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White (*Trifolium repens* L.) and Arrowleaf (*Trifolium vesiculosum* Savi) Clover Emergence in Varying Loblolly Pine (*Pinus taeda* L.) Tree Alley Spacings

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Abstract

Agroforestry systems have the potential to provide year-long income opportunities via the integrated forage or crop, timber, and livestock. Legumes are an attractive alternative option during the growing season when more traditional forages may not be as productive. The objective of this study was to test the establishment of arrowleaf and white clover grown under varying pine tree alley widths. In 2011, existing forage was removed in 15-yr old loblolly pine tree row alleys of different widths (3.7, 4.9, 7.3, and 9.8 m), including an open area. Arrowleaf, as an annual, was replanted in 2012. Seedlings were counted twice/year, while dry matter was measured three times/year. Photosynthetically active radiation (PAR) was measured in all alley widths to compare light penetration through the canopy. Hot and dry conditions occurred throughout 2012, affecting results. In 2012 and 2013, the greatest PAR for most treatments was observed in June. Seedling counts for all treatments were greatest immediately after establishment, and gradually declined throughout the course of the study. Dry matter yields increased throughout the growing season, and were greatest in arrowleaf clover in the open area on all measurement dates; however, increased weed pressure and repeated flooding affected yields. This study demonstrated that clover establishment in shady wooded areas is possible, but only under suitable environmental conditions.

Keywords

White Clover, Arrowleaf Clover, Dry Matter Yield, Seedling Count, Agroforestry

1. Introduction

Arkansas has a thriving timber industry [1] [2], with forests covering nearly 56% (77,130 km^2) of land area. Additionally, Arkansas has substantial livestock production, comprised of mainly poultry (*Gallus gallus domesticus* [L.] and *Meleagris gallopavo* [L.]) and cow (*Bos taurus*)-calf production, primarily located in Northwest Arkansas [3]. Unlike the Mississippi River alluvial floodplain region in eastern Arkansas where row crop agriculture dominates [4], landowners and producers in Northwest Arkansas get their farm income from various products based on diversified land use. Farms in Northwest Arkansas average 72 ha and cow herds average 69 AU or 0.96 AU ha^{-1} [5].

Given the absence of a single steady source of income in these small-scale operations, multi-purpose agro-silvopastoral systems may become more important in the future for economic and ecologic sustainability [6] [7] [8]. For example, pine (*Pinus* L. spp.) plantations are common in Arkansas and, with appropriate management, have the potential for animal grazing until tree harvest [2] [9]. Many of the cow-calf farms are planted with tall fescue (*Lolium arundinaceum* [Schreb.] S.J. Darbyshire) and bermudagrass (*Cynodon dactylon* [L.] Pers.), which are not always ideal for growing under trees due to shading (bermudagrass) or antiquality components (tall fescue) [6] [9] [10] [11]. In addition, nutritional values of these forages are less than optimal during certain times of the year [12] [13] [14]. Possible solutions include planting a legume such as white clover (*Trifolium repens* [L.]), which is high in nutritive value [15], but susceptible to heat and drought stress conditions typical in the mid-south [16].

It is speculated that pine tree plantations could integrate livestock and optimize forage production through legume intercropping [17], thereby improving the forage base for cow-calf operations while maintaining a timber production practice. Additionally, legumes may be less affected by drought and high temperatures when grown in a shaded environment, but effects on seedling emergence and dry matter (DM) production are not known in agroforestry systems [17]. The objective of this study was to test establishment success of an annual [arrowleaf clover (*Trifolium vesiculosum* Savi)], and a perennial legume [white clover] through DM production when grown under loblolly pine (*Pinus taeda* L.) trees with varying alley widths and resultant differences in light penetration through the tree canopy.

2. Materials and Methods

2.1. Site Description and Establishment

This study was conducted at the USDA-ARS Small Farms Research Center near

Booneville, AR (35.073˚N, 93.983˚W), in which 15-yr old east-west oriented loblolly pine tree alleys of varying width (3.7, 4.9, 7.3, and 9.8 m) were used in this experiment (**Figure 1**). Background on this stand and experimental area can be found by [6] and [7]. In fall 2011, alleys were manually cleared of woody vegetation then raked with a HR318 hay rake (Gehl Co., West Bend, WI) to remove pine needles and other litter from the alleys. Subsequently in fall 2011, each plot received the equivalent of 4483 kg lime, 90 kg P, and 90 kg·K·ha^{-1} as a 0-20-20 blend. Plots were immediately disked with a Rhino 50 (Athens Plow Co., Athens, TN) at a maximum depth of approximately 13 cm to avoid damage to pine tree roots, to control weeds, and to prepare a smooth seedbed. Control plots were prepared in a similar fashion and were represented by an open unshaded area. On October 14, 2011, three replications of legumes were planted at 22.4 kg·PLS·ha^{-1} for variety-not-stated (VNS) arrowleaf and 11.2 kg·PLS·ha^{-1} 'Ivory 2' white clover (DLF Seeds, Roskilde, Denmark), using a Brillion broadcast seeder (Brillion Co., Brillion, WI). Seeding rates were approximately doubled to ensure germination and to mimic seeding rates that are likely to be used under these circumstances. Before planting, arrowleaf clover was inoculated with a rhizobium product to facilitate nodulation (N-Dure rhizobium, Verdesian Co., Cary, NC; *Rhizobium leguminosarium biovar trifolii* vesiculosum) at a rate of 241 g per 22.7 kg seeds. White clover seed was delivered with a proprietary coating at approximately 32% of total bag weight. The Brillion seeder was equipped with packing rolls in front and back of the seed box so the soil was sufficiently compacted during the planting process. Arrowleaf clover, as an annual, was replanted October 17, 2012, while white clover, a perennial species, was not replanted except in the 4.9-m alleys, a result of sporadic flooding that killed both species. In February 2012, LightScout 3 light sensors (Spectrum, Inc., Aurora, IL) were installed in each plot for the purposes of measuring photosynthetically active radiation (PAR) throughout the entire growing season in 2012 and 2013. Only six measuring units were available, so one unit was placed in the center of one randomly chosen alley in one replication per treatment.

2.2. Measurements

Seedlings were counted 4 weeks after establishment (November 2011) and again in January 2012, November 2012, and January 2013 using a 0.75 × 0.75 m Vogel grid [18] placed randomly in each plot. Four frequency counts (100 cells total) were made in each legume plot. For the fall measurements, emerged arrowleaf seedlings were counted and for white clover, the number of remaining plants from initial establishment was recorded. Dry matter production was measured within 0.25-m^2 quadrats inside the exclusion cages, leaving a 3.8 cm residue height, with a gasoline-powered hedge trimmer. Plots were clipped approximately every 3 to 4 weeks from April to June in each plot. Samples were placed in paper bags and dried at 50˚C in a forced-air oven to measure DM yield.

2.3. Statistical Analysis

A two-factor analysis of variance was conducted using the PROC MIXED

Figure 1. Satellite imagery from 2012 displaying experimental design of study in Booneville, AR. Imagery copyright Google, Inc., as edited by the authors.

procedure of SAS (version 9.4, SAS Institute, Cary, NC) to determine treatment effects and interactions between alley widths (whole plot) and species (split plot). For all models, each sampling date, alley width, and species were considered fixed effects and replicates were random. Significance was judged at $P <$ 0.05. Data were analyzed separately by date regardless of a lack of significant year effect ($P = 0.67$) to account for the abnormal amount of precipitation and high temperatures in 2012.

3. Results and Discussion

3.1. Weather

In November 2011, Booneville, AR received nearly triple the normal 30-yr average rainfall following clover establishment (**Table 1**), and air temperatures were similarly 20% warmer than average. Except for March and August 2012, total precipitation (653 mm) was 66% less than the 30-yr average (1083 mm; **Table 1**; [19] [20]). Additionally, 2012 was warmer (18.3˚C) than the average in all months except November and was warmer overall by 2.7˚C (**Table 1**). In 2013, total precipitation was 8% greater than the 30-yr average, while the average temperature was 1.3˚C warmer than 30-yr average (15.6˚C; **Table 1**).

3.2. PAR

Photosynthetically active radiation, similar to temperature, followed an annual somewhat parabolic pattern for all treatments in both years of the study (**Figure 2**). In the open area (control), mean monthly PAR was greatest in May 2012

Table 1. Total precipitation and mean air temperatures for Booneville, AR for 2011-2013. Observed values were from SRCC [20] and normals were from NOAA [19].

Month	2011	2012	2013	Normal	2011	2012	2013	Normal
Jan	16.0	22.6	124.2	92.5	2.4	7.2	4.3	3.4
Feb	93.5	89.4	98.0	84.6	6.7	8.3	6.0	6.1
Mar	36.8	226.1	116.1	109.5	11.8	16.3	9.1	10.6
Apr	348.5	42.2	114.0	110.0	17.7	18.7	15.3	15.5
May	200.2	34.5	135.6	145.0	20.4	23.5	20.0	20.2
Jun	18.5	60.2	159.8	105.9	28.3	27.4	25.4	24.3
Jul	35.1	64.0	96.5	86.9	31.2	30.3	26.4	26.9
Aug	133.4	80.0	64.0	72.6	29.9	28.0	26.8	26.6
Sep	68.1	178.6	68.6	104.1	21.2	24.3	24.7	22.3
Oct	104.4	66.0	136.9	120.7	16.7	15.9	16.6	16.5
Nov	344.2	25.9	85.9	122.7	12.5	11.0	10.1	10.1
Dec	142.0	69.6	164.1	110.2	6.9	7.2	4.2	4.3
Total/Mean	1540.5	959.1	1363.7	1264.7	17.3	18.3	16.9	15.6

(1818 μmol·m^{-2}·s^{-1}) and September 2013 (1763 μmol·m^{-2}·s^{-1}). PAR was overall greater in the 9.8-m alley than the 7.3-m alley, which both peaked in June 2012 and June 2013. Similarly, June had the greatest PAR for the 3.7-m alley in 2013, while July had the greatest PAR in 2012 (**Figure 2**). The 4.9-m alley had greater annual PAR than the 3.7-m alley, but less than the 7.3-m alley. Overall, July (512.4 μmol·m^{-2}·s^{-1}) had the greatest mean PAR in 2012, while May (467.4 μmol·m^{-2}·s^{-1}) had the greatest mean PAR in 2013 for the 4.9-m alley (**Figure 2**).

3.3. Seedling Count

Seedling count and DM yield differed ($P < 0.05$) between clover species and alley widths on at least two measuring dates (**Table 2**; **Figure 3** and **Figure 4**). Arrowleaf and white clover differ in shade stress adaptability [21]. In November 2011, seedling count was greatest in the 9.8-m alley for white clover (**Figure 3**), which did not differ ($P > 0.05$) from the counts in the 3.7- and 7.3-m alleys or the open area for white clover. The lowest seedling count in November 2011 (0.54 seedlings·m^{-2}) was observed in the 4.9-m alley for arrowleaf clover, which did not differ from the 7.3- and 9.8-m alleys for arrowleaf clover (0.99 and 0.96 seedlings·m^{-2}, respectively). The relatively high counts for both species in the 3.7-m alley (2.75 and 1.22 seedlings·m^{-2}, respectively) was counterintuitive based on PAR levels, but was likely due to less weed competition or greater moisture retention compared with other treatments [6] [22] [23] [24]. In January 2012, the greatest ($P < 0.05$) seedling count for all treatments (2.02 seedlings·m^{-2}) was measured in the 7.3-m width alley for white clover (**Figure 3**). No seedlings were

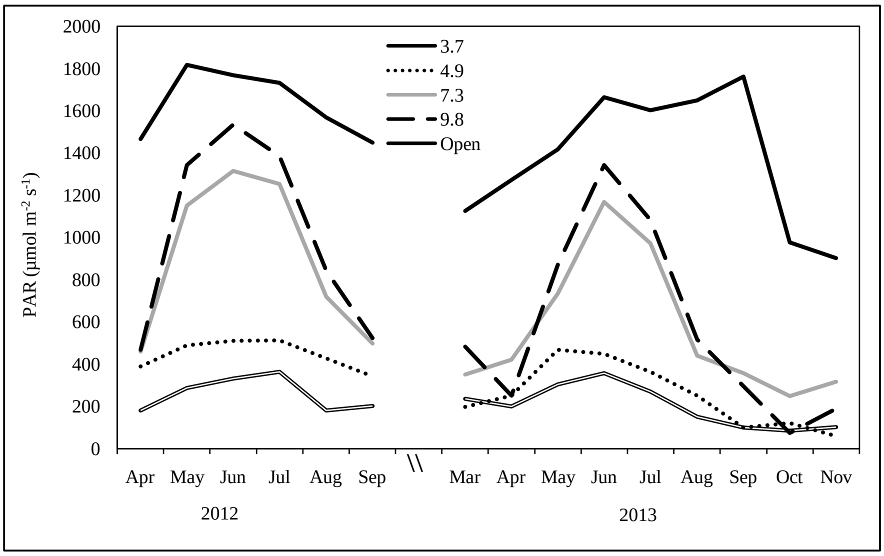

Figure 2. Mean monthly photosynthetically active radiation (PAR) as measured in each alley treatment (3.7, 4.9, 7.3, 9.8 m between pine [*Pinus* spp.] tree rows plus open unshaded area) at the USDA-ARS Dale Bumpers Small Farms Research Center, Booneville, AR.

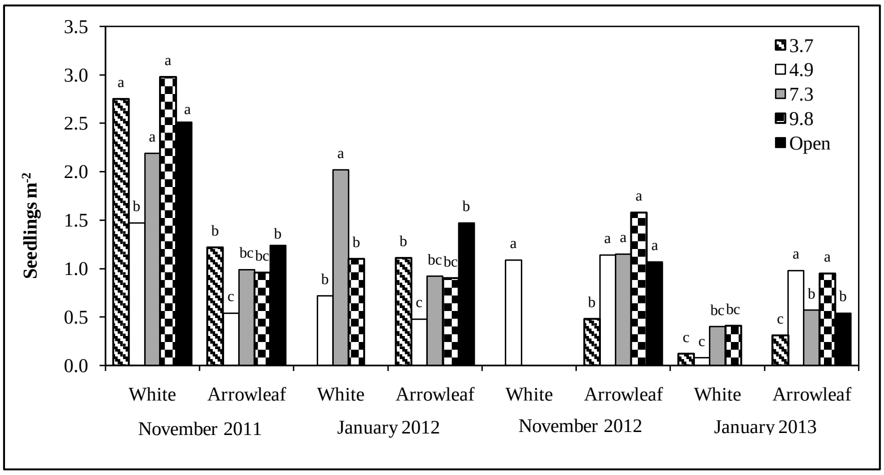

Figure 3. Seedling counts for arrowleaf (*Trifolium vesiculosum* Savi) and white (*Trifolium repens* L.) clovers throughout the study for each alley treatment (3.7, 4.9, 7.3, 9.8 m between pine [*Pinus* spp.] tree rows plus open unshaded area) at the USDA-ARS Dale Bumpers Small Farms Research Center, Booneville, AR. Common letters within a date indicate no differences between meansat $\alpha = 0.05$. The absence of a bar for a date indicates missing data, due to flood and drought.

counted in the 3.7-m alley or open area for white clover due to the extreme precipitation that occurred in November and December 2011 (Table 1). Seedling counts were greatest (1.47 seedlings·m^{-2}) in the open area for arrowleaf clover, which did not differ ($P > 0.05$) from the 3.7-, 7.3-, and 9.8-m alleys (arrowleaf) and 4.9- and 9.8-m alleys (white clover). The lowest seedling counts occurred in the 4.9-m alley for arrowleaf clover. Seedling count was greatest (1.58 seedlings·m^{-2}) in November 2012 in the 9.8-m alley for arrowleaf clover, which did not

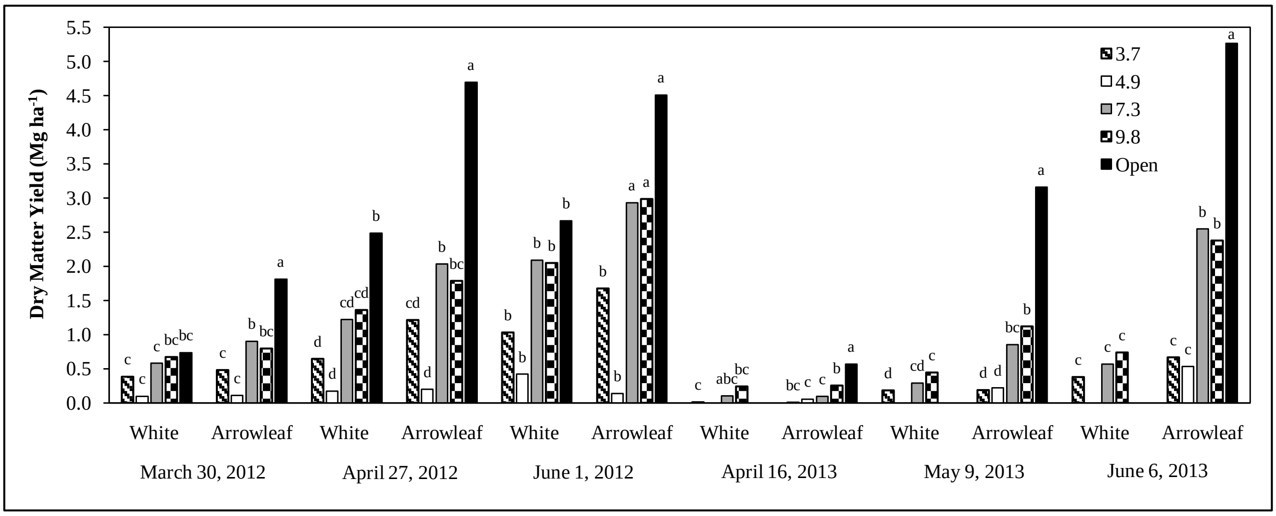

Figure 4. Herbage dry matter yield for arrowleaf (*Trifolium vesiculosum* Savi) and white (*Trifolium repens* L.) clovers for each alley treatment (3.7, 4.9, 7.3, 9.8 m between pine [*Pinus* spp.] tree rows plus open unshaded area) at the USDA-ARS Dale Bumpers Small Farms Research Center, Booneville, AR. Common letters within a date indicate no differences between meansat $\alpha = 0.05$. The absence of a bar for a date indicates missing data, due to flood and drought.

Table 2. Analysis of variance summary of the effects of alley width (3.7, 4.9, 7.3, 9.8 m between pine [*Pinus* spp.] tree rows plus open area) on arrowleaf (*Trifolium vesiculosum* Savi) and white (*Trifolium repens* L.) clover production measured at the USDA-ARS Dale Bumpers Small Farms Research Center, Booneville, AR. *, **, ***, NS, and -- represent $P < 0.05$, 0.01, <0.01, not significant, and missing data, respectively.

Factor	Seedling counts				Dry matter yield					
	Nov 2011	Jan 2012	Nov 2012	Jan 2013	Mar 2012	Apr 2012	Jun 2012	Apr 2013	May 2013	Jun 2013
Width(W)	***	***	***	***	***	***	***	***	***	***
Species (S)	***	***	NS	***	***	***	***	NS	NS	***
W × S	NS	*	--	***	***	***	NS	NS	NS	NS

differ ($P > 0.05$) from the 4.9- and 7.3-m alleys and open area for arrowleaf clover and the 4.9-m alley for white clover. The lowest counts observed in November 2012 (0.48 seedlings·m^{-2}) occurred in the 3.7-m alley of arrowleaf clover. There were 0 seedlings for other treatments of white clover due to severe drought throughout 2012 (Table 1; Figure 3). In January 2013, the greatest ($P < 0.05$) numbers of seedlings for all treatments were counted in the 4.9- and 9.8-m alleys for arrowleaf clover (0.98 and 0.95 seedlings·m^{-2}), respectively). The lowest number of seedlings was counted in the 3.7- and 4.9-m alleys for white clover, which did not differ ($P > 0.05$) from the 7.3- and 9.8-m alleys for white clover and the 3.7-m alley for arrowleaf clover. Because there was no evidence of white clover growth the previous fall, any white seedlings counted possibly emerged from seeds that were shed in 2012 or as growth from dormant stolons. As a perennial, white clover flowers and sets seed continuously during the growing-season [16]. No white clover seedlings were observed for the open area, as the drought likely had a more severe effect than in the shaded alleys where soil conditions remained more favorable for growth.

3.4. Dry Matter Yield

Dry matter production varied among treatments and time of sampling (**Figure 4**). Overall, yield was greatest ($P < 0.05$) in the open area for arrowleaf clover compared to all other treatments for all dates in the study (**Figure 4**). Dry matter yield was greatest in June for most treatments in both years (**Figure 4**). In March 2012, the second-greatest DM yield (0.90 $\text{Mg}\cdot\text{ha}^{-1}$) was measured in the 7.3-m alley for arrowleaf clover, which did not differ ($P > 0.05$) from DM yield in the 9.8-m alleys for arrowleaf and white clover and the open area for white clover. In April 2012, DM yield (2.48 $\text{Mg}\cdot\text{ha}^{-1}$) was second-greatest in the open area for white clover, which did not differ ($P > 0.05$) from the 7.3- and 9.8-m alleys for arrowleaf clover. Although DM yield for the arrowleaf open area (4.51 $\text{Mg}\cdot\text{ha}^{-1}$) was numerically greatest in June 2012, it did not differ ($P > 0.05$) from DM yield in the 7.3- and 9.8-m alleys for arrowleaf clover, which were greater ($P < 0.05$) than all other treatment combinations (**Figure 4**). Throughout all of 2012, the 4.9-m alleys for both clover species yielded the smallest DM, probably due to repeated flooding. Tree-forage interactions influence soil temperature and moisture, leading to differences in susceptibility to environmental extremes such as drought or flooding [21] [25]. Because arrowleaf clover concludes its annual life cycle by the end of June, further sampling did not occur. In 2013, DM yield decreased dramatically, and for the 4.9-m alley and open area for white clover, no growth was observed for the year, most likely due to the drought of 2012. Additionally as a result of the drought, white clover plots became increasingly infested with weeds (data not shown), which also influenced the persistence of this perennial clover. This differs from previous research [26] that confirmed cool-season legumes such as clover are good choices for use as living mulches in controlling weed pressure. By June 2013 (**Figure 4**), the 7.3- and 9.8-m alleys for arrowleaf clover had achieved significant growth, nearly equal to 2012, but yield was still less ($P < 0.05$) than in the open area; however, yields for all other treatments were lesser ($P < 0.05$). As expected, DM production was greater in the open area compared with the alleys, and differences were more pronounced later in the growing-season. This is in contrast to other shade-tolerant species such as *Amphicarpaea bracteata* L. and *Lespedeza virginica* L., which also have high yield and forage quality [21].

4. Conclusion

Based on the results of this study, arrowleaf and white clover can be established in shaded wooded areas under adequate environmental (*i.e.* soil moisture and temperature), but not overly wet conditions. Forage productivity in temperate North America is dictated by not only canopy coverage and climate, but also tree and forage species [27]; thus it is possible arrowleaf clover is better suited to grow among loblolly pine than white clover. However, yields of clover grown in loblolly pine plantation spacings less than 7.3-m are not comparable to clover grown in open areas. Although arrowleaf clover is competitive, it is an annual and must be reestablished every year if plants are not allowed to flower and set seeds. Furthermore, this study showed that white clover production and yield were greatly reduced due to weed pressure during the 2-yr study. Further studies should be conducted to investigate optimal planting dates, other shade-tolerant species, and forage quality that can lead to economic and ecologic resiliency on small-scale operations.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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