DEVELOPMENT OF A FIELD-BASED HIGH-THROUGHPUT MOBILE PHENOTYPING PLATFORM

Jared Barker III¹, Naiqian Zhang^{1*}, Joshua Sharon², Ryan Steeves², Xu Wang², Yong Wei¹, and
Jesse Poland^{2,3}

¹ Department of Biological and Agricultural Engineering, Kansas State University, 129 Seaton
Hall, Manhattan, KS, USA 66506-2906; ² Wheat Genetics Resource Center, Department of Plant
Pathology, Kansas State University, 4024 Throckmorton Plant Sciences Center, Manhattan KS,
USA 66506. ³Department of Agronomy, Kansas State University, Manhattan, KS 66502

* Corresponding author, email address: zhangn@ksu.edu

Keywords: High-throughput phenotyping; GreenSeeker; Crop Circle; Infrared thermometer;
laser distance sensor; LabVIEW

Abstract

In this study, a mobile, field-based, high-throughput phenotyping platform was developed
for rapid measurement of plant characteristics. The platform consisted of three sets of sensors
mounted on a high-clearance vehicle. Each set contained two infrared thermometers (IRT), one
ultrasonic sensor, one Crop Circle multi-spectral crop canopy sensor, and one GreenSeeker crop
sensing system. Each sensor set measured canopy temperature, crop height, and canopy spectral

reflectance of a plant plot. Thus, three plots were measured simultaneously in a single pass. In addition to the sensors, the platform was equipped with a laser distance sensor to measure the height of the sensor beam and an RTK-GPS system that provided precise, accurate position data for georeferencing sensor measurements. Software for collecting, georeferencing, and logging sensor data was developed using National Instruments LabVIEW on a laptop computer. The hardware and software design was modular, allowing easy addition and removal of sensors and flexible system expansion. The fast sampling rates for sensors allowed the phenotyper to operate in field at a ground speed of 3.2 km/h. Two verification tests were conducted to evaluate the phenotyping system. In the first test, data timestamps were analyzed to determine if the system could collect data at the required rates and if the time delays would cause significant position errors. Test results showed that data from all sensors were received within the desirable time frame and the largest position error was 17.9 cm when the phenotyper was moving at a speed of 3.2 km/h. The position errors can be corrected during data post processing. The second test determined whether changes in ambient light and ambient temperature had statistically significant effects on the accuracy of the sensor measurements. For the IRT sensors, a correction method using ground truth temperature measurement made during two periods within a day was recommended to correct the errors in surface temperature measured by the IRTs.

INTRODUCTION

In order to meet food, feed and fiber needs of a growing world population, crop-breeding methods must be improved and new technologies must be developed. One area under focus is the decoding of the genetic basis of complex traits, such as yield and drought stress tolerance, and

predicting these traits from genetic composition of lines or cultivars. In the last three decades, the genomics revolution and advances in gene technology have resulted in a wealth of genomic information (Furbank and Tester, 2011). Genotyping methods have become highly mechanized, uniform across organisms, and relatively low in cost, with cost decreasing every year (Cobb et al., 2013). However, little improvement of methods for collecting plant trait data or phenotyping have occurred in the last 30 years, especially for collecting data for single plots or plants in field-based situations (White et al., 2012). Availability of high-quality phenotypic and corresponding environmental data has become essential to understand the genotype-to-phenotype relationship. Due to lack of phenotypic data, phenotyping is currently considered the major operational bottleneck of genetic analysis (Cobb et al., 2013; Furbank and Tester, 2011). To improve complex traits through genomic selection, phenotypic data from thousands of plant varieties grown in replications under various environmental conditions is necessary, and measurements must be repeatedly taken throughout plant development in order to observe the interaction between the expression of plant traits and the environment (Cabrera-Bosquet et al., 2012; Montes et al., 2007).

Current methods for collecting phenotypic data on field-based plots require a researcher to visit each plot and manually measure specific parameters such as canopy temperature or plant height. For smaller sized trials with hundreds of plots, manual phenotyping is feasible but, depending upon methods of data collection, may be subjective and prone to human error. With larger trials of thousands of plots, manual phenotyping would become laborious, time consuming, and costly. Consequently, many breeding programs only collect yield data at the end of the growing season, missing phenotypic information that can give insight to the in-season performance and adaptation of lines (Furbank and Tester, 2011).

Therefore, a need exists for a flexible, robust, mobile, multi-sensor platform, capable of rapidly and efficiently collecting data from hundreds to thousands of plant plots, that can be used to reliably estimate phenotypic traits. The platform should be capable of handling a variety of sensors that can collect different types of data, such as plant height, canopy temperature, and various spectral readings. Furthermore, the platform should be flexible to allow additions of new sensors as they become available. It is essential for the platform to be equipped with a high accuracy and precision positioning system, such as RTK-GPS, for georeferencing, which is necessary to correctly link data to specific plots or plants.

Various plant phenotyping platforms, including ground-based and aerial-based (manned and unmanned aerial vehicles) platforms, have been developed during recent years. Ground-based platforms were developed for indoor (laboratory and greenhouse) with controlled environment and field applications. These phenotypers also can be categorized as manually driven, vehicle carried, and robotic type, based on throughput and human involvement. Most advanced, high-throughput phenotyping systems have been those developed for transnational seed companies and large public plant research institutions, such as the Australian Plant Phenomics Facility and the European Plant Phenotyping Network (Araus and Cairns, 2014). Most of their phenotyping equipment have been for indoor facilities, such as growth chambers and greenhouses, with precise environmental control (Kumar et al., 2015). An advanced phenotyping system, the LemnaTec Scanalyzer, has been used outdoor over a field of several hundred square meters. However, it required a rail-bound crane system to move the phenotyper (Lemnatec, 2015).

Several field-based, experimental phenotyping platforms have been recently developed. These platforms varied in mechanical design, sensors, data communication, and data acquisition

systems. Mechanical designs ranged from pushcarts to pulled trailers, and from self-propelled vehicles to robots. The designs also varied in sensor position adjustability, from sensors mounted to a fixed platform to sensors mounted on a height-adjustable platform. Sensor position adjustability allowed phenotyping for different crops at different growth stages. The number and types of sensors on these phenotypers varied from one or two sensors of the same type for measuring a single trait on one plot, to multiple sensors of multiple types for simultaneously measuring multiple traits on two or more plots in a single pass. Data communication and acquisition systems ranged from sensor specific data loggers with one or two ports to more general-purpose data loggers with multiple ports , and from general-purpose laptops to industrial computers running a network server equipped with a database. Compared to data loggers, computer based systems were typically more versatile due to their capability of connecting to more sensors of different interface types, whether digital or analog.

White et al. (2013) developed a proximal sensing pushcart consisting of a rectangular frame mounted on two bicycle frames. The cart was equipped with a single set of monochrome camera, ultrasonic proximity sensor, infrared thermometer, radiometer, and a GPS receiver connected to CR1000 and CR3000 data loggers (Campbell Scientific, Inc., Logan, Utah, USA) for collecting data at 1 Hz and 5 Hz, respectively. The pushcart was more labor intensive to operate compared to motorized platforms. Crain et al. (2015) developed a hand-held phenotyper (“PhenoCorn”) with a GreenSeeker, an IRT, and a web cam. The sensors were connected to a laptop through USB ports and was controlled by a LabVIEW program (National Instruments, Austin, Texas, USA). Measurement data were geo-referenced using a GPS receiver with OmniSTAR satellite position correction (Trimble Navigation). The PhenoCorn was designed to measure only one plot of crop and it was labor intensive. Montes et al. (2011) developed a

phenotyping platform to study the use of light curtains and spectral reflectance sensors to simultaneously measure height and spectral reflectance of four rows of maize during the early development stage. These measurements were then used to estimate biomass. This platform was mounted on a mini-tractor system with fixed sensor positions. The platform was able to operate at a speed of 1.0 km/h, but sensor data was not georeferenced with GPS and a 3 second break between measurement sets was required to finalize the recorded data. Busemeyer et al. (2013) developed BreedVision, a phenotyping platform for small grain cereals up to a plant height of 1.6 m. The platform consisted of a variety of sensors, including 3D time-of-flight cameras, color cameras, laser distance sensors, hyperspectral imaging systems, light curtains, and a GPS receiver, all mounted on a tractor-pulled trailer platform that was height adjustable. BreedVision was capable of phenotyping one plot in a single pass. It used an industrial PC with a MySQL database equipped network server for data storage. The interfaces between sensors and the PC system were through USB and Ethernet. Each sensor was connected to the PC through an individual microcontroller. The system developed by Andrade-Sanchez et al. (2014) contained four sets of sensors mounted on a high-clearance vehicle and was capable of measuring four plots of plants simultaneously. Each sensor set included an ultrasonic sensor, two infrared thermometers (one pointing at nadir, one pointing 30° from the vertical axis), and a Crop Circle ACS-470 multi-spectral crop canopy sensor. Three data loggers were used for data collection and storage. This system collected data at 1Hz. Comar et al. (2012) developed a tractor mounted boom platform for high throughput phenotyping wheat cultivars in field conditions. The boom was mounted 1.5 m above the crop canopy. The system used a hyperspectral radiometer and two RGB cameras to observe the canopy from both nadir and oblique views at 57.5° zenith angle. Only one plot could be phenotyped at a time and about 100 micro-plots could be sampled per

hour (Comar et al., 2012). Svensgaard et al. (2014) developed PhenoField, a box shaped phenotyping platform transported via a jib telescopic arm mounted to the back of a tractor. The PhenoField contained a 5 Mpix CCD camera mounted within the box and was illuminated by an LED panel that produced lights of nine wavelengths (from 465 to 850 nm). The unique feature of PhenoField was its complete blocking of ambient wind and ambient light during phenotyping, however this also made it slow to operate, covering only 80 m² per hour. The box structure also prevented flexible system expansion. The “Phenomobile” developed in Australia allowed various types of sensors to be used for field phenotyping. The phenotyper can be driven by three types of drive mechanisms – a pushcart type (“Phenomobile Lite”), a self-propelled high-clearance vehicle, and a robotically driven system. The wheel base and sensor height were adjustable to accommodate different plot width and desirable field of view. Only one plot can be measured in a single path (HRPPC, 2015).

The overall objective of this work was to advance phenomics through the development of a modular sensor platform for field-based, high-throughput phenotyping. It was envisioned that the developed platform would serve as a model and resource for future phenotyping platform designs.

The specific objectives were as follows:

1. Assemble a modular sensor system composed of three sets of GreenSeekers, Crop Circles, infrared thermometers, distance sensors, and two GPS receivers on a high-clearance field vehicle.
2. Develop modular software for interfacing with various types of sensors to collect, geo-reference, format, and save data from the sensors into a convenient format for further processing and analysis.

3. Test and verify that the developed platform timely collects, geo-references, formats, and saves data from the sensors and GPS.

4. Determine if changes in ambient light or ambient temperature significantly affect sensor readings.

MATERIALS AND METHODS

System Design

Basic requirements for the phenotyper included assembly of a mobile sensor platform for three sets of sensors and two GPS units, collection of data from the sensors, and data georeferencing and saving. The system was designed to measure plant canopy temperature, plant height, and canopy reflections at various spectral wavelengths at a rate of 10 samples per second.

The system consisted of sensors, GPS units, and data acquisition hardware mounted on a Bowman Mudmaster sprayer (Bowman Manufacturing Co., Inc., Newport, Ariz., USA) (Figure 1). The spraying boom was replaced with a sensor bar. The phenotyper had a clearance of 1.6 meters, allowing it to pass over a variety of field crops, such as wheat and soybean. The sensor bar can be moved up and down while maintaining level status, thus maintaining sensor orientation relative to the ground.

Figure 1. Phenotyping platform consisting of sensors, GPS units, and data acquisition hardware mounted on a high clearance vehicle

Three sets of sensors were mounted on the sensor bar for simultaneous data collection from three plant plots. The spacing between the sensor sets was adjustable. Each sensor set (Figure 2) contained a GreenSeeker (Trimble, Westminster, Colo., USA), a Crop Circle ACS-470 (Holland Scientific, Inc., Lincoln, Neb., USA), a Banner U-GAGE Q45U Ultrasonic Sensor (Banner Engineering Corp., Minneapolis, Minn., USA), a Micro Epsilon 15:1 CX-SF15-C8 IRT (Micro-Epsilon, Raleigh, N.C., USA), and a Micro Epsilon 2:1 CTH-SF02 IRT (Micro-Epsilon, Raleigh, N.C., USA). These sensors were all active sensors.

Figure 2. One sensor set composed of (left-to-right) two IRTs, one ultrasonic sensor, one Crop Circle, and one GreenSeeker

Within each set, the sensors were spaced to prevent interference with each other. The IRTs were used to measure canopy temperature. The difference between the two IRTs was in their fields of view (FOV). The 15:1 IRT had a circular FOV with a 6.6 cm diameter when placed 1 m from the target, whereas the FOV for the 2:1 IRT was a circle with a 50 cm diameter at the same height (Micro-Epsilon, 2015a; Micro-Epsilon, 2015b). The two IRT sensors were placed side-by-side so that the areas measured by them overlapped.

The GreenSeeker and Crop Circle sensors were used for measuring spectral reflectance from the plant canopy at different wavelengths. Both GreenSeeker and Crop Circle were active sensors. GreenSeeker used lights of two wavelengths (774 nm and 656 nm) so that the measurements could be used to form the NDVI. The Crop Circle used white light that covered the visible band and a portion of NIR so that optical filters at different wavelengths could be used to form other vegetative indices, making the measurement more flexible. The two sensors had different FOVs: At a height of 1 m from the object, the FOV for the GreenSeeker was a

rectangle of 61x1.5cm, and that for Crop Circle was a rectangle of 54x25cm (NTech Industries, Inc., 2008; Holland Scientific, 2015).

A Banner LT3NUQ laser distance sensor (Banner Engineering Corp., Minneapolis, Minn., USA) was mounted on the sensor bar in front of the left wheel track to measure the sensor bar height from the bare ground.

Both the ultrasonic and the laser sensor can be used to measure the distance between the sensor and an object with a reflective surface. In general, the field of view (FOV) of the laser sensor was much smaller than that of the ultrasonic sensor because of the small divergence angle of the laser beam. When placed 1 m from the object, the FOV of the laser sensor was a circle of 0.6 cm in diameter whereas that of the ultrasonic sensor was 40cm in diameter (Banner Engineering, 2015; Banner Engineering, 2005). A larger FOV would allow measurement of average distance between the sensor and a large, uneven surface, such as plant canopy. This was why we used the laser sensor to measure the sensor height from the bare ground and the ultrasonic sensor the distance from plant canopy. Combining these two measurements, the plant height can be determined. Usually, the laser sensor was strongly affected by sunlight, whereas the ultrasonic was more sensitive to temperature variations. Table 1 lists the major specifications of the sensors.

Table 1. Summary of major specifications of the sensors.

Sensor Type	Model	Major specifications	Power Input	Output Signal
Ultrasonic Sensor	Banner U-Gage Q45U	Measurement range: 0.25–3 m	15-24 V DC	0-10 V DC

IRT	Micro Epsilon CX-SF15-C8 15:1	Measurement range: -30–150 °C Spectral range: 8-14µm	5-28 V DC	With USB Kit – RS232, 9600 baud, 8-n-1
IRT	Micro Epsilon 2:1 CTH-SF02	Measurement range: -40–975°C Spectral range: 8-14µm	8-36 V DC	With USB Kit – RS232, 9600 baud, 8-n-1
Spectral Reflectance Sensor	Holland Scientific ACS 470 Crop Circle	Multiple Spectral Wavelengths: 550nm, 670nm, 760nm Field of view: 30° x 14°	11-17 V DC, 350 mA	RS-232, Auto Send Mode, 76800 baud, 8-n-1
Spectral Reflectance Sensor	Trimble GreenSeeker 500 with RT200	Spectral Wavelengths: 770nm, 656 nm Field of view: 61x1.5 cm rectangle for height 61-157cm	12 V DC, 300 mA	RS-232, 38400 baud, 8-n-1
Laser Distance Sensor	Banner LT3NUQ	Measurement range: 0.3-5mwavelength: 658 nm Beam diameter: 6 mm	12-24 V DC, 108 mA	0-10 V DC

228

229 In addition to the sensors, a Trimble AG 25 GPS antenna was mounted on each end of the
230 sensor bar to determine the sensor bar leveling status and to georeference data from each sensor
231 through triangulation. The antennas were connected to two Trimble FmX integrated displays,
232 which processed GPS signals and received RTK corrections from a Trimble AgGPS 542 RTK
233 base station (Trimble, Westminster, Colo., USA).

234 Prior to hardware and software development, selection of the data acquisition system was
235 essential. Two options were considered: 1) a Campbell Scientific C3000 data logger that
236 contained a large number of analog input ports but only four RS-232 ports and 2) a laptop
237 computer with a PC-based data acquisition system that provided extensible communication ports.
238 Due to the requirements for high throughput and a large number of serial/communication ports to

achieve the modular design, the laptop computer option was selected. The data acquisition system used was a National Instruments NI 9207 analog input module, which was connected to the laptop through USB. The software was developed using National Instrument's LabVIEW Professional software package (National Instruments, Austin, Texas, USA).

Several types of hubs were mounted inside a protective box. The hubs extended the number of ports of the laptop computer that ran software to collect sensor and GPS data. Figure 3 illustrates the connections between the sensors, hubs, and the laptop.

Figure 3. Three communication hubs and one analog data acquisition module routing signals from sixteen sensors and two GPS receivers to a personal computer.

The NI 9207 analog input module was used to interface analog voltage signals from the three ultrasonic sensors with the laptop through a USB port. The module had 16 channels, 8 of which were used to measure signals between ± 10 V, and the remaining channels measured current signals between ± 20 mA. This added flexibility to the phenotyper to handle sensors that produce voltage or current signals.

The entire system was powered by two 12 V batteries connected in parallel. A 12 V to 110 V inverter provided power to the laptop. The laptop was mounted on a platform on the right side of the driver's seat. The two Trimble FmX units were located next to the laptop, each connected to a GPS antenna. A Planar PCT2265 22" Touch Screen Monitor (Planar Systems, Inc., Beaverton, Ore., USA) was mounted on a swivel arm. The touch screen monitor equipped the system with a large display for system control and monitoring, allowing the user to quickly and easily navigate the phenotyping software.

Software Development

The LabVIEW phenotyper software consisted of separate modules. Each type of module was responsible for interfacing with and collecting data from a specific type of sensor (National Instruments Corporation 2013). The main program was a collection of continuous loops, each containing one instance of a sensor module, which received data from a specific sensor, displayed the data in custom gauges and indicators, and saved the data into a formatted text file.

Modular Software Design

Sensors

Each sensor software module performed the tasks of interfacing and receiving data from a specific type of sensor. The modules differed in the specifics of communication with their respective sensors due to differences in baud rate and data strings, but the design of each module was very similar.

Each type of sensor module was created within LabVIEW as a virtual instrument (VI) using LabVIEW's Instrument I/O Assistant (IA). The IA created a VI for interfacing with a specific communication port (COM port), to which the sensor was connected for extracting data from the sensor data strings. Prior to using the IA, the COM port was configured to the required settings of the sensor. Once the connection was established within the IA, the data string received from the sensor was parsed to extract the sensor data, such as NDVI or temperature. The generated VI or sensor module was then added into the main program as a subVI.

Within the main program, each sensor module was placed into a loop. The loop rate was important and must be faster than the sampling rate of the sensor. If it was slower, incoming data would accumulate in the buffer and would not be read in real-time, resulting in incorrect georeferencing. The buffer would continue to fill, eventually resulting in data loss.

Positioning System

The source of GPS data was the two Trimble FmX units. The FmX units were configured to output RTK corrected global positioning system fix data (GGA) strings at 5 Hz, the maximum rate. Data from all sensors were collected at a rate of 10 Hz, twice as fast as the incoming GPS data. Therefore, more than one sensor data point could be georeferenced to a single position data point.

The FmX units were connected to the RS-232 hubs via DB-9 connectors, and the hubs were connected to the laptop computer via USB. Four pieces of data were extracted: UTC, elevation, latitude, and longitude. A virtual instrument was created from the generated code and two instances of it were added to the main program, one for each FmX unit.

Main Program

The main program was composed of multiple loops that can be timed and run independently or simultaneously on multi-processor computers. The loops can be divided into three categories: button-servicing loops, sensor loops, and write-to-file loops. A button-servicing loop was for checking the value of user-interface control buttons, such as the main on/off button and buttons for turning on/off individual types of sensors. The button-servicing loop rate was set to 1 Hz. For every sensor, including the GPS receiver, there was a sensor loop that contained a specific sensor module. The timing of the sensor loop was critical because, if it was consistently slower than the rate at which the sensor sent data, the data would not be correctly georeferenced, and would eventually be lost. This occurred when the port was not serviced frequently enough. The sensors and GPS receivers were set to send data at 10 Hz and 5 Hz, respectively. The GreenSeeker loops, Crop Circle loops, and distance sensor loop were set to a maximum of 20 Hz. IRT loops were set to poll IRT sensors at 10 Hz. There were a total of five write-to-file loops, one for each type of sensor and one for GPS receivers. These loops were responsible for writing the sensor and position data to text files.

The front panel of the main program was divided into four sections or tabs: Setup, Monitor, GPS, and Raw Data. The Setup tab contained the control buttons, distance sensor calibration settings, and the field and sensor row spacing settings. The Monitor tab contained the custom gauges for graphically viewing incoming sensor data. The GPS tab showed a graph of

position data points from the left and right GPS receivers. The Raw Data tab contained tables, one for each type of sensor that displayed the five most recent data points.

Because of the fast sampling rates, the phenotyper can move in the field at a ground speed of up to 4.0 km/h while phenotyping three plant plots simultaneously. However, to insure safety and smooth operations, a ground speed of 3.2 km/h has been used in field operations.

Uniqueness of the Platform Design

The phenotyper developed in this study combined the advanced features of existing phenotypers reported in the literature. These features were: (1) it was built on a high-clearance tractor that was easily maneuverable in field; (2) it allowed the use of a broad range of sensors with various types of signals – analog (voltage and current) and digital; (3) it used a fast sampling rate (10 Hz) for the sensors, allowing a high vehicle ground speed (about 3.2 km/h); (4) it allowed simultaneous phenotyping in three plant plots in field; (5) it allowed flexible adjustment of sensor height and sensor position; and (6) it provided real-time monitoring of the sensor outputs via a user-friendly interface. Apparently, both features (3) and (4) contributed to the significant increase in phenotyping throughput.

Perhaps the most significant, unique feature of this phenotyper was its modular design, which allowed (1) fast formation a new phenotyping system, (2) selective control of data collection and recording from specific sensors, (3) easy addition and removal of sensors to/from the system and (4) flexible expansion and upgrading of the system. The large number of communication ports available, either digital or analog, allowed various types of sensors to be introduced into the system. The modular design of the software allowed each sensor to be added to the system as a module. When a sensor was added, the only effort needed was to configure the

sensor based on its signal type. No change on the main program was needed. This feature could not have been implemented using data loggers, which in general were limited in I/O and processing capabilities.

Verification Test Procedure

System Timing and Position Errors

The objective of this test was to evaluate the timeliness of sensor and position data collection in order to determine position error of the sensor data in relation to GPS position coordinates. Delays in reading-in sensor and GPS data, output rate of the sensors and GPS receivers, and ground speed of the phenotyper all affected accuracy of location data that was logged with each sensor data point. In general, sensor data and position data were not received simultaneously because the phenotyping software had no control over when sensors sent data and any delays that existed in the communication hardware. The sensors were configured to send data at a rate of 10 Hz, and the Trimble FmX units were configured to send position data at a rate of 5 Hz, which was the maximum rate. Furthermore, since sensor data was received twice as often as position data, two sensor data points were assigned identical position coordinates. Since the phenotyper moved forward during data collection, the position error due to differences in sampling time were proportional to phenotyper speed.

Because time differences in receiving sensor and positions data were independent of phenotyper speed, this test was performed while the phenotyper was stationary. Three 20-minute trials were conducted. For each trial, the phenotyping software logged the sensor and position data along with several timestamps for each sampling period. These timestamps were the time

when the software was ready to read the data, the time when it actually read or received the data, and the time when it wrote the data to the text file. These timestamps were based on the computer system's millisecond clock available through the operating system.

Determining Effects of Ambient Light and Ambient Temperature

Calibration procedures for the five types of sensor used on the phenotyper can be found in the user manuals of the sensors (Trimble Navigation Limited, 2008; Holland Scientific, 2015; Banner Engineering Co., 2015; Micro-Epsilon, 2015a; Micro-Epsilon, 2015b). This study, however, was concentrated on the effects of ambient conditions on the performance of the sensors.

Throughout the phenotyping season and during a single phenotyping session, sensors were subjected to varying ambient light intensities and ambient air temperatures due to weather, time of day, duration of phenotyping, direction of travel, and sensor position. Thus, it was important to determine the effects of changes in ambient light and ambient temperature on sensor readings.

Because this test was conducted in one day, ambient light and air temperature were strongly correlated. The GreenSeeker, Crop Circle, and laser distance sensor operated in the visible and near-infrared band. Thus, the effect of ambient light condition was considered more influential than ambient temperature to their performance. On the other hand, the ultrasonic sensor was affected more by ambient temperature (Banner Engineering, 2015). The IRT sensors measured radiations in the mid-infrared band (8-14 μm) (Micro-Epsilon, 2013) and, therefore, were not affected by sun light. Based on these considerations, the effect of ambient light intensity was analyzed only for GreenSeeker, Crop Circle, and the laser sensor. For the ultrasonic sensor and the IRTs, only the effect of ambient temperature was analyzed. This test

was performed outdoors, with the phenotyper parked facing south to avoid self-shadowing. The sensor boom was raised to 100 cm above the ground and supported at two ends to maintain constant height. A 182.9 cm by 243.8 cm piece of green rug, made of a non-transparent plastic material, was placed on the ground, centered underneath the left set of sensors. A tarp and canvas sheet was placed underneath the rug to keep it dry. A T-type thermocouple was taped on the rug's surface to measure rug temperature and another T-type thermocouple was used to measure ambient air temperature. Both thermocouples were connected to a Campbell Scientific CR850 data logger (Campbell Scientific, Inc., Logan, Utah, USA), which was programmed to send temperature measurements through the serial port at a rate of 1 Hz. The ambient light intensity was assessed by the illuminance (in lux) received over the rug area. A Sper Scientific 850007 (Sper Scientific, Scottsdale, Ariz., USA) visible light logger was placed on the rug, facing up, pointing at the zenith, to measure the illuminance. This light meter sent light illuminance measurements in lux at a rate of 1 Hz through its RS-232 port.

All sensor data was logged on a laptop computer through a LabVIEW program. Data was collected once every ten seconds. The test was conducted on a clear, sunny spring day with no wind. Test data was analyzed to determine any significant relationships between sensor readings and ambient light intensity or ambient temperature. The test set up is displayed in Figure 4.

Figure 4. Ambient light and temperature test setup

RESULTS AND DISCUSSION

Position Errors

The phenotyping software had no control of when data was generated and sent from the sensors and GPS receivers and when the data arrived at the communication ports. Data can arrive and be read in any order and at any time within controlled intervals. With the phenotyper moving, the difference between the times when the sensor data and the position data were generated can create position errors for the sensor data. Because the exact moments when the sensor and GPS data were generated were unknown, the difference between the times when the phenotyper software received the sensor data and the position data can be used as an approximation.

Results of all three tests indicated that the average difference between the times the sensor data and position data were received was approximately 50 ms (Figure 5), with a standard deviation of approximately 30 ms. If the phenotyper was moving at 3.2 km/h, the position error this time delay caused was approximately 4.5 cm. An average of 50 ms time delay was to be expected since the sensor data was received at 10 Hz and position data at 5 Hz. If the sensor data and position data were perfectly synchronized, the time differences for consecutive samples would alternate between 0 ms and 100 ms, with an average of 50 ms. The minimum time difference found was 0 ms, indicating that instances occurred when sensor data and position data were received at the same time. This finding also confirmed that, on the specific laptop computer used for the tests, multiple loops were running simultaneously. The maximum time difference varied between sensors and trials, but it was typically less than 200 ms. Figure 6 shows acquired NDVI data from the GreenSeeker in an experimental field with 1.2 x 2.85 m blocks. There were two rows of data points in each block because the phenotyper passed each block twice. For each pass, about 17 data points were obtained within each block and the average distance between adjacent data points was 15.8 cm, which was much larger than the average position error of 4.5 cm. With the two data points near the block boundaries removed, we can believe that the remaining 15 data points were from within the block.

Figure 5. Boxplots for signal delays measured for five sensors on the phenotyper.

Figure 6. NDVI measured using the GreenSeeker when the phenotyper passed the field twice.

The position error of each sensor data point can be calculated through interpolation and then used to correct the position at which the data was taken so that each data point can be assigned to the correct field plot. The alternative, also implemented by phenotyping software, was to tag each data point with the most recent position data. By doing this, the time difference between position data and sensor data ranged from 0 to approximately 200 ms, which corresponded to a position error of 17.9 cm at a phenotyper speed of 3.2 km/h. Reducing the phenotyping speed or using a GPS system that is capable of providing position data at a rate of 10 Hz can reduce this error.

Effects of Ambient Light and Ambient Temperature

The effects of ambient light intensity on the readings from GreenSeeker, Crop Circle, and laser distance sensor, and that of ambient temperature on the ultrasonic sensor and the IRTs were tested. The range of ambient light intensity and ambient temperature tested were 0 – 100,000 lux and 5.8 – 22.5 °C, respectively.

GreenSeeker

A linear regression analysis on the effect of ambient light intensity on NDVI readings from the Green Seeker (Figure 7) showed a small negative slope. The slope was -4.58×10^{-7} NDVI/lux and it was proven statistically significant by a near zero p-value for the t-test. This slope can be translated as a reduction of 0.046 in NDVI from night (approximately 0 lux) to the brightest time in the day (approximately 100,000 lux). If measurement was taken between 9 am and 3 pm (with a change of approximately 40,000 lux), the change in NDVI readings, as affected by the ambient light, would be approximately 0.018. Kim et al. (2010) in a similar study found no significant changes in NDVI readings due to changes in artificial illumination, but did observe that, as solar radiation increased, NDVI readings were reduced. The maximum deflection in NDVI due to a change in light intensity of approximately 150,000 lux was 0.08. Their finding was consistent with our observation.

Figure 7. Fitted plot of GreenSeeker data vs. ambient light intensity

Crop Circle

The Crop Circle data string contained five pieces of information: a user selectable vegetation index (VI), a second VI, Channel 1 reflectance (670 nm), Channel 2 reflectance (760 nm), and Channel 3 reflectance (550 nm). The relationship between Channel 1 readings and ambient light intensity (Figure 8) were statistically significant. However, the slope was only - $1.351\text{e-}08$ reflectance/lux, indicating that, from the darkest night to the brightest noon, the difference would only be 0.0013. The results were similar for Channels 2 and 3.

Figure 8. Fitted plot of Crop Circle Channel 1 reflectance (670 nm) vs. light intensity

The NDVI calculated using the reflectance of the 670 nm (red) and the 760 nm (near infrared) wavelengths was found not significantly affected by ambient light intensity.

Laser distance sensor

Figure 9 shows the laser distance sensor readings under different ambient light intensities ranging from 0 to 500,000 lux. It can be seen that, above 10,000 lux, the laser distance sensor cannot consistently detect the distance. When the ambient light intensity was high, the reading defaulted to the maximum distance limit set at 150 cm.

Figure 9. Plots of raw laser distance sensor data vs. light intensity

A linear regression analysis of distance measurement against ambient light intensity was conducted after removing data points that defaulted to 150 cm (Figure 10). Regression results indicated that a statistically significant relationship existed between the measured distance and light intensity. The slope was $4.332\text{e-}05 \text{ cm}\cdot\text{lux}^{-1}$, however, only 5.9% ($R^2=0.0588$) of the change in the distance sensor reading can be explained by light intensity. The mean of the laser distance measurements was 89 cm, with a range between 85 and 92 cm, and a standard deviation of 0.93 cm.

Figure 10. Fitted plot of laser distance sensor data vs. ambient light intensity

Ultrasonic Sensor

The relationship between ultrasonic sensor distance readings and ambient temperature were found to be statistically significant. The slope with respect to ambient temperature was $6.275 \times 10^{-2} \text{ cm} \cdot ^\circ\text{C}^{-1}$. If the temperature increased by 20°C , the distance measured would increase by 1.25 cm.

IRT

Measurement errors of the IRTs were represented by the difference between the surface temperature measured by the IRT and that measured by the thermocouple, ΔT . A ΔT of zero indicated an accurate measurement.

Data used in the analysis was taken between 12:30 PM and 8:30 PM on the day of experiment. During this period, the ambient light intensity varied between 0 and 95,000 lux. The range of ambient temperature was $9.7\text{--}21.2^\circ\text{C}$ (Figure 11). The ambient temperature compensation function of the IRTs was activated during the test.

Figure 11. Surface temperature measured by the 15:1 IRT and the thermocouple with ambient temperature and ambient light monitored

The following trends can be observed from this figure: (1) The IRT seemed to have underestimated the surface temperature when the ambient air temperature was low but overestimated it when it was high. (2) When the air temperature was high, the IRT signal was noisy.

Regression analyses showed a statistically significant effect of ambient air temperature on the measurement errors for both IRTs. When the surface temperatures measured using the two IRTs were plotted against that measured using the thermocouple (blue dots in Figures 12 and 13), the following trends were observed: (1) Both plots showed clear linear patterns. In fact, this was proven by a linear regression analysis, which gave R-square values of 0.9949 and 0.9961 for the 15:1 and 2:1 IRTs, respectively. (2) The overestimation of surface temperature by both IRTs in the high temperature region and underestimation in the low region indicated the needs for adjusting both the slope and intercept of the regression lines.

Figure 12. Surface temperature measured by the 15:1 IRT before (blue dots) and after (green dots) correction. The red line is the ideal 1:1 line

Figure 13. Surface temperature measured by the 2:1 IRT before (blue dots) and after (green dots) correction. The red line is the ideal 1:1 line

Possible causes for these errors may include (1) The emissivity of the measured surface was not correctly selected for sensor setting. (2) The temperature compensation function, which used a temperature sensor embedded in the IRT sensor to measure the sensor temperature, was not able to correct the temperature reading errors completely.

Emissivity of the measured surface was often difficult to determine and its value may vary throughout the experiment. Furthermore, the temperature compensation function of the IRT sensor was internal, over which the operator had no control. Thus, we used “ground truth” measurement – surface temperature measured using a thermocouple embedded on the rug surface - to correct the sensor readings. The ground truth measurement can be done many times.

However, a minimum of two sets of measurement were needed to allow correction of both the slope and intercept of the regression line. To cover a wide range of surface temperature, it was preferred to measure the ground truth temperature during the warmest and the coldest periods within the day.

Figures 12 and 13 show the uncorrected (blue dots) and corrected (green dots) surface temperatures measured by the 15:1 and 2:1 IRTs, respectively. The ground truth surface temperatures used were ten measurements taken by the thermocouple around 3:00 pm and other ten taken around 8:40 pm, the warmest and coldest periods during the experiment in that day. IRT measurements taken during the same periods were used in regression analyses to obtain the corrected slopes and intercepts.

Table 2 lists the statistics of surface temperature measurement error by both 15:1 and 2:1 IRTs, before and after correction. Obviously, the measurement accuracy was greatly improved using this correction method.

Table 2. Surface temperature measurement error statistics for both the 15:1 and 2:1 IRTs

		<i>Error mean (°C)</i>	<i>Root mean square error (°C)</i>	<i>Error minimum (°C)</i>	<i>Error maximum (°C)</i>
15:1 IRT	Before correction	1.99	4.31	-4.75	12.21
	After correction	-0.45	1.29	-6.57	4.85
2:1 IRT	Before correction	6.68	6.49	-3.63	22.62
	After correction	0.02	1.10	-4.76	7.83

Based on the IRT's instruction manual (Micro-Epsilon, 2015a and 2015b), the slope and offset can be adjusted for data post-processing if a digital interface was added to the IRT. However, correction using these fixed values would not compensate for dynamic temperature changes of the sensor body, which was directly affected by ambient air temperature.

In order to reduce the effect of ambient temperature on measurement accuracy, the IRT manufacturer suggested that the IRT sensor be covered by a shield so that the sensor body was blocked from direct sun light. The IRT also provided a warning sign when the ambient temperature exceeded the proper range for measurement. These measures need to be taken to further ensure correct temperature measurement.

The phenotyping platform developed in this study has been demonstrated to accurately log sensor measurements and GPS position information. This platform has been used for phenotyping in wheat and soybean experimental fields during the past three seasons, and has demonstrated great potential for broad application to address a range of questions regarding crop canopy characteristics on small plot research. Such information can be leveraged for a more detailed physiological understanding of crop growth and development in plant breeding programs. For large populations needed for crop genomics studies, these detailed measurements can be rapidly taken on thousands of plots, enabling genomics studies on a scale not previously possible.

SUMMARY AND CONCLUSIONS

A mobile, field-based, high-throughput phenotyping platform was developed. A sensor platform consisting of three sensor sets was assembled and mounted on a high-clearance vehicle. Each sensor set was composed of two infrared thermometers with different field of views, one ultrasonic sensor, one Crop Circle sensor, and one GreenSeeker sensor. Two GPS receivers and a laser distance sensor were also installed. The sensor sets were connected to a laptop computer through a set of hubs. Software to interface with the sensors, collect sensor and position data, and to georeference sensor data was developed using National Instruments LabVIEW. The software featured a modular design approach with each module developed to interface with a type of sensor.

A system verification test was conducted to test the capability of the developed system in timely collecting sensor and position data and to observe the effects of ambient light and temperature on the performance of the sensors.

The following conclusions can be drawn from these tests:

1. The multiple sensor communication hubs and modular design of the software allowed easy addition and removal of various sensor modules and flexible system configuration and alternation.
2. The average difference between the times the sensor data and position data were received was found to be approximately 50 ms, with a standard deviation of approximately 30 ms, which would cause position error of 4.5 cm for the sensor readings if the phenotyper was moving at 3.2 km/h. The maximum time difference varied between sensors and trials, but it was typically less than 200 ms.

3. Test results indicated statistically significant effect of ambient light intensity on readings from the GreenSeekers and Crop Circles sensors and statistically significant effect of ambient temperature on readings from the ultrasonic sensor. Whether or not these effects are significant from an engineering or a scientific perspective should be evaluated based on the specific application of the sensors.
4. When the laser distance sensors was aimed at bare soil, it cannot consistently measure distance when the ambient light intensities was above 20,000 lux.
5. The 2:1 and 15:1 IRT temperature readings were found to be significantly affected by ambient temperature. A correction method using ground truth measurement conducted during the warmest and coldest periods in a day was recommended to reduce the IRT surface temperature measurement error.

ACKNOWLEDGEMENTS

This work was supported through the National Science Foundation (NSF) Plant Genome Research Program (PGPR) (Award IOS-1238187), the Kansas Wheat Commission and Kansas Wheat Alliance and the US Agency for International Development (USAID) Feed the Future Innovation Lab for Applied Wheat Genomics (Cooperative Agreement No. AID-OAA-A-13-00051). The graduate student working in this project was supported by the National Science Foundation, through the Graduate STEM Fellows in K-12 Education (GK-12) Program, under Grant No. DGE-0948019.

REFERENCES

- Andrade-Sanchez, P., M. Gore, J. Heun, K. Thorp, A. Carmo-Silva, A. French, and J. White
2014. Development and evaluation of a field-based high-throughput phenotyping
platform. *Functional Plant Biology*, 41(1), 68-79.
- Araus, J. L. and J. E. Cairns. 2014. Field high-throughput phenotyping: the new crop breeding
frontier. *Trends in Plant Science*, January 2014, Vol. 19, No. 1, 52-61.
- Banner Engineering Co. 2005. LT3 long-range time-of-flight sensor. Minneapolis, MN, USA
- Banner Engineering Co. 2015. U-Gage analog Q45 long-range ultrasonic sensors. Minneapolis,
MN, USA
- Busemeyer, L., D. Mentrup, K. Möller, E. Wunder, K. Alheit, V. Hahn, and A. Ruckelshausen.
2013. Breedvision—A multi-sensor platform for non-destructive field-based phenotyping
in plant breeding. *Sensors*, 13(3), 2830-2847.
- Cabrera-Bosquet, L., J. Crossa, J. von Zitzewitz, M. Serret, and L. Araus. 2012. High-throughput
Phenotyping and Genomic Selection: The Frontiers of Crop Breeding
ConvergeF. *Journal of integrative plant biology*, 54(5), 312-320.
- Cobb, J., G. Declerck, A. Greenberg, R. Clark, and S. McCouch. 2013. Next-generation
phenotyping: requirements and strategies for enhancing our understanding of genotype–
phenotype relationships and its relevance to crop improvement. *Theoretical and Applied
Genetics*, e126(4), 867-887.
- Comar, A., P. Burger, B. de Solan, F. Baret, F. Daumard, and J. Hanocq. 2012. A semi-automatic
system for high throughput phenotyping wheat cultivars in-field conditions: description
and first results. *Functional Plant Biology*, 39(11), 914-924.

649 Crain, J.L., Wei, Y., Barker III, J., Thompson, S.M., Alderman, P.D., Reynolds, M., Zhang, N.,
650 and Poland, J. 2015. Development and deployment of a portable field phenotyping
651 platform. *Crop Science*. Accepted 2015.

652 Furbank, R. and M. Tester. 2011. Phenomics—technologies to relieve the phenotyping
653 bottleneck. *Trends in plant science*, 16(12), 635-644.

654 Holland Scientific. 2015. Crop Circle ACS-470 Active Crop Canopy Sensor. Lincoln, NE, USA
655 HRPPC. 2015. Phenomobile. Retrieved from
656 <http://www.plantphenomics.org.au/services/phenomobile/>

657 Kim, Y., D. Glenn, J. Park, H. Ngugi, and B. Lehman. 2010. Active spectral sensor evaluation
658 under varying condition. *ASABE Paper No.* 1009111.

659 Kumar, J., A. Pratap, and S. Kumar. 2015. Phenomics in Crop Plants: Trends, Options and
660 Limitations, (pp.285-296). New Delhi Heidelberg New York Dordrecht London:
661 Springer

662 Lemnatec. 2015. Field Phenotyping. Retrieved from
663 <http://www.lemnatec.com/applications/field-phenotyping/>
664

665 Micro-Epsilon. 2013. Basics of non contact temperature measurement. Raleigh, NC, USA
666 Micro-Epsilon. 2015a. Operation manual thermoMETER CT/CTF/CTH/CTM-1/CTM-2/CTM-3.
667 Ortenburg, Germany

668 Micro-Epsilon. 2015b. Operation manual thermoMETER CX. Ortenburg, Germany

669 Montes, J., A. Melchinger, and J. Reif. 2007. Novel throughput phenotyping platforms in plant
670 genetic studies. *Trends in plant science*, 12(10), 433-436.

671 Montes, J. M., Technow, F., Dhillon, B. S., Mauch, F., & Melchinger, A. E. 2011. High-
 672 throughput non-destructive biomass determination during early plant development in
 673 maize under field conditions. *Field Crops Research*, 121(2), 268-273.
 674 National Instruments Corporation. 2013. NI 9207. Austin, TX, USA
 675 NTech Industries, Inc., 2008. GreenSeeker model 500 series. Ukiah, CA, USA
 676 Svensgaard, J., Roitsch, T., and Christensen, S. 2014. Development of a mobile multispectral
 677 imaging platform for precise field phenotyping. *Agronomy*, 4(3), 322-336.
 678 Trimble Navigation Limited. 2008. AgGPS 432/442 Receivers. Trimble Navigation Limited,
 679 Sunnyvale, California
 680 White, J. and M. Conley. 2013. A flexible, low-cost cart for proximal sensing. *Crop Science* 53.4
 681 (2013): 1646-1649.
 682 White, J., P. Andrade-Sanchez, M. Gore, K. Bronson, T. Coffelt, , M. Conley, and G. Wang.
 683 2012. Field-based phenomics for plant genetics research. *Field Crops Research*, 133,
 684 101-112.