer and
- 93 winter fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac) broadcast].
- 94 b 95% CI = 95% confidence intervals are displayed in brackets after the mean and have been adjusted with the Sidak multiple
- 95 comparison adjustment.
- 96 °p-values on which the multiple comparison letters are based have been adjusted with the Tukey adjustment.

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Table 7. Main effects of lupine seedling assay on the number of seedlings 4 and 100 days after seeding (das) of 18 lupine seed placed in each tray of soil collected from each of six soil amendment treatment plots and three comparison sites.

Soil amendment	Seedling count 4 das	Seedling count 100 das
Treatment	Estimate [95% Cl ^{b, c}	Estimate [95% CI]
Control	4.58 [2.54, 8.25] b	8.01 [4.79, 13.4] ab
Bark	4.58 [2.54, 8.25] b	6.84 [3.97, 11.8] ab
Brassica	2.19 [0.943, 5.09] b	3.91 [1.98, 7.69] b
Grass	2.99 [1.45, 6.16] b	4.49 [2.37, 8.52] b
Legume	5.38 [3.11, 9.28] ab	8.4 [5.07, 13.9] ab
Ridomil	2.79 [1.32, 5.9] b	5.47 [3.03, 9.88] ab
Pc positive	3.4 [1.16, 9.96] ab	4.49 [1.79, 11.3] ab
Pc negative	12.1 [6.6, 22.3] a	11.4 [5.95, 22] a
Pc unknown	6.79 [3.11, 14.8] ab	11.4 [5.95, 22] a

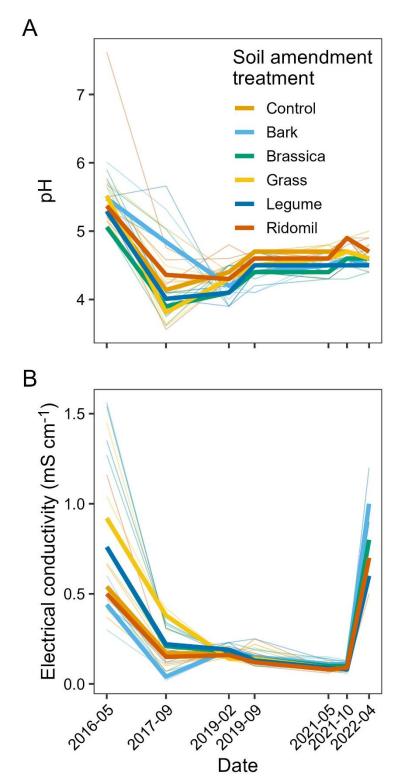
^a <u>Control</u> (Fallow) no treatment; <u>Bark</u> (Ground pine bark) [summer and winter - 8 cm (3 inch) layer of aged, ground pine bark seeded and disked into the soil at the same time as the cover crops]; <u>Brassica</u> cover crop [summer - mustard (*Brassica juncea (L.) Czern.*) seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (*B. napus* L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; <u>Grass</u>

cover crop [summer - brown top millet (*Urochloa ramosa* (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter - annual ryegrass (*Lolium multiflorum* Lam.) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; *Legume* cover crop [summer - 'Iron and Clay' cowpea (*Vigna unguiculata* (*L.*) *Walp.*) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter - crimson clover (*Trifolium incarnatum*) seeded at a rate of 16.8 kg/ha (15 lb/ac)]; *Ridomil* (mefenoxam) [summer and winter - fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac) broadcast]; Pc positive, soil from a site with Phytophthora root rot symptomatic blueberry plants and a history of *P. cinnamomi* infestation; Pc negative, soil from a site with Phytophthora root rot asymptomatic blueberry plants and no history of *P. cinnamomi* infestation; Pc unknown, soil from a site with Phytophthora root rot symptomatic blueberry plants, but with no history of *P. cinnamomi* infestation.

b 95% CI = 95% confidence intervals are displayed in brackets after the mean and have been adjusted with the Sidak multiple comparison adjustment.

112 ° p-values on which the multiple comparison letters are based have been adjusted with the Tukey adjustment.

Figure 1.



Caption Figure 1.

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Figure 1a and b. Time trends of pH and electrical conductivity (EC, mS/cm) values of soil collected from six soil amendment treatments at seven dates. Individual plot's time trends are plotted as thin semi-transparent lines, and medians for each soil amendment treatment are plotted as thick lines. Soil amendment treatments are Control (Fallow) no treatment; Bark (Ground pine bark) [summer and winter - 8 cm (3 inch) layer of aged, ground pine bark seeded and disked into the soil at the same time as the cover crops]; Brassica cover crop [summer mustard (Brassica juncea (L.) Czern.) seeded at a rate of 11.2 kg/ha (10 lb/ac), winter - 'Dwarf Essex' canola (B. napus L.) seeded at a rate of 11.2 kg/ha (10 lb/ac)]; Grass cover crop [summer - brown top millet (*Urochloa ramosa* (L.) T. Q. Nguyen) seeded at a rate of 33.6 kg/ha (30 lb/ac), winter - annual ryegrass (Lolium multiflorum Lam.) seeded at a rate of 100.9 kg/ha (90 lb/ac)]; <u>Legume</u> cover crop [summer - 'Iron and Clay' cowpea (Vigna unguiculata (L.) Walp.) seeded at a rate of 95.3 kg/ha (85 lb/ac), winter - crimson clover (Trifolium incarnatum L.) seeded at a rate of 16.8 kg/ha (15 lb/ac)]; and *Ridomil* (mefenoxam) [summer and winter - fungicide drench applied at a rate of 5 L/ha (3.6 pts/ac) broadcast].