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AGRONOMY & SOILS

Investigation of the Radicle Length Threshold of the Cool Germination Test to Improve Field Predictability

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ABSTRACT

The cotton (*Gossypium hirsutum* L.) cool germination test is conducted to provide information regarding how seed lots will likely perform in suboptimal conditions. One aspect of the cool germination test is the use of a 4-cm radicle length threshold for a seedling to be counted as germinated, with little data to support its use. The objective of this study was to correlate cool germination test results using different radicle length thresholds with in-field emergence parameters. Germination tests were conducted to determine cool germination percentages for 12 seed lots using various radicle length thresholds. Field trials consisting of 12 seed lots were used to determine in-field emergence and vigor under suboptimal conditions. Linear regression was used to generate R^2 values between the percentage cool germination using various radicle length thresholds and in-field emergence parameters. These R^2 values were used to compare how well different radicle length thresholds explained variation in emergence and seedling vigor data. Across site-years and in-field parameters the radicle length threshold that maximized R^2 was under 3 cm, with variation between parameters and field sites observed. The 4-cm radicle length threshold used commercially in cool germination testing did not maximize R^2 value at any location for any field data collected. These results suggest that using a shorter radicle length threshold than the 4-cm standard when conducting the cool germination procedure would improve predictability of a seed lot's performance in field conditions that are suboptimally cool.

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The development of a uniform, rapidly growing stand is the first step to high yielding, profitable cotton (*Gossypium hirsutum* L.) (Snider et al., 2022). Many studies have found that a significant reduction in cotton stand can result in a lower yield (Bednarz et al., 2005; Christiansen and Rowland, 1981; Harrison et al., 2009; Hofmann et al., 1988). The development of a suitable cotton stand can help mitigate effects caused by early-season pests including thrips and seedling diseases (Colyer and Vernon, 2005; Krob et al., 2022). Cotton germination and emergence are influenced by a complex interaction of many factors, some of which are still not fully understood.

Planting conditions play a significant role in the emergence of cotton seedlings, as does seed quality. Cottonseed has limited energy to promote emergence, which is why cotton needs to be planted shallow, approximately 1.3 to 2.5 cm deep, compared to other crops (Reddy et al., 2020). Planter settings, including the proper use of downforce and closing wheels that enable seeds to be planted at the ideal depth and maintain seed to soil contact, have been shown to significantly impact cotton emergence, especially in reduced tillage scenarios (Virk et al., 2021; Way et al., 2018). Bare soils with conditions that lead to poor soil aggregate structure, such as low organic matter and conventional tillage, along with substantial amounts of precipitation around the time of planting, lead to the possibility of soil crusting (Varco, 2020). This crusting of the top layer of soil can significantly inhibit the ability of cottonseed to emerge (Bilbro and Wanjura, 1982). In response to these conditions, a planting technique of "hill-dropping" can be implemented to place seed together within a hill to combine the seed's ability to break through this layer (Collins et al., 2009).

Weather at, and soon after planting, has been shown to significantly impact cotton emergence. Weather also influences some of the early-season pests that impact cotton emergence and early-season growth including the seedling disease complex (Rothrock and Buchanan, 2020). The ideal conditions for cotton emergence are at least 5 d of warm temperatures with good moisture that result in at least 13.7 to 27.6 growing degree days (GDD) accumulation; however, these conditions can be rare within the cotton planting window (Edmisten and Collins, 2024a). Cotton planted when soil temperature is below 17.5 °C has been shown to emerge slower and have lower plant stands (Reddy et al., 2020). Cotton is commonly exposed to suboptimal temperatures during planting, especially at northern latitudes (Gipson, 1986), due to a limited planting window. In North Carolina (NC), cotton planting begins approximately 25 April and ceases by the end of May; planting after this date significantly increases the risk of lower cotton yields (Edmisten and Collins, 2024a). Additionally, 25 May is the first crop insurance deadline for cotton planting in NC, after which, coverage declines until the end of May. Planting beyond 31 May is not covered by crop insurance, and therefore not recommended (USDA-RMA, 2018). Crop insurance deadlines occur noticeably sooner in NC than in most other cotton producing states in the U.S. This is likely due to a reduction in the amount of heat units that can be acquired to grow a fully mature crop (Peng et al., 1989). In addition to a narrow planting window, the average size of farms in the U.S. has been steadily increasing over the past several decades, resulting in more acreage that must be planted within the same short planting window (USDA-NASS, 2024a). A short planting window in the northern-most region of the Atlantic coastal cotton belt, compounded by larger acreage producers, creates a scenario where cotton is more likely to be planted when suboptimal cool conditions occur, so growers can plant their cotton acreage prior to crop insurance deadlines.

Seed quality plays a critical role in the development of a rapidly emerging stand of any crop, but especially for cotton as cottonseed vigor has been observed to be noticeably lower than that of other commercial row crops. Bourland et al. (2019) reasoned that cotton is a perennial plant that originated in subtropical regions where high vigor seed was not essential and, therefore not an adaptation that was prioritized through natural selection. Additionally, the indeterminate growth habit of cotton negatively affects seed quality and vigor. Variation in boll development influences when and how environmental stress impacts the seed (Helmer and Abdel-Al, 1965). Boll opening over an extended period also impacts how long the seed is exposed to conditions known to cause seed deterioration (Woodruff et al., 1967). Another reason cotton seedlings are likely less vigorous than other domesticated crops is that cottonseed is not the primary agricultural use of this commodity, so less human-induced artificial selection has occurred to enhance seed properties (USDA-NASS, 2024b).

Factors affecting growth and development within one generation of cotton affect the quality of seed from that crop, which inevitability impacts the following year's cotton crop by impacting emergence, early-season growth, and yield (Kamran et al. 2020). Although many of the factors that influence cottonseed quality are not fully understood, factors known to affect seed quality can be divided into pre- and post-harvest conditions. Fall temperatures, relative humidity, and precipitation during seed development are pre-harvest factors that have been shown to influence seed quality (Buxton et al., 1978; Wang et al., 2019; Woodruff et al., 1967). Post-harvest conditions such as seed moisture, mechanical damage from harvest and ginning, storage conditions, the de-linting process, and amount of time in storage have also been shown to influence seed quality (Afzal et al., 2020; Delouche, 1981; Kamran et al., 2020).

Cottonseed is one of the most expensive inputs that growers make in a cotton crop. Since the incorporation of transgenic technologies for insect and herbicide tolerance associated with cotton, the average price for cottonseed to plant is reported to be approximately \$300 ha^{-1} (Washburn, 2024). It is important for farmers to know the investment they are making in cottonseed has the best opportunity to emerge, grow rapidly, and reach its genetic potential. One way this is accomplished is by testing for seed quality (Edmisten and Collins, 2024b). Although there are many metrics to quantify seed quality, the primary two are the standard germination test, also known as warm germination, and the cool germination test (AOSA, 2002, 2018).

The standard germination test provides information regarding how cottonseed will perform in nearly optimum conditions. This involves using germinators set at either at 30 °C continuously, or 16 h of 30 °C and 8 h of 20 °C each day for a maximum of 12 d (AOSA, 2018). This test is used throughout the U.S. cotton industry and as the legal standard to determine if seed meet the minimum germination levels to be sold, per state and national seed laws. Optimum conditions do not always occur in the normal planting window, especially in the northern regions of the cotton belt, where periods of cool, wet weather during planting are not uncommon.

Seed vigor has been defined as the ability of a seed lot to exhibit high germinability and emergence over a range of environmental conditions (Niles, 1967). The cool germination test is the most widely used predictor of seed vigor (Savoy, 2005). It provides another parameter in addition to the standard germination test to explore seed quality. This value gives an indication of how the seed will perform under stressful, cool conditions, although it is known to be a relatively weak predictor of plant stands when seed is planted in suboptimal conditions. The cool germination test is conducted at a constant 18 °C for 7 d (AOSA, 2002). Although this is not the optimum temperature for cotton germination, it provides valuable information about how cottonseed will perform in conditions that are not ideal. Growers can use this value to loosely compare seed lots and to determine which lot would perform better if planting must continue while conditions are thermally suboptimal. To be counted as germinated and reported in the results of the cool test, seedlings must have a radicle length of at least 4 cm or longer, as measured from the tip of the radicle to the point of cotyledon attachment, also known as the hypocotyl. This measurement is used because seedlings with radicles longer than 4 cm are said to be stronger or more vigorous (Delouche and Baskin, 1970; McCarty and Baskins, 1978). Although there are little data supporting the use of this 4-cm radicle length threshold as a criterion within the cool germination test, it is the standard threshold for conducting the cool germination test nationwide (AOSA, 2002). The cool germination test is known to be a relatively weak predictor of field performance, with low repeatability as test results can vary widely over time, over multiple samples collected from the same lot, and between labs (Shmidt et al., 2023). As such, there is no minimum, legal standard, or criteria for cool germination test results in cotton.

Due to the relatively low repeatability of cool germination test results, as well as weak predictability of actual field performance, the use of the 4-cm radicle length criteria should be reviewed and investigated. The objective of this research was to determine if using a shorter radicle length threshold while conducting the cool germination test could improve predictability of field performance when cotton is planted into known suboptimally cool conditions.

MATERIALS AND METHODS

Twelve seed lots consisting of multiple cotton varieties from various companies were sampled based on differences in cool germination percentage. These seed lots were previously analyzed by the NC Department of Agriculture and Consumer Services and were chosen to ensure a wide range of cool germination percentages were represented (Table 1). Per agreement with the seed companies, the variety names are not disclosed to prevent inferences relating seed quality to specific varieties or germplasms, as these variables are generally unrelated. During the off seasons, seeds were stored in a cool (18.3 °C), dry seed storage facility.

Table 1. Germination data of selected cotton varieties

Seed Lot[z]	Warm Germination	Cool Germination[y]
	--------------------%--------------------	
1	75	25
2	89	28
3	92	52
4	84	53
5	96	56
6	84	45
7	83	61
8	75	25
9	80	43
10	95	79
11	67	25
12	92	75

[z]Seed lots were chosen from different varieties across multiple different companies

[y]Cool Germination based on AOSA rules with 4-cm radicle threshold

In the fall of 2022, cool germination tests were conducted on the 12 seed lots in L. Snyder's seed quality lab at NC State University, in accordance with the Association of Official Seed Analysts (AOSA) rules (AOSA, 2002). These rules include the use of rolled paper towels (38# Regular Weight Seed Germination Paper, Anchor Paper Company, St. Paul, MN) enclosing 50 seeds per replication and four replications per seed lot for a total of 200 seeds assessed for each seed lot. Each replication used four germination towels, two underneath the seeds and two above, which maintained seed placement and consistency of exposure to moisture and temperature.

Deionized water was added at a rate of 2.25 mL per g of towel. These rolled towels were placed upright in an unsealed metal container. The germinator (GR-36L, Percival Scientific, Perry, IA) was set at 18 °C and allowed to acclimate until temperature remained stable. These tests were conducted at 18 ± 0.5 °C for 7 d as measured by the germinator. After 7 d, the towels were removed from the germinator and seedlings were observed and evaluated to calculate germination percentage. The length of the radicle, as measured from the point of cotyledon attachment to radicle tip, was recorded for each seedling. These lengths were then categorized and used to determine the percent germination (percentage of seedlings that met the minimum radicle length criterion and were therefore considered to be germinated) using various radicle length thresholds. These thresholds were categorized in 0.5-cm intervals ranging from 0 cm, or germinated with radicle length less than 0.5 cm, to 5 cm.

Field experiments were conducted during 2022 at the Peanut Belt Research Station (PBRS) near Lewiston, NC (36.13°N, -77.17°W) and at the Upper Coastal Plain Research Station (UCPRS) near Rocky Mount, NC (35.89°N, -77.68°W). In 2023, field trials were conducted only at UCPRS. In 2022, the two trial sites in Lewiston, Lewiston-Early and Lewiston-Late, were planted 28 April and 5 May 2022, respectively. The 2022 site at UCPRS, 2022 Rocky Mount, was planted 10 May 2022. In 2023, the two sites at UCPRS, Rocky Mount-Early and Rocky Mount-Late, were planted 24 April and 2 May 2023, respectively (Table 2). Although these planting dates are relatively early in the planting window for cotton, it was important that this study be conducted where and when conditions were sub-optimally cool for cotton planting so that differences in cool germination percentage between the seed lots would be expressed. All locations were conventional tilled, and seed were planted 1.5 cm deep on 91-cm bedded rows. Seed for each of the 12 seed lots were planted at 11.5 seed row m^{-1}, using commercially available vacuum planters (John Deere, Moline, IL). Plots were randomized in a randomized complete block design including four replications.

Weather was variable between sites and planting dates. Table 2 shows the number of GDD with base temperature of 15.6 °C accumulated within 5 d of planting for each site year. The daily number of GDD was calculated by using the formula:

$$Growing\ Degree\ Days = [(T_{max} + T_{min})/2] - 15.6$$

where T_{max} and T_{min} were the maximum and minimum daily temperatures in °C, respectively, and 15.6 °C was the threshold temperature for growth. This temperature was evaluated based on the planting conditions ratings scale used by the NC Cotton Planting Conditions Calculator developed by NC Cooperative Extension and the NC Climate Office (https://products.climate.ncsu.edu/ag/cotton-planting/). This provides a general rating for planting conditions based on 5 d after planting (DAP) GDD accumulation (Table 2). In 2023, there was a hail event at 15 DAP that affected the earlier planting in Rocky Mount. Due to hail damage and standing water, this site was abandoned beyond this date.

Seedling emergence was evaluated at 7, 14, and 21 DAP and was measured by counting emerged seedlings within a 3-m length of row for both rows per plot, these two measurements were averaged and recorded as plants row m^{-1}. Seedling emergence was counted when cotyledons were visible and above the soil line. Crop biomass was collected at 21 DAP by harvesting the above-ground biomass for 10 plants $plot^{-1}$. Within each plot, the 10-plant sample was formed from five representative plants from each

Table 2. Planting date and planting conditions for 2022 and 2023

Year	Site	Planting Date	5 DAP GDD Accumulation[z]	Planting Condition[y]
2022	Lewiston Early	28 April	18.50	Adequate
2022	Lewiston Late	4 May	27.45	Good
2022	Rocky Mount	10 May	24.70	Adequate
2023	Rocky Mount Early	24 April	14.26	Marginal
2023	Rocky Mount Late	2 May	16.69	Adequate

[z]Abbreviations: DAP, days after planting; GDD, Growing Degree Days with base temperature of 15.6 °C.

[y]Planting Condition from North Carolina Cotton Planting Conditions Calculator.

row within the plot. The sample was then dried in an oven at 60 °C for 3 d to account for potential differences in water content.

Field and germination data were analyzed through JMP Pro 17 (JMP, Version 17. SAS Institute Inc., Cary, NC) software. An analysis of variance was conducted on the field emergence and crop biomass data, with variety and site-year treated as fixed effects, and replication as a random effect. Linear regression was used to correlate cool germination percentage using a specific radicle length threshold as the predictor with field emergence and crop biomass as the response variables. The value for each seed lot used in the regression for field emergence and crop biomass was an average of that seed lot across the four replications within each site-year. R^2 values are shown from those correlations and were used to compare different radicle length thresholds for the cool germination test. The difference between the maximum R^2 and the R^2 at 4 cm (industry standard) was calculated for each parameter at each site-year using the equation:

$$\Delta R^2 = R^2_{max} - R^2_{standard}$$

where R^2_{max} and $R^2_{standard}$ represent the maximum R^2 for a particular parameter at a certain site-year and R^2 using the standard 4-cm radicle length threshold for the same parameter and site-year evaluated, respectively.

RESULTS AND DISCUSSION

For each emergence timing and crop biomass, the main effect of site-year was significant, so data are shown by site-year. The correlation between percentage cool germination at all radicle length thresholds and field emergence at each timing was significant at the $\alpha \leq 0.05$ level for all site-years. All site-years were also significant for correlations between cool germination percentage and crop biomass at all radicle thresholds except for the 2023 Rocky Mount-Late site, which was not significant at any of the radicle length thresholds ($\alpha > 0.05$).

7 DAP Emergence. At Lewiston-Early during 2022, the maximum R^2 value for the 7 DAP emergence count was 0.662 and was observed using the 0.5-cm radicle length threshold. The maximum R2 for 7 DAP emergence at the Lewiston-Late site was 0.643 and was observed when the radicle length threshold was 1 cm. The maximum R^2 for 7 DAP emergence at the Rocky Mount location during 2022 occurred when using the 1.5-cm radicle length with an R^2 of 0.582. In 2023, the maximum R^2 was 0.574, achieved at the Rocky Mount-Early site using the 0.5-cm radicle length threshold. Conversely, the $R^2$was maximized for the Rocky Mount-Late location between 2- to 2.5-cm radicle length threshold with an R^2 of 0.326 (Table 3).

Table 3. R^2 values of cool germination results using different radicle length thresholds and 7 DAP emergence by location

	7 DAP Emergence R^2 Values[z]				
	--------2022--------			--------2023--------	
RLT[y]	**Lewiston Early**	**Lewiston Late**	**Rocky Mount**	**Rocky Mount Early**	**Rocky Mount Late**
0	0.659	0.598	0.503	0.574	0.230
0.5	0.662[x]	0.605	0.506	0.576[x]	0.234
1	0.627	0.643[x]	0.576	0.569	0.283
1.5	0.567	0.606	0.582[x]	0.519	0.309
2	0.520	0.553	0.545	0.473	0.326[x]
2.5	0.495	0.529	0.531	0.436	0.326[x]
3	0.484	0.517	0.496	0.407	0.306
3.5	0.481	0.500	0.459	0.403	0.285
4	0.466	0.477	0.417	0.373	0.255
4.5	0.427	0.425	0.347	0.324	0.228
5	0.368	0.381	0.303	0.289	0.177

[z]DAP, days after planting.

[y]RLT, Radicle length threshold for cool germination test (cm)

[x]Denotes maximum R^2 corresponding to a particular radicle length threshold at each location

In all site-years, the currently used 4-cm threshold did not maximize the correlation between cool germination and 7 DAP field emergence. Additionally, R^2 values correlating cool germination percentage to 7 DAP plant emergence declined similarly when using radicle lengths larger than 2.5 cm at all site-years (Table 3). The maximum R^2 value was greatest at the Lewiston-Early 2022 and Rocky Mount-Early 2023 sites, which were the locations that had the lowest 5 DAP GDD accumulation each year. These sites represent the most challenging planting conditions each year, with the Lewiston-Early 2022 and Rocky Mount-Early 2023 sites accumulating 18.5 and 14.26 GDD within 5 DAP, respectively (Table 2). This response is consistent with the findings of Smith and Varvil (1984) suggesting that cool germination tests are used to distinguish which seed lots are suitable for planting in suboptimal conditions.

14 DAP Emergence. In 2022, the maximum R^2 for 14 DAP emergence was 0.542 at the Lewiston-Early site, using the 1-cm radicle length threshold. The maximum R^2 for at the Lewiston-Late site was 0.535 using the 1.5-cm radicle threshold. At the Rocky Mount site, the maximum R^2 was 0.467 using the 2.5-cm radicle threshold. In 2023, the maximum R^2 was 0.572 from the Rocky Mount-Early site using the 1-cm radicle length. The Rocky Mount-Late site resulted in a maximum R^2 value of 0.383 using the 1-cm radicle length.

Similar to the 7 DAP emergence data (Table 4), 14 DAP emergence R^2 values declined when using radicle length thresholds larger than 2.5 cm. The sites where the cool germination test best correlated with field emergence at 14 DAP were the same as those observed for the 7 DAP data and included the Lewiston-Early site in 2022 and the Rocky Mount-Early site in 2023. Similar to the 7 DAP data, currently used 4-cm radicle length threshold did not result in maximum R^2 for 14 DAP emergence at any of the tested locations.

21 DAP Emergence. In 2022, the 21 DAP emergence data (Table 5) show that the maximum R^2 value was 0.772 obtained from the Lewiston-Early site, using the 1-cm radicle length. At the Lewiston-Late site in 2022, the maximum R^2 was 0.445 using the 1.5-cm radicle length requirement; whereas at the Rocky Mount site in 2022, the maximum $R^2$was 0.451 when using the 2.5-cm radicle length requirement. In 2023, a hailstorm occurred on 8 May causing severe stand loss in the Rocky Mount-Early site, resulting in abandonment of this site before the 21 DAP timing. The Rocky Mount-Late site, however, did survive and data were collected on this site. At this site, the maximum R^2 was 0.394 using the 1-cm radicle requirement.

Similar to both the 7 and 14 DAP emergence data, the location that had the highest correlation between cool germination percentage and plant stands

Table 4. R^2 values of cool germination results using different radicle length thresholds and 14 DAP emergence by location

	14 DAP Emergence R^2 Values^z				
	-----2022-----			-----2023-----	
RLT^y	Lewiston Early	Lewiston Late	Rocky Mount	Rocky Mount Early	Rocky Mount Late
0	0.533	0.470	0.330	0.530	0.358
0.5	0.538	0.475	0.332	0.527	0.357
1	0.542^x	0.526	0.409	0.572^x	0.383^x
1.5	0.474	0.535^x	0.457	0.562	0.374
2	0.421	0.500	0.457	0.516	0.364
2.5	0.391	0.495	0.467^x	0.482	0.346
3	0.372	0.493	0.437	0.436	0.348
3.5	0.369	0.478	0.399	0.394	0.362
4	0.352	0.472	0.362	0.348	0.351
4.5	0.327	0.428	0.303	0.272	0.323
5	0.300	0.407	0.267	0.221	0.296

^{z}DAP, days after planting.

^{y}RLT, Radicle length threshold for cool germination test (cm)

^{x}Denotes maximum R^2 at each location

Table 5. R^2 values of cool germination results using different radicle length thresholds and 21 DAP emergence by location

	21 DAP Emergence R^2 Values[z]			
	----------2022----------			---------2023 ---------
RLT[y]	Lewiston Early	Lewiston Late	Rocky Mount	Rocky Mount Late
0	0.705	0.378	0.322	0.370
0.5	0.714	0.382	0.324	0.370
1	0.772[x]	0.424	0.399	0.394[x]
1.5	0.699	0.445[x]	0.446	0.387
2	0.601	0.426	0.442	0.374
2.5	0.561	0.432	0.451[x]	0.357
3	0.561	0.431	0.42	0.355
3.5	0.557	0.412	0.375	0.359
4	0.555	0.407	0.34	0.349
4.5	0.524	0.369	0.279	0.319
5	0.500	0.349	0.242	0.292

[z]DAP, days after planting.

[y]RLT, Radicle length threshold for cool germination test (cm)

[x]Denotes maximum R^2 at each location

was the Lewiston-Early site in 2022, which had the lowest 5 DAP GDD accumulation, and therefore the poorest rated planting conditions of all the sites that were tested (Table 2). Additionally, all locations at 21 DAP, maximum R^2 values were observed when using radicle length thresholds shorter than 2.5 cm. At 21 DAP, the conventionally used 4-cm threshold did not result in maximum R^2 at any of the sites evaluated (Table 5).

Crop Biomass. In 2022, the maximum R^2 value for crop biomass was 0.471 when using the 1-cm radicle length threshold at the Lewiston-Early site. The maximum R^2 achieved at the Lewiston-Late site was 0.181 using the 3-cm radicle length threshold. The maximum R^2 at the Rocky Mount site was 0.321 at the < 0.5-cm radicle length threshold (Table 6). In 2023, no data were collected for the early planted site in Rocky Mount due to the hailstorm. The correlation

Table 6. R^2 values of cool germination results using different radicle length thresholds and 21 DAP crop biomass by location

	21 DAP Biomass R^2 Values[z]			
	----------2022----------			---------2023 ---------
RLT[y]	Lewiston Early	Lewiston Late	Rocky Mount	Rocky Mount Late
0	0.469	0.152	0.321[x]	N.S.[w]
0.5	0.466	0.153	0.319	N.S.
1	0.471[x]	0.159	0.260	N.S.
1.5	0.446	0.169	0.230	N.S.
2	0.406	0.169	0.220	N.S.
2.5	0.385	0.172	0.213	N.S.
3	0.365	0.181[x]	0.220	N.S.
3.5	0.351	0.173	0.235	N.S.
4	0.330	0.169	0.244	N.S.
4.5	0.282	0.147	0.242	N.S.
5	0.260	0.130	0.242	N.S.

[z]DAP, days after planting.

[y]RLT, Radicle length threshold for cool germination test (cm)

[x]Denotes maximum R^2 at each location

[w]N.S., not significant at $\alpha < 0.05$ level

between cool germination percentage and crop biomass at the Rocky Mount-Late site in 2023 was not statistically significant at the $\alpha \leq 0.05$ level. In general, the R^2 between cool germination and the crop biomass field data was substantially lower than the R^2 between cool germination and field emergence. This is similar to results found in 2019 by Virk et al., who reported that early season vigor is impacted by many factors other than seed quality including cultivar, leaf area index, and growth conditions.

ΔR^2. The difference between maximum R^2 and the R^2 at the currently used 4-cm threshold (ΔR2) was calculated for each parameter at each location to determine how much variation was not being explained by using the current methodology of the cool germination test. This value varied drastically by site-year and parameter. Depending on site-year and parameter, the radicle length threshold with the maximum R^2 for each location explained between 3 and 22% more variation in the field data than the cool germination percentage using the 4-cm threshold (Table 7), with an average of 12% greater variation accounted for. Overall, the sites with the largest difference between the two R^2 were noted at the 2022 Lewiston-Early and 2023 Rocky Mount-Early sites (Table 7). These sites had the lowest 5 DAP GDD accumulation per year (Table 2) when compared to other sites within each year. The parameter that had the largest difference between maximum R^2 and the R^2 at 4 cm across all locations was 7 DAP emergence, where the radicle threshold that maximized R^2 values explained anywhere from 7 to 20% more variation in field emergence compared to the 4-cm threshold (Table 7).

Although the length threshold that resulted in maximum R^2 was not the same across all site-years or for a particular parameter measured, these data illustrate drastic differences in the amount of variation in field data that is explained simply by shortening the radicle length threshold used for counting percentage of germinated seedlings when conducting the cool germination test.

CONCLUSIONS

The radicle length threshold that maximized the relationship to field parameters was variable by site and parameter tested. However, in all locations, maximum R^2 values were observed when using radicle length thresholds under 3 cm, across all data collected, and under 2.5 cm for all emergence parameters. Overall, the R2 values for emergence tended to be higher for the sites that had the lowest 5 DAP GDD accumulation per year, and in these site-years, the optimum radicle length ranged from 0.5 to 1 cm with R2 values ranging from 0.471 to 0.772 (Tables 3, 4, 5, 6), which explained more variation in field emergence data compared to other research using the cool germination test with the standard 4-cm radicle length threshold (Kerby et al., 1989). Overall, the relationship between radicle length and emergence was greater than the relationship between radicle length and crop biomass at each site-year, which has been demonstrated in previous studies. Although seed quality can have an influence on crop biomass, many other factors can influence crop biomass and confound the relationship between radicle length and crop biomass. In 2023, the relationship between radicle length and crop biomass at the Rocky Mount-Late site was not significant, but this site received the lowest 5 DAP GDD accumulation for any of the sites where crop biomass was collected. Although conditions at planting were adequate for this site, conditions improved after emergence (data not

Table 7. ΔR^2 by site-year for field emergence and crop biomass[z]

	$\Delta R2^y$				
	---2022---			---2023---	
Parameter	**Lewiston Early**	**Lewiston Late**	**Rocky Mount**	**Rocky Mount Early**	**Rocky Mount Late**
7 DAP Emergence	**0.196**	**0.166**	**0.165**	**0.201**	**0.071**
14 DAP Emergence	**0.538**	**0.475**	**0.332**	**0.224**	**0.032**
21 DAP Emergence	**0.190**	**0.063**	**0.105**	**–[x]**	**0.045**
Crop Biomass	**0.474**	**0.535[x]**	**0.457**	**–[x]**	**N.S.**

[z]Abbreviations: DAP, days after planting; N.S., not significant at the $\alpha < 0.05$ level

[y]ΔR^2 = (Maximum R^2 – R^2 standard using 4 cm radicle length threshold) for a specific parameter at a singular site-year

[x]No data collected due to effects of hailstorm at this location

shown), potentially reducing the impact of radicle length on crop biomass.

These data demonstrate that a radicle length threshold should be used when conducting the cool germ test to maximize correlation to field emergence, versus counting only sprouted seed. Additionally, this study illustrated that in the conditions tested, which were suboptimally cool, but not uncommon for cotton planting, the conventionally used 4-cm cool germination threshold did not result in the maximum relationship to field emergence and therefore, was not an accurate predictor of field performance. Data comparing various radicle length thresholds within the cool germination test have not been previously reported. This study indicated that the cool germination test can be more predictive of actual field emergence or seedling vigor, based simply on changing the radicle length threshold used to determine percentage germination within the cool germination test.

This study was conducted on a relatively small sample of seed lots and only compared a few sites in NC across two years. To determine the appropriate radicle length threshold to be used in the cool germination test, future studies should be conducted with representative sites across all cotton production areas within the U.S. and in a broader range of planting conditions. These studies should include many different varieties and seed lots with multiple companies represented to explore whether this relationship holds across a range of environments and other seed quality characteristics. Other studies need to be conducted to determine how changing the radicle length threshold within the cool germination test would impact the repeatability and implementation of the cool germination test. For example, using a radicle length threshold shorter than the standard 4 cm would increase the reported cool germination percentage, therefore changing how cool germination test results need to be interpreted. If a different radicle length threshold were to be established, studies would need to be conducted to assess the potential impact of changing the cool germination test on the amount of variation within replications of a cool germination test and how it could impact the large amount of variation that exists between seed labs that are conducting these germination tests (Shmidt et al., 2023).

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