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ARTICLE



Estimation of plant health in a sorghum field infected with anthracnose using a fixed-wing unmanned aerial system

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ABSTRACT

Diseases cause enormous losses of yield and quality for crop producers worldwide. To meet future food demands, crops are bred for resistance to as many of these maladies as possible. One such disease, anthracnose [*Colletotrichum sublineola*], is a fungal disease of great importance to sorghum [*Sorghum bicolor*, L. Moench] production because it causes significant annual economic losses in the crop. Breeding for anthracnose resistance requires time-consuming phenotyping, which is subjective and conditional to the evaluator. It is possible that quantitative assessment using high-throughput methodologies to estimate the trait may be more effective. In this study, we present an in-depth statistical analysis of fixed-wing, unmanned aerial system (UAS) evaluation of anthracnose incidence and severity in sorghum using normalized difference vegetation index (NDVI). In early phases of infection, correlations between ground-truth and UAS estimates of anthracnose were moderate but they increased substantially by the end of the season ($r = -0.55$ to -0.95). Additionally, both metrics had moderate-to-high repeatabilities throughout the growth period ($R = 0.60$ – 0.90), indicating they were consistently able to differentiate genotypes. Finally, we found that the UAS-derived measurements ($R^2 = 0.377, 0.473$) were better associated with ground-truth measurements ($R^2 = 0.278, 0.347$) for grain yield under anthracnose pressure. The results of this study indicated that fixed-wing UAS could potentially be effective for evaluating anthracnose disease present in sorghum, and the greater range of the UAS allowed the effective evaluation of larger numbers of plants than ground truth or traditional remote sensing methods.

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High-throughput phenotyping; phenomics; plant breeding; plant pathology; remote sensing; sorghum; sorghum anthracnose

1. Introduction

To meet the food production expectations, cereal crop yields must increase at a rate of at least 2.4% per year (Cairns et al. 2013; Godfray et al. 2010). Unfortunately, numerous crop diseases reduce yield potentials throughout the world (Strange and Scott 2005). Phenotyping for disease has historically been difficult and has served as a barrier to the improvement of crop resistance (Furbank and Tester 2011). High-throughput techniques, particularly those that utilize remote sensing, could reduce this disease phenotyping bottleneck, which would help crop improvement programs achieve the required rate of genetic gains for future production (Araus and Cairns 2014; Furbank and Tester 2011; Shi et al. 2016; Tester and Langridge 2010). The capability of unmanned aerial systems (UAS) to cover large areas in a relatively short length of time makes them an appealing option for plant breeders (Chang et al. 2017; Malambo et al. 2018; Pugh et al. 2017; Shafian et al. 2018; Shi et al. 2016). However, no previous studies have conducted an in-depth evaluation of fixed-wing UAS for their ability to measure large numbers of plots in a disease-evaluation nursery and make decisions based upon the results.

Previous studies have shown the efficacy of remote sensing to estimate damage caused by other biotic and abiotic stressors in crops, and these same concepts could apply to the phenotyping of crop diseases (Caturegli et al. 2016; Valasek et al. 2016). A common remote sensing technique to assess plant stress is normalized difference vegetation index (NDVI), which is a rating that is often used as a measurement of plant health and photosynthetic integrity (Jones and Vaughan 2010; Rouse et al. 1974). The NDVI has been previously evaluated and implemented as an estimator of many parameters of interest, including disease presence, photosynthetic activity, abiotic stress, and others (Anyamba and Tucker 2005; Gamon et al. 1995; Kumar et al. 2016; Rouse et al. 1974).

Disease progress curves have been utilized by crop scientists for many years and have been applied in breeding programs previously (Hess, Bandyopadhyay, and Sissoko 2002; Li and TeBeest 2009; Néya and Le Normand 1998; Ngugi et al. 2000). The area under the disease progress curve (AUDPC) can provide a quantitative assessment of disease severity within each individual genotype (Jeger and Viljanen-Rollinson 2001). In much the same way that individual assessments of disease severity could be useful, disease progress curves generated using high-throughput techniques could produce new information for breeders.

Sorghum serves as an excellent case study by which to evaluate UAS for their ability to phenotype disease. While there are several diseases of significance in sorghum, anthracnose (*Colletotrichum sublineola* P. Henn., Kabát and Bubák) is arguably the most important (Li and TeBeest 2009;

Moore, Ditmore, and TeBeest 2010; Tebeest, Kirkpatrick, and Cartwright 2004). In sorghum, the disease infects all above-ground parts of the plant (stalk, leaves, peduncle, and panicle), producing visible damage (Moore, Ditmore, and TeBeest 2010; Warren 1986). Anthracnose can cause sorghum yield reductions of > 50% (Ali 1987; Li and TeBeest 2009; Thomas 1996). As such, anthracnose serves as an excellent case study to assess UAS for their ability to phenotype this disease.

Genetic resistance is the primary means of controlling the disease. Because there are many different strains of *C. sublineola*, sorghum breeders must continuously breed for resistance in their material (Leslie 2002). Traditional methods of phenotyping sorghum for the presence and severity of the disease in the field are ultimately effective but are also quite laborious and prone to a significant amount of measurement error attributable to their inherent subjectivity. Thus, the objectives of this study were to i) evaluate the relationship between remotely sensed NDVI data collected using a fixed-wing UAS and ground-truth anthracnose disease rating, and ii) evaluate each of the various measurements in this study for their relationship to final grain yield.

2. Materials and methods

2.1. Germplasm and experimental design

The germplasm used for the evaluation of anthracnose comprised a set of 542 experimental grain sorghum hybrids; this set included 15 hybrids that were replicated two to seven times throughout the trial to account for spatial variability within the field. Thus, the total number of field plots was 597. The replicated hybrids were used for the calculation of least-square means, variance components, and repeatability. Each hybrid was planted in a 6.71 m test plot with 1.22 m alleys between them. The entire set of 597 field plots was used for correlative analysis. Included in the material were five susceptible genotypes, two resistant genotypes, and one moderately resistant genotype that were to serve as experimental controls. The experimental test was planted in one trial, which was in College Station, TX, on 31 March 2017. Standard agronomic practices for grain sorghum were used in this study, with the exception that plants were inoculated with *C. sublineola* to ensure disease infection.

2.2. Anthracnose inoculation

To prepare anthracnose inoculum, the methods described by Prom et al. (2009) were used. First, nine single-spored isolates of *C. sublineola* were inoculated onto ½ X potato dextrose agar plates with streptomycin (0.1 mg/ml) and were incubated for two weeks at ambient room temperature (~ 25° C)

(Guthrie et al. 1992). One hundred agar plates were prepared for each of these isolates. After incubation, spores were removed from the plates by placing approximately 5 mL of water on them and gently dislodging them with a glass rod. Approximately 600 mL per isolate were collected in this manner, and each product was brought to 1000 mL by adding distilled water. This spore suspension was used to inoculate autoclaved sorghum seeds.

The autoclaved seeds were prepared as follows: 3000 cc volume of sorghum seeds and 1.5 L of distilled water were placed into a 46 cm × 38 cm × 13 cm autoclavable plastic tray and were covered with aluminum foil. This mixture was autoclaved for 1 h, with rapid exhaust (i.e. 1 min on the dry cycle). After autoclaving, the tray was emptied into a 132 L translucent plastic bag and the bag was spread across the working area to increase its surface area for rapid cooling. After cooling, 250 mL of inoculum was added to each bag and was shaken thoroughly to mix. Bags of inoculum were incubated open and spread out in a warm room (~ 28° C) out of direct sunlight for 3–4 days prior to application in the field. Each bag was inoculated with one isolate, but prior to field inoculation, inoculum of different isolates was uniformly mixed.

Inoculation of sorghum plants in the test plots occurred 49 days after planting (DAP). To ensure even distribution of the inoculum, about two to three plants were inoculated per meter by directly sprinkling inoculum into the whorl of the plants. During the growing season, anthracnose developed on the inoculated plants and spores arising from these infections then spread throughout the trial via splash dispersal, a process whereby a fungal pathogen spreads via water droplets (Madden 2009).

2.3. Field measurements of disease incidence and grain yield

Ground-truth measurements of anthracnose incidence and severity in the sorghum plots were conducted via a subjective visual rating within the research plot (Table 1). Disease presence was determined depending upon the proportion of the plants in each plot that exhibited any anthracnose symptoms, i.e. necrotic lesions, grey diseased tissue, etc. These measurements were recorded on five different dates during the growing season (Table 2). As the sorghum plots were inoculated with anthracnose at the beginning of the growing season, it was reasonably expected that most of the diseased tissue within those plots was specifically attributable to anthracnose. Grain yield was measured on the genotypes that were replicated throughout the trial by hand harvesting the plot and threshing it in an Almaco plot thresher (ALMACO, Nevada, Iowa).

2.4. UAS data collection and data processing

UAS flights were conducted 24 times from March to August in 2017. This study used five datasets collected on June 16, June 23, June 29, July 13, and July 25,

Table 1. Field Measurements of Anthracnose Incidence and Severity This table summarizes the 1–9 visual rating (Rating) that was used to estimate disease incidence and severity (Description) in sorghum research plots in College Station, TX in 2017.

Rating	Description
1	Disease inconspicuous or present on an occasional plant, only speckling occurs
2	Disease is present and has up to 50% prevalence on all plants with low severity on each plant; apparently causing little damage
3	Disease is present and over 50% prevalence on all plants with low severity on each plant; apparently causing little damage
4	Disease is present with 100% prevalence on all plants; some severity which apparently is causing some damage
5	Disease is severe with 100% prevalence on all plants; estimated leaf area destroyed up to 25%; disease appears to be of economic importance
6	Disease is severe with 100% prevalence on all plants; estimated leaf area destroyed is between 25 to 50%; disease is of economic importance
7	Disease is severe with 100% prevalence on all plants; estimated leaf area destroyed is between 50 to 75%; disease is of economic importance
8	Disease is severe with 100% prevalence on all plants; estimated leaf area destroyed is above 75%; disease is of economic importance
9	Disease is severe with 100% prevalence on all plants; leaf area destroyed is 100%; death of leaves or plants due to disease

Table 2. Summary of Flight and Ground-Truth Dates The dates for the ground-truth (GT) data taken during this study as well as when the normalized difference vegetation index (NDVI) was taken via an unmanned aerial system (NDVI Date). The ground-truth date is also shown as the number of days after planting (DAP), and this value will be used for the rest of this study to refer to the associated GT and UAS dates. In addition, the growth stage of the sorghum as described by Vanderlip and Reeves (1972).

GT Date	GT Date (DAP)	NDVI Date	Sorghum Growth Stage
June 14 th , 2017	75	June 16 th , 2017	Half-bloom
June 21 st , 2017	82	June 23 rd , 2017	Soft Dough
July 3 rd , 2017	94	June 29 th , 2017	Hard Dough
July 18 th , 2017	109	July 13 th , 2017	Physiological Maturity
July 27 th , 2017	118	July 25 th , 2017	Physiological Maturity

which were acquired using a Tuffwing[®] UAV Mapper fixed-wing platform outfitted with a red-green-blue (RGB) and multispectral camera. The RGB camera used was a Sony[®] A6000 with 16-mm Pancake Lens. A RedEdge multispectral sensor, manufactured by MicaSense (Seattle, Washington, USA), was mounted to collect five bands (Red, Green, Blue, Red-edge, Near-infrared) spectral imagery. Immediately after collecting these data, the raw imagery was uploaded to a university data-sharing hub (UASHub) and was made available for processing. The UAS images were processed via Agisoft Photoscan Pro software (Agisoft LLC, St. Petersburg, Russia) with Structure from Motion (SfM) to generate the Digital Surface Model (DSM) and orthomosaic image (Chang et al. 2017; Mesas-Carrascosa et al. 2015). For precise geo-referencing of the orthomosaic images, 16 Ground Control Points (GCPs) were installed in the study area and the coordinates of these GCPs were measured by real-time kinematic (RTK) global positioning system (GPS). The coordinates were

inputted to Agisoft Photoscan Pro when orthomosaic and DSM were generated. The positions of the GCPs were manually elected on one image and automatically projected to the remaining images in ESRI ArcGIS 10.3.1 software. The spatial resolution of the orthomosaic image was ~ 7 to 8 cm. All orthomosaic images were geo-referenced with less than 4 cm accuracy, which meant a pixel size of 0.5.

The mosaicked images for the experimental field were then clipped with the 597 research plots within it, which formed a region of interest (ROI) and 4.7 square meters (6.0 m \times 0.78 m) for each. To prevent plot edge effects from influencing calculations, the plots were reduced by 0.2 m on each edge using an ArcGIS buffer tool. Data from the multispectral cameras were converted into NDVI to estimate the incidence of anthracnose disease. The NDVI was calculated based upon the equation

$$NDVI = \frac{\rho_{NIR} - \rho_{Red}}{\rho_{NIR} + \rho_{Red}}$$

where ρ_{NIR} and ρ_{Red} are the reflectance of the near-infrared and the visible red bands, respectively.

2.5. Data analysis and statistics

The AUDPC is an informative metric that can be used to temporally estimate the severity of disease in crops (Jeger and Viljanen-Rollinson 2001). The AUDPC for anthracnose incidence within the experimental material was calculated in Microsoft Excel software using the equation:

$$AUDPC = \sum_{i=1}^n \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where n = total number of observations, y_i = disease intensity or incidence at the i th observation, and t = time at the i th observation. This was calculated using the date of inoculation, and areas under the disease curves were calculated for each ground-truth date.

Sorghum disease estimates and AUDPCs collected and calculated via ground-truth and via the UAS were checked for outliers using the Huber test in JMP Pro 12.2.0 software SAS Institute Inc. (1989–2017). Least-square means were calculated for the ground-truth measurements and the UAS-derived NDVI estimates in JMP using an analysis of variance (ANOVA, all fixed effects). The same model was used for a restricted maximum likelihood (REML) analysis that was conducted within environments using Fit Model (all random effects) in JMP. The statistical model that was used to perform these analyses was:

$$Y = \alpha_i + \gamma_t + \delta_k + \varepsilon$$

where Y = NDVI or Ground-truth estimates of anthracnose disease, α = genotype (i), γ = row (l), δ = range (k), and ε = error, where the row and range are spatial adjustments of what are sometimes referred to as columns and rows, respectively (D'Agostino, Sullivan, and Beiser 2006). For the REML results, effects that had negative variance components were subsequently removed from that model. The percentage of total genotypic variation as well as the repeatability (R) estimates was calculated using this model. Repeatability estimates were calculated using the equation:

$$R = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

where σ_g^2 = the genotypic variance, and σ_e^2 = the error variance (Nakagawa and Schielzeth 2010). The least-square means and variance components were obtained using the replicated hybrids within the trial.

Variance components and repeatability estimates were obtained for NDVI and the daily visual rating scores. Using all plots, Pearson's correlation coefficients (r) were calculated between ground-truth estimates of each parameter and the UAS-derived estimates SAS Institute Inc. (1989–2017). In addition, simple linear regression was performed between the four estimates of disease and grain yield using the replicated hybrids in JMP. This analysis was conducted to better understand which measurement would be most useful to predict yield losses attributable to anthracnose.

3. Results and discussion

3.1. Anthracnose infection and development

The general progression of anthracnose throughout the trial can be clearly observed via the visual rating (Ground-truth) and NDVI-derived estimates (Figure 1, Figure 2). Overall, anthracnose incidence and severity increased as the growth period progressed; the change was much more pronounced in the NDVI data (Figure 2). The reason for this is not entirely clear, but it was likely attributable to the inherent nature of NDVI. An NDVI rating is a vegetation index that provides an assessment of overall plant health and is not necessarily specific only to anthracnose presence. Thus, NDVI values started quite high on the first flight date (75 DAP) but fell markedly by the end of the season (118 DAP). This was likely because the starting flight date was close to the peak growth period for sorghum and, therefore, corresponded with the time when the NDVI values were expected to be highest. Nevertheless, the overall progression of disease and variability of the disease response in the experimental material were favorable for further analyses (Figure 1, Figure 2).

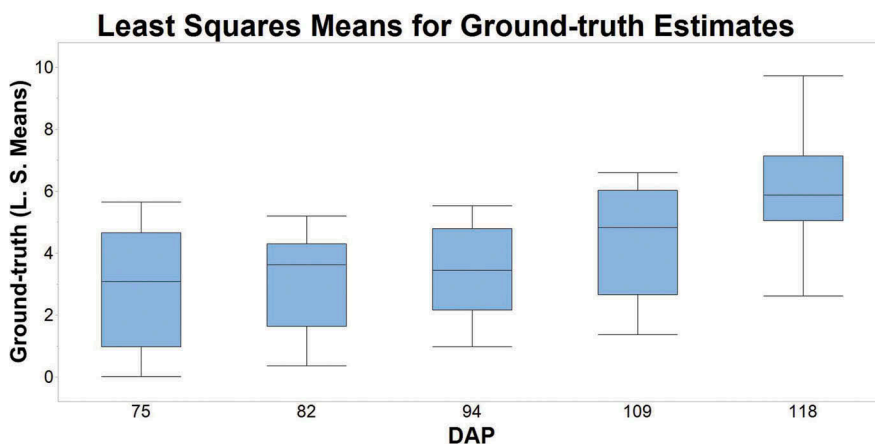


Figure 1. Least Squares Means for Ground-truth Estimates of Anthracnose Disease This figure shows the general progression of anthracnose estimates via visual rating (Ground-truth). Estimates are presented as least squares means (L. S. Means) across the five measurement dates, which are presented as the days after planting (DAP).

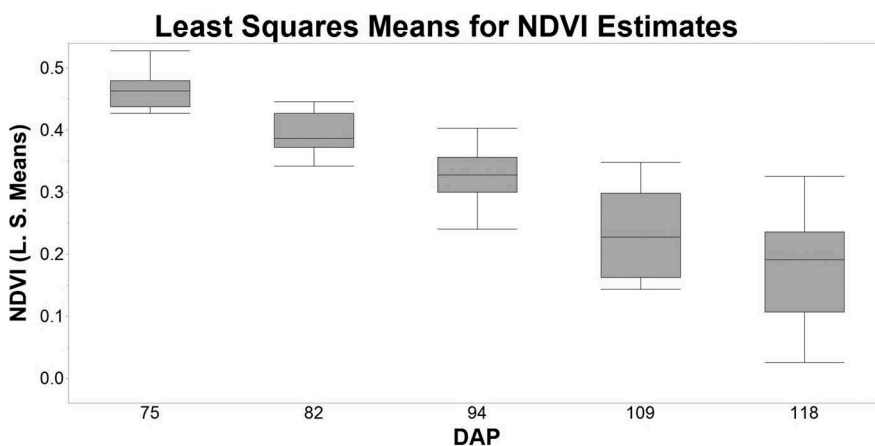


Figure 2. Least Squares Means for NDVI Estimates of Anthracnose Disease This figure shows the general progression of anthracnose estimates via normalized difference vegetation index, or NDVI. Estimates are presented as least squares means (L. S. Means) across the five measurement dates, which are presented as the days after planting (DAP).

3.2. Correlations between ground-truth and UAS estimates of anthracnose incidence and severity in sorghum

Pearson's correlations (r) between ground-truth and NDVI measurements generally increased during the growth period (Table 3). When comparing ground-truth measurements with the UAS-derived NDVI measurement, the correlations were low at the beginning of the season and rapidly increased until reaching a maximum of > -0.90 by the end of growth (Table 3). Similarly, the relationship between the AUDPC generated by the NDVI

Table 3. Correlations for Sorghum Disease Estimates Pearson's correlation coefficients (r) between measurements of disease obtained from either ground-truth or a UAS. The ground-truth measures include visual ratings of disease incidence within the plots (Ground-truth) as well as the area under the disease progress curve (AUDPC-GT). The normalized difference vegetation index (NDVI) and the area under the disease progress curve using NDVI data (AUDPC-NDVI) were derived from the UAS platform. Areas under the disease progress curves were calculated using

the equation: $AUDPC = \sum_{i=1}^n \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$, n = total number of observations, y_i = disease intensity or incidence at the i th observation, and t = time at the i th observation. Measurements were taken on five flight dates and their closest corresponding ground-truth dates during the growth period, shown as days after planting (DAP). All correlations were statistically significant at the $p < 0.001$ level.

DAP	Ground-truth vs. NDVI	AUDPC-GT vs. NDVI	Ground-truth vs. AUDPC-NDVI	AUDPC-GT vs. AUDPC-NDVI
75	-0.53	-0.53	-0.53	-0.53
82	-0.69	-0.69	-0.58	-0.58
94	-0.74	-0.74	-0.63	-0.65
109	-0.83	-0.81	-0.72	-0.74
118	-0.94	-0.91	-0.80	-0.82

data (AUDPC-NDVI) and the two ground-truth measurements became stronger during the growth period; however, the correlation was not as strong between those metrics.

The correlations in this study were negative because resistant plots had higher NDVI values and AUDPC-NDVIs; conversely, susceptible plots had higher ground-truth values and AUDPC-GTs (Table 3) (Gamon et al. 1995; Jones and Vaughan 2010). Because anthracnose was inoculated early (approximately at panicle initiation), most of the foliar damage to the plants that was observed was caused by the disease as opposed to foliar damage by other diseases (Gamon et al. 1995; Jones and Vaughan 2010). In addition, the presence of anthracnose was repeatedly confirmed on the plants by researchers on the ground. Evaluation dates later in the season (later DAPs) probably have a stronger relationship as the disease is more obvious and the range of variation between plots is greater. This expectation is borne out by the progression of correlation coefficients, as the correlations at the beginning of the season between all ground-truth methods and their respective NDVI measurements were low (< -0.55), and then progressively improved to end season values that ranged between -0.80 s and -0.90 s, depending upon the traits being correlated (Table 3). Additionally, upon examination of the raw imagery for each flight date, it was much easier to identify susceptible plots on later flight dates (109 and 118 DAP) than it was on the earlier dates (75 and 82 DAP).

Several prior studies have used NDVI data to estimate disease presence in crops (Franke and Menz 2007; Kumar et al. 2016; Pretorius et al. 2017). In Franke and Menz (2007), NDVI results were used to classify the disease severity of research plots infected with powdery mildew (*Blumeria graminis*)

and leaf rust (*Puccinia recondita*) in wheat (*Triticum aestivum*). The first date in that study had the lowest overall accuracy at 56.8%, which gradually increased to 88.6% by the last date (Franke and Menz 2007). Pretorius et al. (2017) and Kumar et al. (2016) both associated NDVI and disease incidence by identifying quantitative trait loci (QTL) that were co-identified using ground-truth estimates. In Kumar et al. (2016), a high negative correlation coefficient (-0.91) was observed between the spot blotch (*Bipolaris sorokiniana*) severity measured by field researchers and the NDVI measured at the same growth stage in wheat. Additionally, a QTL for spot blotch resistance was identified in the same interval using the NDVI and ground-truth measures of disease severity (Kumar et al. 2016). Similarly, strong relationships between NDVI and final wheat leaf stripe rust (*Puccinia striiformis*) severity were observed in Pretorius et al. (2017), and four QTL were identified for the trait as well as for NDVI, two of which were common.

3.3. Genotypic variance explained and repeatability for ground-truth and UAS measurements of anthracnose incidence in sorghum

Ground-truth anthracnose estimates could discern significant genotypic variance between plots from the first date onward (Figure 3). The genotypic variance explained increased across the course of the growth period. Additionally, the error variance abruptly decreased from the first to the second date (75 and 82 DAP, respectively) and further decreases were

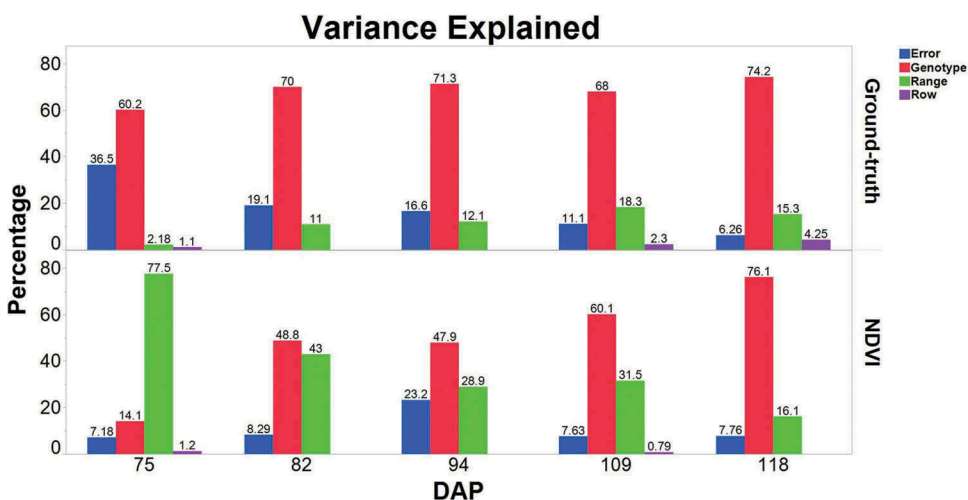


Figure 3. Variance Explained for Ground-truth and NDVI Estimates of Disease The differences in the amount of variance that were explained by visual scores (Ground-truth) and normalized difference vegetation index, or NDVI estimates of disease presence in hybrid sorghum grown in College Station, TX in 2017. Measurements were taken on five flight dates and their closest corresponding ground-truth dates during the growth period, shown as days after planting (DAP).

more gradual (Figure 3). In contrast, the UAS-derived NDVI measurements of genotypic variation were minimal but increased as the plants matured (Figure 3).

The ground-truth method was superior for early estimates of disease incidence and severity in the sorghum research plots (Figure 3). However, it was matched by the UAS later in the season, wherein the differences between plots were much easier to discern for the NDVI (Figure 3). This indicated that researchers who wished to accurately assess the disease presence within their plots using a fixed-wing UAS would need to wait for full development of the disease. The genotypic variance for the ground-truth data was not temporally linear; it varied between measurement dates (Figure 3). These fluctuations within the ground truth data were likely attributable to human error.

Spatial variation was also detected in the analysis. Interestingly, a very high amount of the variance for the NDVI data recorded at 75 DAP was explained by the range effect; one possible explanation for this is that it could be attributable to an inability of the multispectral sensor to measure the leaves that were lower in the canopy within each plot, as the typical progression of anthracnose disease begins at the bottom of the sorghum plant and moves upwards (Figure 3). Thus, while researchers on the ground might have been able to spot lesions and other signs of foliar damage on the lower leaves of a sorghum plant early on, remote-sensing techniques could not. The technique also could not differentiate between anthracnose susceptible and resistance responses. In the host pathosystem, some resistant lines may have necrosis or reddening of the leaves without the presence of acervuli, or fungal fruiting bodies, and the presence of acervuli is what indicates the successful reproduction of the pathogen. Another possible explanation for the high range effect might be that the NDVI measured differences in the field that were outside the purview of this study, including nutrient availability, water holding capacity, insect pressure, and others (Figure 3). The high range effect decreased on subsequent dates, perhaps as the spatial differences within the field began to be outweighed by differences in susceptibility between plots (Figure 3). Further experiments will be necessary to fully determine which of these explanations, if any, is correct.

For ground-truth repeatability, the estimates were moderate early but quickly increased to high as the season progressed (Table 4). A similar trend was observed in the NDVI data, with exception of the 94 DAP flight, where the repeatability dropped to a value that was closer to the estimate at 75 DAP (Table 4). Because repeatability is calculated from the genotypic and error variances, it is likely that the score was reduced on account of how close these two values were to each other on that flight date (Figure 3). The reason for the increased error and the reduced genotypic variance on that date is unknown, but possible reasons include extenuating weather and flight

Table 4. Repeatability Estimates The repeatability (R) estimates for a visual rating (Ground-truth) and normalized difference vegetation index (NDVI) calculated across the course of five flight dates and their closest corresponding ground-truth dates in sorghum. The dates where measurements were taken are shown as days after planting (DAP). Estimates were calculated using the equation, $R = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$, where R = the repeatability score, σ_g^2 = the genotypic variance, and σ_e^2 = the error variance.

DAP	Ground-truth	NDVI
75	0.62	0.66
82	0.77	0.86
94	0.81	0.67
109	0.86	0.89
118	0.92	0.91

conditions as well as image overlap issues. Nonetheless, UAS-based NDVI repeatabilities recovered and repeatabilities for the remainder of the trial were similar regardless of whether the NDVI or the visual rating was used (109 and 118 DAP) (Table 4). These results demonstrated that the UAS was as consistent as the ground-truth methodology later in the season but was less consistent during early stages of growth.

3.4. Ground-truth and UAS-derived measurements of anthracnose and their relationship with grain yield

Based upon simple linear regressions, NDVI measurements were superior for predicting final yield losses attributable to disease; indeed, the ground-truth methods (Ground-truth) lagged far behind their counterparts in this capacity (Figure 4). The daily scores were less effective than the AUDPCs that were generated from them; the individual scores were also statistically inferior and were subject to more error as they had higher root mean square error (RMSE) values (Figure 4). Thus, plant breeders who wish to predict yield loss that can be attributed to disease within their plots would perhaps be best served using the AUDPC-NDVI.

Previous studies have shown a strong relationship between disease severity and grain yield (Gaunt 1995; Savary et al. 2000). However, no studies have evaluated fixed-wing UAS for their ability to predict yield losses attributable to disease. The results of this study suggest that the AUDPC-NDVI measurement was a better indicator of disease severity during the course of the entire season as it had a stronger relationship with yield; however, it is important to note that this phenomenon could also be attributed to the fact that NDVI measures overall plant health as previously mentioned (Jones and Vaughan 2010; Li and TeBeest 2009; Rouse et al. 1974; Thomas 1996). Therefore, it is not surprising that it is a more useful predictor of yield than measurements that only estimate the presence of disease. Further study will be required to determine the exact relationship between NDVI and final grain yield in sorghum in the presence of anthracnose infection.

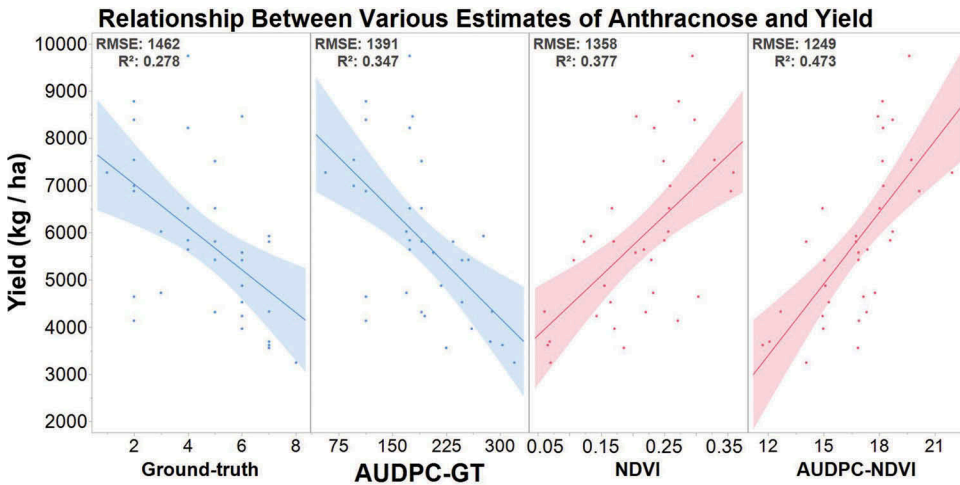


Figure 4. Regression of Disease Measurements and Yield The relationship between various measurements of disease presence and severity and final grain yield (kg/ha) in sorghum. Ground-truth measurements include the subjective visual rating (Ground-truth) that was taken daily, as well as the area under the disease progress curve generated using the same data (AUDPC-GT). Unmanned aerial system (UAS) measurements include normalized difference vegetation index (NDVI) and the AUDPC generated using NDVI (AUDPC-NDVI).

4. Conclusion

This study represents the first statistical evaluation of a fixed-wing UAS for its ability to estimate anthracnose disease incidence and severity in sorghum. Based on the results herein, UASs can potentially serve as estimators of anthracnose incidence and severity in sorghum, so long as the measurements are taken later in the growth period. Conversely, ground-truth estimates taken using a subjective visual rating are superior during early periods of growth. In addition, ground-truth methodologies can explain a higher amount of genotypic variation during early stages of growth but are quickly matched by NDVI. However, the relationship between the UAS-derived NDVI measurements and final grain yield is stronger than the correlation between yield and ground-truth disease estimates. Thus, researchers can reliably use NDVI and AUDPCs generated using NDVI as predictors of yield loss attributable to anthracnose, provided that those measurements are used at the end of growth period. Of course, this can also be attributed to the fact that NDVI measures differences between genotypes that may not be attributable to anthracnose only, as that measurement is an estimate of overall plant health. Future studies should elucidate this relationship and determine how and when these technologies should be implemented in breeding programs aimed at developing resistance to diseases. Nonetheless, this study serves as a proof-of-concept that fixed-wing UAS can be used to estimate anthracnose disease presence in sorghum breeding programs.

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