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Research Articles

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OUTSTANDING STUDENT AWARD WINNER

Colored Nonenhanced Head Computed Tomography Decreases Assessment Time Without Compromising Diagnostic Accuracy from Trainees to Expert Neuro-radiologists in the Setting of Acute Ischemic Stroke

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ABSTRACT

PURPOSE: To improve diagnostic speed and accuracy for the detection of acute ischemic stroke utilizing colored nonenhanced head computed tomography (NECT) images. **METHODS:** This multi-reader prospective observational study included 100 consecutive adult patients with concern for acute ischemic stroke from 2/1/2018 to 3/17/2018 with grayscale NECT and CT angiogram (CTA) head images and a reference-standard confirmatory brain MRI. Grayscale NECT and CTA head images were collected and colored NECT head images were generated using a custom, fully automated software designed to color the intracranial contents on the head CT images and increase the conspicuity of ischemic strokes. Two randomized imaging sets were generated from each patient's CT exam, including Grayscale and Color+Grayscale images. Two radiology residents and two expert fellowship trained neuroradiologists independently assessed each imaging set, separated by at least two weeks. The mean accuracy, sensitivity, specificity, and time of assessment were compared between individual readers and between the two reader groups, in a multivariate model. **RESULTS:** Among the 4 readers, the mean accuracy/sensitivity/specificity for correctly diagnosing acute ischemic stroke were 72%/46%/87% using grayscale NECT images only and 69%/36%/86% using Color+Grayscale NECT images (p=0.08/p=0.006/p=0.858). The mean time of assessment with grayscale NECT images only was 59 seconds, which was decreased by an average of 19 seconds when using Color+Grayscale NECT images (p<0.001). **CONCLUSION:** Improving diagnostic speed and maintaining diagnostic accuracy using colored NECT images could be widely applicable among many radiologists without additional patient expense or radiation exposure, while potentially improving patient outcomes.

KEYWORDS: ischemic stroke, CT, color

INTRODUCTION

Stroke is the 5th most common cause of death in the United States and a leading cause of serious long-term disability (CDC, 2015). Every year, more than 795,000 people in the United States have a stroke and up to 2 million people are evaluated for acute stroke-like symptoms (Mozaffarian, 2016). This leads to an annual cost to the U.S. healthcare system of $36.5 billion (CDC 2015). Strokes are responsible for 942,000 hospitalizations, 73,000 nursing home admissions, and 3.7 million medical office visits (CDC, 2013; Caffrey, 2012; CDC, 2009-2010).

Strokes fall into two distinct categories, including hemorrhagic strokes, which account for 17%, and ischemic strokes, which account for 83% of all strokes. Hemorrhagic and ischemic strokes are managed quite differently, and time is critical in improving the outcomes of both stroke subtypes. The American Stroke Association recommendation for patients with stroke-like symptoms includes a rapid assessment with nonenhanced computed tomography (NECT) head imaging, the fundamental purpose of which is to identify the cause of the clinical symptoms Jauch, 2013). NECT head imaging is 99%

accurate for identifying hemorrhagic strokes and only 50-70% accurate for identifying ischemic strokes; therefore, American Stroke Association guidelines recommend thrombolytic therapy in patients with stroke-like symptoms in the absence of hemorrhage on NECT head images, based on a clinical assessment algorithm that includes the National Institutes of Health Stroke Scale (NIHSS) (Sabarudin, 2014).

The goal of this prospective study is to improve diagnostic speed and accuracy in the detection of acute ischemic stroke in the setting of a stroke code by evaluating paired grayscale and colored NECT head and grayscale CTA head images. Improving diagnostic accuracy and speed in this setting utilizing color enhancement techniques would be widely applicable, especially among high-volume diagnostic radiologists, who may have less experience with the interpretation of stroke codes Thus, there is identifiable potential to significantly reduce interpretation times while maintaining accuracy. As time to diagnosis and treatment is crucial for preservation of neurological functionality as well as accreditation of stroke centers, the clinical utility of a streamlined diagnostic algorithm is obvious.

MATERIALS AND METHODS

Patient Population

This single-center, multi-reader, and prospective observational study was approved by the Institutional review board (IRB) and is HIPAA-compliant. The study included 100 consecutive adult stroke code patients from 2/1/2018 to 3/17/2018 with grayscale NECT and CTA head images and a reference-standard confirmatory brain MRI. Patients without a confirmatory MRI, patients with an MRI greater than 4 weeks of confirmation of ischemic stroke, and/or patients with a documented hemorrhagic stroke on NECT, were excluded. Patient demographics, stroke symptoms, NIHSS, time of onset, and stroke type were recorded.

Imaging Acquisition

CT images were acquired consecutively within the Department of Radiology at UMMC. Grayscale NECT images were obtained with multidetector CT scanners using the institutional stroke code imaging protocol which includes grayscale NECT and CTA head images. Axial image sequences were downloaded and exported to OsirixMD, an offline DICOM viewer (Pixmeo, Bernex, Switzerland). Using OsirixMD software, all patient images were de-identified by author E.V. per standard techniques.

Color Enhanced Detection (CED) Software Colorization Process

Grayscale NECT images were collected, and colorized NECT images were generated using a custom, semi-automated color-enhancing software algorithm developed in collaboration with ImageIQ (Cleveland, OH). The color was introduced to volume-rendered, post-processed images using Color Enhanced Detection (CED) software to enhance the display of each image. This custom software was specifically designed to color the intracranial contents of the head CT images and increase the conspicuity of ischemic strokes by assigning specific colors to brain attenuation (Hounsfield units) measurement ranges. Two randomized imaging sets were generated from each patient's CT head exam, including Grayscale (containing only grayscale NECT and CTA head images, **Figure 1a**) and Color+Grayscale (containing paired, colored, and grayscale NECT and grayscale CTA head images, **Figure 1b**).

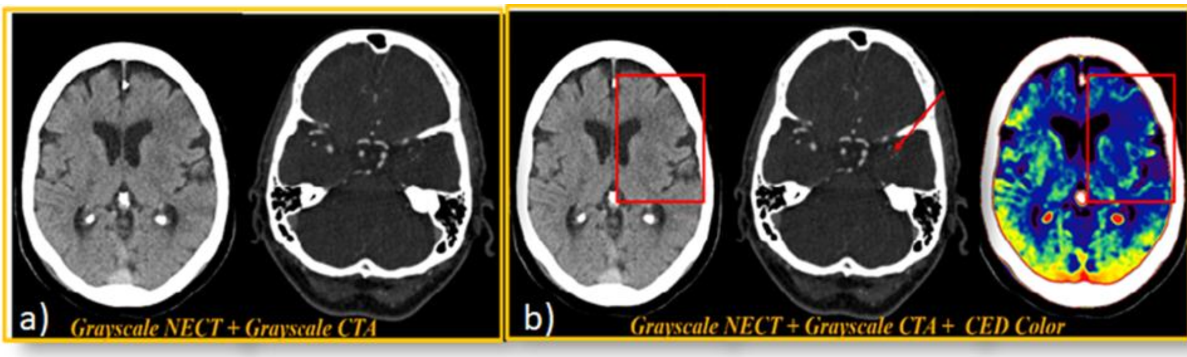

Figure 1. a) Grayscale (containing only grayscale NECT and CTA images) images evaluated in the Grayscale-only reading session. b) Color+Grayscale (containing paired colored and grayscale NECT and grayscale CTA images) images with the acute infarct outlined within the area of concern outlined by the red box with a subtle occlusion of

Multi-reader Assessment of Grayscale and Color+Grayscale Head CT images

Four readers (two senior-level radiology residents and two expert neuroradiologists) were given brief training on how colored images are developed and were subsequently trained using a small training cohort to become comfortable with the interpretation of combined grayscale and colored CT images before assessing the prospective clinical cohort. Each reader independently assessed each reading set in two independent reading sessions (Grayscale and Color+Grayscale). Reading sessions were separated by at least 2 weeks to minimize recall bias. Time from initial viewing to the entry of the final interpretation of an individual de-identified image series was assessed. All diagnostic interpretations and each time of assessment were recorded by author E.V.

Statistical Analysis

Statistical analysis was conducted by the Center for Biostatistics and Bioinformatics at UMMC. For stroke code patients with confirmatory MRI imaging, true positive, false positive, true negative and false negative cases were established to assess the mean accuracy, sensitivity, specificity, and assessment time between grayscale NECT and CTA head images and paired Color+Grayscale NECT and grayscale CTA head images. Reader assessments were analyzed both collectively and by reader experience in a multivariate model.

RESULTS

Population Characteristics

Patient demographics, clinical history, and presenting symptomology are summarized in **Table 1.** The mean age of the study population was 61 years (range: 19-102 years) with a slight Caucasian and male predominance (51% and 53%, respectively). The average presenting NIHSS score, which is the most common clinical diagnostic method for rapid assessment of stroke severity, was 7 (range: 0-38, scale 0-42). Of patients with clinical histories provided before imaging studies (90%), 74 patients (74%) presented with a focal neurological deficit (i.e. focal weakness, loss of sensation, slurred speech, etc.) and 17 patients (17%) presented with altered mental status. Only 6% of patients presenting with acute stroke-like symptoms had a reliable symptom onset and most patients (94%) woke up with stroke-like symptoms or were unsure when symptom onset occurred.

Table 1. Summary of patient demographics and clinical status upon presentation with acute stroke-like symptoms

Patient Demographics and Clinical Presentations	
Patient Characteristics	**Prospective Cohort (N=100)**
Age	61 (range: 19-102)
Male	53
African-American	49
NIHSS	7 (range: 0-38)
History	
None Provided	10
Provided	90
Symptoms	
Focal deficits	74
Altered mental status	17
Other	2
Symptom Onset	
< 4.5 hours	1
> 4.5 hours	5
Other	94

Multi-reader Assessment Analysis

The overall assessments as a collective group and for the individual readers are depicted in **Figure 2**. As a group, there was no significant difference in accuracy or specificity for correctly diagnosing acute ischemic stroke with a percent difference of 2.8% (p=0.080) and 0.4% (p=0.858), respectively (**Fig. 2a**). However, diagnostic accuracy within the study cohort was consistent and near the upper limit of the reported average accuracy in acute ischemic stroke detection. Similar results were seen on an individual basis, although Reader 1 (senior-level resident radiologist) did show a significant improvement in both diagnostic accuracy and sensitivity in acute stroke detection, with a 7.2% and 20.5% increase, respectively (**Fig. 2b**).

When analyzing the interpretative results based on reader experience, the mean accuracy for correctly diagnosing acute ischemic stroke using Grayscale NECT and CTA head images by neuroradiologists of 76% was significantly higher than that of the senior-level resident radiologists' accuracy of 67%, as expected (p < 0.001). Conversely, when interpreting paired Grayscale+Color NECT images and the grayscale CTA images, the difference in diagnostic accuracy was not statistically significant between expert neuroradiologists and the senior-level resident radiologists (70% and 68%, respectively; p= 0.711, p=0.142).

Time of Assessment Analysis

The analysis of the mean assessment time for each reader and the group of readers is depicted in **Figure 3**. There was a significant decrease in assessment time for interpreting Grayscale+Color images. The addition of the Colored NECT images decreased assessment time by nearly 20 seconds per exam. Furthermore, Reader 3 (senior-level resident radiologist) was nearly one minute faster in interpretation without compromising diagnostic accuracy—a very valuable amount of time saved for a busy general radiologist who may not be as comfortable interpreting stroke code images.

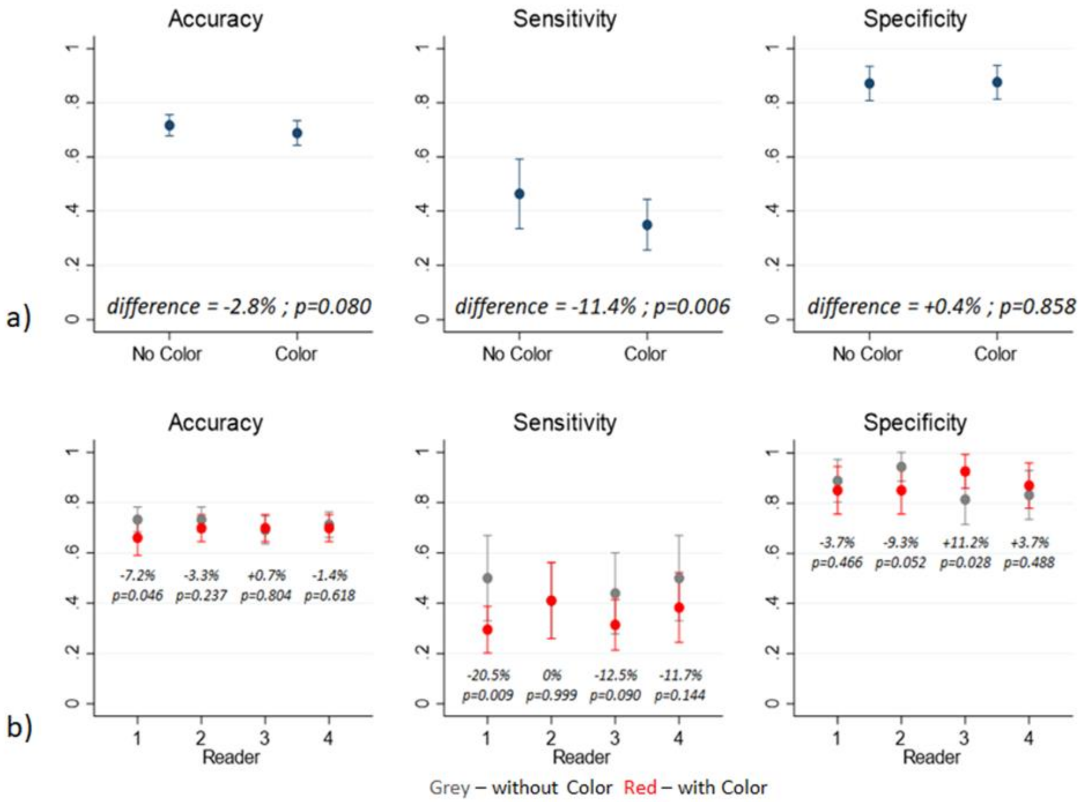

Figure 2. Accuracy, Sensitivity, and Specificity for the diagnosis of acute ischemic stroke in the setting of a Code Gray for the readers a) as a collective group and b) on an individual basis. Readers 1 and 2 are expert fellowship trained attending neuro-radiologists and readers 3 and 4 are trainee resident radiologists.

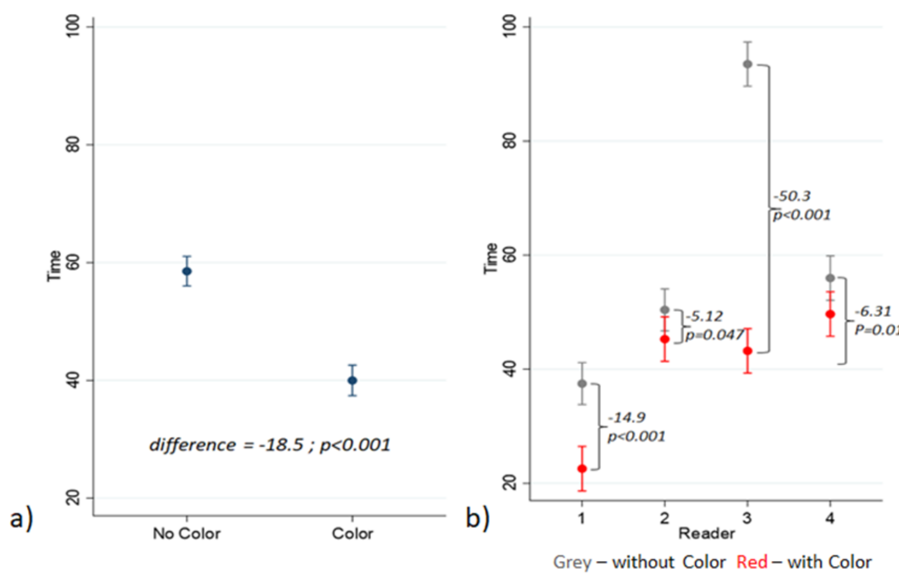

Figure 3. Time of assessment between Grayscale and Grayscale+Color as a) a collective group and b) individual readers. Readers 1 and 2 are expert fellowship trained attending neuro-radiologists and readers 3 and 4 are trainee resident radiologists.

DISCUSSION

Stroke is a complex spectrum of pathology requiring complex clinical care. Diagnostic speed is a critical element that may often dictate which initial intervention a stroke patient will be eligible to receive.

Although the initiation of a stroke code depends on clinical presentation and physical exam, imaging is required for diagnosis. NECT head imaging is the workhorse of stroke imaging, largely due to its quick acquisition time, wide availability, and relatively low cost. In addition, NECT is extremely accurate in excluding intracranial hemorrhage, which is the most crucial aspect of the initial imaging assessment, especially when a patient presents within the time window to receive pharmacologic thrombolytic therapy.

Imaging features of ischemic infarct can be variable on CT and can drastically change with increasing time from initial insult. NECT imaging features of acute ischemic infarct include parenchymal hypoattenuation, loss of gray-white matter differentiation, cytotoxic edema, and/or the hyperdense vessel sign with or without sulcal and/or ventricular effacement. Early CT images have been shown to demonstrate parenchymal hypoattenuation in greater than 80% of patients, cytotoxic edema in approximately 40%, and hyperdense MCA (vessel) in nearly 50% of patients, in some published cohorts (von Kummer, 1994). Importantly, early manifestations of ischemic infarct are often difficult to differentiate with grayscale imaging, as demonstrated by the reported average diagnostic accuracy of 50-70%, and the modest inter-rater reliability, particularly among physicians with limited experience in acute ischemic infarct detection (Jauch, 2013 and Wardlaw, 1999).

Increasing conspicuity of ischemic infarct imaging manifestations has been a common area of interest within the literature but has proven to be a very difficult feat to achieve. The authors believe, however, that the addition of color enhances the acute ischemic infarct. NECT imaging features and can potentially improve diagnostic accuracy, even though accuracy was merely maintained among the four readers within this limited cohort. It has been shown that color increases attention to information, hence increasing the probability of information being stored in memory (Farley, 1976; Hoeffner, 2004). It has also been shown that inter-observer reliability of detecting signal changes on Fluid-Attenuated Inversion Recover (FLAIR) sequences within acute diffusion-weighted image (DWI) lesions is improved with the addition of color (Pulli, 2012; Kim, 2014).

With the effort that has been put into improving diagnostic accuracy, there has been little effort to directly increase diagnostic speed. As formerly mentioned, delivering an accurate diagnosis as quickly as possible is critical for stroke management, and resident radiologists, not expert neuroradiologists, are often responsible for making the initial imaging diagnosis. Our study demonstrates that the addition of colored NECT head images can significantly increase diagnostic speed by potentially halving assessment time and maintaining, or even improving diagnostic accuracy in non-expert readers, which could have profound implications for improving patient outcomes.

Improving diagnostic speed and maintaining diagnostic accuracy by the addition of colored grayscale NECT head images could be widely applicable and invaluable to many radiology practices with varying degrees of expertise and could potentially improve patient outcomes without additional patient expense or radiation exposure.

ACKNOWLEDGEMENTS

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DISCLOSURES

Financial Disclosures: Author AS is the patent holder to the Color Enhanced Detection Software and the President of Color Enhanced Detection, LLC

Conflicts of Interest: Author AS is the patent holder to the Color Enhanced Detection Software and the President of Color Enhanced Detection, LLC.

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Effects of Spring and Fall Cover Crops on Weed Density and Yield of Sweet Potato Cultivars

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ABSTRACT

This study investigated the use of fall cover crops and spring buckwheat monoculture to suppress weeds and improve yields in sweet potato cultivation. Buckwheat emerged as a highly effective summer cover crop, providing robust weed suppression throughout the growing season. Its ability to shade weeds, compete for resources, and release allelopathic compounds contributed to its effectiveness. The selection of fall cover crops influenced weed suppression, with rye mixed with clover showing limited differences in weed densities, while rye mixed with hairy vetch reduced Palmer amaranth coverage. Sweet potato cultivars demonstrated varying levels of weed competition, with Beauregard exhibiting higher weed coverage compared to Heart-O-Gold and 529. These findings highlight the importance of selecting suitable cover crops and cultivars based on their competitive abilities and weed tolerance for effective weed management. Overall, incorporating appropriate cover crops and cultivars holds promise for suppressing weeds and improving sweet potato yields.

KEY WORDS: crop cover, monoculture, weed density, sweet potato

INTRODUCTION

Mississippi ranks second in terms of acreage and third in total sweet potato (*Ipomea batatas*) production in the United States. In 2019, approximately 11 thousand hectares of sweet potatoes were planted in Mississippi, with an estimated value of $107 million (USDA, 2019). Weed interference, particularly by Palmer amaranth (*Amaranthus palmeri*), poses a significant threat to sweet potato crops, leading to substantial yield reductions (Smith et al., 2018).

To minimize losses due to weed interference, it is crucial to maintain a weed-free environment for at least 2 to 6 weeks after transplanting sweet potatoes (Seem et al., 2003). Integrated management strategies that incorporate cover crops and competitive cultivars have been explored to control weeds in sweet potato production. Cover crops such as rye and radish, commonly planted during the fall, have shown promise in suppressing weeds. Radish, a Brassica species, contains isothiocyanates (ITCs) derived from glucosinolates, which exhibit pesticide properties and herbicidal activity (Norsworthy and Meehan, 2005; Malik et al., 2008). These ITCs also possess allelopathic effects that inhibit weed seed germination. Species such as redroot pigweed (*Amaranthus retroflexus* L.), dandelion (*Taraxacum officinale* Weber), yellow nutsedge (*Cyperus esculentus* L.), sicklepod (*Senna obtusifolia* L.), and palmer amaranth [*Amaranthus palmeri* (S.) Wats.] have been successfully suppressed in the presence of brassica as a cover crop (Norsworthy, 2003; Norsworthy and Meehan, 2005). Rye plants, on the other hand, compete effectively with weed species due to the allelopathic effects of benzoxazinoid compounds present in rye grains (Macias et al., 2005). Giant foxtail (*Setaria faberi* Herrm.), common lambsquarters (*Chenopodium album* L.), pigweeds (*Amaranthus* spp.), horseweed [*Conyza canadensis* (L.) Cronq.], and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] are among the weed species effectively suppressed by these compounds (Burgos and Talbert, 1996; Przepiorkowski and Gorski, 1994).

Legume cover crops, often mixed with grasses, have the potential to suppress weeds and enhance nitrogen fixation and soil organic matter, leading to increased overall yield (SARE, 2007). Clover (*Trifolium* spp.) and vetch (*Vicia* spp.) are examples of legume cover crops used in various crop production systems for their weed suppression and nitrogen fixation abilities (Akemo et al., 2000; Daniel et al., 1999; Mohler and Teasdale, 1993; Norsworthy et al., 2010). However, legume cover crops decompose rapidly compared to other cover crops, which may result in increased weed emergence if not managed properly throughout the growing season (SARE, 2007). Mixing legume cover crops with cereal grains is recommended to optimize their weed suppression and reduce reliance on purchased fertilizers (Brennan et al., 2009; Reberg-Horton et al., 2012).

During the summer months, when fallow fields may face weed infestations, buckwheat (*Fagopyrum esculentum* M.) serves as an excellent short-season cover crop. It helps prevent weed growth, break disease cycles, and improve soil tilth (Kumar et al., 2011; Björkman et al., 2008; Clark, 2007). Buckwheat exhibits allelopathic activity due to the presence of compounds like rutin, gallic acid, and quercetin (Iqbal et al., 2003; Golisz et al., 2007; Kalinova, 2004; Tominaga and Uezu, 1995). In the United States, buckwheat has been successfully used to suppress weed species such as Canada thistle (*Cirsium arvense* (L.) Scop.), quackgrass (*Elymus repens* (L.) Gould.), and Powell amaranth (*Amaranthus powelli* S. Wats.) (Bicksler and Masiunas, 2009; Golisz and Gawronski, 2003; Kumar et al., 2009). The reseeding ability of buckwheat further contributes to its cost-effectiveness as a weed control option.

The choice of sweet potato cultivars also plays a significant role in weed management, as different cultivars exhibit varying degrees of weed suppression. The predominant cultivar in the United States is Beauregard, but studies have shown that it is not highly tolerant to weed interference, resulting in yield reductions (Schultheis et al., 1999; LaBonte et al., 1999). Therefore, implementing sustainable weed control practices, such as cover crop management, can help farmers increase their total yield and optimize herbicide usage. This research aims to investigate the effects of cover crops on weed density and yield in three different sweet potato cultivars.

MATERIAL AND METHODS

The experiment was conducted in 2019 at the Pontotoc Ridge-Flatwoods Branch Experiment Station in Pontotoc, Mississippi, United States (34.14097 N, 89.007232 W). The research site is characterized by sandy loam soil, and the growing season experiences an average monthly precipitation of 7.366 mm. The high and low temperatures range from 33 to 15 °C.

The experimental design employed a randomized complete block with four fall cover crop treatments, each replicated four times. The cover crop treatments consisted of the following mixtures: rye (*Secale cereal*) + clover (*Trifolium* spp.); rye + vetch (*Vicia* spp.); rye + radish (*Raphanus raphanistrum*) + vetch; and a weedy fallow treatment. Prior to sowing the spring cover crop treatments, the fields were plowed in the spring and summer with either a monoculture of buckwheat (*Fagopyrum esculentum*) or a weedy fallow, with four replications. After the main crop harvests, the crop residues were tilled to create a fine seedbed for the entire trial area before planting the spring cover crop treatments. Approximately a week after cover crop biomass sampling in the following spring, an application of 2.24 kg a.e. ha-1 glyphosate was made to the entire trial site to control weeds and terminate the cover crops. The trial site was then disked and cultivated approximately four weeks before transplanting sweet potatoes, which took place in May 2019. Standard production practices for processing sweet potatoes, including nutrient fertilization and other field management techniques, were followed.

The selection of cover crop species aimed to represent different plant functional groups, including broadleaf, grass, and nitrogen-fixers (Polygonaceae, Brassicaceae, Poaceae, and Fabaceae) (Lavorel et al., 1997). The plots were planted sequentially within each of the four blocks, with the ground being harrowed immediately before planting buckwheat. The cover crop served as the primary factor, while the split-plot factors consisted of three sweet potato cultivars: Beauregard, Heart-O-Gold, and 529. All cover crops were planted in November 2019, except for buckwheat, which was planted in March 2020. The main plots measured 6.096 m by 30.48 m, and the split-plots measured 6.096 m by 0.9144 m. Sweet potatoes were transplanted at a density of 13 plants per 0.000557418 ha-1 in May 2019 using a John Deere 6410 tractor pulling a 2-row 12" butterfly transplanter. The plant spacing within the rows was 45.72 cm. Each plot was broadcasted with a mixture of three weed species (yellow nutsedge, large crabgrass, and palmer amaranth) at a density of 20 plants m-2 for each weed species.

Data collection involved weed counts (% plants per plot) for each weed species, as well as the total yield recorded at harvest. Weed control was assessed not only for the planted weed species but also for the dominant native population. The biomass of the buckwheat cover crop was harvested eight weeks after planting. Biomass was collected from each plot using a two-foot² area and then dried at 65°C for four days to determine the dry weight.

Statistical analysis was performed using JMP 13.0.0 software (SAS Institute Inc., Cary, NC). The data were subjected to ANOVA, and the treatments were arranged in a completely randomized block design with four replications. Means were separated using the Student's t-test adjustment test at a significance level of 0.05. The standard error of the difference between treatments was utilized to distinguish treatment means.

RESULTS

In the presence of buckwheat as a summer cover crop, weed density was significantly lower compared to all fallow treatments. The analysis of fall cover crops revealed significant differences, leading to a separate analysis of the data. The effects of cover crop treatments were found to be significant for smallflower morningglory and Palmer amaranth densities. Furthermore, the cultivars Heart-O-Gold, 529, and Beauregard had a significant impact on the density of carpetweed and large crabgrass in the third week after transplanting. However, these cultivars did not show an influence on yellow nutsedge, Palmer amaranth, and smallflower morningglory. The fall cover crops did not affect the biomass of buckwheat.

Among the weed species, large crabgrass exhibited the highest density at 6% (Fig. 1), but no significant differences were observed among the fall cover crops. Only smallflower morningglory and Palmer amaranth showed variations between the cover crops. Smallflower morningglory reached a density of 3% in the presence of the rye + vetch cover crop, which was not significantly different from rye + clover. However, rye + vetch + radish and fallow treatments demonstrated less than 1% density for this weed species. Palmer amaranth had a density of 2.5% at 21 days after sweet potato transplanting when the combination of rye + vetch + radish was used, and it showed similar results to fallow and rye + clover cover crops. On the other hand, rye + vetch had less than 1% infestation of Palmer amaranth. Yellow nutsedge and carpetweed exhibited weed densities of less than 2%, and the fall cover crops did not affect the density of these weed species.

The choice of sweet potato cultivar had a significant impact on carpetweed and large crabgrass (Fig. 2). The cultivar Beauregard exhibited a 1% density of carpetweed. In contrast, cultivars 529 and Heart-O-Gold had approximately 2% density and were statistically similar to each other. When Beauregard was used, large crabgrass showed a higher density, reaching 6%, which did not differ significantly from Heart-O-Gold. The cultivar 529 had a 2% lower density of large crabgrass compared to the other cultivars. However, the cultivars did not affect the density of smallflower morningglory, Palmer amaranth, and yellow nutsedge, with all weed species reaching a density of less than 3%.

There were no significant effects of fall cover crops on the dry biomass of buckwheat. Although no differences were observed, the rye + vetch + radish and fallow treatments resulted in a higher weight (1.6 kg ha-1) compared to rye + clover and rye + vetch treatments (1.6 kg ha-1) (Fig. 3).

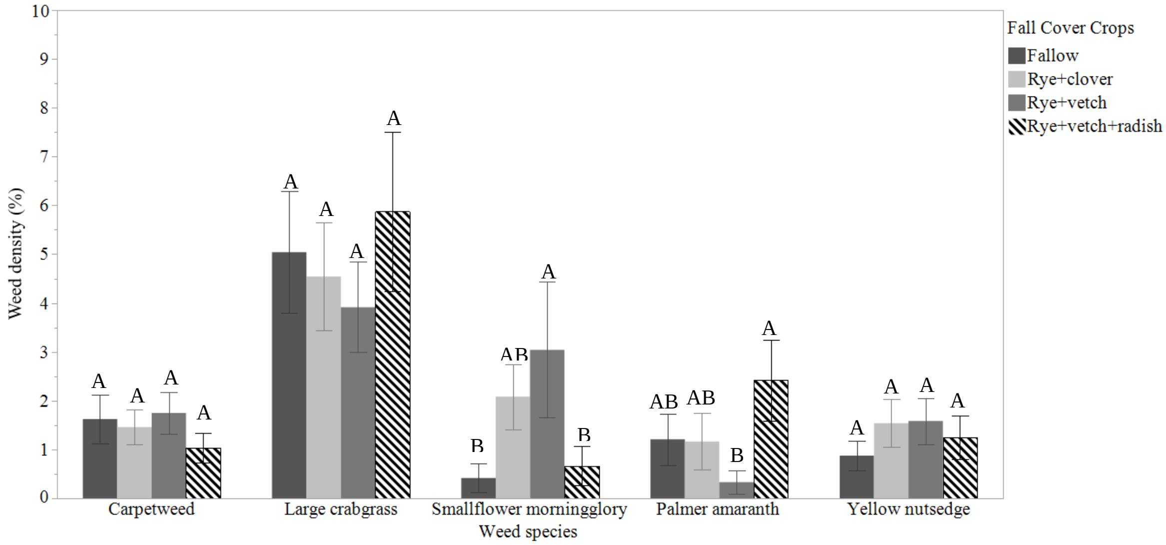

Figure 1. Effect of different fall cover crops on weed density (%) at 21 days after transplanting.

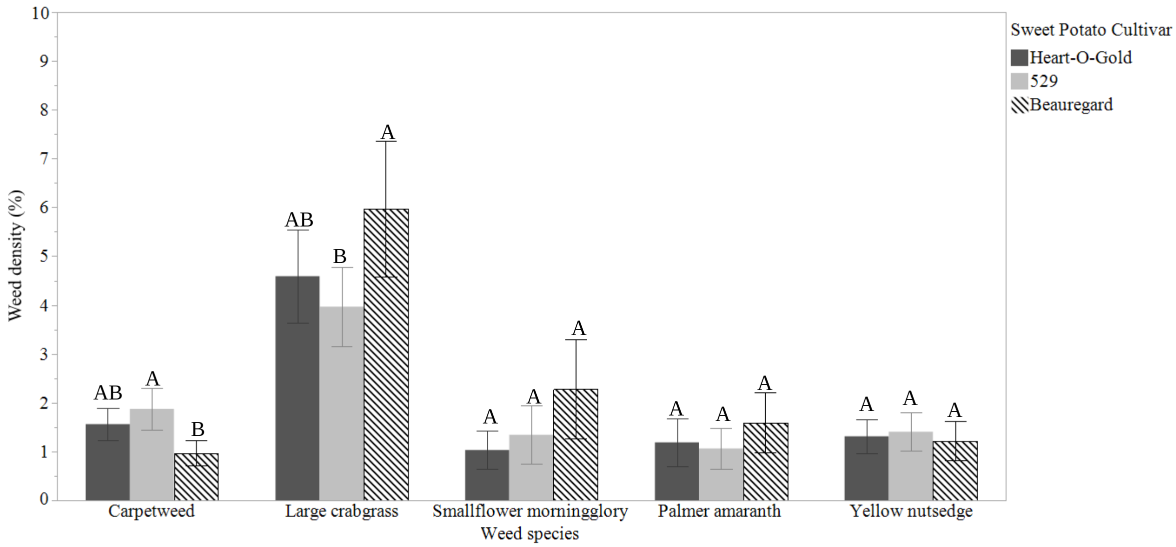

Figure 2. Effect of sweet potato cultivars Heart-O-gold, 529, and Beauregard on weed density (%) at 21 days.

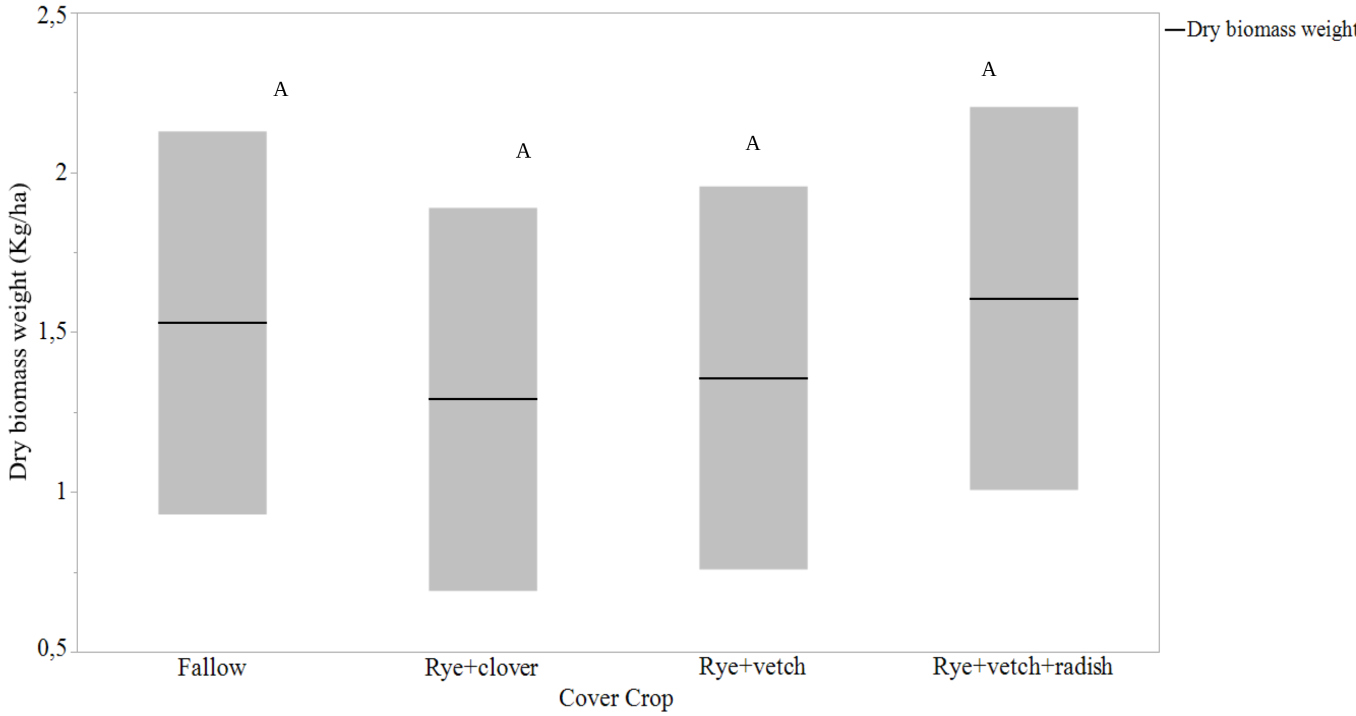

Figure 3. Effect of different cover crops on buckwheat dry biomass weight (kg ha^{-1}). Biomass was determined by harvesting all aboveground buckwheat in 2 ft^2 plot, drying, and measuring dry weight.

DISCUSSION

The aim of this study was to evaluate the effectiveness of a fall cover crop mixture consisting of five species from four different plant families, along with a spring buckwheat monoculture, in preventing weed emergence and achieving higher total yields in three sweet potato cultivars. Emphasis was placed on identifying characteristics that could reduce weed coverage and enhance sweet potato yield. The findings of this study reaffirm the potential of buckwheat as a summer cover crop that effectively suppresses weeds in all treatments. Previous research by Björkman et al. (2013) indicated that cooler spring temperatures result in slower buckwheat growth, making it less competitive against weeds. However, during summer growth, buckwheat produces abundant biomass and provides adequate weed suppression, consistent with our findings. Another study by Kumar (2009) reported significant weed suppression in buckwheat monoculture, reducing hairy galinsoga seed production by 98% and weed coverage by 90% to 99%. These results suggest that buckwheat is particularly effective in suppressing summer weeds. Our study identified early June as the optimal planting date for buckwheat to maximize weed suppression. Under established buckwheat cover, weeds experienced minimal growth and were largely absent in most treatments from June to September.

The presence of buckwheat as a cover crop can be attributed to several primary factors contributing to weed suppression. Shading, resource competition, and allelopathy effects have been proposed as key mechanisms by Falquet et al. (2015). Buckwheat establishes quickly in the field, limiting weed access to light and impeding their growth in the absence of resource competition. Additionally, allelochemicals released by buckwheat, especially during the early stages, have been shown to possess phytotoxic activity in the soil (Tsuzuki et al., 1987; Kalinova et al., 2005; Golisz et al., 2007; Kalinova et al., 2007). Studies have demonstrated that these allelochemicals can inhibit the root and shoot elongation of various weed species, such as ryegrass and lettuce (Kalinova et al., 2005). Buckwheat plays a vital role in preventing weed emergence throughout the growing season, even after its termination, as its allelochemicals persist in the soil. Our results confirm this, as weed emergence was absent in the buckwheat plots from June to September.

The selection of fall cover crops significantly influences weed suppression, and species choice plays a crucial role in designing an effective and sustainable weed management system. Clover has been recognized as a strong competitor with other cover crops and the main crop (Bottenberg et al., 1997; Brandsaeter et al., 1998; Lotz et al., 1997; Weber et al., 1999). In our study, when rye was mixed with clover, no significant differences were observed in carpetweed, large crabgrass, and yellow nutsedge. However, higher densities of smallflower morningglory and Palmer amaranth were recorded. On the other hand, the combination of rye and hairy vetch has been reported to exhibit weed suppression through physical barriers that prevent light from reaching the soil and weeds (Liebman and Davis, 2000; Teasdale and Mohler, 2000). In our study, the rye + vetch mixture resulted in reduced coverage by Palmer amaranth but increased density of smallflower morningglory. No significant differences were observed for carpetweed, large crabgrass, and yellow nutsedge. Morningglory species tend to grow toward other plants to capture more sunlight, competing with the main crop (Price and Wilcut, 2007). Once established, they become challenging to control due to their sizeand rapid growth.

Our findings are consistent with a study conducted by Wortman et al. (2012), where the use of cover crop species from the Fabaceae and Brassicaceae families resulted in antagonistic interactions between mustard, hairy vetch, and field pea when used in mixtures. In our study, the rye + vetch + radish mixture did not exhibit significant differences in carpetweed, large crabgrass, and yellow nutsedge. However, Palmer amaranth coverage showed a significant increase, while smallflower morningglory was suppressed in the presence of this mixture. Meyers et al. (2010) demonstrated that sweet potato yields decreased by 36% to 81% when 0.5 to 6.5 Palmer amaranth plants were present per meter of row. The complexity of diverse cover crop mixtures can result in reduced weed suppression activity, as establishing and managing such mixtures can be challenging, particularly when species have different seed sizes, growth rates, life histories, or termination requirements (Wortman et al., 2012; Creamer et al., 1997). Furthermore, there is a possibility of antagonistic interactions between specific cover crop species or with subsequent main crops (Snapp et al., 2005).

Cultivar selection can also influence weed competition in the field. Allelopathic sweet potato cultivars or clones with architecturally diverse canopies may exhibit higher competitive ability and weed tolerance. In our study, except for carpetweed, the Beauregard cultivar showed greater weed coverage compared to Heart-O-Gold and 529, although there were no significant differences between the latter two cultivars. Beauregard, the most commonly grown cultivar in Mississippi, tends to have slower vine growth and a thinner canopy relative to other cultivars grown in the U.S. Seem et al. (2013) reported that sweet potato vine cover was 40% higher during the late stages compared to early transplanting, indicating that Beauregard may be a weaker competitor against weeds when planted earlier. The better development of Beauregard plants during late transplanting dates could be attributed to higher temperatures, promoting more rapid growth (Seem et al., 2013). However, the presence of the 529 cultivar resulted in lower coverage of large crabgrass but increased coverage of carpetweed.

Overall, this study highlights the importance of selecting appropriate cover crop mixtures and sweet potato cultivars to effectively manage weeds. Buckwheat serves as a valuable cover crop for summer weed suppression, while considerations such as shading, resource competition, and allelopathy contribute to its effectiveness. Understanding the interactions between cover crops, cultivars, and weed species can aid in designing sustainable weed management systems and optimizing sweet potato yields.

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Understanding the Microbial Profile of Raw Goat Milk and Farmer Food Safety Perceptions in Mississippi

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ABSTRACT

Dairy goat populations have been growing in the US making goat milk production an opportunistic field to pursue. Currently, 30 states allow the sale of raw goat milk, including Mississippi, which imposes strict regulations limiting sales to very small farmers (n<9 goats). While the consumption of raw milk poses a threat to consumers due to human illness, the direct ban of products would also be detrimental for local farmers who lack the resources needed to pasteurize their goods. The objectives of the study were to determine incidence of pathogens and fecal indicators in raw goat milk and to survey farmers' practices to identify deficiencies and opportunities for improvement in safety. Milk samples were obtained from Mississippi dairy goat farmers who used their personal milking techniques to collect the samples. They were tested for *Salmonella* spp., *Listeria monocytogenes*, *E. coli*, and *Staphylococcus* spp. and the quantification of total bacterial count. All samples (n=30) were free from *Salmonella* spp. and *Listeria* but *Staphylococcus* spp., total coliforms, *Enterobacteriaceae*, and *E. coli* were found in 21, 12, 10, and 4 samples, respectively. The survey displayed the farmers' need for more educational opportunities on safe milk handling, with emphasis on the effectiveness of handwashing, as only 44.8% of surveyed producers wash their hands between animals during milking. Survey results showed that 80.8% of producers would invest more in their farms if it allowed more freedom to advertise and sell their products, creating a strategic opportunity for extension outreach to help with market expansion and sustainability.

Keywords: Goat Milk, Food Safety, Microbial Profile

INTRODUCTION

Despite cow's milk being predominantly consumed around the world, other species contribute to the dairy production industry including sheep, water buffalo, yak, camel, mare, and goat (Park and Haenlein, 2006). Caprine, or goat milk production, in particular, is a growing industry that has been essential to the wellbeing of millions of people worldwide and plays a crucial role in the economies of many countries (Silanikove et al., 2010). While the dairy goat industry is still relatively new in the United States, demand for dairy goat milk, yogurt, cheese, and other products is rising in demand (Lu and Miller, 2019). Goat milk has been considered by some to have attractive nutritional and health benefits as it consists of a variety of proteins, fatty acids, and minerals and is less allergenic and easier to digest compared to traditional cow's milk (Clark and Garcia, 2017; Silanikove et al., 2010). Despite the perceived health advantages, the microbial safety of raw goat milk is the most important factor that comes into play as it could affect the health of consumers. Raw milk is a nutrient-dense product with a neutral pH thus providing an ideal environment for pathogenic and spoilage microbial organism growth (Gonzales-Barron et al., 2017; Von Neubeck et al., 2015; Li et al., 2008).

While there are a wide variety of factors that can create variations in the microbial composition of milk, like the facility design, animal management, and bedding materials, the most important factors are the influence of milking practices and milk handling. Some of the most common pathogens identified in raw milk products, including goat milk, include *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* (Dhahir et al., 2020; Gonzales-Barron et al., 2017; Berge and Baars, 2020). These microorganisms are strongly associated with fecal and environmental contamination and can cause foodborne illnesses when raw milk is consumed without pasteurization (Berge and Baars, 2020). Common sources of contamination can be due to the udder skin, air quality, silage and feeds, crosscontamination of feeds by manure, poor-quality bedding, milking practices, contaminated milking equipment, or infected milk, as it comes from the udder, like in the case of mastitis infections (Verdier-Metz et al., 2009; Gonzales-Barron et al., 2017). Despite all the avenues of contamination, effective pre-milking sanitation practices can reduce the number of bacteria on the teat skin, and therefore decrease the bacterial load that could otherwise adulterate milk and decrease milk quality and food safety (Baumberger et al., 2016).

The objectives of this study were to: 1) examine the microbial profile and prevalence of common foodborne pathogens in milk from local Mississippi dairy goat farms and 2) understand dairy goat farmers' knowledge of food safety and their milking practices. This research serves as an exploratory study and these objectives should be investigated further in the future.

MATERIALS AND METHODS

Milk collection: Five Mississippi farms were identified to participate in the study on a volunteer basis. Raw milk was collected by the farmers into sterile 50 mL centrifuge tubes. Typically, milk samples were comingled from 2 to 3 goats (n= 26) and some milk samples were from individual goats (n= 4). The samples were collected in duplicate and were replicated once a week for three consecutive weeks (n= 6/farm) with some variation based on farmer schedules. The farmers were instructed to use their normal milking techniques to collect and store the milk as if it were to be consumed. The farmers were then instructed to store the samples in their household refrigerator until collection, this time typically ranged from 1 to 16 hours. They were then held on ice until arrival at the laboratory, transportation varied from 30 minutes to three hours. Analyses were initiated 24 to 36 hours after sample collection. Overall, 30 caprine milk samples were collected over a two-month period from October to November 2021.

Milk analysis: The microbial analysis of the goat milk samples in conventional methods and Petrifilm™ use, was evaluated in duplicate based on the protocols described by Freitas et al. (2009) and Brazil (2003). Milk samples were examined for *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus* spp. on Xylose Lysine Deoxycholate Agar (XLD), Modified Oxford Agar (MOX), and Mannitol Salt Agar (MSA) (ThermoFisher Scientific, USA), respectively. Total coliforms, *Escherichia coli*, *Enterobacteriaceae*, psychrotrophic plate count, and mesophilic plate count were determined using the Petrifilm™ culture system including *E. coli* Coliforms (EC) Petrifilm™, Enterobacteriaceae (EB) Petrifilm™, and Aerobic Count (AC) Petrifilm™, respectfully (3M™ Petrifilm™, USA). Enrichment medias included *Listeria* and Rappaport-Vassiliadis (RV) enrichment broths (ThermoFisher Scientific, USA) that were incubated at 36 ± 2°C for 24 hours. Detection and enumeration of organisms were performed by serial dilutions of milk samples with spread-plating of .1mL onto XLD, MOX, and MSA agars for presumptive *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus* spp.. Milk samples were plated directly and diluted with dilutions using a 1:10 ratio in Butterfields Phosphate Buffer (ThermoFisher Scientific, USA). All Petrifilm™ plates were hydrated by adding 1 mL of the dilution and incubated at 36 ± 2°C following the protocols described by Freitas et al. (2009) as well as at the recommended 32± 2°C as suggested by Petrifilm™ for dairy products. The psychrotrophic bacteria were incubated at 4 ± 2°C. The samples were plated on the respective agar media using the spread plate method and incubated at 36 ± 2°C for 48 hours before enumeration. The pH of individual milk samples was also taken.

Survey data collection: For collecting data on farm management and milk handling practices, a knowledge-based survey was designed through Qualtrics (www.qualtrics.com). The survey was comprised of seven sections (background, milking practices, management practices, products and market, food safety background, food safety knowledge and risk perception, and demographics) and took approximately 13 minutes to complete. The survey was developed based on literature and included some previous USDA survey questions. The survey was shared through five social media groups targeting American dairy goat producers. Overall, 85 American dairy goat farmers responded, this data is part of a separate study. Analysis for the purpose of this paper was narrowed down to Mississippi dairy goat farmers 18 years of age or older who completed 45% or more of the survey (n= 29). The survey protocol was reviewed and deemed exempt by

Mississippi State University's Institutional Review Board (Supplemental-Appendix) (protocol #21-455).

Statistical analysis: The experiment was a completely randomized design. Prevalence of various pathogens were determined. All samples were collected in duplicate at three different time points. One-way analyses of variance (ANOVA) was used to analyze data between sampling periods for each farm followed by least squares differences comparisons. The microbial results were analyzed using SAS for Windows (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Survey responses were analyzed in SPSS for Windows (version 28.0.0.0, SPSS Inc., Chicago, IL) to produce descriptive statistics.

RESULTS

Milk analysis: Staphylococcus spp. occurred in the majority (70%) of the samples. Due to the time of year for data collection, October to November, does may be milked less frequently at lactations end as part of the dry-off process. Drying-off is the time where involution occurs within the mammary gland and the animal is milked less frequently to allow for proper cell death and regrowth of mammary tissue (Silanikove et al., 2013). *Staphylococcus aureus* is noted to grow well in milk secretions collected during the late lactation period before involution begins (Oliver, 1991). *Staphylococci* can be presumptively identified via Gram staining and catalase determination of isolates; however, further testing needs to be conducted to confirm an isolate as *Staphylococcus aureus*. *Salmonella* spp. or *Listeria monocytogenes* were not detected in any milk samples (limit of detection 10^2 CFU/mL) (Table 1). *Salmonella* was not detected in the samples even after sample enrichment. Coliforms were observed in samples from all but one farm. According to the Cornell University's Milk Quality Improvement Program (2008), coliform counts of less than 50 CFU/mL should be the goal in raw milk products, but less than 25 CFU/mL is achievable. Alternatively, the Pasteurized Milk Ordinance (PMO) sets a standard of 10 CFU/mL for pasteurized milk (FDA, 2015). Six of the 12 samples that contained coliforms were below the suggested 50 CFU/mL but only three were below the PMO's required 10 CFU/mL. *E. coli* and coliforms are a part of the *Enterobacteriaceae* family so it can be assumed that the bacterial occurrences denoted by *Enterobacteriaceae* may coincide with the EC Petrifilm™ (which enumerates all coliforms as well as pathogens such as *Salmonella*, *Shigella* and Yersini) or indicate the presence of a different intestinal bacteria (Table 1) (Bhunia, 2018). *Enterobacteriaceae* occurred in 10 samples (33%) (Table 1). There was a total of 4 *E. coli* occurrences (13%) on the EC Petrifilm™. Two of these occurrences were below the 50 CFU/mL limit as suggested by Cornell University's Milk Quality Improvement Program while the other two were above. Further confirmation testing needs to be conducted to determine the specific serotype of the *E. coli* present as most species are not harmful while others, such as O157:H7, are pathogenic.

The total number of bacteria which grow aerobically at a given temperature is known as the 'standard' or 'aerobic total plate count' when measured using the 3M Petrifilm™ ACP method. While analyzing the data, we found comparable results for the total plate count from the 3M Petrifilm™ ACP method, as compared to the total bacterial count from TSA plates. Both of these methods can be used to target mesophilic bacteria. Mesophiles thrive in moderate temperatures between 20-45℃ and can include bacteria such as *Listeria monocytogenes, Staphylococcus aureus,* and *Escherichia coli,* along with a wide variety of others. In this study, only the AC Petrifilm™ was enumerated for the total bacterial count. Petrifilm™ was incubated at 36 ± 2℃ following the protocols described by Freitas et al. (2009) as well as at the recommended 32± 2℃ as suggested by Petrifilm™ for dairy products (Figure 1). Freitas et al. (2009) determined that higher incubation temperatures could be utilized without significant differences for raw products. For Grade "A" Milk, raw milk's legal limit for total plate count is 100,000 CFU/mL or 5 log CFU/mL (PMO, 2015). A study by Yamazi et al. (2013) indicated that 6 log CFU/mL typically indicates poor hygienic practices during milk production. Poor cooling could be another reason high counts could occur. Due to random variables between farms like the locations, management techniques, and sampling timepoints in the month, the farms could not be statistically compared. Therefore, the analysis conducted is between each farm's three sampling periods (Figure 1). From all the farms, only one sample from farm E (S3) was over the 5 log CFU/mL limit approaching 6 log CFU/mL (Figure 1). Farm A biologically displayed a decrease in mesophilic count over the testing period, but samples were not statistically different ($P > .05$). Farm B was the only farm that displayed significant differences between all sampling periods showing a growth ($P < .05$) of mesophilic bacteria in sample two and a decrease ($P < .05$) in sample three with means of 2.3 log CFU/mL, 3.0 log CFU/mL, and 1.8 log CFU/mL, respectively. The decrease in colony-forming unit counts could be due to the survey being released prior to the completion of sampling, thus influencing producers to use better practices to produce satisfactory results. Neither farm C nor farm D displayed any significant differences ($P > .05$) in mesophilic counts between the individual farms' sampling periods. Farm D did note that they did not use hygienic practices due to a misunderstanding of instructions. Farm D was also observed to have an outdoor milking area compared to farm C which was fully enclosed. Farm E did display a growth in mesophilic counts during the third sampling period, resulting in the highest ($P < .05$) mean of 5.4 log CFU/mL. It is important to note that some samples from farms D and E contained observable amounts of hair, dirt, and debris in the collection tube. This is a significant deficiency in sanitary and hygienic practices considering the potential liability if a consumer were to fall ill. It is also an indicator that the proper hygienic measures such as wiping away dirt on the dry udder and pre-dipping may not have been utilized before milking. Only 2 of the 30 samples (7%) were filtered. One of the filtered samples (farm E S3) was the closest to unhygienic standards nearing 6 log CFU/mL while the other filtered sample was lower than 3 log CFU/mL (farm A S2). The farm E S3 sample approaching 6 log CFU/mL may indicate the filter may have been contaminated. The non-selective TSA served as an observational media as we could differentiate some colony types based on color and morphology. Besides seeing the selected bacterial colonies, *Bacillus* species and spore formers were observed as well, along with bacterial colonies of varying yellow, pink, and grey colors. *Bacillus* and spore formers have the potential to be food poisoning causative agents so further identification would be beneficial to classify the specific *Bacillus* species present in the samples (Magnusson et al., 2007). Aerobic Petrifilm™ for psychrotrophic bacteria showed no growth in all samples after incubation (data not shown). While more samples would strengthen the relationship, the data show a positive correlation ($r = .56$, $P < .05$) between high mesophilic counts and high pH values. A study by Ogola et al. (2007) also displayed high pH in relation to elevated somatic cell count noting it as an indicator for mastitis, which is often caused by *Staphylococcus* spp. and other pathogenic bacteria (Figure 2).

Table 1. Prevalence of *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus* spp., total coliforms, *Escherichia coli*, and *Enterobacteriaceae* in raw goat milk samples (n = 30) from five dairy goat farms in Mississippi over a two-month period.

	Farm A	Farm B	Farm C	Farm D	Farm E	Totals
Salmonella spp.						
Negative	6 (100%)	6 (100%)	6 (100%)	6 (100%)	6 (100%)	30 (100%)
Positive	0	0	0	0	0	0
Listeria monocytogenes						
Negative	6 (100%)	6 (100%)	6 (100%)	6 (100%)	6 (100%)	30 (100%)
Positive	0	0	0	0	0	0
Staphylococcus spp.						
Negative	0	4 (66.7%)	3 (50%)	0	2 (33.3%)	9 (30%)
Positive	6 (100%)	2 (33.3%)	3 (50%)	6 (100%)	4 (66.7%)	21 (70%)
E. coli						
Negative	4 (66.7%)	6 (100%)	6 (100%)	5 (83.3%)	5 (83.3%)	26 (86.7%)
Positive	2 (33.3%)	0	0	1 (16.7%)	1 (16.7%)	4 (13.3%)
Coliforms						
Negative	4 (66.7%)	6 (100%)	4 (66.7%)	1 (16.7%)	3 (50%)	18 (60%)
Positive	2 (33.3%)	0	2 (33.3%)	5 (83.3%)	3 (50%)	12 (40%)
Enterobacteriaceae						
Negative	3 (50%)	6 (100%)	6 (100%)	2 (33.3%)	3 (50%)	20 (66.7%)
Positive	3 (50%)	0	0	4 (66.7%)	3 (50%)	10 (33.3%)

The total number of bacteria which grow aerobically at a given temperature is known as the ‘standard’ or ‘aerobic total plate count’ when measured using the 3M Petrifilm™ ACP method. While analyzing the data, we found comparable results for the total plate count from the 3M Petrifilm™ ACP method, as compared to the total bacterial count from TSA plates. Both of these methods can be used to target mesophilic bacteria. Mesophiles thrive in moderate temperatures between 20-45℃ and can include bacteria such as *Listeria monocytogenes, Staphylococcus aureus,* and *Escherichia coli*, along with a wide variety of others. In this study, only the AC Petrifilm™ was enumerated for the total bacterial count. Petrifilm™ was incubated at 36 ± 2℃ following the protocols described by Freitas et al. (2009) as well as at the recommended 32± 2℃ as suggested by Petrifilm™ for dairy products (Figure 1). Freitas et al. (2009) determined that higher incubation temperatures could be utilized without significant differences for raw products. For Grade “A” Milk, raw milk’s legal limit for total plate count is 100,000 CFU/mL or 5 log CFU/mL (PMO, 2015). A study by Yamazi et al.

(2013) indicated that 6 log CFU/mL typically indicates poor hygienic practices during milk production. Poor cooling could be another reason high counts could occur. Due to random variables between farms like the locations, management techniques, and sampling timepoints in the month, the farms could not be statistically compared. Therefore, the analysis conducted is between each farm's three sampling periods (Figure 1). From all the farms, only one sample from farm E (S3) was over the 5 log CFU/mL limit approaching 6 log CFU/mL (Figure 1). Farm A biologically displayed a decrease in mesophilic count over the testing period, but samples were not statistically different ($P > .05$). Farm B was the only farm that displayed significant differences between all sampling periods showing a growth ($P < .05$) of mesophilic bacteria in sample two and a decrease ($P < .05$) in sample three with means of 2.3 log CFU/mL, 3.0 log CFU/mL, and 1.8 log CFU/mL, respectively. The decrease in colony-forming unit counts could be due to the survey being released prior to the completion of sampling, thus influencing producers to use better practices to produce satisfactory results. Neither farm C nor farm D displayed any significant differences ($P > .05$) in mesophilic counts between the individual farms' sampling periods. Farm D did note that they did not use hygienic practices due to a misunderstanding of instructions. Farm D was also observed to have an outdoor milking area compared to farm C which was fully enclosed. Farm E did display a growth in mesophilic counts during the third sampling period, resulting in the highest ($P < .05$) mean of 5.4 log CFU/mL. It is important to note that some samples from farms D and E contained observable amounts of hair, dirt, and debris in the collection tube. This is a significant deficiency in sanitary and hygienic practices considering the potential liability if a consumer were to fall ill. It is also an indicator that the proper hygienic measures such as wiping away dirt on the dry udder and pre-dipping may not have been utilized before milking. Only 2 of the 30 samples (7%) were filtered. One of the filtered samples (farm E S3) was the closest to unhygienic standards nearing 6 log CFU/mL while the other filtered sample was lower than 3 log CFU/mL (farm A S2). The farm E S3 sample approaching 6 log CFU/mL may indicate the filter may have been contaminated. The non-selective TSA served as an observational media as we could differentiate some colony types based on color and morphology. Besides seeing the selected bacterial colonies, *Bacillus* species and spore formers were observed as well, along with bacterial colonies of varying yellow, pink, and grey colors. *Bacillus* and spore formers have the potential to be food poisoning causative agents so further identification would be beneficial to classify the specific *Bacillus* species present in the samples (Magnusson et al., 2007). Aerobic Petrifilm^{TM} for psychrotrophic bacteria showed no growth in all samples after incubation (data not shown). While more samples would strengthen the relationship, the data show a positive correlation ($r = .56$, $P < .05$) between high mesophilic counts and high pH values. A study by Ogola et al. (2007) also displayed high pH in relation to elevated somatic cell count noting it as an indicator for mastitis, which is often caused by *Staphylococcus* spp. and other pathogenic bacteria (Figure 2).

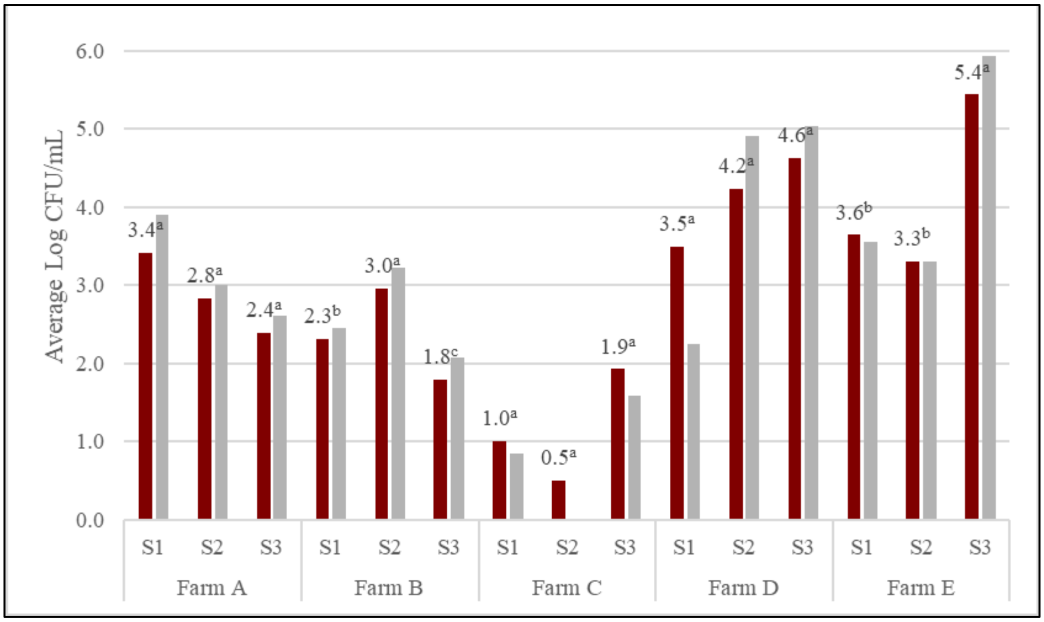

Figure 1. Mesophilic plate count (average log CFU/mL) of raw goat milk samples (n = 30) from five dairy goat farms in Mississippi over a two-month period where S1, S2 and S3 refer to the sampling interval. In each sampling period, the first column represents incubation at 36 ± 2℃ while the second column was incubated at 32 ± 2℃.

[a-c] Different superscripts indicate mean differences between sampling periods at each farm based on ANOVA LSD post-hoc test (*P* <.05).

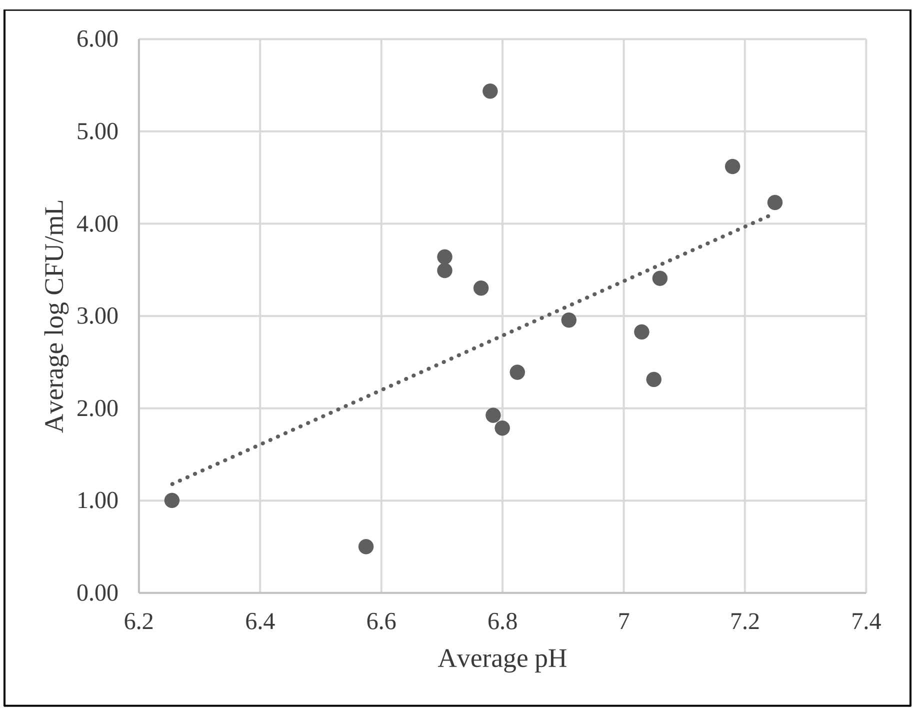

Figure 2. Average mesophilic plate count (log CFU/mL) in correlation to raw goat milk pH (n=30) (r=.56, *P*<.05).

Survey analysis: There was a total of 33 Mississippi dairy goat farmers who participated in the survey, of those 29 were utilized based on the inclusion criteria. The demographics indicated that 73.1% of the Mississippi producers who responded to the survey were female. The majority (82.8%) of the producers were Caucasian while 3.4% of individuals surveyed identified as African American and 3.4% identified as American Indian or Alaskan Native. Regarding industry purpose, the most popular reason for keeping goats was milk production (96.6%). In addition, more than half of respondents kept goats for breeding (58.6%), hobby (58.6%), and for showing (51.7%). When asked to select utilized milking practices, the majority (69%) of farmers indicated hand milking was their primary technique for milk collection, however, more than half utilize milking machines (55.2%). When asked to select all the handwashing practices the producer used, 82.8% of producers indicated they wash their hands before milking, 65.5% after milking, 44.8% between animals, 6.9% wear gloves during the milking process, and 6.9% do not wash at all during the process. This is important as bacteria on the hands can easily contaminate the milk supply during the hand milking process and can also lead to the spread of infections, like mastitis, among animals especially when handwashing is not used intensively. These results alone are a clear indication of room for improvement within the dairy goat milk industry. One publication that can aid farmers in making improvements on the farm is the Dairy Goat Management Best Practices Guide which was developed by the University of Wisconsin. This document can be found on North Carolina State University's small ruminant extension page under books (https://smallruminants.ces.ncsu.edu/).

The majority of producers indicated they "always" utilized milking procedures such as wiping off the dry udder (89.7%), pre-dipping the teats (59.3%), forestripping to remove any initial bacteria (64%), drying the teat before milking (71.4%), and dipping the teat after milking (78.6%). However, some producers do not pre-dip (25.9%), forestrip (20%), or dry the teat before milking (21.4%). Galton et al. (1986) reports that pre-dipping with sanitizers can reduce bacteria counts before milking by 44% alone and by 85% when combining sanitizers and drying the teat. It is also a requirement in the Grade "A" Pasteurized Milk Ordinance that teats are supposed to be treated with a sanitizing solution and dried before the time of milking (FDA, 2015). This simple practice could be one step towards lowering bacterial counts and becoming a registered Grade "A" dairy goat producer.

Pasteurization equipment was mainly inaccessible (70.4%) to producers due to the lack of dairy industry cooperatives in Mississippi and high investment costs but when asked if they would be willing to invest in the equipment if it allowed more opportunities to advertise and sell products, producers were split down the middle with 25.9% saying no, 29.6% saying yes, and 44.4% saying maybe. Similar results were observed when they were asked about their interest in pasteurization workshops. Conversely, 59.3% of producers were interested in safe handling workshops, 33.3% stated they may be interested, and 7.4% said no. The majority (57.7%) of producers agreed that pasteurization affects the nutrient content of the milk. While we did not further explore understanding how producers believe it is different, the United States Food and Drug Administration emphasizes that pasteurization causes little to no influence of nutrients in milk (FDA, 2018). A study by Pestana et al., (2015) concluded that while pasteurization does have a very slight effect on milk as it can increase the pH and decrease the total fat and solids, the effects create no considerable difference in the milk product as a whole. As a thermal treatment, it also has the potential to create organoleptic changes within the product at ultra-high temperatures which can create off-flavors and off-odors (Melini et al., 2017).

In Mississippi, the sale of raw milk is legal with some stipulations. These regulations require that the milking location must be in a clean environment, have a cement or comparable floor, must be enclosed by a wall or screen to prevent insects, and must have a fly strap (MS Code § 75-31-65, 2018). It also requires sterile containers to be used in the milking process and during milk storage. Farmers were asked to rank the importance of these regulations with 0 being extremely unimportant and 100 being extremely important. On average, producers regarded sterile equipment as the most important and cared less about the fly strap being in the milking place (Figure 3). Since some of these regulations are seen as less important by producers, it would be interesting to further explore these sites to see if they should be regulated areas or if legislation should shift their attention to other potential sources of contamination.

The majority of producers were lacking training in topics such as hazard reduction (81.5%), hygienic milking practices (77.8%), and food safety (66.7%). The survey also indicated that 88.9% of producers do not conduct microbial analyses or quality testing of their milk products. This knowledge gap creates an opportunistic scenario to improve producer expertise through university extension and other trainings and events. The majority of respondents (80.8%) indicated that they would invest more money into their operation if they were able to advertise and sell their products more freely. Training events designed to increase the safety of products could not only help farmers financially but could bring a new market to Mississippi's economy and take advantage of the booming popularity of natural preservative-free or minimally processed products. Besides extension, the Raw Milk Institute (www.rawmilkinstitute.org) is one source that provides free videos using science-based food safety principles to improve the safety and quality of raw milk products (Raw Milk Institute, 2020). They provide farmers with the Raw Milk Institutes' common standards to help farmers reduce the risk of illness from raw milk consumption. These standards include providing the farmer with resources to develop a Risk Analysis and Management plan, conduct monthly coliform and standard plate count testing, and gather all the proper documentation needed for selling raw milk for human consumption.

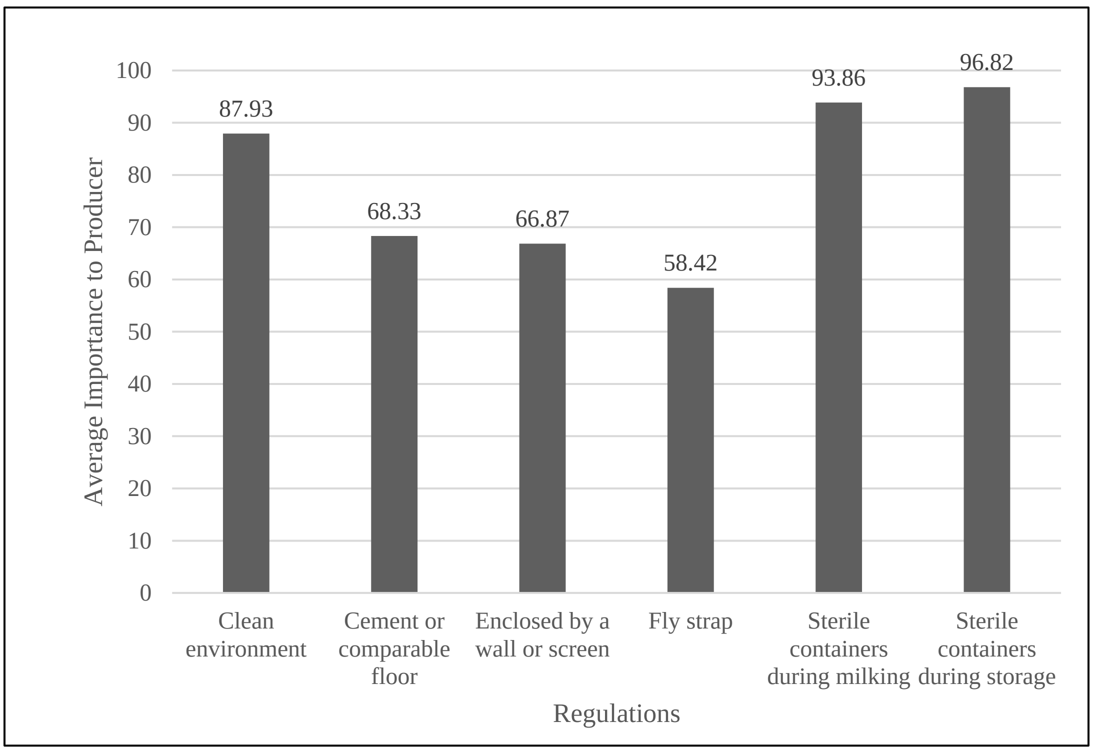

Figure 3. Perception of importance on a rating scale of 1 to 100 of Mississippi's raw milk regulations reported by 29 MS dairy goat producers.

DISCUSSION

To get a more well-rounded observation of the milk quality and pathogen presence, samples should be taken in the early and middle stages of lactation to compare with the late lactation results presented in this exploratory study. Taking environmental samples would be another alternative route of investigation to identify the sources and routes of milk contamination.

In the future, research should be conducted into assessing the microbiome of raw milk. This will aid in furthering our understanding of what serotypes of bacteria may be present in raw goat milk as well as assess the types and extent of probiotics that may be present in farm-fresh dairy goat milk. Future observations of diet could also show an impact on the bacterial presence in the milk. This will give raw milk consumers a better idea of what they are consuming, allowing them to better weigh their risks and benefits, and allow producers to better understand their product. Raw milk products from cows and goats alike have been implicated in a variety of pathogen outbreaks over the years. Between 2002 and 2008, five raw milk outbreaks in the United States were linked back to *Campylobacter* spp. (Pyz-Łukasik et al., 2015). In a CDC study from 2007 to 2012, 81 outbreaks from 26 states were associated with unpasteurized milk. From these outbreaks, the second-highest causative agent was shiga-toxin producing *E. coli* (STEC) (17%) (Mungai et al., 2015). Most recently in 2021, a California goat dairy issued a recall of unpasteurized, raw milk due to illegal levels of *C. jejuni* (Beach, 2021). Listeria has also been indicated as a causative agent in other dairy related outbreaks in products such as ice cream and butter (Pouillot et al., 2016; Maijala et al., 2001). In the future, producer perceptions of pasteurization should be addressed using tailored training programs focused on pre- and post-processing milk quality and marketability. Besides training, the state of Mississippi could work more closely with producers to make corrective actions on the farm to promote improvement within the local dairy goat community. This could lead to the potential for less restrictive farm regulations and move the emphasis of safety from environmental factors to the actual milk product.

CONCLUSION

The microbiological analysis of the milk showed that producers still have room for improvement to create a safe milk product. It is important to consider that often the bacterial presence in milk becomes more common as producers dry off their does. Survey results show the opportunity and need for additional involvement from university extension and other training programs. The importance of producer handwashing practices during the milking procedure should be communicated to establish proper food safety practices. Limitations of the study included the overlapping time frame of milk sample collection and survey collection as the survey could have influenced the farmers milk collection methods. To the knowledge of the authors, this study is the first of its kind to engage the participation of Mississippi dairy goat farmers while assessing food safety practices, making it relevant for both research and policy-making purposes.

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AUTHOR DISCLOSURES:

The authors have not stated any conflicts of interest.

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Supplement

Questions from the raw milk survey distributed to Mississippi farmers.

Background

- Are you a dairy goat producer?
- Are you 18 or older?
- Is your dairy goat operation located in Mississippi?
- Where is your operation located if not in Mississippi?
- What is your purpose in the goat industry? Select all that apply.
- How many goats do you currently house? Please answer all that apply.
- What dairy breeds do you keep? Select all that apply.

Milking Practices

- How often do you milk your goats?
- How important are these procedures to you?
- What milking systems do you typically use? Select all that apply.
- How often are these procedures used (per milking process)?
- How often do you wash your hands during the milking process? Select all that apply.
- How often do you filter your milk?
- Do you use a cooling system during milking? (I.e. bulk tank cooling)
- Do you milk in an air conditioned area?
- When do you sanitize your milk tank or bucket? Select all that apply.
- How often do you sanitize your milking claw? Select all that apply.
- When sanitizing your equipment, what products do you use? Select all that apply.
- Do you ever use a telephone, cell phone, smart phone, tablet, laptop or computer while milking goats?
- How often do you participate in Dairy Herd Improvement testing?
- How often do you conduct Somatic Cell Count tests?
- How long do you usually store your milk before using?

Management Practices

- What housing type do you use for your goats? Select all that apply.
- How often are bedding areas cleaned?
- Do you have a separate quarantine area for new or sick animals?
- What feeding routines do you utilize? Select all that apply.
- Please select your kidding seasons: Select all that apply.

Products and Market

What products do you make with your goat milk? Select all that apply.

What products do you sell that are made with your goat milk? Select all that apply.

How often do you sell your products through these avenues:

On the farm, Farmers market, Social Media (Facebook, Instagram, etc.), Online (Website, blog, etc.), Local store, I do not sell my products, I give away my products, Other (Please explain)

How often do you reach your consumer through these avenues:

On the farm, Farmers market, Social Media (Facebook, Instagram, etc.), Online (Website, blog, etc.), Local store, I do not sell my products, I give away my products, Other (Please explain)

Would you invest more money in your operation if you were able to advertise and sell more freely?

Are you a registered goat dairy?

Do you have access to pasteurization equipment? If so, where?

Would you be willing to invest in pasteurization equipment if it allowed for more opportunities to advertise and sell your products?

Rank these obstacles with 1 being your biggest operational challenge.

Keeping a clean milking environment, Having a cement or comparable floor in the milking area, Having an enclosed wall or screen in the milking area to prevent insects, Keeping milking machines sterile, Access to pasteurization equipment, Ability to advertise products, Investment costs to be an operating goat dairy

Food Safety Background

Please answer the following questions regarding trainings and risk analysis by answering yes or no.

Do you have a risk analysis and management plan for your milk products?

Have you taken any hazard reduction trainings?

Have you taken any hygienic milking procedures trainings?

Have you taken any food safety trainings?

Do you have a Standard Operating Procedure for milking?

Please answer the following questions regarding the time frame of milk analysis testing.

How often do you have a microbial analysis of your milk products?

How often do you conduct milk quality testing of your milk products?

Are you interested in workshops in pasteurization?

Are you interested in workshops in safe milk handling?

How often do you and your family drink raw goat milk?

For what reasons do you drink raw milk? Select all that apply.

Do you use raw milk in recipes when preparing meals?

After handling raw milk, do you usually continue what you're doing, or do you first rinse your hands with water, or wipe them, or wash them with soap?

Food Safety Knowledge and Risk Perception

How much do you know about bacteria in raw milk?

Select the pathogenic bacteria you are familiar with. Select all that apply.

Pathogenic bacteria can cause foodborne diseases. (T/F)

Raw milk can harbor a variety of pathogenic bacteria. (T/F)

Pasteurization affects the nutrient content of milk. (Strongly agree to strongly disagree)

Ideally, milk should be stored below 40 degrees F. (T/F)

Somatic Cell Counts will increase to fight off mastitis-causing pathogens like Staphylococcus aureus. (T/F)

How common do you think it is for people in the United States to get food poisoning due to the following factors?

Food prepared at home, food prepared at a restaurant, food prepared in industry

Do you think contamination of food by bacteria is a problem?

How likely do you think it is that raw milk has bacteria that could make you sick?

Demographics

What is your gender?

What is your age?

Are you of Hispanic, Latino, or Spanish origin?

How would you describe yourself? Please select all that apply.

What is the highest degree or level of school you have completed?

Is your highest degree or level of school completed related to agricultural sciences (food science, animal and dairy science, etc.). If yes, please state your completed major and concentration.

What is your marital status?

What is your current employment status?

What is your annual household income from all sources?

Automating An Environmental Heat Flux Simulation Workflow

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ABSTRACT

A considerable amount of research has been performed by various groups with the goal of detecting objects of interest in various environments. These objects can be above ground, partially buried, or fully buried. One of the detection methods that has piqued the interest of researchers is Automated Target Recognition (ATR) algorithms that can pick out the locations of such objects from infrared (IR) sensor imagery once it was recognized that such objects absorb and emit heat at rates that distinguish them from their surrounding environments. In order to obtain training data for ATRs, one option is to gather large volumes of field data from many locations, but such efforts are resource and labor intensive. Another option is to generate synthetic training data that simulates heat flux in real environments. Several research groups in the past have implemented a combination of both, using field data to inform and verify simulation tools that model heat flux, energy absorption, and IR emission from various parts of the environment, as well developing simulation workflows that model real environments. Such workflows are often developed for the purpose of simulating a single environment. Our goal is to modify and generalize such a workflow so that simulation parameters and the location of object of interest can be changed by non-experts or automated processes. In addition; this study is also focused on developing an efficient methodology to simulate a large variety of scenarios to produce varied sets of synthetic training data for the ATRs. The HPC simulations involved in generating the training data for ATRs require the exchange of data between several high-fidelity physics models. Efficient techniques have been implemented to ensure that the inputs to each component are consistent and correct. A more generalized workflow has been developed that is able to simulate complex scenarios with new LiDAR data, weather data, vegetation placements, target placements, and as output produce synthetic IR imagery useful for training ATRs. The synthetic IR images are automatically tagged with bounding boxes so that minimum post processing is required when the images are used for training the ATRs. The synthetic IR imagery was passed on to researchers developing ATRs and used to train the algorithms. The parts of the workflow that are streamlined can now be executed with relative ease on a high-performance computing environment and components of the workflow can now be more easily updated and modified in the future.

KEYWORDS: Automatic Target Recognition, Environmental Simulation, Sensor Simulation, High Performance Computation, Configuration and Input File Management, Synthetic Infrared Imagery

INTRODUCTION

A considerable amount of research has been performed by various groups with the goal of detecting objects of interest in various environments. These objects can be above ground, partially buried, or fully buried. One such detection method is training Automated Target Recognition (ATR) algorithms that can pick out the locations of such objects from infrared (IR) sensor imagery. This works because objects absorb and emit heat at rates that distinguish them from their surrounding environments. In order to train ATRs, one must gather large volumes of varied IR imagery. One method to do so is to generate synthetic IR imagery using environmental simulators. Such simulators exist at the U.S. Army Corps of Engineers Engineer Research and Development Center (USACE ERDC) and are used in a workflow that simulates incident solar radiation warming the surfaces of the soil and vegetation of the simulated scene, heat flux propagating through the volumes of the soil and vegetation, and IR radiation emitted from warm surfaces propagating through the scene and being captured by an IR sensor. This work is an effort to improve this workflow so that it could both be used with ease by ATR researchers and be automated for parameter studies.

WORKFLOW DETAILS

The three simulators used in the workflow are Advanced Hydrology (AdH), VegTherm, and QuickCast. AdH simulates heat flux transmission through the soil, the absorption of solar radiation at the soil surface, and heat loss via IR radiation emission at the surface and VegTherm does much the same but for the vegetation in the scene. QuickCast is a raytracer that simulates solar radiation incident to the soil and vegetation surfaces and IR radiation re-emitted by those surfaces toward an IR sensor. A shell script developed specifically for this study changes the position of the sun in the sky for each simulated hour and coordinates transmission of data between AdH, VegTherm, and QuickCast. The general concept of the workflow is depicted in Figure 1.

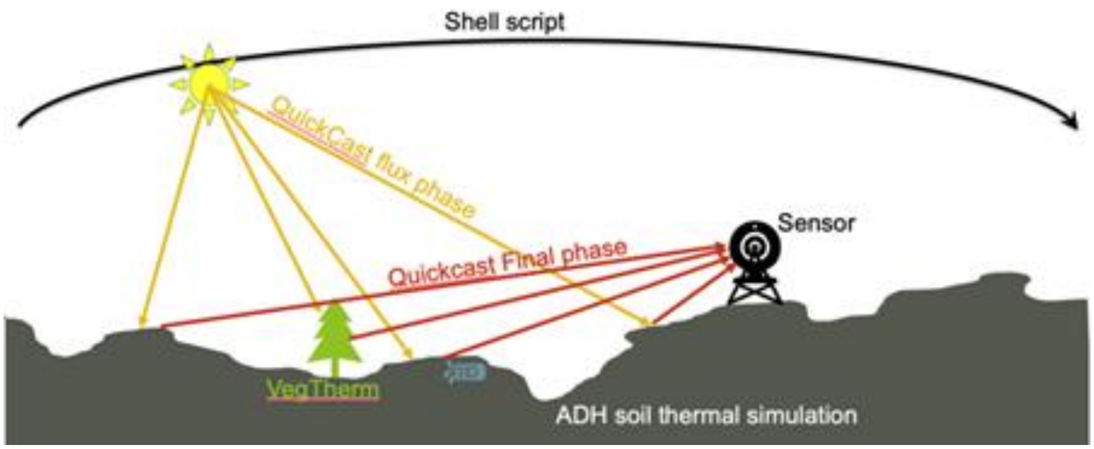

FIGURE 1: WORKFLOW GENERAL CONCEPT

QuickCast generates an enormous number of rays based on the image size, so for efficiency these geometric computations are run only once outside of the loop that simulates the passage of time. The intensity of radiation flux each of the rays carries does vary based on the solar position, so inside the time loop, a QuickCast flux phase passes incident solar radiation flux data to AdH and VegTherm which pass surface temperature data back to a QuickCast final phase using plaintext data files. QuickCast then produces the sensor data for the simulated hour of the day.

WORKFLOW IMPROVEMENTS The simulation workflow was originally designed for a verification study, so it needed to be improved to increase its utility for researchers unfamiliar with the fine details of running these three high fidelity physics simulations, to reduce user error, and to enable automation during parameter studies. A problem that has arisen in the 10 years since the verification study was performed is that some of the parameters common to the configuration files of the three simulators have become inconsistent. In order to make such details consistent in novel simulations using the same workflow, a new suite of python scripts was developed that read configuration information from a master configuration file and generated both configuration files for the individual simulators and a workflow coordinator shell script that sets up the workspace and runs the simulation on High Performance Computers (HPC). The setup of new simulations using new data files was made much easier using the setup scripts and master configuration file while keeping parameters shared between the three simulators (e.g., the time span being simulated) consistent. A browser-based configuration file assistant was also created to assist the user in generating a complete and syntactically correct master configuration file.

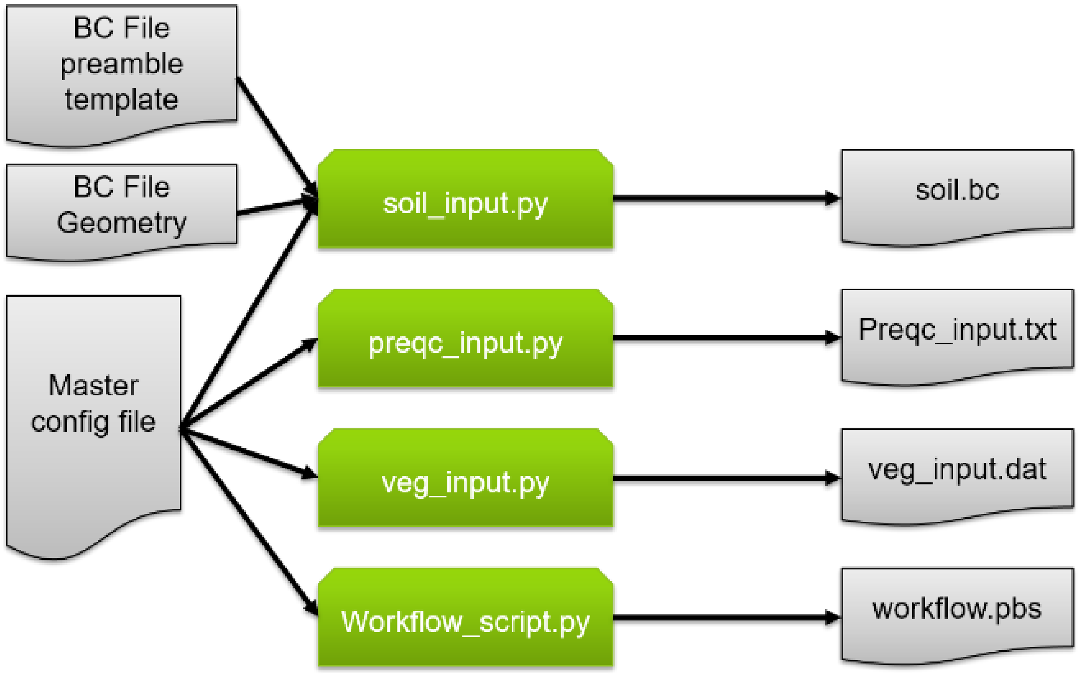

FIGURE 1: CONFIGURATION FILE GENERATING SCRIPTS

ONGOING WORK

These python scripts and the master configuration file form the basis of a new version of the simulation workflow being used by the developers of the next generation of the VegTherm and QuickCast simulators. The configuration file assistant itself is being turned into web-based portal for setting up and running these kinds of simulations. There still remain many issues that affect the accuracy and flexibility of simulation in the input data and configuration files. Some of these issues can be addressed through python scripts but some may require changes to the workflow that creates the input files for scene geometry (i.e., landscape, vegetation, and sensor placement). Increasing the utility of the scene generation workflow for non-experts and making it more automatable will also have a greater benefit to ATR researchers, since it is during scene generation that the detection target can be moved around to different locations in the scene. The positions and types of vegetation in the scene is also determined in this stage. Giving end users a simple interface to vary these parameters of the simulation will allow the production of the greatest variety in synthetic imagery for training the ATRs.

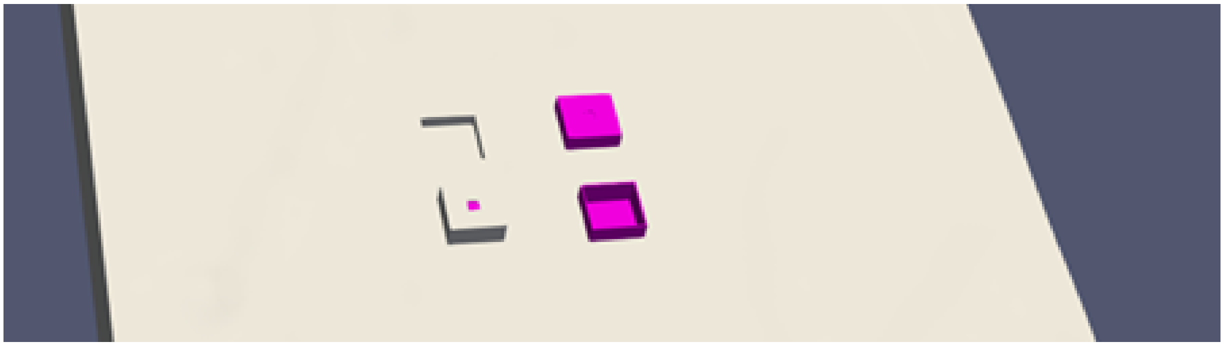

FIGURE 3: PROBLEMS EMBEDDING MULTIPLE TARGETS

Another goal of improving the scene generation workflow is to gain the ability to place multiple detection targets in a scene. The current scene generator is limited to placing one detection target in the scene and attempts to place more than one resulted in missing geometries and mislabeled surfaces, as seen in Figure 3. In addition, the convoluted web of software needed to work around the difficulties in placing that one target in a scene currently prevents us from adding the flexibility we desire. This is caused by the software we use for manipulating the geometry of the scene and for producing 3D meshes of the scene. Introducing a more robust or more lightweight software to replace it will enable the development of capabilities that are required.

ACKNOWLEDGEMENTS

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Description of an Ancient Roman Two-sided "H Comb"

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ABSTRACT

An ancient wooden comb, donated to one of the authors, was photographed, analyzed, and examined closely for any signs of head lice or their eggs. The comb was determined to be a two-sided "H comb" dating to 250-400 C.E. Although no evidence of human lice was found in the teeth of the ancient comb, small bits of debris, perhaps human skin, and hair were recovered.

Keywords: Head lice, nit combs, ancient combs, wooden combs, H combs

INTRODUCTION

For thousands of years, humans have used wooden combs to clean and detangle hair, and also remove head lice and their eggs (Zias and Mumcuoglu 1989, Palma 1991, Araujo et al. 2000). Head lice combs, very similar in appearance to modern louse or "nit" combs were already being used for delousing in Egypt during Pharaonic times (approximately 6,500 years ago) (Kamal 1967). Sometimes, upon present-day examination of these ancient combs, head lice (or parts thereof) have been recovered from debris found between the fine teeth of wooden combs dating as far back as the first century, Before Current Era (B.C.E) (Mumcuoglu and Zias 1988), and in fifth/sixth century Current Era (C.E.) combs excavated in Antionoe, Egypt (Palma 1991). Here we report results and analysis of one such ancient wooden comb.

MATERIALS AND METHODS

An ancient wooden hair comb was donated to the second author (MB) by a professional colleague for use in her Egyptian History class at the University of Louisiana Monroe. At that point, the comb was believed to be a 4-5 century C.E. Egyptian comb, perhaps used for delousing. The comb was then loaned to the first author (JG), an entomologist, for microscopic examination for remnants of head lice or their eggs. In the lab, the comb was carefully measured, photographed, and then flossed between the wooden teeth with commercially available dental floss. During the flossing procedure, 70% ethanol was sprayed with a spray bottle over the teeth and floss into a large petri dish positioned below it. Subsequently, alcohol in the petri dish was carefully examined with a microscope for presence of hair, skin, lice, or lice eggs. In addition, photographs of the comb were sent to Barbara Birley (Curator, the Vindolanda Trust, Chesterholm Museum, U.K.) and Dr. Suzana Hodak (Coptologist, The University of Münster, Germany) for examination and possible dating.

RESULTS AND DISCUSSION

The comb was most likely made of boxwood from the Mediterranean area, a type of wood known for not splitting (Figure 1). Careful measurement revealed the comb was 66.7 mm tall, 49.6 mm wide, and 8 mm thick. The top section of the comb contained 16 teeth (about one tooth per mm), 4 of which were broken, while the bottom contained 27 teeth (about ½ tooth per mm) with 23 of them broken. Although combs of this form were not designed exclusively and primarily to comb out lice, they were certainly sometimes used for that purpose. In fact, modern "nit" combs have this exact same shape and size (Anonymous 2022).

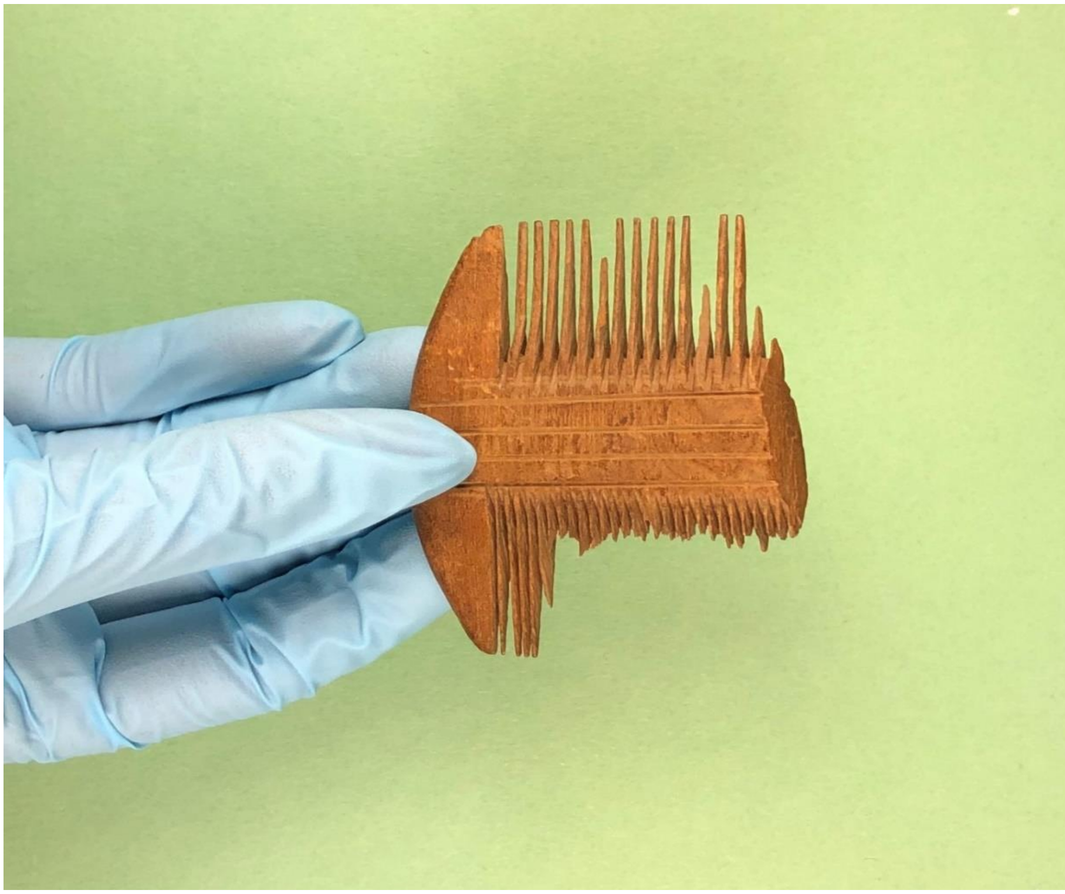

Figure 1. Ancient Roman two sided "H comb."

Photographic analysis of the comb by two experts revealed the following: Ms. Birley commented that the comb appeared to be of typical Roman style as seen at the archaeological site, Vindolanda (U.K.). Dr. Hodak said the comb was definitely post-Christian, and when compared with other such pieces, it likely dates to the period 250-400 C.E. (Late Antiquity). Microscopic examination of the comb teeth and alcohol resulting from flossing with dental floss (Figure 2) revealed a few pieces of debris (possibly human skin) and two pieces of what appeared to be human hair (Figures 3-4). Although one piece of skin and embedded hair resembles an insect head and antennae (Figure 4), the object was not of insect origin. There was no evidence of human head lice or their eggs in the comb. Nonetheless, it was intriguing to examine the comb and imagine all the people whose hands have touched it for over 1,500 years.

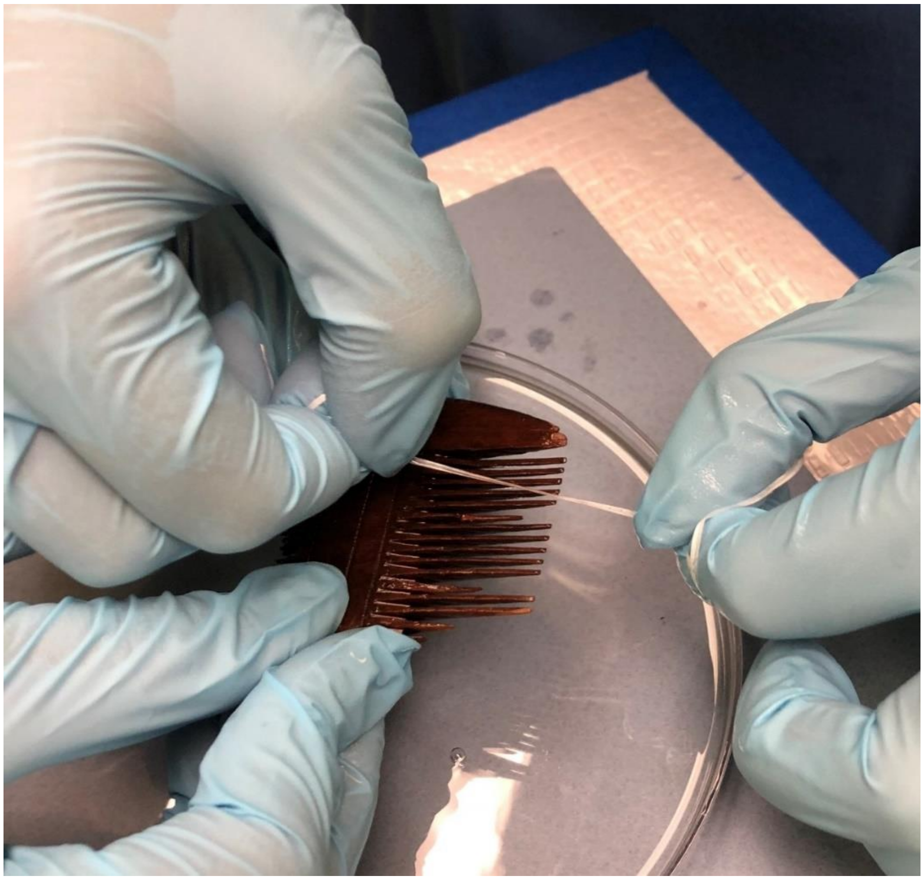

Figure 2. Cleaning between the teeth of comb.

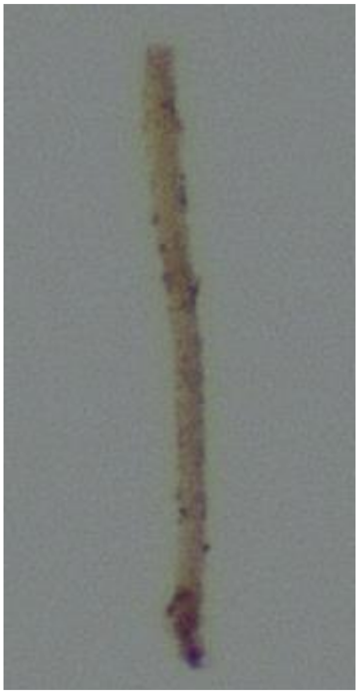

Figure 3. Hair found between teeth of comb.

Figure 4. Piece of skin and hair found between the teeth of comb.

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We thank Barbara Birley (Curator, the Vindolanda Trust, Chesterholm Museum, U.K.) and Dr. Suzana Hodak (Coptologist, The University of Münster, Germany) for their analysis and comments concerning this ancient comb. Also, Afsoon Sabet, Kristy McAndrew, and Abby Jones, Mississippi State University graduate students, aided with flossing teeth of the ancient comb.

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Optimal Planting Density For Thinned Shortleaf Pine Plantations In The Western Half Of The Southeastern United States

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ABSTRACT

Shortleaf pine (*Pinus echinata* Mill.) has been planted in the western part of the Southeastern United States. The United States Department of Agriculture (USDA) Forest Service Forest Vegetation Simulator (FVS) is the only currently widely available comprehensive yield prediction system for these plantations. As an initial attempt to determine optimal thinning regimes, predictions were obtained for planting densities of 988, 1,483, and 1,977 seedlings per hectare (400, 600, and 800 seedlings per acre) for site indexes of 13.72, 18.29, and 22.86 m (base age 25) (45, 60, and 75 ft, respectively). Total discounted reforestation costs were calculated using a 5% interest rate. Projections were obtained for 80 years and a thinning "trigger" of 27.55 square meters per hectare [120 square feet of basal area (at a height of 4.5 feet aboveground) per acre] was used along with a residual stand density of 16.07 square meters per hectare (70 square feet per acre). Financial assessments were conducted by combining the discounted reforestation costs and FVS projections with one of three sets of stumpage revenues; Current, Reasonable, and Optimum.

For lower site qualities (e.g. SI 13.72), when using this particular thinning regime, positive financial returns were not observed regardless of the stumpage prices. On higher quality sites (e.g. SI 22.86), given FVS projections and the selected thinning regime, positive financial returns will likely be observed. For medium quality sites (e.g. SI 18.29), if stumpage markets improve (e.g. Reasonable and Optimum sets of stumpage revenues), growth rates on these sites may be enough to produce positive financial returns. For this thinning regime (27.55 trigger and 16.07 residual), an optimal planting density is likely around 1,236 to 1,359 seedlings per hectare (500 to 550 seedlings per acre). However, a much more complete assessment of thinning regimes needs to be conducted and calibrated FVS projections should be used.

Keywords: Shortleaf pine, Optimal Planting Density, Stumpage Revenue, Forest Vegetative Simulator

INTRODUCTION

The historical range of shortleaf pine (*Pinus echinata* Mill.) includes much of the western half of the southeastern United States as well as the southeastern part of Missouri and a small area in the southwestern part of Illinois (Burns and Honkala 1990). According to the Shortleaf Pine Initiative (http://shortleafpine.org/), shortleaf was often found in open woodlands in both pine dominated and mixed pine-oak forests. Historically, in some Western Gulf locations it was the dominant pine species (Clabo and Clatterbuck 2015, Bragg 2016). Shortleaf pine ecosystems are often highly fire-dependent (Clabo and Clatterbuck 2015). The extensive shortleaf pine ecosystem has lost over 50% of its former acreage over the last 30 to 40 years (Clabo and Clatterbuck 2015, Guldin 2019). Contributing factors include massive pine beetle outbreaks in poorly managed stands, changes in timber management practices, altered fire regimes (Guldin 2019), disease, and land use changes.

Coordinated efforts are beginning to restore the shortleaf pine ecosystem such as by groups like the Shortleaf Pine Initiative. Plantations will likely be an active part of the ecosystem restoration process (Guldin 2019). Therefore, managers will need tools to examine how different management options will likely impact financial returns helping to initially offset costs associated with the ultimate creation of a more open woodland setting. Those wanting to restore and/or manage for other components of the ecosystem such as wildlife, water quality, etc., will benefit from knowing how various planting densities in combination with various thinning regimes may impact stand development.

According to a recent Forest Inventory and Analysis (FIA) estimate (USDA Forest Service 2022), there are 98,882 hectares of shortleaf pine plantations in the western half of the southeastern United States, while northwestern Arkansas and southeastern Oklahoma have considerable amounts of naturally-regenerated stands (Table 1). Shortleaf pine stands are also relatively abundant in Alabama, Mississippi, Missouri, and Texas. A small amount of planting has occurred in the southern parts of Illinois and Indiana.

Table 1. Hectares of shortleaf pine forest types as defined by the USDA Forest Service Forest Inventory and Analysis (FIA) program (USDA Forest service 2022).

State	Plantation	Naturally-Regenerated	Total Hectares
Alabama	1,256	45,152	46,408
Arkansas	40,611	474,790	515,400
Louisiana	-	14,977	14,977
Mississippi	4,244	71,255	75,499
Missouri	34,959	62,871	97,830
Oklahoma	9,233	202,876	212,109
Tennessee	6,398	18,942	25,340
Texas	2,181	109,612	111,793
Total	98,882	1,000,475	1,099,357

Several studies (Williston 1972, Watson et al. 1973, Schubert et al. 2004, Amishev and Fox 2006, Dipesh et al. 2015, Lynch et al. 2016, Hooker et al. 2020, among others) have found that shortleaf pine generally has slower growth rates relative to the more frequently planted loblolly (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm). However, Hooker et al. (2020) showed that at age 3 shortleaf had greater height growth than slash pine on 2 of 3 sites, but lagged behind loblolly pine on all 3 sites. Shortleaf is generally considered to outgrow the two other pines by age 40 or 50 and hence this species will likely have longer rotations (Smalley 1986, Clabo and Clatterbuck 2015). The author also observed that shortleaf pine lagged behind loblolly pine at age 16 in northern Mississippi old-field plantations found on the same site as part of a study described by Kushla (2009, 2010). Hence, landowners may sacrifice some financial return when establishing shortleaf pine plantations as part of an ecosystem restoration program.

However, shortleaf does have some advantages relative to loblolly and slash pine. These advantages include better stem form (Will et al. 2013) that often helps to produce greater merchantable volume. Shortleaf is particularly resistant to fusiform rust (*Cronartiium quercuum* f. sp. *fusiforme*) (Clabo and Clatterbuck 2015) and is often less susceptible to damage resulting from ice, snow, and cold temperatures (Will et al. 2013, Clabo and Clatterbuck 2015). Shortleaf often can ultimately attain greater standing basal areas per hectare (Smalley 1986). Slower stand development can be beneficial for wildlife habitat and for grazing. Since crown closure will occur at older ages relative to loblolly or slash pine, understory vegetation production will likely be greater in shortleaf pine plantations at earlier ages and for an extended period of time.

Planting Density

There are few specific recommendations about the optimal planting density in terms of shortleaf pine growth and yield. Recommendations are essentially that the planting density depends on local market conditions. Even fewer studies have addressed directly the financially optimal planting density. VanderSchaaf et al. (2018) examined the financial returns of shortleaf pine plantations using the Forest Vegetation Simulator growth and yield model (Dixon 2002, Keyser 2008). Planting densities of 741, 1,236, and 1,730 seedlings per hectare were examined. Given the financial market conditions at the time, for higher quality sites a planting density of 741 seedlings per hectare was recommended, while for moderate sites a planting density near 1,236 seedlings per hectare was recommended.

Thinning

As trees develop, they grow larger in size, and at some point, site resources of light, moisture, and nutrients become limiting leading to intense competition for these resources. At this point, trees start to lose their vigor and trees often become more susceptible to droughts, diseases and insects, wildfires, and density-dependent mortality starts to occur. Thus, thinning is a very important component of forest management. Thinning provides a means to remove diseased, forked, or poorly formed trees, maintain tree vigor helping to reduce the impacts of insects, diseases, droughts, and wildfires. Thinning can also allow landowners to alter species compositions, this alteration of species is often related to wildlife habitat objectives. Financially, thinning provides early financial returns on investment, allows for the financial capture of mortality, and to maintain and/or increase growth rates to produce larger, more valuable trees.

Several studies have examined the impacts of thinning "triggers" and residual basal area targets on the growth and yield of shortleaf pine plantations. Brinkman and Rogers (1967) recommended thinning back to 14.92 square meters in a first-thinning around age 35, and to thin back to around 18.37 square meters during a second-thinning which should be near age 50 in Missouri old-field plantations, with a final harvest around age 65. In contrast, Phipps (1973) showed in two southern Indiana plantations that when conducting a first and second thinning around ages 14 to 22, with a minimum of a five-year thinning interval, a much greater residual thinning target of around 22.96 to 27.55 square meters produced the greatest cubic foot volume growth rates at ages 29 and 32. Rogers (1983) presented Gingrich style stocking charts and recommended thinning back to a stand density around 60% of full stocking.

On the Shortleaf Pine Initiative website (https://shortleafpine.org/growing-shortleaf-pine/stand-management/thinning), J. Blanton (Blanton) recommends first thinnings should occur when basal area per hectare approaches 32.14 square meters, and the residual basal area should be 18.37 square meters. As trees become older and greater in size, thinning should occur before the basal area reaches 39.03 square meters, and for maximum diameter growth, the desired residual basal area would be approximately 22.96 square meters.

The Southern variant (SN) of Forest Vegetation Simulator (FVS) covers forest areas in the southern United States including all Western Gulf states (Dixon 2002, Keyser 2008) and can be used to predict shortleaf pine stand development. Similarly, shortleaf pine plantations can be modeled within Illinois, Indiana, and Missouri using the Central States variant (CS). Both variants are a distance-independent individual tree model. However, the MANAGED keyword that essentially tells FVS that projection of plantations is desired by modifying the large-tree diameter growth models, is not available for shortleaf in either variant.

Based on verification analyses of actual shortleaf pine plantations, VanderSchaaf et al. (2018) found that unless FVS is calibrated to an actual forested condition, FVS will often underpredict stand development up to around age 30. Nonetheless, uncalibrated FVS projections can allow for some initial investigation of the optimal silvicultural regime in relation to planting density, thinning "triggers" and residual stand densities ("targets"), and the timing and number of those thinnings. The objective of this current analysis is to determine the financial returns of various combinations of planting density, a single thinning trigger of 27.55 square meters per hectare, a single residual thinning stand density of 16.07 square meters per hectare, and the timing and number of those thinnings.

METHODS

As an initial attempt to determine optimal thinning regimes, predictions from the time-of-planting were obtained for densities of 988, 1,483, and 1,977 seedlings per hectare for site indexes of 13.72, 18.29, and 22.86 m (base age 25). A single thinning regime was examined. Whenever a stand's trajectory attained or exceeded a basal area per hectare of 27.55 square meters, a thinning from below was conducted back to a basal area per hectare of 16.07 square meters. Clabo and Clatterbuck (2015) consider this an applicable thinning regime. Within FVS, projections were obtained every 2 years up to 80 years of plantation age.

Reforestation costs were based on Maggard (2021) for operations conducted during 2020. Across all planting densities, a mechanical site preparation cost of \$395.37 per hectare (Northern Coastal Plain) was assumed and a first-year herbaceous weed control treatment at a cost of \$134.40 (Northern Coastal Plain) per hectare was assumed. A hand planting cost of \$0.17 was assumed and seedling costs per thousand were of bareroot source at \$50.00 per thousand. Price per thousand was based on Arkansas Department of Agriculture Tree Seedling Prices for 2022 – Advanced Generation Shortleaf Pine. An interest rate, or alternative rate of return, of 5% was used. For planting densities of 1,977, 1,483, and 988 seedlings per hectare, discounted reforestation costs were \$958.27, \$849.55, and \$740.82 per hectare, respectively.

Minimum merchantability limits were consistent with standard FVS SN protocol and stump height was set to 0.3048 m. Minimum merchantable pulpwood diameter at breast height (1.37 m above ground level, DBH) was 10.16 cm, and upper stem diameter outside–bark (DOB) was 10.16 cm. Chip–n–saw specifications were minimum DBH of 20.32 cm and a DOB of 10.16 cm. Sawtimber specifications were minimum DBH of 27.94 cm and a DOB of 17.78 cm. Volumes were calculated using the SpMcDBH keyword within FVS. Three sets of stumpage values were used entitled the Current, Reasonable, and Optimal stumpage prices. The Current prices are based on Mississippi 4th quarter 2021 (Measells 2021) and are 3.60, 12.20, and 23.20 per ton for pulpwood, chip-n-saw, and sawtimber, respectively. The Reasonable prices are based on the Current stumpage prices but adjusted upwards, and are 7.50, 20.00, and 30.00 per ton for pulpwood, chip-n-saw, and sawtimber, respectively. The Optimum prices are 10.00, 22.50, and 35.00 per ton for pulpwood, chip-n-saw, and sawtimber, respectively. Although not observed currently in Mississippi, there are some locations within the southeastern United States where the Optimum set of prices is being observed. To convert volumes to tons, it was assumed that each cubic foot of wood and bark weighs 58 lbs (26.31 kg - Miles and Smith 2009).

RESULTS AND DISCUSSION

Consistent with expectations, as site quality and planting density increase, total cubic meter volume increases (Fig. 1). For a planting density of 1,977 seedlings per hectare, and when using a basal area target of 27.55 square meters per hectare, first thinnings occurred at ages 32, 26, and 20 for site indexes of 13.72, 18.29, and 22.86 m (base age 25), respectively (Table 2). For a planting density of 988 seedlings per hectare, first thinnings occurred at ages 46, 38, and 32 for site indexes of 13.72, 18.29, and 22.86 m (base age 25), respectively. Across all planting densities and site qualities, these thinnings occur at relatively lengthy ages. VanderSchaaf et al. (2018) stated that, based on observations of other growth and yield studies, unless calibrated using observed growth measurements, FVS may underpredict stand development of shortleaf pine plantations up to around age 30. Unfortunately, there is currently no MANAGED keyword available for shortleaf pine to inform FVS that projections are for plantations and hence growth and yield should be enhanced. For a 16-year old old-field shortleaf pine plantation in northern Mississippi planted at 1,537 seedlings per hectare (Kushla 2009, 2010) with a site index of around 15.24 (base age 25), the observed basal area and total cubic meter volume per hectare was 33.98

square meters and 187.0 cubic meters, respectively. These values even exceed those of the FVS projections from the high quality site (SI 22.86) at a planting density of 1,977 seedlings per hectare. The predicted values were 22.50 square meters per hectare of basal area and a total cubic meter volume of 162.9 per hectare. Thus, operationally, stand development may be faster than that predicted when using uncalibrated projections from FVS, and therefore in operation in the "Real World," the first thinnings may occur at younger ages.

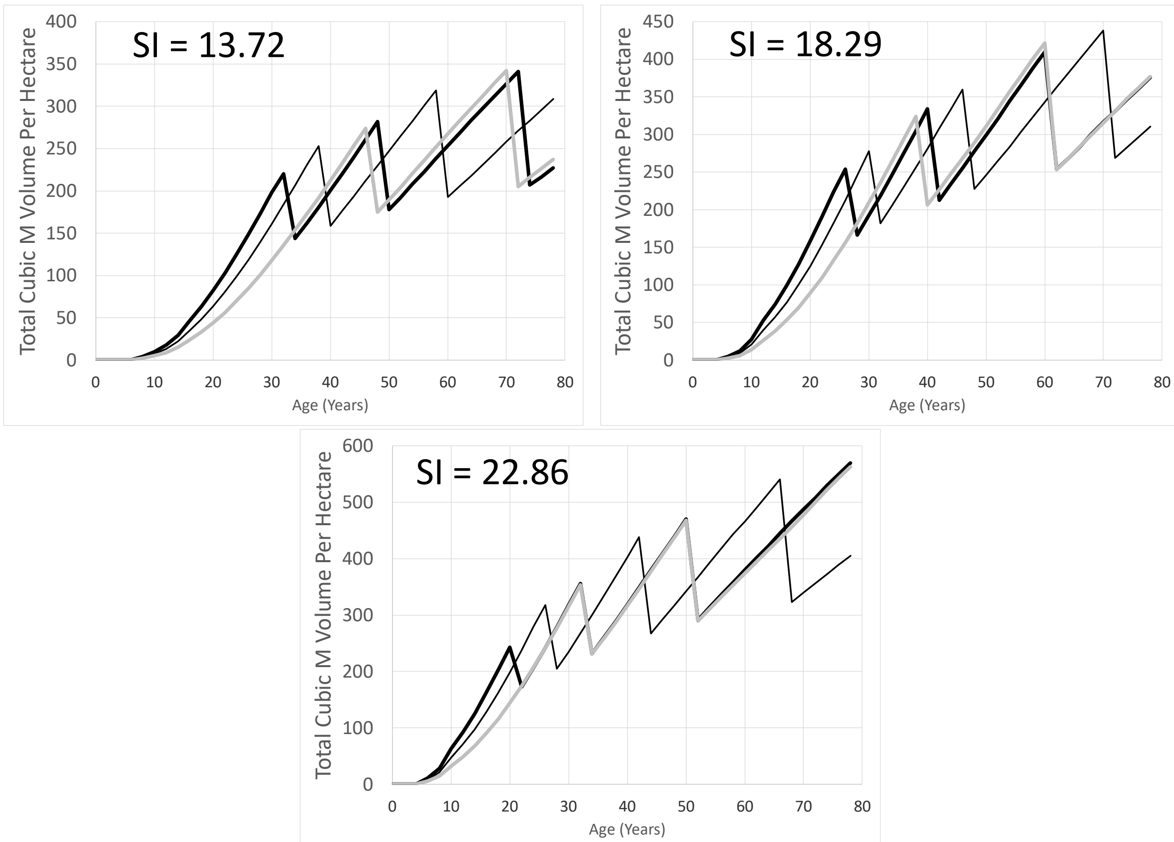

Fig. 1. Total merchantable cubic meter volume (minimum DBH of 10.16 cm to a 10.16 cm top Diameter Inside Bark) projections by planting density and site index (SI – 13.72, 18.29, and 22.86 m – base age 25). The bold black lines are projections for 1,977 seedlings per hectare, the black lines are projections for 1,483 seedlings per hectare, and bold gray lines are projections for 988 seedlings per hectare.

Number of Thinnings

Assuming a rotation age of 80 years, the total number of thinnings ranged from 2 to 4 depending on the planting density and site quality (Table 2). For a planting density of 1,977 seedlings, FVS predicted enough growth and yield such that at least 3 thinnings were observed regardless of site quality. However, for lower planting densities, based on FVS projections, not only will the timing of first thinnings be later for lower site qualities relative to higher site qualities, but in general there will be fewer thinnings for lower site qualities across an 80-year rotation.

Table 2. Timing of the first thinning (First), timing of all thinnings (Allthin), and the total number of thinnings (Numthin) across an 80 year rotation by planting density per hectare (SPH) and site index (SI - base age 25 in meters). The optimal Soil Expectation Value (SEV) and the age at which it occurs is also presented for three sets of stumpage values. Where: C – Current, R – Reasonable, and O – Optimum stumpage values.

					Optimal SEV Age			Optimal SEV Value ($/hectare)		
SPH	SI	First	Allthin	Numthin	C	R	O	C	R	O
988	13.72	46	46, 70	2	70	50	50	-500.09	-338.29	-257.85
	18.29	38	38, 60	2	60	60	60	-236.26	28.84	185.72
	22.86	32	32, 50, 78	3	52	52	52	140.28	597.80	858.94
1,483	13.72	38	38, 58	2	74	70	70	-518.60	-320.62	-213.57
	18.29	30	30, 46, 70	3	62	60	60	-162.27	224.74	432.48
	22.86	26	26, 42, 66	3	56	54	54	262.90	862.12	1,188.01
1,977	13.72	32	32, 48, 72	3	74	50	50	-676.08	-437.94	-306.63
	18.29	26	26, 40, 60	3	62	60	60	-382.42	6.89	237.76
	22.86	20	20, 32, 50, 78	4	54	54	54	50.06	668.10	1,031.54

Soil Expectation Value (SEV)

Based on projections from FVS, and regardless of planting density, when using a thinning "trigger" of 27.55 square meters per hectare and thinning back to 16.07 square meters, lower site qualities (e.g. SI 13.72) may not produce positive financial returns, even when stumpage values could be considered "Optimum" (Table 2, Figs. 2 to 4). VanderSchaaf et al. (2018) also found that unthinned stands on lower quality sites did not produce positive financial returns given market conditions. They also noted though, based on verification analyses of observed growth and yield, that FVS likely underpredicts stand development when using uncalibrated projections. For medium quality sites (e.g. SI 18.29), at Current stumpage values within Mississippi, and given FVS projections and the selected thinning regimes, investment in shortleaf pine plantations may not produce positive financial returns (Table 2, Fig. 2).

If markets improve (e.g. Reasonable and Optimum sets of stumpage revenues), growth rates on these sites may be enough to produce positive financial returns (Table 2, Figs. 3 and 4). On higher quality sites (e.g. SI 22.86), given FVS projections and the selected thinning regime, positive financial returns will likely be observed (Table 2, Figs. 2 to 4).

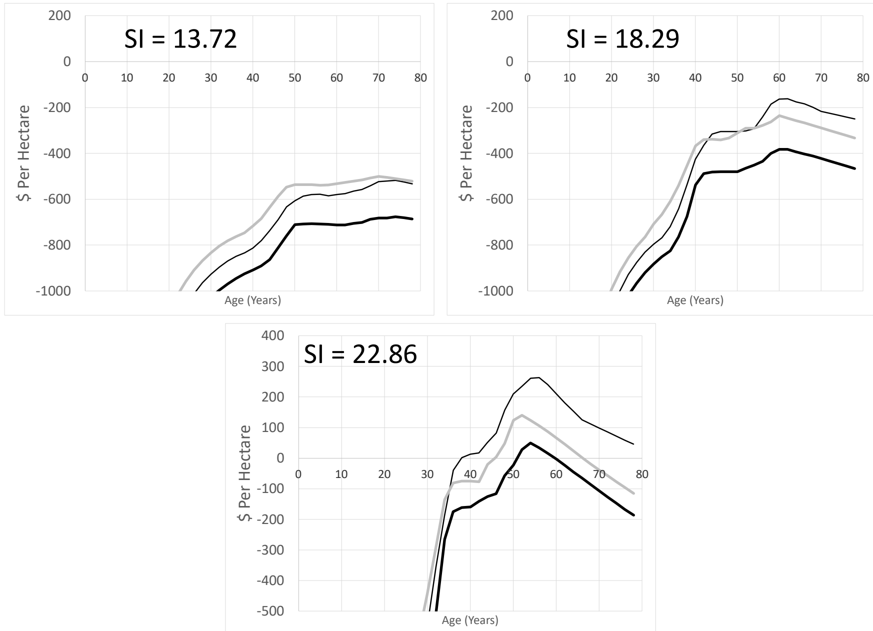

Fig. 2. Dollars per hectare (Soil Expectation Value) projections by planting density (988, 1,483, and 1,977 seedlings per hectare) and site index (SI – 13.72, 18.29, and 22.86 m – base age 25) using Current stumpage revenues of $3.60, $12.20, and $23.20 per ton for the pulpwood, chip–n–saw, and sawtimber product classes, respectively. The bold black lines are projections for 1,977 seedlings per hectare, the black lines are projections for 1,483 seedlings per hectare, and bold gray lines are projections for 988 seedlings per hectare**.**

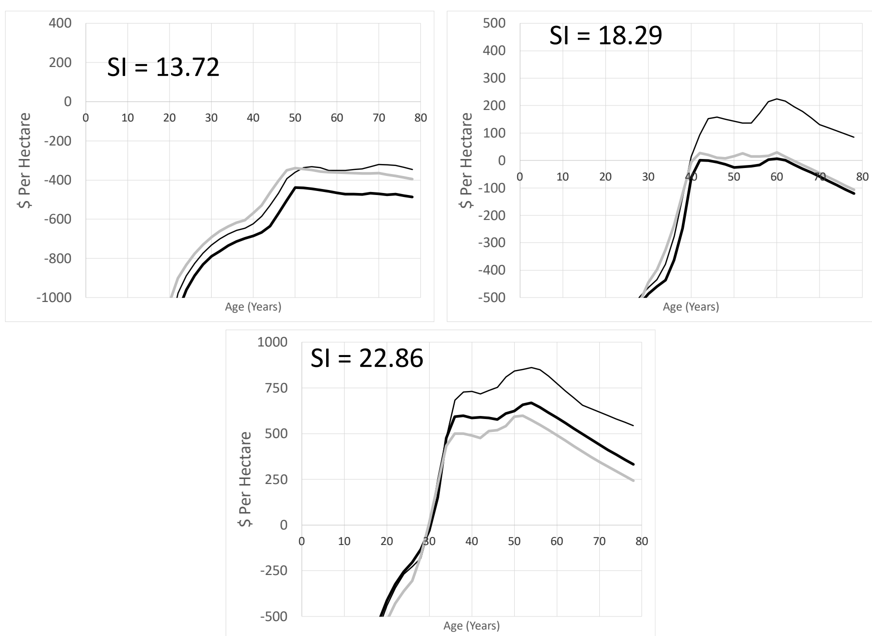

Fig. 3. Dollars per hectare (Soil Expectation Value) projections by planting density (988, 1,483, and 1,977 seedlings per hectare) and site index (SI – 13.72, 18.29, and 22.86 m – base age 25) using the Reasonable stumpage revenues of $7.50, $20, and $30 per ton for the pulpwood, chip–n–saw, and sawtimber product classes, respectively. The bold black lines are projections for 1,977 seedlings per hectare, the black lines are projections for 1,483 seedlings per hectare, and bold gray lines are projections for 988 seedlings per hectare.

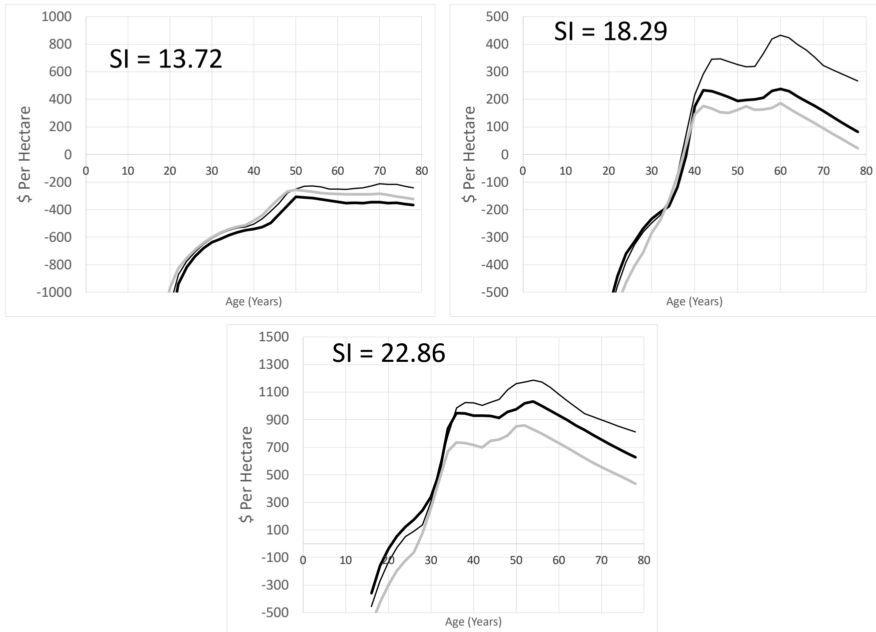

Fig. 4. Dollars per hectare (Soil Expectation Value) projections by planting density (988, 1,483, and 1,977 seedlings per hectare) and site index (SI – 13.72, 18.29, and 22.86 m – base age 25) using the Optimum stumpage revenues of $10, $22.50, and $35 per ton for the pulpwood, chip–n–saw, and sawtimber product classes, respectively. The bold black lines are projections for 1,977 seedlings per hectare, the black lines are projections for 1,483 seedlings per hectare, and bold gray lines are projections for 988 seedlings per hectare.

Given the current thinning regime (thinning at a basal area target of 27.55 square meters per hectare with a residual basal area per acre of 16.07 square meters), growth projections from FVS, and based on causal observation of Table 2 and Figs. 2 to 4, the optimal planting density is likely less than 1,483 seedlings per hectare but greater than 988 stems per hectare. Landowners may want to target around 1,236 to 1,359 seedlings per hectare. Clabo and Clatterbuck (2015) state in Tennessee usually around 1,236 to 1,977 seedlings per hectare are planted. For unthinned plantations, VanderSchaaf et al. (2018) recommended a similar range of planting density for medium quality sites but a lower planting density of 741 seedlings per hectare on higher quality sites. Pelkki (1997) found that the financially optimal planting density was 1,236 seedlings per hectare. He only examined planting densities ranging from 1,236 to 4,942 seedlings per hectare. A research study established in 2002 on four sites in southeastern Oklahoma used a planting density of 1,344 seedlings per hectare at all four sites while a recent shortleaf pine provenance trial in Virginia established in 2011 used a planting density of 1,537 seedlings per hectare (Creighton and Bitoki 2012). Similarly, a study in north-central Mississippi established in 2005 (Kushla 2009) used a planting density of 1,537 seedlings per hectare. Three study sites established in December of 2015 in East Texas used a planting density of around 1,495 seedlings per hectare (Hooker et al. 2020).

Future work should concentrate on examining a greater variety of thinning targets and residual stand densities. There are many thinning "triggers," including different basal area targets, as well as Reineke's Stand Density Index (Density Management Diagrams), Gingrich Guides (e.g. Rogers 1983), etc. Other studies have suggested greater target basal areas (Blanton) and residual stand densities for plantations (Phipps 1973, Blanton) relative to the particular thinning regime (27.55 trigger and 16.07 residual) used within this assessment. A more comprehensive study would increase our knowledge about optimum thinning targets and residual stand densities. It also would likely be advantageous to "calibrate" FVS based on observations from actual plantations. As mentioned in VanderSchaaf et al. (2018) and observed here, uncalibrated projections from FVS for shortleaf pine plantations appear to produce underpredictions of actual growth and yield.

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