Optimizing Temperature Requirements for Clover Seed Germination

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Core Ideas

• Temperature significantly affects the time and rate of clover (Trifolium spp.) germination.
• Germination and vigor were evaluated from -1.0 to 48.0 °C at approximately 1.0 °C increments.
• Tested cultivars maintained 80% + germination from 4.9 to 28.2 °C, on average.
• Temperature range for germination was much wider than reported in previous literature.
• Data will be useful in updating planting recommendations for producers in the US Southeast.

ABSTRACT

There are many reports of failed clover (Trifolium spp.) establishments each year. Temperature is one of the primary factors affecting germination and significantly affects the time and rate of clover seed germination. The objectives were to determine (i) the temperature and time required to reach maximum germination and (ii) range in which germination will occur for six coated clover cultivars adapted to the US Southeast. Seed germination and vigor were evaluated in Petri-dishes incubated over thermal gradients ranging from -1.0 to 48.0 °C at approximately 1.0 °C increments where germination was counted every 24 h for 7 consecutive days. Base temperatures were calculated for each cultivar. Three parameter logistic growth models were used to find the minimum and maximum temperatures at which these cultivars could maintain 80% germination. Lorentzian distribution models were used to establish the temperature and time to maximum germination. These cultivars maintained at least 80% germination from 4.9 to 28.2 °C on average, although some germination was observed outside these extremes. The range at which these clovers could germinate was much wider than previously reported. Excluding ‘Barduro’, maximum germination occurred at 34.2 to 52.8 h after planting at optimum temperatures (10.9–17.2 °C). Barduro germinated so rapidly in the warmer temperature range that the full data set could not be used, as it did not conform to the same regression. Future trials will begin measuring germination at 12 h to better capture early trends in germination. These data will be useful in updating planting recommendations for producers in the US Southeast.

Abbreviations: GDD, growing degree day.

Clovers (Trifolium spp.) are an important component for improving the nutritive value of forages and the seasonal distribution of forage quantity in forage–livestock grazing systems in the southeastern United States. Trifolium is one of the two most important legume genera for forage–livestock systems because of the diversity of available species, wide adaptation to different soils and climates, and general ability to reseed (Van Keuren and Hoveland, 1985). Annual, cool-season clover species are used more readily than perennials because of their persistence and ability to complement warm-season perennial grass pastures (Evers, 2011). Most often, annual clovers are interseeded with cool-season annual grasses into dormant warm-season grass stands in the fall to provide winter grazing for livestock. Clovers biologically fix atmospheric N, have an inherently greater nutritive value than warm-season grasses, and help the sward outcompete undesirable winter weed species (Hoveland and Evers, 1995).

Unfortunately, there are numerous reports of failed cool-season clover stands each year because of poor establishment (Butler et al., 2014). In the Southeast, the performance of an annual crop is highly dependent on planting date (Van Keuren and Hoveland, 1985). Many producers fail to plant a winter annual crop until late fall or early winter, when planting conditions are less than ideal (i.e., dry soils, cooler temperatures). Temperature, light, moisture, and oxygen are all important factors in seed germination (Garcia-Huidobro et al., 1982). According to Kendall and Stringer (1985), temperature significantly affects the time and rate of clover seed germination, which ultimately impacts the compatibility of clovers to grow with other crops, ability to outcompete weed species, or quickly take advantage of ideal planting conditions. Every plant species has an optimum germination temperature and range within which germination will occur.

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Previous research indicates that cool-season legumes have an optimum germination temperature of approximately 20°C (i.e., Brar et al., 1991; Butler et al., 2014). Monks et al. (2009) reported that the germination of annual *Trifolium* species declined when temperatures exceed 20°C whereas perennial species did not decline until temperatures reached 35°C. In fact, most annual clovers maintained 70% germination at 25°C (Lonati et al., 2009). Subterranean (*T. subterraneum* L.), arrowleaf (*T. vesiculosum Savi*), and rose (*T. hirtum*) clovers were most sensitive to temperatures above 25°C (Brar et al., 1991). Kendall and Stringer (1985) reported that germination of arrowleaf, cup (*T. cheiridium* L.), low hop (*T. procumbens* L.), and subterranean clovers were most inhibited at 35°C (1985). Conversely, crimson (*T. incarnatum*) and Persian clovers (*T. resupinatum* L.) were able to maintain optimum germination at 35°C (Kendall and Stringer, 1985). At 5°C, germination was inhibited in buffalograss (*T. glomeratum* L.), crimson, and cluster clovers (*T. glomeratum* L.), but least inhibited in cup, Persian, and subterranean clovers (Hoveland and Elkins, 1965; Young et al., 1970). Similarly, Lonati et al. (2009) reported that little hop and subterranean clovers annual clovers maintained 100% germination at 35°C.

When clovers were grown in optimum temperatures (5–25°C), annual clovers required less time for radicles to exceed the diameter of the seed compared with perennial white clover (*T. repens*; Lonati et al., 2009). Butler et al. (2014) discovered that germination rate of annual clovers increased with increasing temperatures up to 25°C, then decreased. Interestingly, germination time also decreased with decreasing seed size (Moot et al., 2000). Hampton et al. (1987) did not report a difference in germination time for white or red clover (*T. pratense* L.).

Optimum temperature ranges were not conclusive after most of these reported experiments (i.e., Brar et al., 1991; Kendall and Stringer, 1985; Lonati et al., 2009). The tested cultivars did not reach 0% germination at temperature extremes in their studies. For these data, research only evaluated clover seed germination at temperatures in 5°C increments, disallowing accurate isolation of the optimum temperature range for clovers to germinate. Many investigators did not begin to measure germination until 48 to 72 h after planting and reported nearly complete (often exceeding 75%) germination at that first time point. It is difficult to determine the accuracy of thermal time required for germination without measurements from earlier time points in the “optimum” temperature range (near 20°C). Furthermore, most of this previous research has been performed on species or cultivars that are not adapted to the Southeast. Isolating the minimum, maximum, and optimum temperatures at which clover species can germinate would allow researchers to fine-tune planting recommendations to ensure successful stand establishment. The objectives of this experiment were to determine (i) the temperature and time required to reach maximum germination and (ii) the range in which germination will occur for six clover cultivars adapted to the Southeast.

**MATERIALS AND METHODS**

**Clove Species**

This experiment was conducted with coated seed for ‘Dixie’, ‘AU Robin’, and ‘AU Sunrise’ crimson clover, ‘FIXatioN’ halansa clover (*T. michelianum* Savi), ‘Barduro’ red clover, and ‘Neches’ white clover. AU Sunrise, AU Robin, and Dixie are all adapted to the Southeast but differ in their maturity. AU Sunrise will mature 5 to 18 d before AU Robin, which matures earlier than Dixie (Young-Mathews, 2013). FIXatioN is marketed as the most cold-tolerant annual clover (Grassland Oregon, 2018). Neches is a perennial developed to survive the harsh conditions in the South, whereas Barduro is a biennial red clover that is extremely heat and drought tolerant (Barenbrug USA, 2018a, 2018b).

**Thermal Gradient Table**

Two thermal gradient tables were used to determine the temperature and time for optimum clover germination using equipment and methodology similar to the methods of Grey et al. (2016). Tables were constructed from solid aluminum blocks that measured 2.4 m long × 0.9 m wide × 7.6 cm thick and weighed 470 kg. Beneath the table, 1.0-cm holes were drilled on each end of the aluminum blocks so fluid could be pumped into the table. A water bath unit was set at each end of the tables. When germination was tested from 7.0 to 48.0°C, the chilling unit was set at 5.0°C and the heating unit was set to 36.0°C. Each unit contained an independent, 1:10 mixture of ethylene glycol and water, pumped at 3.8 L per min to generate the thermal gradient. This allowed temperatures to increase in 1.0°C increments every 10 cm along the length of the table for a total of 24 columns. Constant temperatures were maintained in each 10-cm column across nine rows along the width of the table. This resulted in 216 total cells used to evaluate germination in the warmer temperature range. When germination experiments were repeated to test vigor from −1.0 to 12.0°C, the chilling unit was set to −19.0°C and the heating unit was set to 1.0°C. To prevent lines from freezing, a 3:1 mixture of ethylene glycol to water was used in the chilling and heating units. The thermal gradient was less consistent in this cooler temperature range, so temperatures increased 0.5 to 2°C every 10 cm. Only 17 temperature columns were used in this colder evaluation, so only 153 total cells were used.

Duplex, insulated PR-T-24 wire (Omega Engineering, Stamford, CT) was used to construct thermocouples to be mounted underneath the table from the hot to cold ends. The wire was inserted vertically into an 8 mm wide by 7-cm deep hole on the underside of the table, allowing the thermocouple to be approximately 5 mm from the table’s surface, at 10-cm intervals, along the length of the table. The temperatures were continuously monitored for each thermocouple and recorded at 30-min intervals with a Graphite data logger (MicroDAQ.com Ltd., Contoocook, NH). All temperature data was downloaded weekly into a spreadsheet format to a flash drive. The data indicated a continuous temperature gradient ranging from −1.0 to 12°C and 7.0 to 48.0°C along the length of the table, for the respective germination evaluations under cold and warm temperature ranges.

Germination paper (SDB 86 mm, Anchor Paper Co., St. Paul, MN) was used to line sterile plastic 100 × 15 mm Petri dishes (Fisher Scientific Education, Hanover Park, IL) for each clover cultivar. Twenty-five seeds for each cultivar were evenly distributed on the paper before 10 mL of distilled water was added. One Petri dish was added to each cell on the table, so cultivars were placed along the thermal gradient. This resulted in 17 dishes per replication in the colder temperature evaluations and 24 dishes per replication for warmer temperature evaluations. Three replications were conducted for each cultivar, resulting in 3075 seeds per cultivar.

**Data Collection**

Seeds were considered germinated when the radicle extended more than 5 mm beyond the seed. Beginning 24 h after initiation, germinated seeds were counted then removed every 24 h for 7 d.
Counts were always made from the cold to warm end of the tables and were conducted in less than 1 h each day. Percent germination was calculated daily and cumulative germination was measured for each Petri dish over the 7-d trial period. Data loggers recorded the temperature of each cell for the duration of the experiment. This data included minimum and maximum (±0.5°C) temperatures for each thermocouple measured over each respective 7-d germination period.

Mathematical Calculations and Statistical Analyses

An analysis of variance was applied to each data set combined over experimental units and experimental replication in time. Average temperature was the arithmetic mean of all temperatures for a cell over the duration of each test. Minimum and maximum temperatures were the lowest and highest temperature measured in that cell during the 7-d test.

Base Temperatures

The base temperature for each clover variety was calculated in Excel (Microsoft Corporation, Redmond, WA) using the equation (Angus et al., 1980):

\[ T_b = -\frac{B_o}{B_1} \]  

where \( T_b \) is base temperature, \( B_o \) is the intercept, and \( B_1 \) is the slope of the linear relationship between the average temperature and the germination rate (inverse of germination percentage). Only data from temperature cells that remained below 10°C on average were used in this evaluation to avoid overestimating the intercept when analyzed in SAS 9.4 (SAS Inc., Cary, NC). The base temperatures used for the growing degree day (GDD) calculations made in Excel are reported in Table 1.

Minimum and Maximum Temperatures for Seed Germination

A three-parameter logistic growth function was used with nonlinear regression to model the minimum and maximum temperatures needed to achieve 80% germination (Eq. [2]) SigmaPlot (SigmaPlot 13.0. SPSS Inc., Chicago, IL). The algebraic form of the function is:

\[ Y = \frac{\beta_0}{1 + \left(\frac{1 - P}{P}\right)^{\beta_1 + \beta_2(x - XP)}} \]  

where \( \beta_0 \) is the upper asymptote (maximum germination), \( \beta_1 \) is the overall slope estimate, and \( XP \) is the estimate of \( X \) (temperature) when \( Y \) (germination) is at \( P \)% of the maximum germination, which was set to 80% in this analysis because that was the acceptable minimum germination in these ideal conditions. The minimum and maximum temperatures were determined independently because of the differing slope directions on each temperature extreme. A separate curve was fit for each cultivar, then 95% confidence limits of the parameters were used to determine statistical differences in the three model parameters (\( \beta_0, \beta_1, \) and \( XP \)).

Growing Degree Days

Minimum and maximum temperature data were used to determine thermal time or GDD accumulation. These data are readily available to growers through online weather station networks (i.e., www.georgiaweathernetwork.com). The calculation for GDD accumulation is:

\[ t_s = \sum_{i=1}^{n} \left( \frac{T_{i,\text{max}} + T_{i,\text{min}}}{2} - T_b \right) \]  

where \( t_s \) is the sum of degree days for \( n \) days, \( T_{i,\text{max}} \) and \( T_{i,\text{min}} \) are the daily maximum and minimum temperature of day \( i \), and \( T_b \) was the appropriate base temperature for each cultivar (McMaster and Wilhelm, 1997).

Maximum Seed Germination

A three-dimensional nonlinear regression was conducted in SigmaPlot to establish the relationship between time, temperature, and germination. The nonlinear relationship between time and temperature were regressed on seed germination to determine if there were differences in cultivars. Lorentizian distribution models:

\[ z = \frac{a}{\left(1 + \left(\frac{x - x_0}{b}\right)^2\right)^\frac{1}{c} - \left(1 + \left(\frac{y - y_0}{c}\right)^2\right)^\frac{1}{c}} \]  

were fitted to the germination data of each cultivar. Temperature \( x \) and time \( y \) data were used to develop the parameters \( (a, b, \text{ and } c) \) to predict the temperature \( x \) and time \( y \) that produced the maximum germination \( z \) for each cultivar. According to Motulsky and Christopoulos (2003), the Lorentizian distribution has a wider and more comprehensive analysis if there are outlying data points than other models in the category of r-distribution.

RESULTS AND DISCUSSION

Base Temperatures

For the non-annual clover cultivars, Neches had the greater \( T_b \) at 1.34°C, with Barduro at 0.62°C (Table 1). These species required greater \( T_b \) than the annuals. The \( T_b \) required for annual clovers was –0.58 to 0.42°C, whereas AU Robin required the lowest \( T_b \) of all species evaluated in this study.

These \( T_b \) follow those previously reported in literature. Moot et al. (2000) reported average \( T_b \) of 0.7°C for perennial (i.e., red and white) clovers and 0°C for annual clovers (i.e., sub). Likewise, the \( T_b \) for balansa clover was 0°C (Nori et al., 2014) and near 2°C for white clover (Black et al., 2006; Lonati et al., 2009). It was hypothesized that \( T_b \) was related to seed size such that lower temperatures were required for smaller seeded species (Moot et al., 2000). There was

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Table 1. Base temperatures \( (T_b) \) calculated for growing degree day calculations for coated clover (Trifolium spp.) cultivars.

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth cycle</th>
<th>Cultivar</th>
<th>Seed no. by mass</th>
<th>( T_b )†‡ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balansa</td>
<td>Annual</td>
<td>FixatioN</td>
<td>424</td>
<td>0.08</td>
</tr>
<tr>
<td>Crimson</td>
<td>Annual</td>
<td>Dixie</td>
<td>88</td>
<td>0.42</td>
</tr>
<tr>
<td>Crimson</td>
<td>Annual</td>
<td>AU Robin</td>
<td>167</td>
<td>–0.58</td>
</tr>
<tr>
<td>Crimson</td>
<td>Annual</td>
<td>AU Sunrise</td>
<td>89</td>
<td>–0.42</td>
</tr>
<tr>
<td>Red</td>
<td>Perennial</td>
<td>Barduro</td>
<td>216</td>
<td>0.62</td>
</tr>
<tr>
<td>White</td>
<td>Perennial</td>
<td>Neches</td>
<td>673</td>
<td>1.34</td>
</tr>
</tbody>
</table>

† Base temperatures were calculated using the equation: \( T_b = -B_o/B_1 \) where \( B_o \) is the intercept and \( B_1 \) is the slope of the linear relationship between the average temperature and the germination rate (inverse of germination percentage).
‡ \( n = 2025. \)
no apparent relation between these factors in this study as Neches (smallest seed) required the greatest $T_p$ whereas Dixie (largest seed) fell intermediate to other species and cultivars (Table 1).

**Minimum Temperatures for Seed Germination**

The three parameter estimates from the logistic growth curves were used to evaluate the minimum temperature for seed germination between $-1.0$ to $12.0^\circ$C (Table 2, Fig. 1). From the model, estimated maximum germination ($\beta_0$) was greatest for the crimson and Barduro clover (>90% germination), lowest for Neches (81%), whereas FIXatioN was not different from any species. The slope ($\beta_1$) was steepest for AU Sunrise (2.5), but only differed statistically from Dixie (0.9). Although the statistical differences were not well defined, the temperature at which the germination fell to 80% of the maximum germination ($XP$) was greatest for Neches ($6.7^\circ$C) and lowest for AU Sunrise ($3.4^\circ$C). FIXatioN, a clover marketed for germinating in cold temperatures, did reach 80% germination at a lower temperature than Dixie or Neches, but not the other three clovers.

The asymptote, temperature at which germination fell below 80°C, is not clearly reported in literature, as experiments did not evaluate germination below 0°C or at a resolution that allowed an accurate calculation of this point. Furthermore, the cultivars used in this evaluation possess greater germination at lower temperatures than reported in literature. Hoveland and Elkins (1965) reported 20% germination from crimson clover grown at $4.5^\circ$C for 12 d. Literature also reported that balansa clover fell to 45% germination at $5^\circ$C (Nori et al., 2014) and red clover achieved only 70% germination at $10^\circ$C (Brar et al., 1991). Conversely, when Lonati et al. (2009) tested germination for annual clover species at $46^\circ$C, the germination was near or equal to 100%. Still, germination of white clover under the same conditions was only 67.9%. Those studies all used different cultivars than those used in this current evaluation. Seed of cultivars originating in northern locations are generally more tolerant of low temperatures than cultivars selected under natural selection in southern locations (Hoveland and Elkins, 1965). However, that was not seen in the present study. This may be because these newer cultivars are more tolerant of temperature extremes or the thermal gradient table simply provided a more accurate environment to locate this asymptote without confounding effects such as water availability.

**Maximum Temperatures for Seed Germination**

Similarly, the three parameter estimates from the logistic growth curves were used to evaluate the maximum temperature for seed germination in the warmer temperatures ($7.0$–$48.0^\circ$C) (Table 2, Fig. 2). There was no difference in maximum germination ($\beta_0$) for the clovers. The slope ($\beta_1$) was greater in the red and white clover than the annual clovers. Although it was not different from Neches (−0.4), Barduro has the steepest slope (−0.5). The slope was less pronounced in AU Robin (−0.2), but it was not different from the other four annual clovers. The temperature at which the germination fell to 80% of the maximum germination ($XP$) was greatest in Barduro ($37.4^\circ$C), a surprising 8 to 14°C greater than the $XP$ for the other clovers. FIXatioN ($29.4^\circ$C) followed Barduro, but it was not different from AU Robin and Dixie.

There is less information on maximum temperatures than minimum temperatures for clover seed germination in the literature. ‘Arlington’ red clover still maintained 70% germination at $30^\circ$C (Brar et al., 1991). Kendall and Stringer (1985) reported that germination was reduced most in subterranean, hop, and arrowleaf clovers at $35^\circ$C and least in Persian and crimson clovers. More specifically, the maximum temperature for Persian clover was calculated to be $45^\circ$C, while other clovers (i.e., arrowleaf) was only $34^\circ$C (Nori et al., 2014). Similar species germinated at maximum temperatures of $25.4$ to $27.0^\circ$C in a study conducted by Lonati et al. (2009). Monks et al. (2009) found that annual clovers declined after peaking at $20^\circ$C whereas perennial clovers did not decline until $35^\circ$C. Likewise, Young et al. (1970) reported that clover germination was only reduced at $30^\circ$C.

**Table 2. Seed germination and logistic growth parameter estimates for coated clover (Trifolium spp.) cultivars using a thermal gradient assay.**

<table>
<thead>
<tr>
<th>Temp. range</th>
<th>Species</th>
<th>Cultivar</th>
<th>$CL_{95%}$ $^a$</th>
<th>$\beta_0$</th>
<th>$CL_{95%}$</th>
<th>$\beta_1$</th>
<th>$CL_{95%}$</th>
<th>$XP$ $^b$</th>
<th>$CL_{95%}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1.0 to 12.0</td>
<td>Balansa</td>
<td>FIXatioN</td>
<td>42.6 (1.5) b</td>
<td>88.2 (2.3) ab</td>
<td>1.4 (0.4) ab</td>
<td>4.5 (0.2) cd</td>
<td>7.0 to 48.0</td>
<td>Crimson</td>
<td>AU Robin</td>
</tr>
<tr>
<td></td>
<td>Crimson</td>
<td>AU Robin</td>
<td>48.1 (1.5) a</td>
<td>94.8 (1.1) a</td>
<td>1.4 (0.2) ab</td>
<td>3.9 (0.2) de</td>
<td></td>
<td>Crimson</td>
<td>AU Sunrise</td>
</tr>
<tr>
<td></td>
<td>Crimson</td>
<td>Dixie</td>
<td>41.0 (1.5) b</td>
<td>94.5 (1.8) a</td>
<td>0.9 (0.1) b</td>
<td>5.8 (0.3) ab</td>
<td></td>
<td>Red</td>
<td>Barduro</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>Neches</td>
<td>28.6 (1.5) c</td>
<td>80.9 (1.9) b</td>
<td>1.6 (0.3) ab</td>
<td>6.7 (0.2) a</td>
<td></td>
<td>White</td>
<td>Neches</td>
</tr>
<tr>
<td>7.0 to 48.0</td>
<td>Balansa</td>
<td>FIXatioN</td>
<td>46.6 (1.1) c</td>
<td>96.3 (1.6) ns</td>
<td>−0.3 (0.0) bc</td>
<td>29.4 (0.6) b</td>
<td></td>
<td>Crimson</td>
<td>AU Robin</td>
</tr>
<tr>
<td></td>
<td>Crimson</td>
<td>AU Robin</td>
<td>55.6 (1.1) a</td>
<td>98.2 (6.0) ns</td>
<td>−0.2 (0.1) c</td>
<td>26.1 (2.2) bc</td>
<td></td>
<td>Crimson</td>
<td>AU Sunrise</td>
</tr>
<tr>
<td></td>
<td>Crimson</td>
<td>Dixie</td>
<td>47.4 (1.1) c</td>
<td>99.6 (7.0) ns</td>
<td>−0.3 (0.1) bc</td>
<td>23.1 (2.0) c</td>
<td></td>
<td>Red</td>
<td>Barduro</td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>Barduro</td>
<td>64.0 (1.1) a</td>
<td>93.8 (1.0) ns</td>
<td>−0.5 (0.1) a</td>
<td>37.4 (0.3) a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>Neches</td>
<td>36.8 (1.1) d</td>
<td>86.9 (1.6) ns</td>
<td>−0.4 (0.1) ab</td>
<td>26.0 (0.5) c</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$CL$_{95\%}$: 95% confidence limit.

$^b$Parameter estimates calculated by nonlinear regression equation:

$$Y = \frac{1}{1 + \left( \frac{P}{P} \right) e^{-\beta_0 - \beta_1 \times (X - XP)}}$$

where $\beta_0$ is the upper asymptote (maximum germination), $\beta_1$ is the overall slope estimate, and $XP$ is the estimate of $X$ (temperature) when $Y$ (germination) is at $P$ percent of the maximum germination. $P$ was set to 80%.

$^c$Values for each parameter within a column and temperature range followed by the same letter are not significantly different at $\alpha = 0.05$. 
Fig. 1. Effect of temperature on cumulative germination of six coated clover (Trifolium spp.) cultivars using a three-parameter logistic growth model and a thermal gradient assay at -1.0 to 12.0 °C (n = 1275).

Table 3. Temperature ($x_0$) and time ($y_0$) to maximum seed germination using Lorentzian regression† for coated clover (Trifolium spp.) cultivars using a thermal gradient assay.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivar</th>
<th>$x_0$</th>
<th>$y_0$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>CL95%</th>
<th>CL95%</th>
<th>CL95%</th>
<th>CL95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balansa</td>
<td>FIXatioN</td>
<td>12.1</td>
<td>(1.6) bc§</td>
<td>50.7</td>
<td>(3.6) b</td>
<td>49.2</td>
<td>(2.5) b</td>
<td>15.8</td>
<td>(2.5) b</td>
<td>43.7</td>
</tr>
<tr>
<td>Crimson</td>
<td>AU Robin</td>
<td>15.9</td>
<td>(2.5) ab</td>
<td>47.3</td>
<td>(2.9) bc</td>
<td>53.9</td>
<td>(3.1) b</td>
<td>26.1</td>
<td>(7.6) a</td>
<td>35.0</td>
</tr>
<tr>
<td>Crimson</td>
<td>AU Sunrise</td>
<td>13.4</td>
<td>(1.7) b</td>
<td>52.8</td>
<td>(2.2) b</td>
<td>64.4</td>
<td>(4.0) a</td>
<td>17.4</td>
<td>(3.6) ab</td>
<td>26.3</td>
</tr>
<tr>
<td>Crimson</td>
<td>Dixie</td>
<td>17.2</td>
<td>(1.7) a</td>
<td>52.2</td>
<td>(1.9) b</td>
<td>59.5</td>
<td>(3.4) ab</td>
<td>23.2</td>
<td>(5.5) ab</td>
<td>26.0</td>
</tr>
<tr>
<td>Red</td>
<td>Barduro¶</td>
<td>9.6</td>
<td>(1.8) c</td>
<td>74.0</td>
<td>(5.6) a</td>
<td>48.1</td>
<td>(4.4) b</td>
<td>7.4</td>
<td>(6.1) b</td>
<td>43.1</td>
</tr>
<tr>
<td>White</td>
<td>Neches</td>
<td>10.9</td>
<td>(2.5) bc</td>
<td>34.2</td>
<td>(10.4) c</td>
<td>50.1</td>
<td>(5.9) b</td>
<td>14.4</td>
<td>(3.1) b</td>
<td>60.8</td>
</tr>
</tbody>
</table>

† Lorentzian regression equation for estimating time and temperature to maximum seed germination:

$$z = \frac{a}{1 + \frac{(x - x_0)^2}{b}} + \frac{b}{1 + \frac{(y - y_0)^2}{c}}$$

where temperature (x) and time (y) data were used to develop the parameters (a, b, and c) to predict the temperature ($x_0$) and time ($y_0$) that produced the maximum germination (z) for each cultivar.

‡ CL95: 95% confidence limit
§ Values for each parameter within a column followed by the same letter are not significantly different at α = 0.05.
¶ Only includes data collected from <10° C.
Fig. 3. Temperature and time to maximum seed germination using Lorentzian regression on six coated clover (*Trifolium* spp.) cultivars using a thermal gradient assay. Legend colors: blue hue represents lower germination with green to yellow hues indicate increased germination.

Dixie: $z = \frac{1}{1 + \left(\frac{x - 12.1}{7.4}\right)^2 \times \left(\frac{y - 47.3}{35.0}\right)^2}$

Fixation: $z = \frac{1}{1 + \left(\frac{x - 10.9}{14.4}\right)^2 \times \left(\frac{y - 19.2}{60.8}\right)^2}$

AU Robin: $z = \frac{1}{1 + \left(\frac{x - 15.9}{26.1}\right)^2 \times \left(\frac{y - 47.3}{35.0}\right)^2}$

Barduro: $z = \frac{1}{1 + \left(\frac{x - 15.9}{26.1}\right)^2 \times \left(\frac{y - 47.3}{35.0}\right)^2}$

AU Sunrise: $z = \frac{1}{1 + \left(\frac{x - 13.4}{17.4}\right)^2 \times \left(\frac{y - 52.8}{26.3}\right)^2}$

Neches: $z = \frac{1}{1 + \left(\frac{x - 12.1}{15.8}\right)^2 \times \left(\frac{y - 50.7}{43.7}\right)^2}$
All of these previously published experiments only measured germination in 5°C increments, which disallowed researchers to accurately isolate this asymptote as well. Additionally, these studies did not test germination at temperatures great enough to reduce germination to zero. Some level of germination was reported for all six clovers above 40°C in the present study, at least 10°C warmer than previous evaluations.

**Maximum Seed Germination**

Only non-zero germination data were used to determine maximum seed germination (Table 3, Fig. 3). Moreover, Barduro clover germinated so rapidly in the warmer temperature range that the full data set could not be used, because it did not conform to the same regression. Future trials should begin measuring germination at 12 h to better capture these early trends in germination.

Dixie, though not different from AU Robin, required the greatest temperature for germination (17.2°C). Alternatively, Barduro reached maximum germination at the lowest temperature (9.6°C; not different from FIXatioN and Neches). Barduro required the most time to reach maximum germination (74.0 h), but this is an artifact of the limited data set used in this particular evaluation. Neches reached maximum germination the fastest (34.2 h) and was only similar to AU Robin.

These data best follow the results of Kendall and Stringer (1985), who wrote that maximum germination of annual clovers occurs at 15°C. Otherwise, these results do not follow the trends reported in literature for both optimum time and temperature for maximum germination. Most trials agreed that optimum clover germination was centered on 20°C and germination was not typically measured before 3 d after planting. For instance, Butler et al. (2014) reported the optimum temperature for warm- and cool-season legumes was 20°C and maximum germination occurred at 72 h after planting, which was the first time point germination was measured.

**CONCLUSIONS**

The objectives of this trial were to determine (i) the temperature and time required to reach maximum germination, and (ii) the range in which germination will occur for six clover cultivars adapted to the Southeast. If Barduro is excluded from the evaluation, these clovers reached maximum germination at 34.2 to 52.8 h after planting at optimum temperatures (10.9–17.2°C). These six varieties maintained at least 80% germination from 4.9 to 28.2°C on average, although some level of germination was observed beyond these extremes. The range at which these clovers could germinate was much wider than reported in previous literature. These clovers can possibly be planted at much warmer temperatures than previously thought, so producers may be able to plant in early fall when weather conditions are more conducive for germination. Alternatively, these species could possibly be planted later in the winter (i.e., in the instance of a failed winter cash crop) and germination may still be expected based on these results. Of course, additional work will be needed to determine the conditions under which late plantings in cooler conditions could still result in an agronomic benefit. It is important to remember that these results are based on otherwise ideal conditions (i.e., moisture) for germination. These data will be useful in planning field trials for further evaluations of thermal requirements for seed germination and eventually updating planting recommendations for producers in the Southeast.

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**REFERENCES**


