Architectural Phenotype Measurements Utilizing High Throughput Phenotyping in Solanaceae Varieties

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ABSTRACT

As populations rise and climate change threatens crop yields in food producing regions, efficient breeding of crops that are resistant to the extreme weather events while still maintaining their yield is important to address the threat to food security. Current methods of evaluating crop breeding lines are labor-, time- and cost- intensive, hindering the development of breeding improved varieties of crops. Even with advances in plant genotyping, the current methods of phenotype evaluation are too impractical to provide the rapid progress required to meet the ever-growing food supply demands. Without the development and implementation of new high-throughput phenotyping technologies, plant breeders will not be able to develop new varieties of crops quickly and efficiently to meet climate change concerns or provide food security for an increasing population.

The overall goal of this project was to develop and implement an automated high-throughput phenotyping (HTPP) system to increase the rate of breeding crop cultivars. This project focused on the development of a custom sensor platform and processing software for tomato and pepper crops in the Solanaceae family due to their nutritional and economic importance and their similarities in important phenotypes for breeders, growers, and consumers. The objectives of the portion of the project specific to this thesis were to (i) develop software and hardware needed to integrate a real-time, proximal plant architecture sensor with sensing systems controls and integrated GPS, (ii) develop methods to measure architectural plant phenotypes (height, width, and volume) of different genotypes in the Solanaceae family utilizing 3D model data from a time of flight camera and compare the results in terms of throughput and spatial and temporal resolution with more traditional methods of measurement and to create a visual tool to show the growth of the crops over the course of an entire season.

A phenotyping platform was developed on using a high clearance tractor (model Classic Spider, LeeAgra, Inc. Lubbock, TX, USA), typically used in farming for crop spraying. A custom arch was built to hold nine color cameras, nine infrared cameras, and three time of flight cameras along with custom lighting modules and a translucent cover to provide consistent lighting throughout the day while in motion. A Real Time Kinematic Global Positioning Systems (RTK-GPS) was integrated with the time-of-flight camera to efficiently collect data and catalog breeding genotype while selectively viewing the plots of interest for this project.

In this study, three-dimensional model data of a variety of genotypes was collected over the course of three growing seasons. Automated algorithms were developed to create 3D models (point clouds) utilizing the time-of-flight output data and to sorted by genotype plot (eight plants of the same genotype were grouped together in the field). Methods were developed to remove noise and extraneous data and then create a single point cloud spanning the entirety of the plot. These methods were automated and applied to all three years of the data so that the final plot 3D models could be used for architectural phenotype measurements. The study resulted in a very large set of 3D point clouds and corresponding architectural phenotypes for full plots (over 4,000 plots in total) of breeding genotypes for the three years of study.

Methods of measuring height, width, and volume phenotypes from the point clouds were developed and automated. This resulted in numerous measurements of height and width at the plant scale for each plot as well as an overall volume measurement for the entire plot over the course of the season. Through comparison with traditional measurement methods, no significant statistical difference was found between the heights measured by breeders and the heights measured utilizing the automated point cloud data. This study shows the potential for automated high throughput phenotyping methods to accelerate genomic breeding in crops to create superior varieties.

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1. INTRODUCTION

With the ever-increasing global population, crop production must at least double by 2050 to satisfy the growing food demands (Araus & Cairns, 2014). Farming more crops on more land and raising more livestock is not considered a long-term option because of the increase in unsustainable resource usage (Njoroge et al., 2018). While many areas are already struggling to increase their yield by that amount, climate change and global warming pose a threat to food security worldwide. The frequency and severity of droughts have increased globally, compromising crop yields (Spinoni et al., 2014). While some cultivars have benefited from the increase of CO_2 in the atmosphere, there are vastly more instances of currently used crop varieties performing poorly to the changes in climate (Furbank & Tester, 2011).

Scientists across the world are working to improve crop breeding and production with new genomic tools and techniques (Araus & Cairns, 2014). Developments in DNA sequencing have improved the speed and efficiency of genotyping costs (White et al., 2012). There are many examples of entire genomes sequenced for plants, some even publicly available (Furbank & Tester, 2011). However, these new plant breeds are limited by our ability to understand how their performance in terms of yield and growth are affected by their environment compared to other varieties. Even genomic tools such as marker-assisted recurrent selection (MARS) and genomic selection require phenotypic information to provide relevant performance results (Jannink et al., 2010). Phenotyping is an essential part of understanding how genotype, environment, and field management practices affect crop health and performance (Roitsch et al., 2019). Phenotyping constitutes an important bottleneck to be addressed if food production is to expeditiously meet the demands of a growing population and changing climate. While some phenotyping techniques used in green houses with conveyor belt transporters moving pots through imaging equipment have been successful at identifying phenotypes, greenhouse conditions do not represent the outdoor growing environments that majorly impact plant growth and structure (Comar et al., 2012). Traditional methods of phenotyping in the field are time as well as labor intensive and often require multiple destructive harvests throughout the season (Furbank & Tester, 2011). They are also often subject to human bias and judgement due to their subjective nature (Martinez-Guanter et al., 2019) and are frequently limited to simplistic measurement techniques that can be easily done manually in an outdoor environment.

High throughput phenotyping, or the ability to identify and categorize plant characteristics nondestructively, quickly, and objectively, can help address the bottleneck issue with gathering the location-specific phenotypic feedback needed in plant breeding. Automated phenotyping utilizing remote sensing can estimate plant traits such as yield, plant height, Leaf Area Index, canopy temperature, nitrogen uptake and many more without destroying plant material, additionally allowing this data to be collected over time to understand the overall history of the plant is beneficial (Jansen et al., 2014; Sun & Wang, 2019; Vázquez-Arellano et al., 2018; Wang & Chen, 2020).

The goal of this project is to develop and implement an automated high-throughput phenotyping (HTPP) system to increase the rate of breeding crop cultivars. This project focuses on tomato and pepper crops in the Solanaceae family due to their similarities in important phenotypes for breeders, growers, and consumers. Beyond this project, the plan is to adapt HTPP methods and technologies to other crops.

2. LITERATURE REVIEW

2.1. General Background

The United Nations estimates the world population will increase to between 9.4 and 10.1 billion by 2050 (UNDESA, 2019). With climate change affecting the yield of many crops around the globe, the need to improve the yield of our food production is necessary. But this challenge is a multifaceted issue with factors like land availability, resource limitations and time sensitivity. Increasing land usage for crop production will lead to increases in greenhouse gases in the atmosphere which will exacerbate the effects of climate change (Tilman et al., 2011). Scientists are faced with the challenge of increasing food production while reducing the amount of resources needed and the impact on the environment.

California, the United States' largest producer of specialty crops, has been significantly affected by climate change, especially in terms of water availability. California produced more than 50 billion dollars' worth of specialty crops in 2019, the most of any state. Vegetable production is a 13-billion-dollar industry in the US, with California producing more than half (7.3 billion dollars) of the US's fresh and processed produce. California is the number one producer of tomatoes in the United States and number two producer of peppers. These crops represent a significant portion of the tomato and pepper products sold in the United States as well as the California's agricultural economy (USDA_NASS, 2016). Approximately 90% of the processing tomatoes grown in the United States are from California. However, like many crop varieties, the yield, plant growth and development of the Solanaceae family has been affected by climate change. Breeding varieties with more adaptable stress tolerances, and better yield in arid environments via breeding is being researched but understanding and testing how these new varieties perform in increasingly warmer and drier regions is essential to maintaining or increasing their production levels (Tolosa & Zhang, 2020).

2.2. Remote Sensing

To address the issue of a lack of phenotypic information, the application of robotics, remote sensing, and imaging technologies have been used to detect information about cultivars and improve the efficiency of crop production. From robotic platforms to UAVs, both government and privately funded groups have worked to develop methods for collecting data to detect and categorize plant phenotypes (Araus & Cairns, 2014). With recent advancements in the understanding of biological features in plants as well as the capabilities of data analysis and processing tools, high throughput phenotyping, or the rapid detection and characterization of plant traits, via remote sensing has the potential to accelerate breeding processes (Gupta & Varshney, 2013). High throughput phenotyping utilizing partially, or fully automated systems can provide detailed and complex analysis of plant structure, growth, and morphology without damaging the plant (Humplík et al., 2015). Tools like Artificial Neural Networks (ANN), Support vector Machined (SVM) and models implementing Fuzzy Logic (FL) based rule systems have greatly increased the accuracy of the results from image data, sometimes even surpassing traditional methods of measuring (Njoroge et al., 2018) .

2.3. Phenotyping Platforms

The best method to collect image or other types of data for high throughput phenotyping varies from crop to crop and the desired traits. Unmanned Aerial Vehicles, custom platforms, tractors, and other systems have been used to collect data (Araus & Cairns, 2014). For example, researchers from Arizona, focusing on developing a phenotyping platform, created one that consisted of four sets of sensors to measure Pima cotton phenotypes across four rows at once (Andrade-Sanchez et al., 2014). A semi-automatic phenotyping system was designed with two RGB cameras and a hyper spectral radiometer to monitor small plots of wheat cultivars and provide information about plant canopy architecture (Comar et al., 2012). Heliaphen is an outdoor high throughput phenotyping platform that consists of a fully autonomous robot that monitors water levels in plants based on the weight of their pot as well as having four digital cameras to take photographs of the plant to monitor characteristics like leaf area and seed production. Those characteristics are then utilized to estimate leaf transpiration or stress responses in order to simulate the plant varieties' response to different environmental conditions and yield production (Gosseau et al., 2019). The Terra phenotyping Referencing (Terra Ref) Project compared NDVI measurements of Durum wheat varieties utilizing both UAV's and ground based phenotyping platforms in order to understand drought tolerance across genotypes (Condorelli et al., 2018).

2.4. Three-Dimensional Phenotyping

Plant architecture is an important set of plant phenotypes because it provides useful insight about the plant's growing conditions as well as information regarding the developmental stage of a plant's life cycle (Paulus et al., 2014). The size and shape of varieties can give breeders insight on which cultivars are best suited for different environments (Chéné et al., 2012).

While 2D imaging is extremely useful for certain phenotypic and environmental trait measurements, it lacks some information when it comes to plant architecture. Because of the nature of many plants, occlusions and shadows can greatly decrease the accuracy of 2D measurements. Three-dimensional models of plants, created from 3D reconstruction techniques, that utilize depth information can overcome many of the limitations 2D information has (Paulus et al., 2014). Using remote sensing to extract phenotypic data has shown promising results. For example, as part of the Smart tools for Prediction and Improvement of Crop Yield project (SPICY), researchers developed an imaging unit that could create a 3D reconstruction model of the plant canopy and detected architectural features as well as other statistical information from the raw 2D images collected in a greenhouse (Van Der Heijden et al., 2012). Researchers were able to obtain volume, mass and berry counts utilizing 3D models via remote sensing (Herrero-Huerta et al., 2015). Vineyard characteristics like grapevine classifications, vine dimensions and crop field conditions were determined utilizing 3D Digital Surface Models (de Castro et al., 2018).

However, many 3D sensors are expensive and not practical for commercial purposes. LiDAR creates high resolution 3D models but has a large investment cost for models with a larger field of view. 3D model creation from 2D RGB images has a significant amount of post processing time and computational requirements. While RGB imaging is typically less costly than 3D, it often requires a priori knowledge of the object of interest and environment which can be time consuming to collect (Chéné et al., 2012). This has led to an increase in use of low cost off the shelf depth sensors on phenotyping systems. Three-dimensional modeling with off the shelf sensors has shown promising results in agriculture as well as other scientific fields. While more costly laser systems are more reliable and accurate, lower cost depth cameras coupled with the RGB information still make these sensors a good option compared to expensive alternatives (Paulus et al., 2014).

The Microsoft Xbox Kinect (Microsoft Kinect V2, Microsoft Corp., Redmond, WA, USA) has proved to be useful as a short-range 3D/4D camera for a variety of feature detection applications (Mankoff & Russo, 2013). The Kinect v2 has become more popular in agricultural settings due to its technical capabilities like speed and accuracy at an affordable price point (Vázquez-Arellano et al., 2016). Paulus et al. (2014) compared the results of high precision laser scanners to RGB-D cameras like the Kinect in measuring sugar beet leaves and wheat ears and found that these low-cost sensors were capable of replacing their expensive counter parts for many phenotyping scenarios. Using the Microsoft Kinect, point clouds of maize in fields were developed to extract multiple height measurements from individual corn plants as well as develop raster crop height models (Hämmerle & Höfle, 2016). In another study the Kinect was used to create 3D reconstruction models of weed infested maize fields to estimate the volume of the weeds (Andújar et al., 2016). The Kinect was also used in greenhouses to take 360-degree views of pepper plants to create a fully comprehensive pepper plant model to analyze features of the plant. (Sun & Wang, 2019).

3. BACKGROUND

This paper is focused on utilizing 3D models to track architectural plant phenotypes in varieties of Solanaceae vegetable plants. The focus of this study was to develop and deploy a ground based remote sensing phenotyping system and implement automated methods of capturing and processing large quantities of data per day to accelerate the breeding of more adaptable crop varieties to combat the effects of climate change. Some of the phenotypes of interest of breeders of Solanaceae varieties can be seen in Table 1.

Table 1 High-throughput plant phenotyping target traits in tomato and pepper for plant breeders

Exterior Traits	*Interior Traits*
Plant size (height and width)	Fruit Yield
Plant shape (ratio of height/width)	Proportion of ripe fruit in total yield
Plant growth habit	Fruit color
Plant fruit carrying ability	Fruit size
Canopy light interception	Fruit shape
Leaf temperature	Days to first: open flower, green fruit, mature fruit
Plant biomass	Sunburned fruit

3.1. Phenotyping Platform

A previous version of phenotyping systems developed at UC Davis for Solanaceae plants was focused more on high-resolution close-up imaging of eggplants (Nguyen et al., 2016).While the models created from that project were incredibly detailed, photorealistic and useful, the phenotyping tractor had to stop at every plant to capture a large number of images of each plant versus continuously driving through the field and post data collection processing the data to create the models was extensively time and labor intensive. For the collection of large amounts of phenotypes of replicated field trials with many genotypes, this time and labor requirement wasn’t feasible.

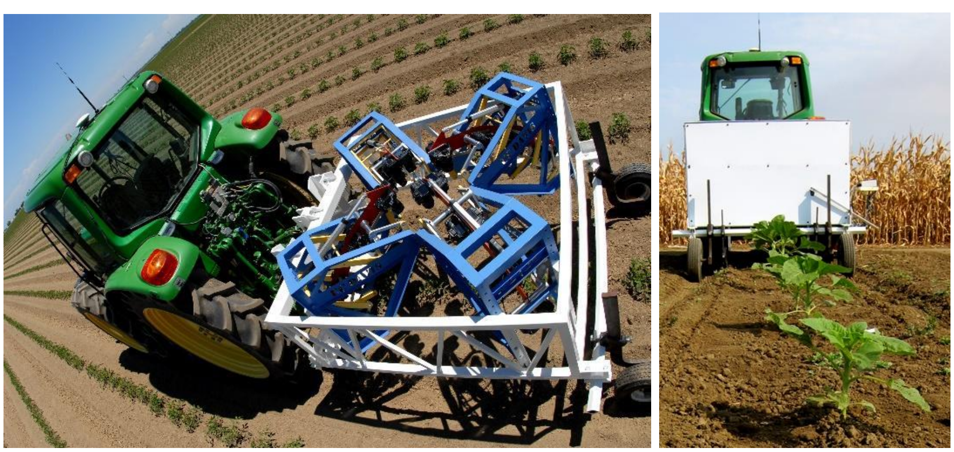


Figure 1 Previous iteration of UC Davis Phenotyping Platform

The goal for this study was to utilize 2D images to create high resolution 3D reconstruction models focused on phenotypes related to plant height, leaf segmentation, leaf counting and leaf area. Because of the high resolution of the system and its field of view width limitations, each camera was located extremely close to the plant once the plant was mature.

Several modifications and design decisions were made during the development of this phenotyping tractor to reduce the limitations and improve upon previous designs. The new design utilized two types of imaging sensors (the Xbox Kinect v2 and Canon T6 cameras) splitting the plant architecture tasks from other imaging tasks with a sensor more suited for three-dimensional modeling. The phenotyping system for this project was built on a high clearance tractor (model Classic Spider, LeeAgra, Inc. Lubbock, TX, USA) to ensure data could be collected all season without disturbing or interfering with the plants. This new design also allowed for the platform to move continuously while collecting data rather than stopping at each plant. This decreased the time it took to traverse the field while still capturing useful data.

Figure 2 UC Davis phenotyping platform "The Spider" in field

Sensor placement in the previous iteration of phenotyping systems from UC Davis was constrained by row width, however due to the increased height of the spider, the camera and sensor placement in the new design was not constrained by the crop row width. Instead, this system extended the sensor mounting positions over adjacent rows allowing each 2D RGB camera to be about 225 cm and the Kinects to be 150 cm away from the center of the target row, Figure 3. This design also helped better satisfy the breeder's requirements for capturing data of the plants at various stages of growth, including full height plants at maturity.

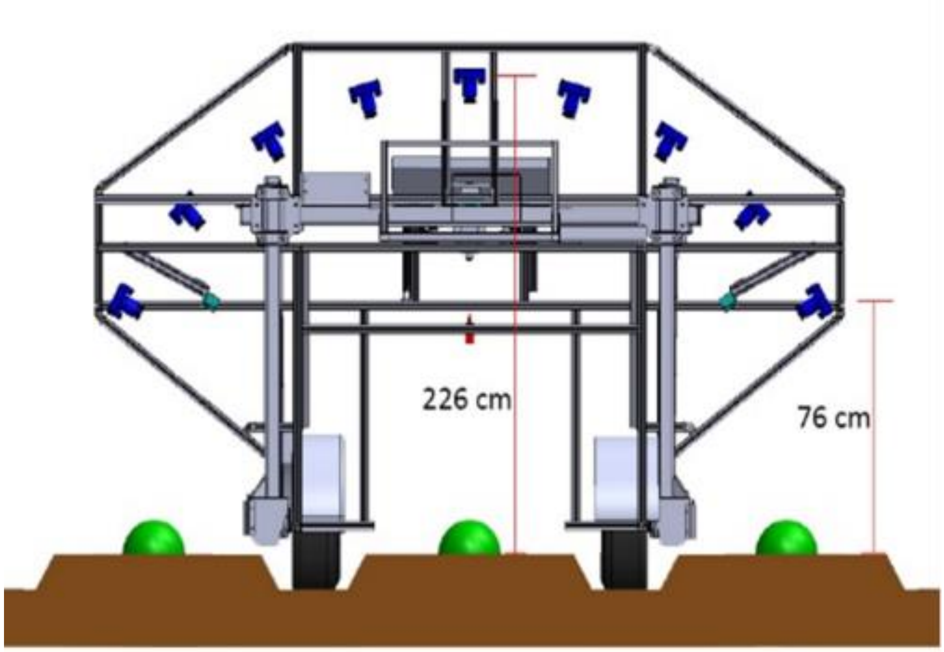


Figure 3 Rendering of the phenotyping platform with distance of the furrows (where the plants are located) to the heights of the sensors.

Another important feature of this version of the phenotyping tractor was the lighting. Ambient light conditions vary throughout the day and year which causes issues for most imaging sensors (Andújar et al., 2016). To address these challenges, the entirety of the sensor system was covered with translucent white corrugated plastic precisely cut to fit to the frame holding the sensors. This translucent cover dispersed the ambient light, reducing shadows from the tractor and other objects, like the sensors themselves. To ensure there was enough light illuminating the plants for in-motion image capture without motion blur, custom LED lighting modules were made and mounted on the sensor frame to allow constant bright lighting conditions.

3.2. Real-time Kinematic Global Positioning Systems (RTK GPS)

Because of the number of plants in the field and the number of sensors on the tractor, it was essential to only collect data for the plants of interest and to automate the data collection process. Previous iterations of this machine collected data continuously, resulting in large amounts of unwanted image and other data of the soil in between crop rows. However, automating the data collection process presented a number of challenges. The plants in this study were planted 12 inches apart, with eight plants per plot, and a 36-inch separation along the row between plots. A Global Positioning System (GPS) was used to track the location of the vehicle and the plants during measurements. To understand where the images and data points were taken, the GPS needed to have more precision than most low-cost consumer-grade GPS options. Fortunately, georeferencing in agriculture for applications like centimeter precision robotic weeding and self-driving tractors have been made possible with Real Time Kinematic Global Positioning Systems (RTK-GPS). RTK-GPS has the ability to give real-time GPS coordinates with centimeter accuracy and has proven effective in mapping transplanted row crops (Sun et al., 2010).

3.3. Phenotyping with a Ground-based vs. Aerial Platform

UAV's (Unmanned Aerial Vehicles) have increased in popularity in civil applications including agriculture in the last decade. As their overall popularity and production have increased, UAV have become more affordable to everyone including researchers (del Cerro et al., 2021). UAV's have some benefits over ground-based options, rugged terrain does not pose a problem and they can travel at much higher speeds, maneuvering through fields faster than many platforms. There is also a plethora of software that have user friendly autonomous route planning. With all these benefits, the UAV is an attractive option for many phenotyping projects. However, for this project UAV's benefits unfortunately do not outweigh the costs. One of the phenotypes of interest for this project was the day of the appearance of the first flower on each plant. UAVs are often not close enough to the plant to have a high enough resolution to capture details like flowers which are often smaller than a few centimeters in width. In addition, the limitations on weight capacity and electrical power limit the number of sensors the UAV can carry whereas a ground-based platform typically does not have such limitations. Lastly, UAVs cannot control or supplement the lighting. While the Kinect v2 outperforms the Kinect v1 in low and inconsistent lighting, it does still measure distances for point clouds more accurately and precisely in consistently well-lit conditions (Zennaro et al., 2015). For measurements like degree of sunburn on the fruit, the color and level of the lighting changes how this appears to a camera. The ground-based phenotyping platform was covered in translucent plastic that dispersed ambient light and the platform also providing its own supplemental light source, ensuring the lighting between plants, fields, and days were consistent, shadow-free, and of sufficient brightness for capturing high quality images.

Specific to UC Davis, the UC Davis Airport is located next to campus near many of the agricultural research fields involved in plant breeding. This limits when drones and other aircrafts can collect data in research fields limiting the availability of data collection times due to Federal Aviation Agency (FAA) regulations.

4. OBJECTIVES

The objective of this project is to develop and implement an automated high throughput phenotyping system to increase the rate of breeding new crop cultivars. This project focuses on the tomato and pepper crops in the Solanaceae family because it is the third most economically important food crop plant taxon and due to their similarities in important phenotypes for breeders, growers, and consumers. This document focuses on the collection of plant color and three-dimensional depth (RGB-D) data and the creation of an automated methods for identifying and measuring architectural plant phenotypes.

Objective 1: Sensor implementation

- Design, fabricate and evaluate the software and hardware needed to integrate a proximal plant architecture sensor with sensing system controls and GPS

Objective 2: Develop and Evaluate Software solutions to detect and identify phenotypes of interest

- Develop a method to measure height, width, and volume of different genotypes of pepper and tomato plants utilizing 3D point cloud data from a time-of-flight camera and compare with manually taken measurements. In addition, create a graphics that shows the change in these measurements over a season
    - Objective 2a: Estimate Height
        - Estimate plant/canopy height. This includes analysis of 3D cloud data from both a plant-level and plot-level basis, relating time of flight point cloud units to breeder's measurement units and comparing the estimated plant heights to manually collected heights at the end of season. Analysis of the automated phenotype data that shows the height change over an entire season of growth will be conducted.
    - Objective 2b: Estimate Width
        - Estimate plant/canopy width and compare with end of season manual width measurements. Unlike height, the width measurement is dependent on the 3D model's pixel density and utilizes pixels at the edge of the point cloud rather than the center
    - Objective 2c: Estimate Volume

- Estimate plant volume and use tools like smoothing to reduce noise and obtain better volume calculations.

5. MATERIALS AND METHODS

5.1. Field Design

This project was done on a variety of tomato and pepper genotypes as part of a study to examine drought response in Solanaceae varieties, so the fields were divided into drought and non-drought treatment blocks using a split-plot design. In 2018 and 2019, there were three fields of plants as seen in Figure 1, two tomato fields and one pepper field. The tomato fields had 24 rows of plants with four guard rows (one on each end of the field and two in the middle of the field). Each plot had eight plants (two guard plants, one on each end of the plot, i.e., plants 1 and 8). There were 16 plots per row for the peppers and 12 plots per row for the tomatoes. There were 16 pepper genotypes and 30 tomato genotypes with four replicate plots of each genotype per treatment block. In adjacent to the field a secure and lockable building was added to store the phenotyping platform when not in use.

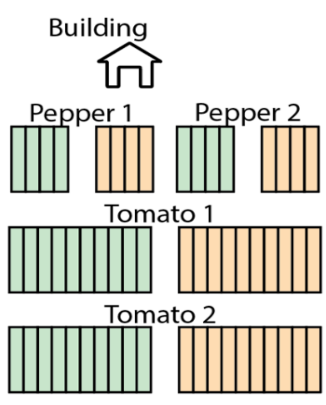


Figure 4. Field layout for 2018 and 2019 trials. Green represents the control group (non-drought) and yellow represents the plants with drought treatment

5.2. Phenotyping Platform Design

5.2.1. Hardware

The data for this project was collected using the High Throughput Phenotyping machine developed at UC Davis called "the Spider." The base of the Spider was a Lee Agra Spider tractor (model Classic Spider, LeeAgra, Inc. Lubbock, TX, USA) typically used for spraying. A custom sensor mounting arch was mounted behind the vehicle driver's seat and in front of the engine. The sensor mounting arch held 18 Canon DSLR cameras, three Microsoft Xbox Kinect V2 and eight lighting modules. The Spider also had an RTK GPS receiver mounted, which communicated with the RTK GPS base station located at the UC Davis Western Agricultural for Agricultural Equipment.

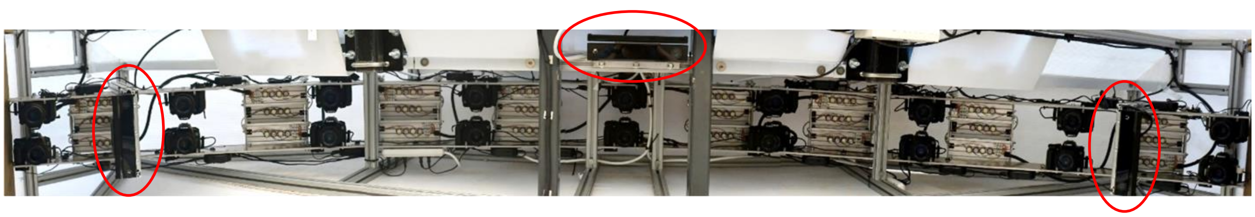


Figure 5. View of the arc mounted on the Spider taken from the ground looking up at the sensor placement (black objects). The Kinects are circled in red.

5.2.2. GPS

At the beginning of each season the GPS coordinates of start and end of all of the plots were recorded. These GPS coordinates were used as data collection control points and included in the software controlling the cameras (2018, 2019) and Kinect (2019). This allowed the cameras and sensors to only record data when located inside the plots, which eliminated image and depth data of guard plots and the road in between rows and decreased the amount of raw data collected and made data sorting more efficient. To achieve this, a ruggedized real-time-kinematic GPS (model 542 RTK-GPS, Trimble Inc., Sunnyvale, CA). was used. The receiver was mounted on the top of the Spider platform and connected to the base station located at the UC Davis Western Center for Agricultural Equipment.

5.2.3. Microsoft Xbox Kinect v2

The Spider was equipped with three Microsoft Kinect V2 sensors. The Kinect is a 3D sensor made by Microsoft with a 1920 x 1080 resolution RGB camera as well as an infrared camera (512x 424-pixel resolution) and infrared emitter. The maximum data collection rate of the Kinect is 30 Hz and it has a 3D depth range of 50 cm to 450 cm. Microsoft has an official developer's kit for researchers to use the Kinect for non-gaming purposes, however the Kinect sensors on the tractor used libfreenect2 (Xiang, 2016), an open sourced software, to collect and store the data to create point clouds.

One of the Kinect sensors was placed directly above the crop row that the tractor straddles to collect data in the nadir direction and the other two were placed above adjacent rows at a 45-degree angles from the nadir direction, also viewing the same crop row that the tractor straddles. The two side angle Kinects were designed, as a precaution, to provide an alternative view of the same plants in case a feature of interest was occluded from the view of the top Kinect by a part of the plant (e.g., a flower hidden below a leaf so it is not visible from above, but is visible from the side). For the phenotypes studied in this document, feature occlusion was not an issue and therefore did not require the data from the side Kinects. All three Kinect sensors were connected via USB 3.0 port to a computer running the Microsoft Windows operating system. A custom program was created to allow the Kinect sensors to be turned on and off manually or automatically according to GPS location by software.

5.2.4. Kinect-GPS Integration

During the data collection process the three Kinects were connected to a computer via USB 3.0 ports. This allowed custom software to turn on and off the Kinects. The RTK-GPS outputted a PTNL-PJK string which includes UTC time, Northing (meters) and Easting (meters). An algorithm then used the location to calculate the time it would take for the platform to enter the next plot and then turn on the time-of-flight camera and other sensors. The workflow for this process can be seen in Figure 6.

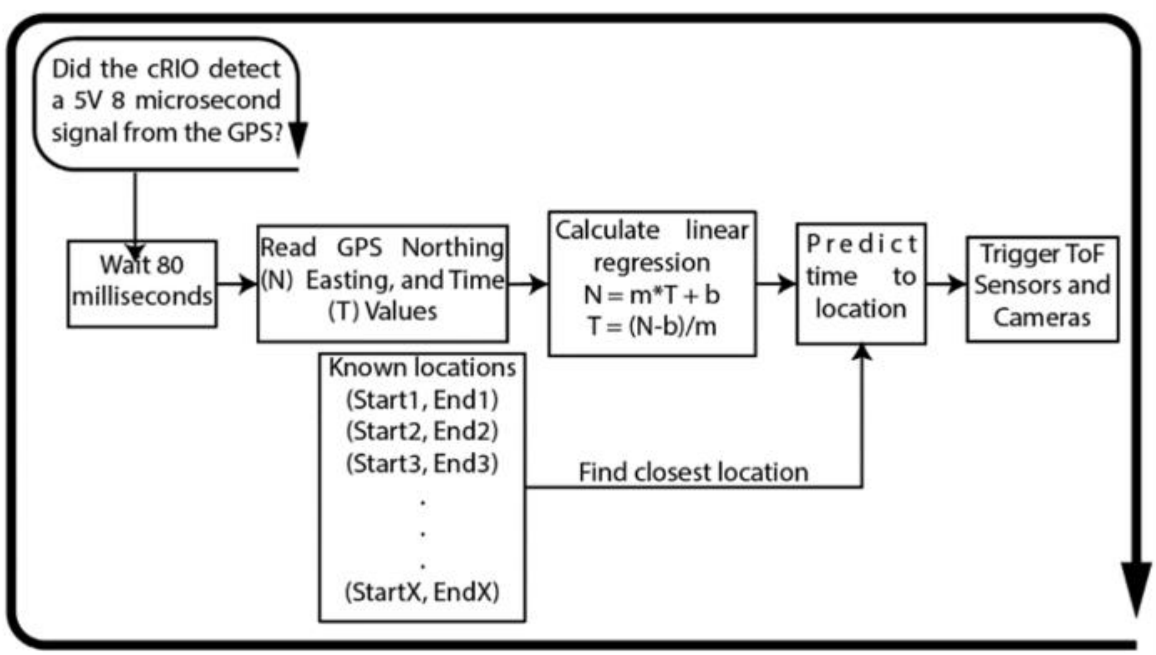


Figure 6 RTK GPS HTPP Platform integration algorithm flow chart

The RTK GPS could not be directly connected to the Kinects and required a device to read the start and stop times based off the RTK-GPS message. A National Instruments USB-6501 digital I/O device, was used to allow the computer to capture a digital GPS timing pulse the computer could utilize for accurate geolocation sensing. Custom software was created that utilized the GPS signal to turn on and off the data collection from the three Kinects was implemented to allow data to be collected only when within a genotype plot and to create the metadata needed to keep track of which the data was collected in.

5.3. Data Collection

To collect data, the Spider was driven through all the rows in the research plots twice a week from June to September. On each day of data collection one pass was made by the Kinects to collect data for each plot.

5.3.1. Validation Trial

In 2020, the field consisted of three rows of pepper plants and three rows of tomato plants. There was only one genotype of pepper and one genotype of tomato plant. Each row had 16 plots with an additional two guard plots on either end of the row. The Spider ran through the field on three dates since this was not a full trial.

5.3.2. Validation Hand Measurements

In 2020 a smaller scale trial was conducted to validate methods developed for phenotype analysis. Measurements like height or width per plant instead of by plot were not collected in 2018 or 2019 but were important to understanding how accurate the measurements collected from the Kinect were. There were six rows of plants (three for peppers and three for tomatoes) with no variation in genotype.

To compare the Kinect results with hand measurements, the height, width, and volume data were collected by hand. To measure height, a yard stick was placed next to the main stem of the pepper plant and the height was written as the tallest point around the yard stick. The width was taken at the visually widest part of the plant perpendicular to the drive direction of the tractor with a measuring tape across the width of the plant. Measuring the volume of the plant required destructive measuring techniques and therefore were only taken once during this trial (at the end).

5.3.3. 2018 and 2019

In 2018 and 2019 there were two tomato fields (North and South) along with a pepper field. The pepper field had 24 rows with eight guard rows. Each row had 16 plots with eight plants per plot (2048 plants in total). There were 16 genotypes represented with four replicate plots for each genotype. The tomato fields each had 24 rows with 12 plots per row. Each plot consisted of eight plants with 30 different genotypes of tomatoes studied. 2018 was the first year that data was collected using the new model of the Spider with the new sensor arch mounting design.

6. Software

Because of the large amounts of data collected per day (about one terabyte of data from all the sensors was collected per day), automated methods of sorting, processing, and analyzing the data needed to be developed to produce phenotype measurements in a useful timeframe. The overall flow of processing data starting with the data collection and ending with the measurement analysis can be seen in Figure 7.

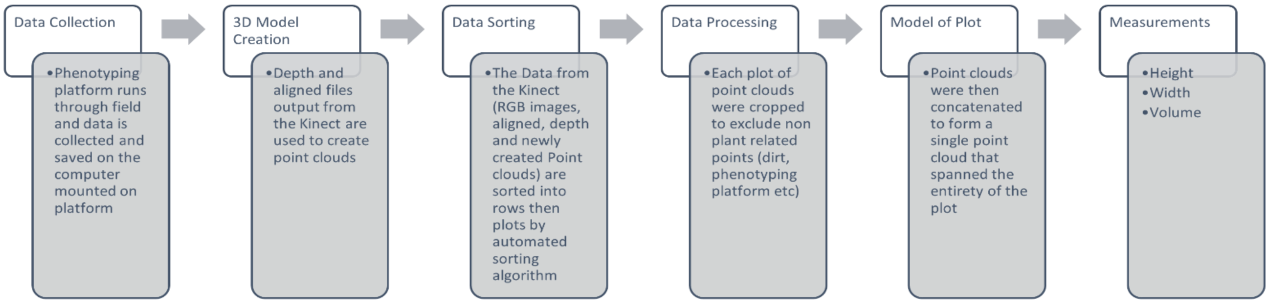


Figure 7 Workflow of automated phenotyping process

6.1.1. Point Cloud Creation

The point clouds for the plants within each genotype plot were created using depth and aligned data files output from the Kinect. Depth frames were one channel 8-bit grayscale image that contained all the distance information from the Kinect as seen in Figure 8.

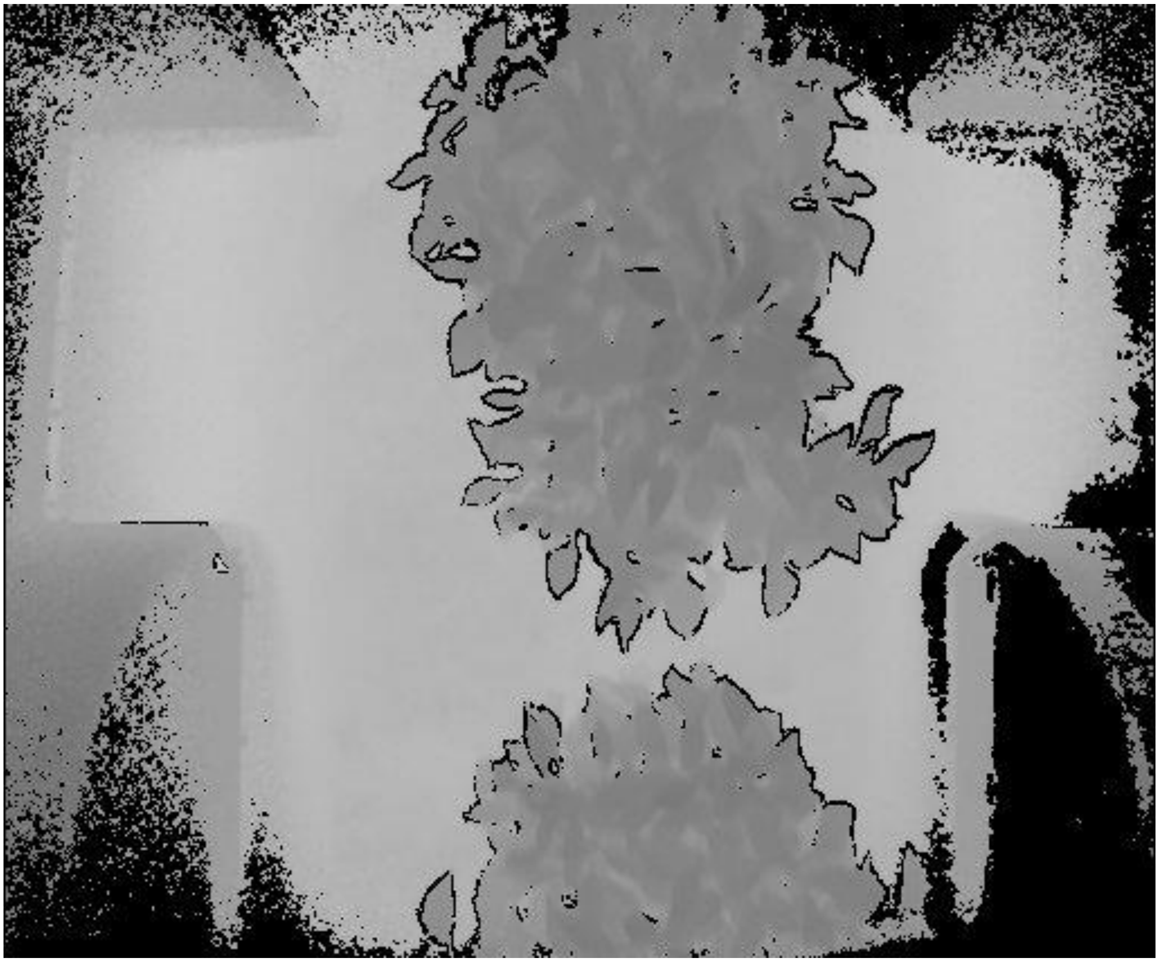


Figure 8 Depth file output from Kinect

The aligned files were image registration files that associated the RGB values with the corresponding depth value to create an RGB point cloud as seen in Figure 9.

Figure 9 Aligned file output from the Kinect

The depth and aligned data then were processed into point clouds with libfreenect, an open-sourced software package as seen in Figure 10.

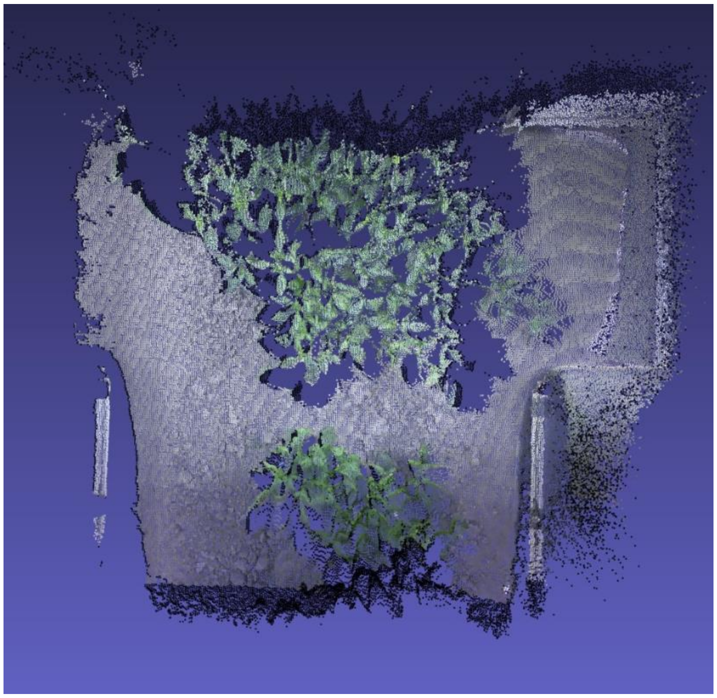


Figure 10 Point cloud created from depth and aligned files

6.1.2. Plot Sorting

In 2018, the GPS based data collection was not yet integrated with the Kinect. This meant the Kinects were manually turned on and off at the start and end of the run in between rows. This required a more labor-intensive approach to sorting. First the data was sorted into rows based on the time difference between data points. After the data was sorted into rows, the rows were then sorted into plots. Because the plots were all the same size in the field and the drive speed of the tractor remained fairly constant, the data was divided evenly among the plots and visually checked.

In 2019 the National Instruments digital I/O device was implemented with the RTK-GPS to turn on and off the Kinect based on the GPS coordinates of the spider. This turned sorting into an automated process. To sort the 2019 field data, first the data was divided and sorted into rows. Because the driving time between rows was longer than the driving time between plots, frames that had large time differences were assumed to be the last and first frame of two different rows. Once the data was sorted into rows, the time stamps of the data in the rows were compared. The time in between frames in a plot was about 0.4 seconds. The time in between frames that were the last and first frames of adjacent plots was about 2 seconds. Using this information, the frames were sorted by plot. Using a field map with the corresponding locations and labels, the plots were matched with their plot number and genotype. Due to the nature of how the RTK GPS was integrated with the phenotyping platform and its sensors, absolute location was not used to mark the start and end of the plots and not saved for each frame hence the use of time to sort.

6.1.3. Point Cloud Concatenation and Plot Creation

The point clouds for each plot were cut and connected to make a single point cloud of an entire plot. The frame rate of the Kinect was fast enough, and the platform travel speed was slow enough at about 3.2 km/h to ensure the entirety of the plot was visible in the point cloud data collected.

To determine which point clouds and what section of the point clouds were best to concatenate to form a full plot, three different sized cylindrical containers (trash cans) were placed in an empty plot and data was collected for those containers during the field trials as seen in Figure 11. Because the containers had a known and simple geometry, it was easier to visually check the point cloud overlaps and ensure that there were not any obvious errors in plot making. The containers were a known size (height, width, and volume) as well as a known distance apart, these were used to calibrate the program to decide what point clouds to crop and combine to create a single point cloud of the full plot. Once the parameters for creating the single point cloud per plot were determined, the full plot point cloud for every sorted date was created.

Figure 11 RGB image of cylindrical containers placed in the field during the field trials

The point cloud for each plot of plants consisted of 10-15 individual point clouds collected at 0.4 second intervals as the platform traveled across the plot. The 3D points that were closest to center of the crop row, directly underneath the Kinect, had the least amount of noise and the highest density of points and was therefore utilized in the phenotype analysis. The rest of the points in the clouds that consisted of points at the edges of the planting bed near the platform or were noisy were removed from the analysis. The edges of the platform and furrows were consistent in location so those locations were identified and saved, then included in the plot creation software to remove the non-plant features in the models.

Figure 12. Point cloud created from the Kinect pre editing process

Noisy points were considered points with no adjacent points within 10 cm. Software was developed to identify these points based on their isolation and remove them to prevent inaccurate phenotype measurements. The cropped point clouds were then concatenated to create one larger point cloud of the entire plot as seen in Figure 13.

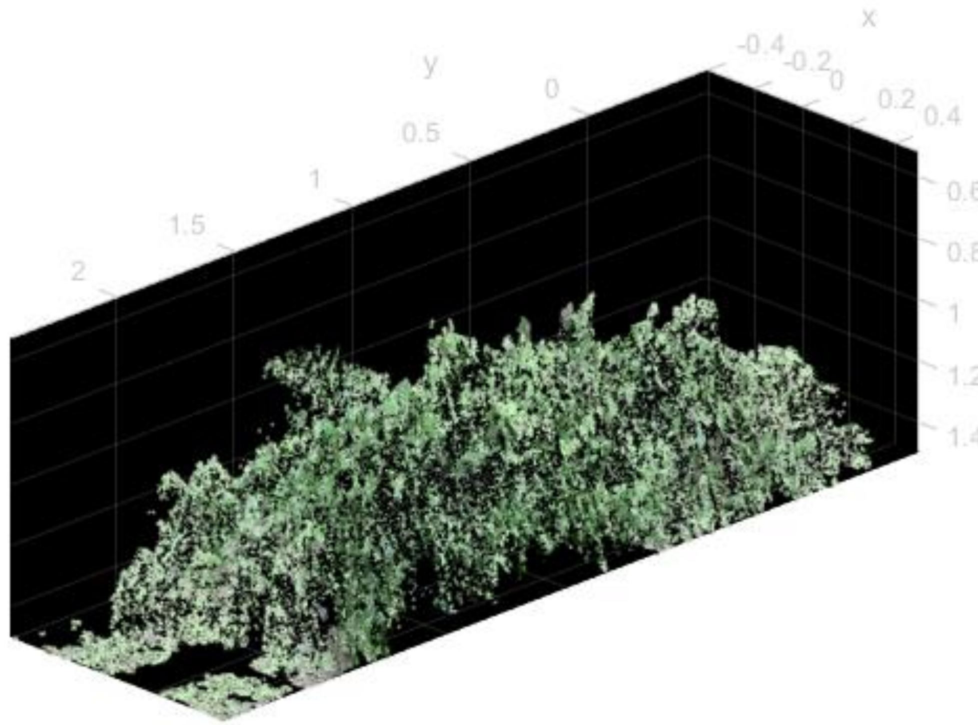


Figure 13 Point cloud of the full plot

This final point cloud does not contain any of the tractor components or furrow included so that any measurements taken are of the plants.

6.1.4. *Calibration Data*

The point cloud data is output in meters with the Kinect as the origin of the local reference frame. For width phenotypes, no additional coordinate system calibration of the data was needed. However, the raw height measurement output by the Kinect was the distance from the Kinect's sensor to the top of the plant, where a height of zero would be a plant that touches the Kinect. To translate that data into a form more suitable to compare with traditional methods of measuring height, a calibration equation was created utilizing three different sized cylindrical containers that were painted matte green and placed in the field during data collection as seen in Figure 11. The 3D models of these containers were reconstructed from the Kinect data as seen in Figure 14.

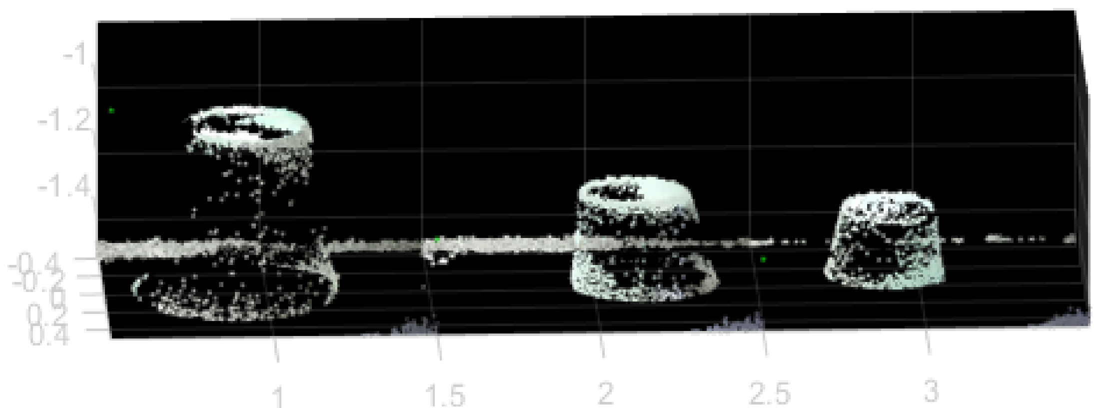
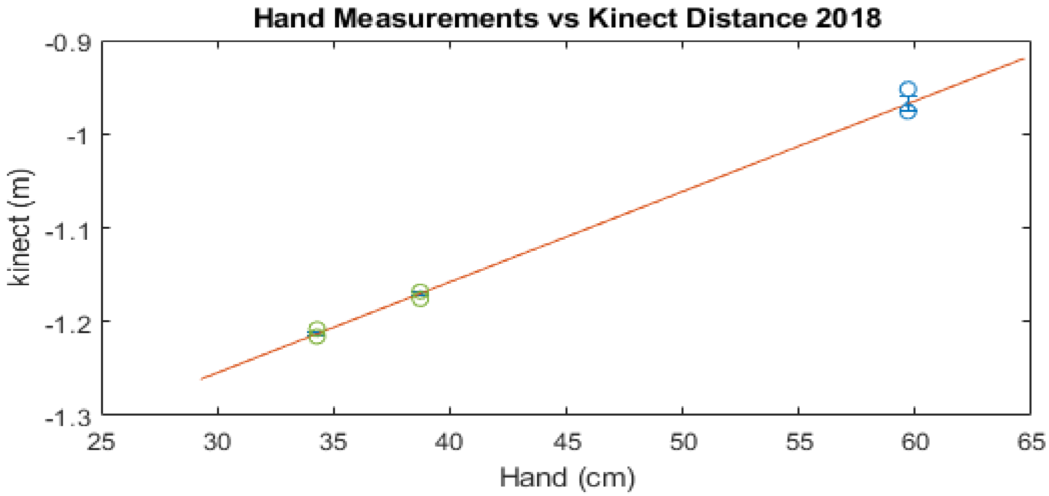
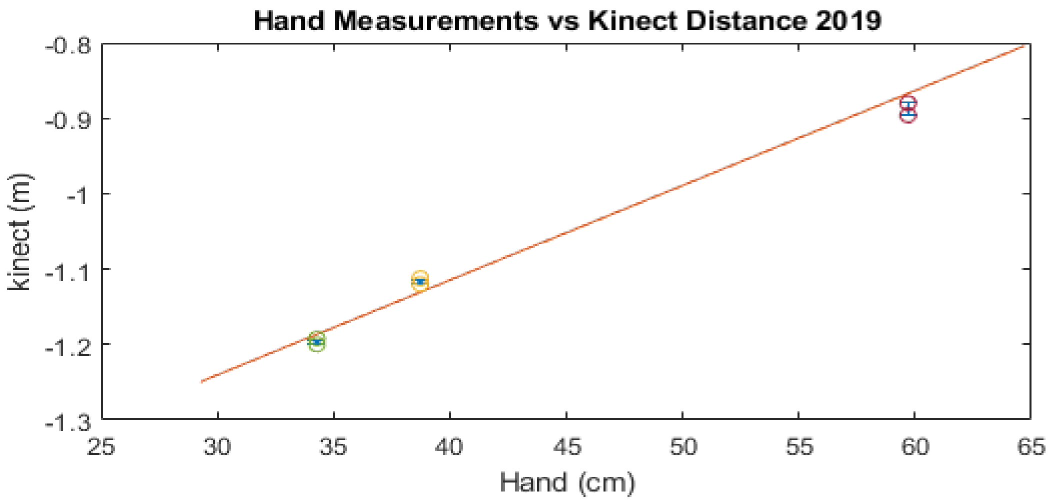


Figure 14 Containers placed in field to calibrate the height equation for the point cloud measurements

Each of these containers has a known height, which was used to make a calibration equation to transform the Kinect data into a breeder-compatible form. A new calibration equation was calculated from the container data collected every year. This was necessary because while the containers remained consistent from year to year, the height of the furrows and other field factors varied.


Hand Measurements vs Kinect Distance 2018
kinect (m)
-0.9
-1
-1.1
-1.2
-1.3
25
30
35
40
45
50
55
60
65
Hand (cm)


Hand Measurements vs Kinect Distance 2019
kinect (m)
-0.8
-0.9
-1
-1.1
-1.2
-1.3
25
30
35
40
45
50
55
60
65
Hand (cm)

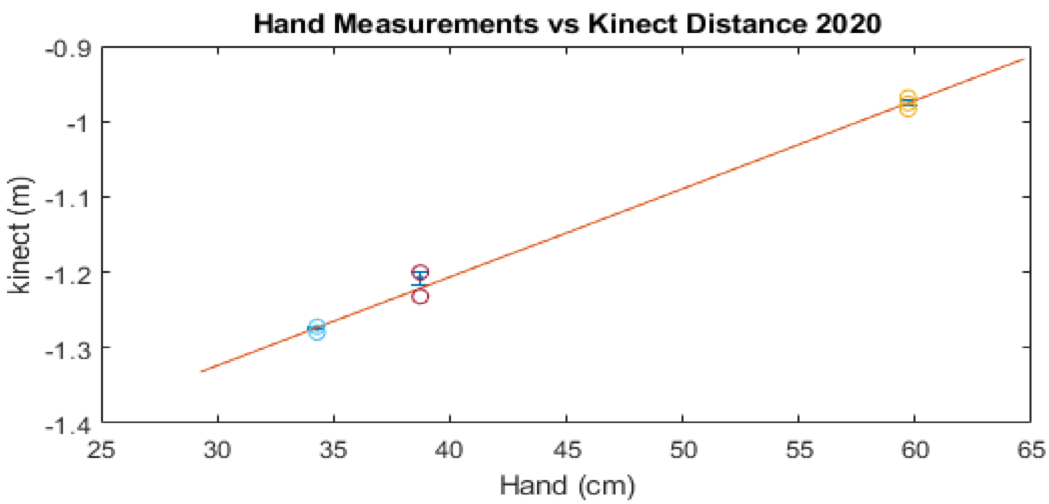

Hand Measurements vs Kinect Distance 2020
kinect (m)
-0.9
-1
-1.1
-1.2
-1.3
-1.4
25
30
35
40
45
50
55
60
65
Hand (cm)

Figure 15 Regression for containers placed in the field for height measurements

From the measurements taken from the cylinders by hand and the measurements from the Kinect, the following calibration equations were calculated:

$$y = mx + b \tag{1}$$

Table 1. Slope and intercept values for calibration equation from Kinect heights to real world heights.

	Slope, m (cm/m)	**Intercept, b (m)**
2018	103.45	154.85
2019	79.56	128.68
2020	85.32	142.93

The values for the slope and intercept as seen in Table 1 were then used each year to calculate height and volume.

6.1.5. Automated Height Measurements

For height measurements, the 3D point cloud was converted into a 2D projection. Defining height to be the z-axis and the tractor travel direction as the y-axis, the x-axis was the direction perpendicular to the crop row. For height, as measured with a single point measurement by the breeder using a ruler, the point cloud x-axis did not provide any useful information, so the points were projected onto the y and z plane to create a 2D image. Then any additional noise points were removed. At this point, the local maximums at evenly spaced intervals (height maximums) were identified. During the development of this algorithm one plot was used to test the intervals and maximum identifying software. After several iterations with parameter changes (like size of interval and sensitivity of maximum location software) the heights were identified in locations that corresponded to where a human would estimate a height measurement. Once parameters were established this method was tested on numerous plots over all the dates to verify and find tune the parameters.

In the beginning of the season there were about three points identified per plant and towards the end of the season as the plants grew larger there were about five points per plant. This resulted in about 20-40 height measurements per plot per day from automated height data.

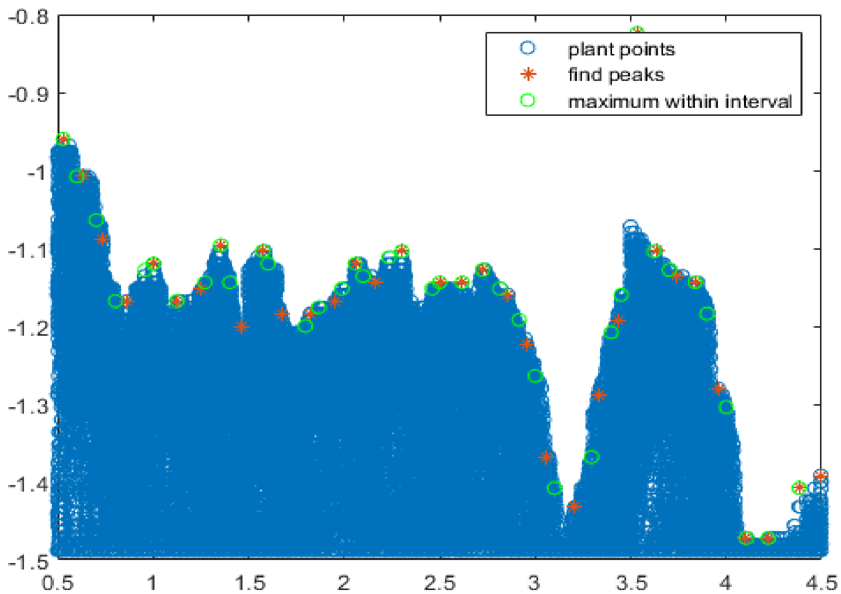


Figure 16. Two-dimensional representation of the point cloud with potential height measurements

The automated height data was compared with the plant breeders' manual measurements collected from two plants per plot on a single day during the season. In 2019 a Tukey multiple range test was conducted on the genotype heights between the automated Kinect method and the manual method.

6.1.6. Automated Width Measurements

For the width phenotype, the 3D point cloud data did not have to be processed as was done for the height because the width was measured along the x-axis. To calculate width, the difference between the widest points were calculated at eight sections along the plot length. Due to the differences in height across all the genotypes and the dates, taking a width for the same height for each plant did not provide a representative comparison to traditional measurement methods. Additionally, the projection method used for heights could not be adjusted and applied to width because the width is dependent on the height so removing the Z data would give inaccurate results. Using the base of the plant as zero and the tallest point of the plant as 100, the width was calculated using the data from the 30th percentile and above. Each plot had eight plants resulting in eight width measurements per plot.

6.1.7. Automated Volume Measurements

Volume for the plots were estimated using the same methods as the height measurements but over all the points instead of selected maximums. The volume of the plants was calculated by measuring the height of each point in the point cloud that was plant matter. The same method used in the plant height measurement of converting the Kinect height data of the plant relative to the Kinect sensor location into the absolute height relative to the soil was implemented here, except this was done for all the points in the point cloud without merging the points onto a 2D plane. The absolute heights were then multiplied by the ground-level area that each point represented. The resolution of the Kinect infrared camera is 512 x 424 pixels. When converted to real world units, each pixel images an area of 0.036 mm^2 at the soil level. After each height measurement was then multiplied by this point area and the volume values were then summed to get the total volume of the entire plot.

A visual representation of this process can be seen Figure 17 and Figure 18.

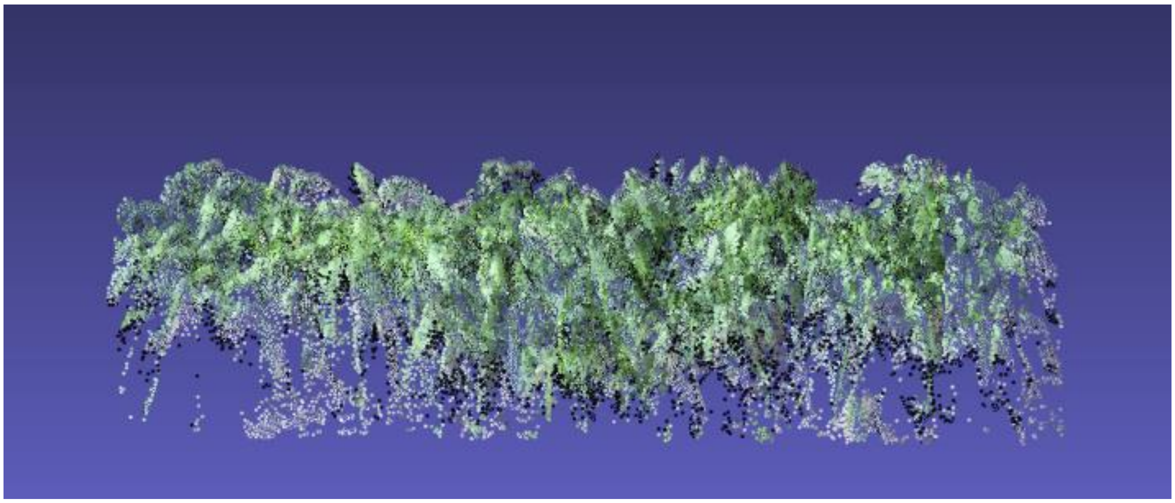


Figure 17 Point cloud of a pepper plant plot from Kinect data

Where Figure 17 is an example of an entire point cloud plot and Figure 18 is a small subset of the point cloud with each bar representing a point's height and the area it covers.

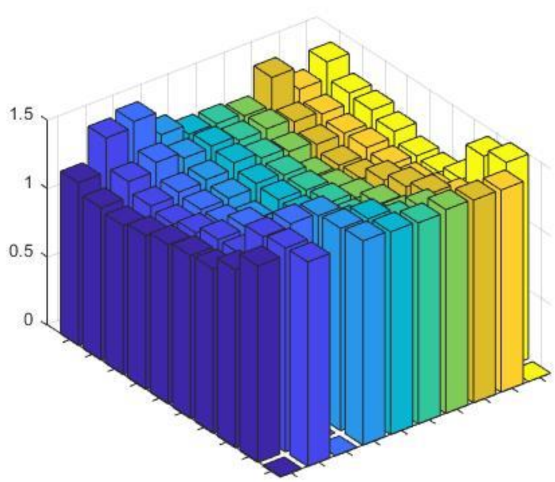


Figure 18 Visual representation of the area covered by each point in the point cloud. Data taken from a small subset of the points from the figure above

7. RESULTS

7.1. Automated Height Phenotypes

Heights were calculated on a plant-by-plant basis for the validation data and plot by plot basis for the 2018 and 2019 data to make the data easier to compare to the manual measurements that were taken. For the validation data, a single date of measurements were collected and for 2018 four dates were collected and 2019 five dates were collected.

7.1.1. Peppers

The validation data shows how the automated results compare to the manual measurements that were taken. As seen in Figure 19, the hand measurements have less information about the shape and overall height of the plot compared to the automated results from the Kinect.

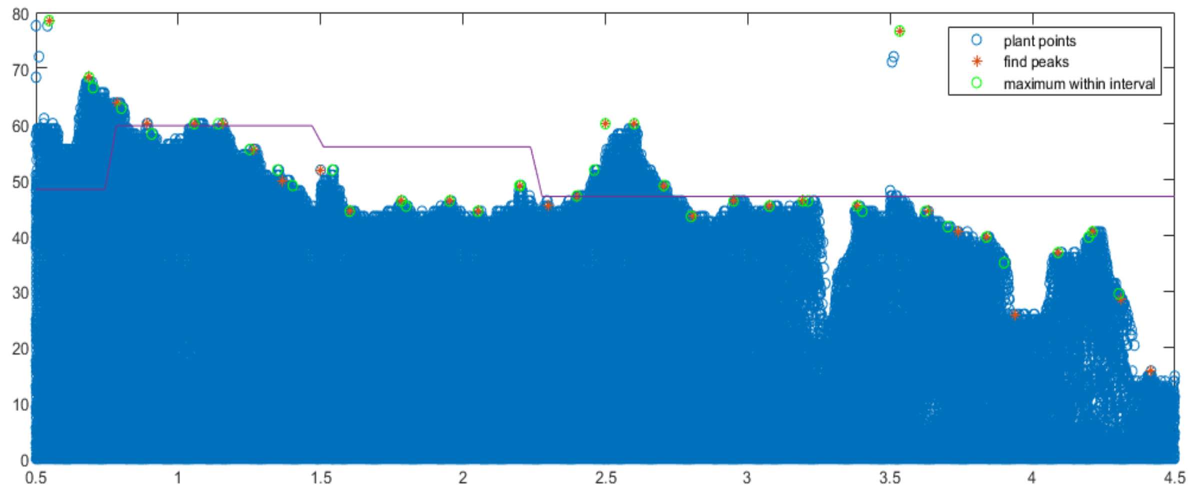


Figure 19 Two-Dimensional presentation of a pepper plot from the validation data in 2020

Because heights were taken of each plant in 2020, the height data per plant collected by the Kinect was compared to the data collected manually on a plant-by-plant basis rather than plot-by-plot as seen in Figure 20.

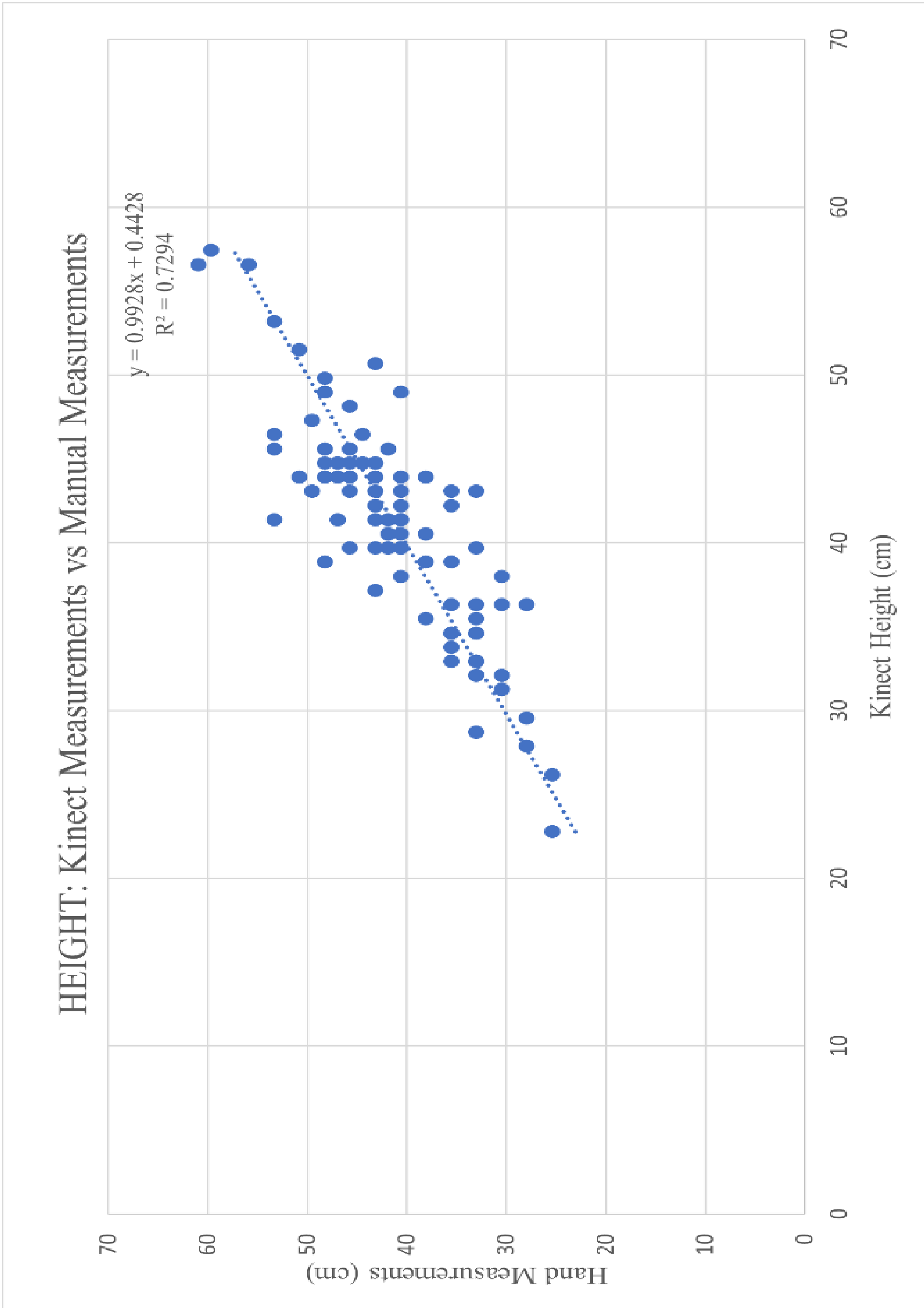


Figure 20 Scatter plot with the Kinect measurements plotted against the hand measurements.

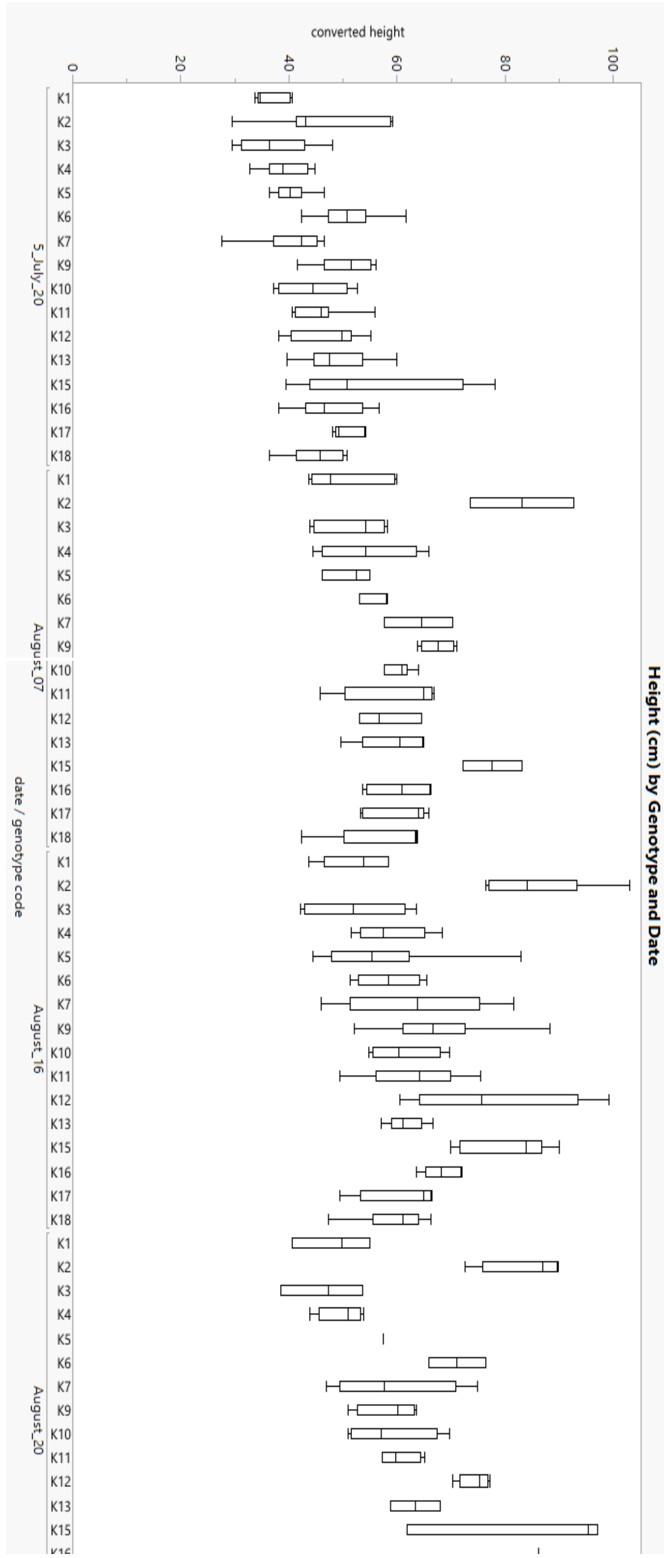


Figure 21 2018 Automatically generated height phenotypes of pepper plants calculated from the 3D Kinect data across the 2018 season by genotype and date

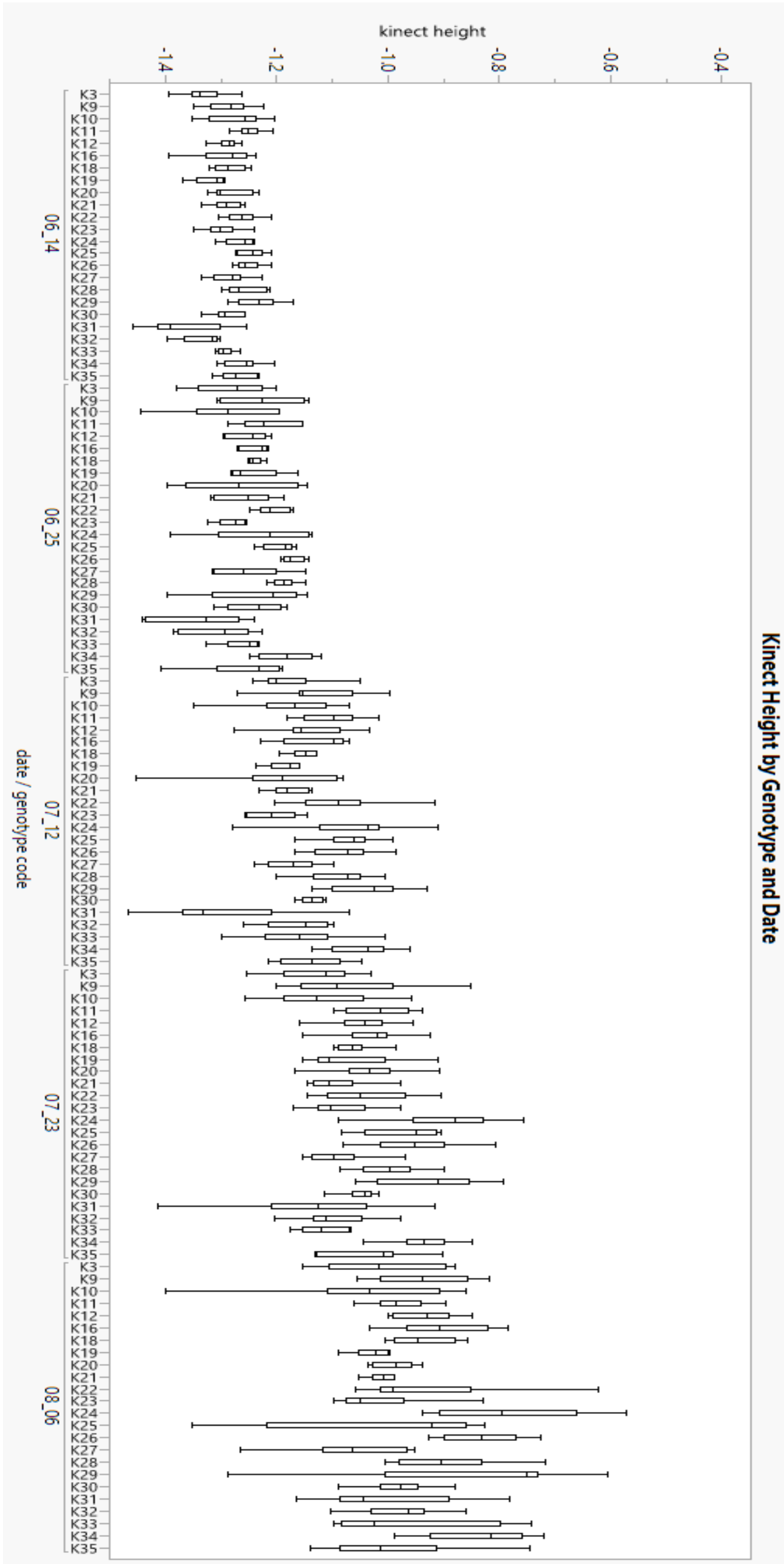


Figure 22. 2019 Automatically generated height phenotypes of pepper plants calculated from the 3D Kinect data across the 2019 season by genotype and date.

In 2019 there were more measurement dates as well as more data per genotype due to improved field conditions. Expectedly, as the season progresses the overall height of the plants increases.

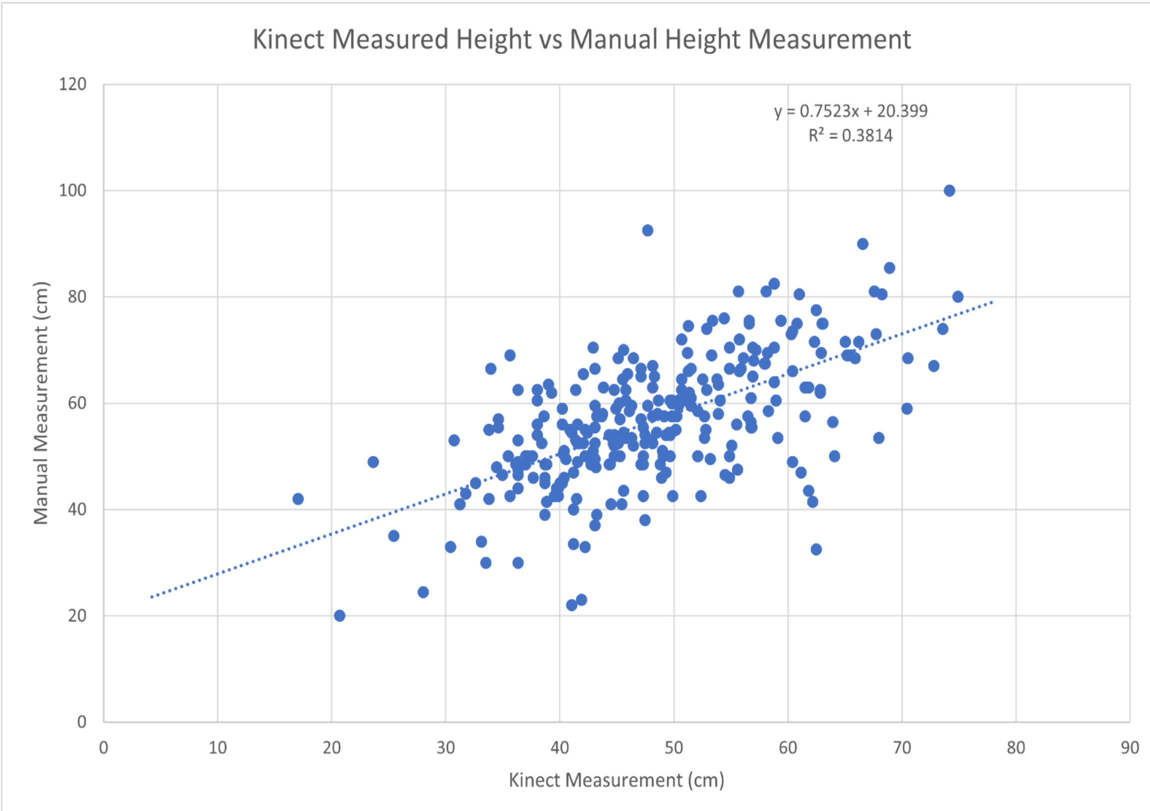


Figure 23 Average Kinect Height measurement vs Average of the Manual Measurements

Because the breeder only collected manual height measurements of the plants on a single date, there is not an effective way to compare Kinect generated heights of plants over the entire season and across the full range of heights of juvenile to mature plants to a more traditional measurement. Additionally, due to the labor-intensive nature of the task and the size of the trial, the breeder only measured the heights of two plants per plot manually, whereas with the Kinect point cloud had multiple height measurements per plot rather than just two. This high temporal and spatial resolution of data obtained by the Kinect is simply not realistic for traditional measuring methods.

A Tukey Multiple Range test was conducted on the 2019 data to compare the mean height of each genotype calculated from the Kinect and the data collected manually in the field (the same data displayed in Figure 23). Except for one genotype, none of the genotypes have a p value that indicates there is a significant difference between the Kinect measurements and the measurements taken manually as seen in Table 1.

Table 2 Tukey test conducted on the Kinect data and the manually collected data

Genotype	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
K03	0.4082	3.674877	-14.2883	15.1047	1
K09	3.56725	3.594101	-10.8062	17.94071	1
K10	13.09221	4.018327	-2.9778	29.16223	0.392
K11	3.2799	3.594101	-11.0936	17.65337	1
K12	15.29924	3.594101	0.9258	29.6727	0.0195
K16	9.50729	3.674877	-5.1892	24.20379	0.8984
K18	13.86908	3.674877	-0.8274	28.56559	0.1043
K19	4.12444	3.594101	-10.249	18.4979	1
K20	5.55782	3.594101	-8.8156	19.93129	1
K21	9.07463	3.594101	-5.2988	23.4481	0.9244
K22	4.3266	3.594101	-10.0469	18.70007	1
K23	6.57463	3.594101	-7.7988	20.9481	0.9998
K24	7.10874	3.846619	-8.2746	22.49208	0.9998
K25	11.83972	3.594101	-2.5337	26.21319	0.3639
K26	6.88998	3.594101	-7.4835	21.26344	0.9994
K27	5.96092	3.594101	-8.4126	20.33438	1
K28	9.20694	3.594101	-5.1665	23.5804	0.9097
K29	10.69156	3.594101	-3.6819	25.06502	0.6315
K30	9.62945	3.674877	-5.0671	24.32596	0.8824
K31	7.02663	4.187001	-9.718	23.77121	1
K32	10.99645	3.674877	-3.7001	25.69296	0.6165
K33	11.48945	3.674877	-3.2071	26.18595	0.5008
K34	19.23279	3.594101	4.8593	33.60625	0.0001
K35	8.61507	3.674877	-6.0814	23.31157	0.9733

In the 2020 trial, the height of each plant was taken in the plot instead of measuring only two plants per plot as was the tradition of the breeder. This allowed us to plot the hand measurements directly over the two-dimensional Kinect side view of each plot as seen in Figure 15.

7.1.2. Tomatoes

Kinect height data was collected for the tomato genotypes on the same days as the pepper plants. Unfortunately, there was no height data collected manually by the breeder for the tomato genotypes, but the methodology is the same for each crop. The processed Kinect height data is shown in Figure 17.

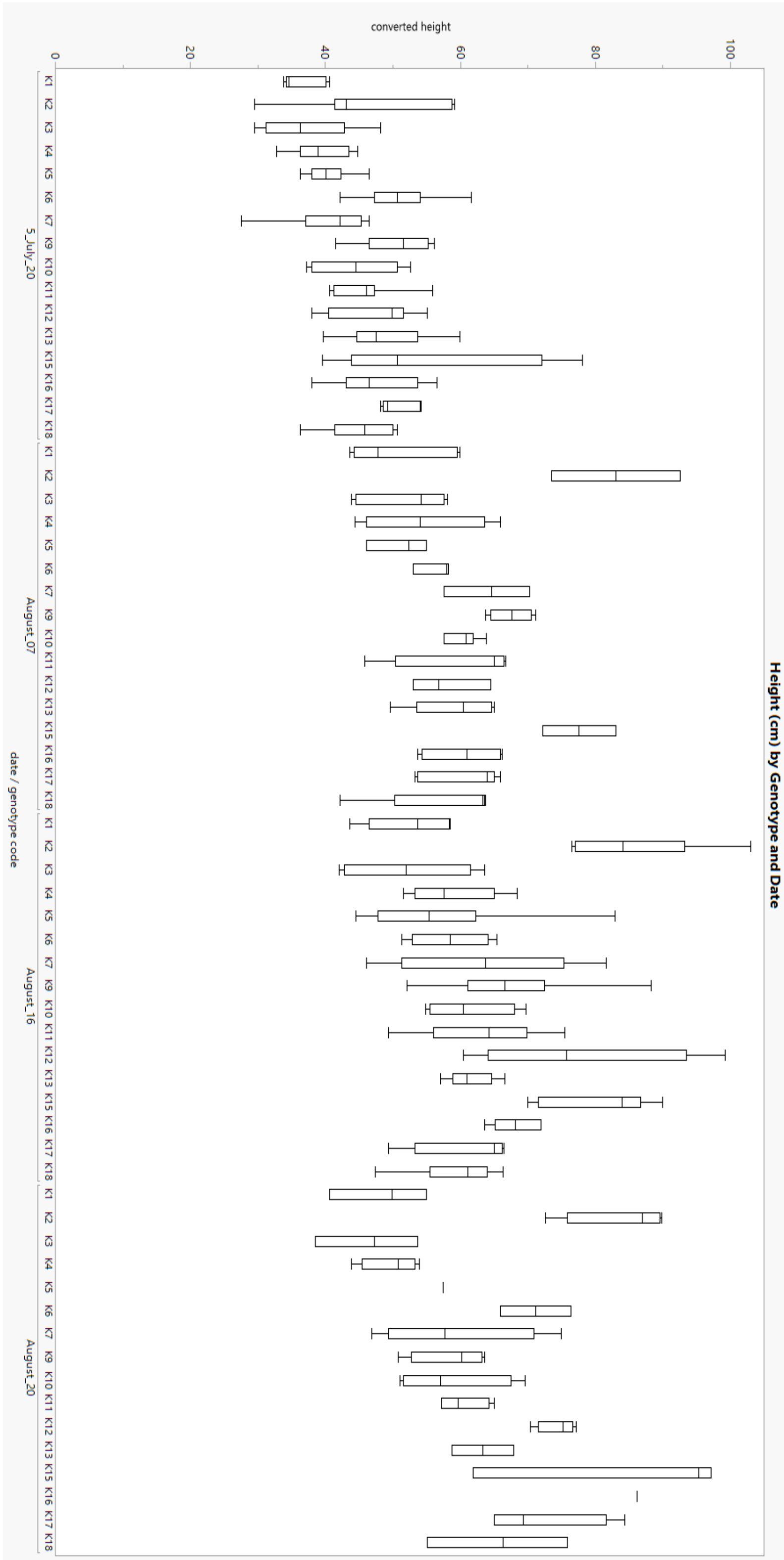


Figure 24. 2018 Automatically generated height phenotypes of tomato plants calculated from the 3D Kinect data across the 2018 season by genotype and date.

The tomato varieties show a different growth pattern than the pepper varieties. Peppers generally get taller as the season progresses and once they reach a certain height stop growing. Processing tomatoes, which are grown in the field with no trellises, generally grow large while producing fruit. Once their fruit reach a critical weight, the tomato plant cannot withstand the weight and collapses. The fruit continues to grow and ripen after this collapse until harvest.

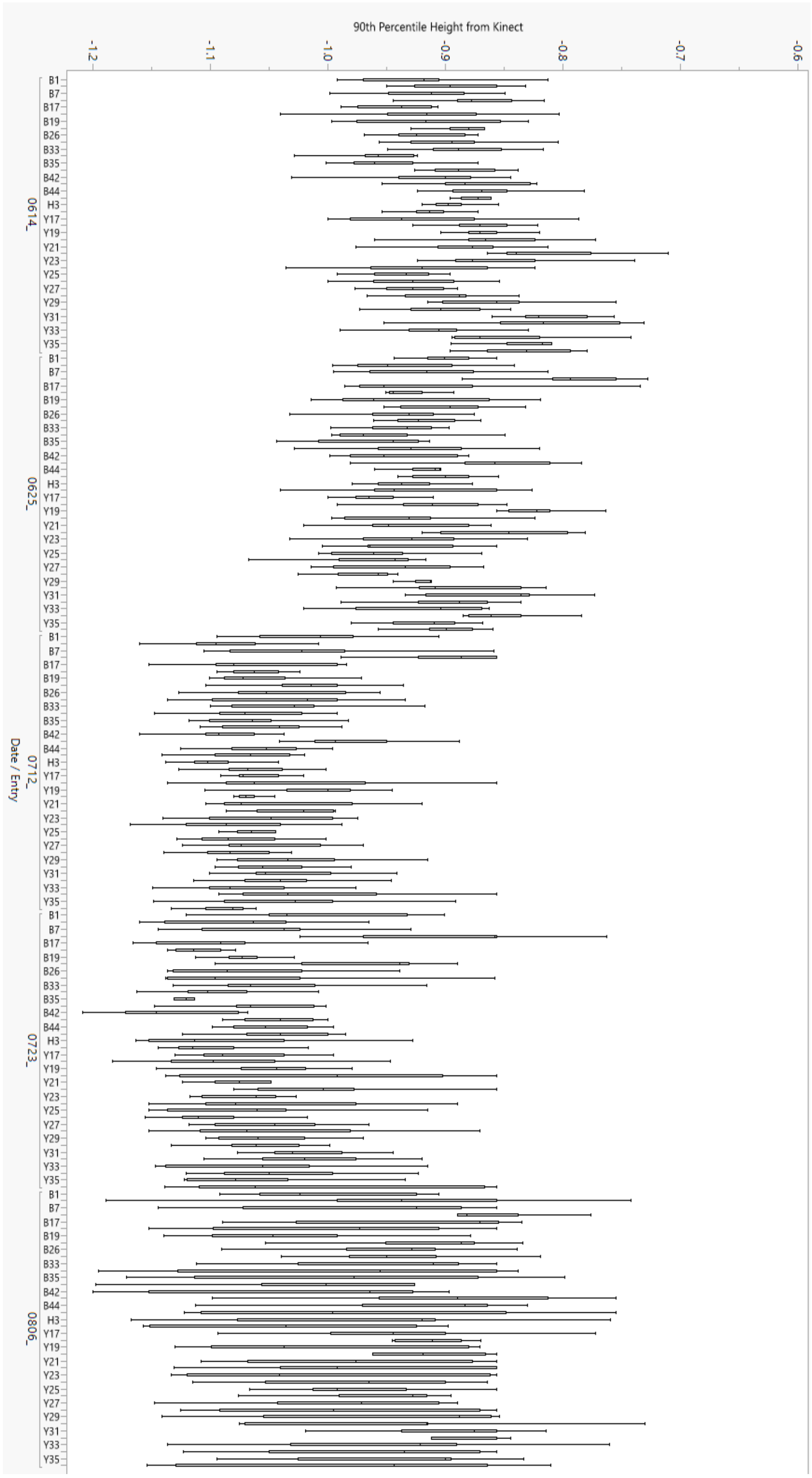


Figure 25. 2019 Automatically generated height phenotypes of tomato plants calculated from the 3D Kinect data across the 2019 season by genotype and data

7.2. Automated Width Phenotypes

7.2.1. Peppers

Width was estimated utilizing autonomous methods for the genotypes in 2018, 2019 and 2020 for validation data. A plant-by-plant comparison of width can be seen in Figure 26. Measuring width in the field in a way that allowed for a meaningful comparison with the Kinect width was difficult. As the plants grow, they merge making the boundary of one plant difficult to distinguish. These challenges were increasingly obvious when determining where to measure width utilizing the point clouds due to the nature of remote sensing. In the field, the person first determines the beginning and end of the plant and then decides where they believe the widest point of the plant is and measures across that distance perpendicular to the row direction to the nearest half inch. With the point cloud data, there is guess work to understanding where the plants start and end. It is relatively easy to find the widest length across the plants in the plot but there is no way to determine if that width was the same location as where the manual measurement was taken.

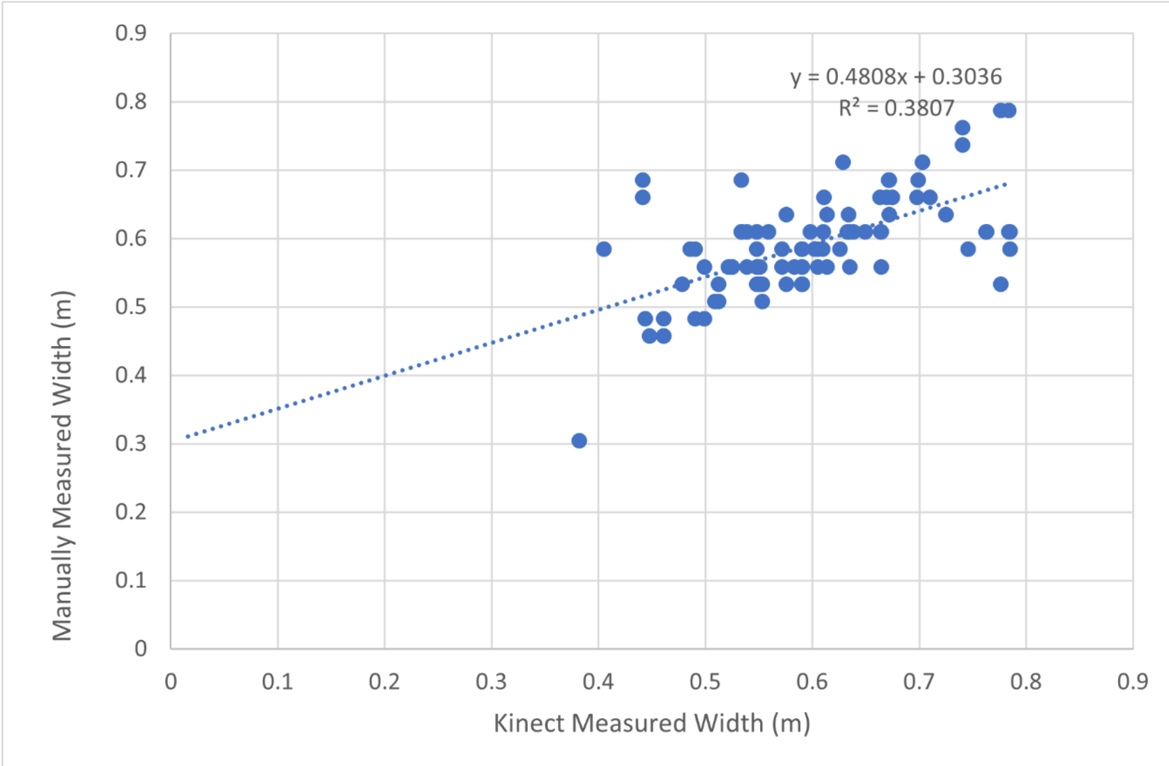


Figure 26 2020 Kinect Measured widths vs manually measured widths

In 2020, manually measured data was collected for each plant to compare with the width calculated from the Kinect data. An overall width comparison on a plot-by-plot basis can be seen in Figure 27.

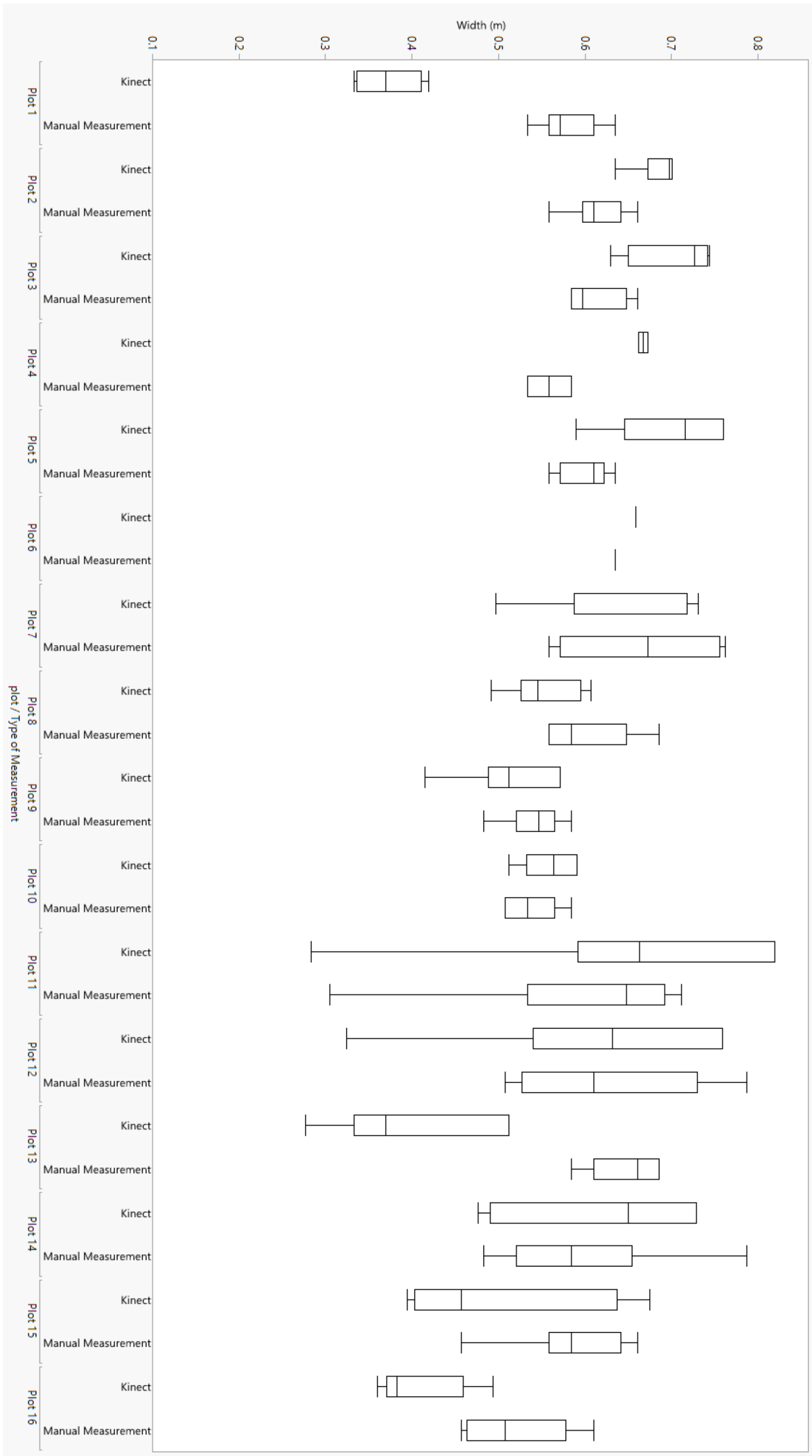


Figure 27. 2020 Kinect measured widths and hand measured widths

Unfortunately, there was no hand-measured data collected by the breeder to validate the 2018 and 2019 Kinect generated widths against, but like height, the automatically generated width data calculated from the point clouds has many measurements per genotype as well as across the season.

Plant width was calculated from the point cloud data across four dates for 2018 and five dates for 2019. In general, most pepper genotypes increased in width across the season, some more than others. Other genotypes had more variation on the width of the plants in the same genotype as seen in Figure 28 and Figure 29.

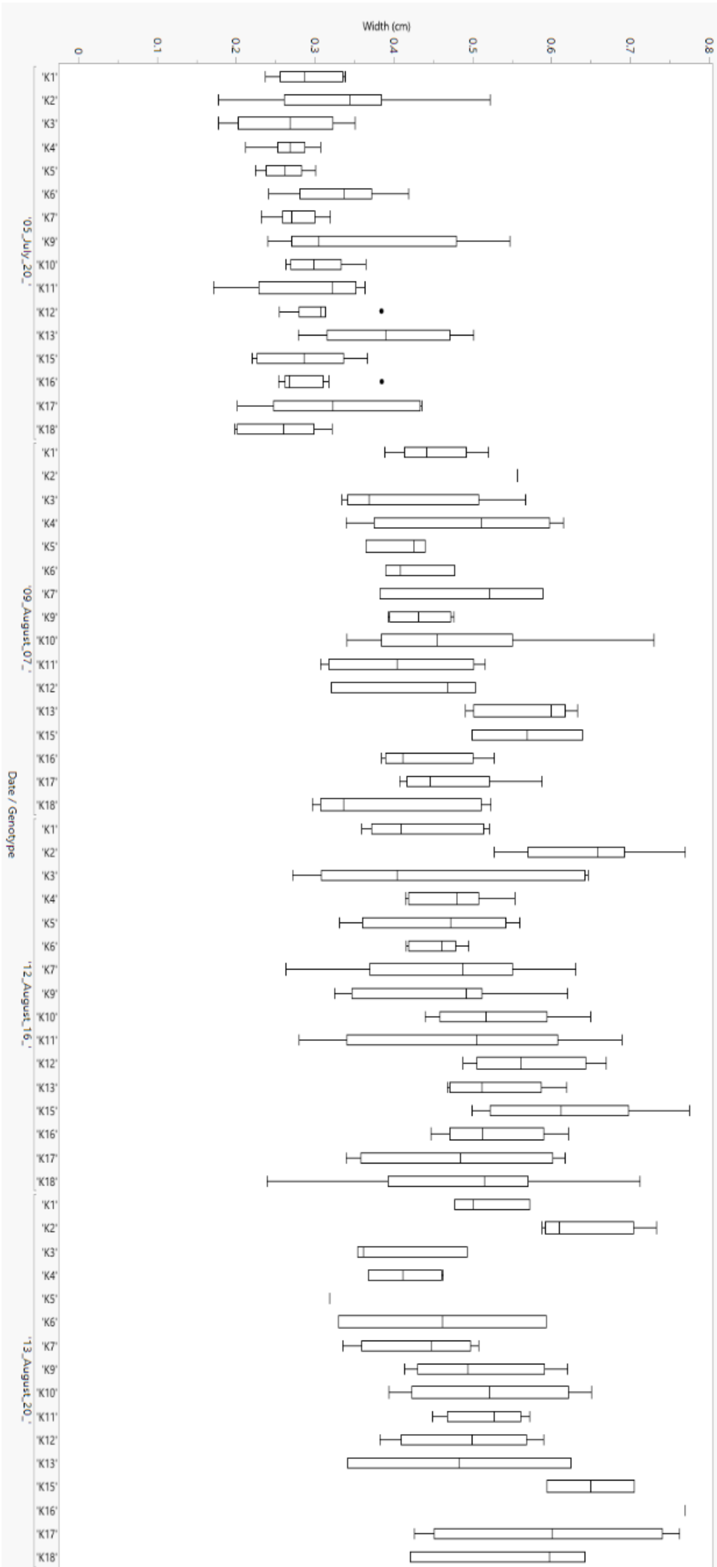


Figure 28. 2018 Automatically generated width phenotypes of pepper plants calculated from the 3D Kinect data across the 2018 season by genotype and date.

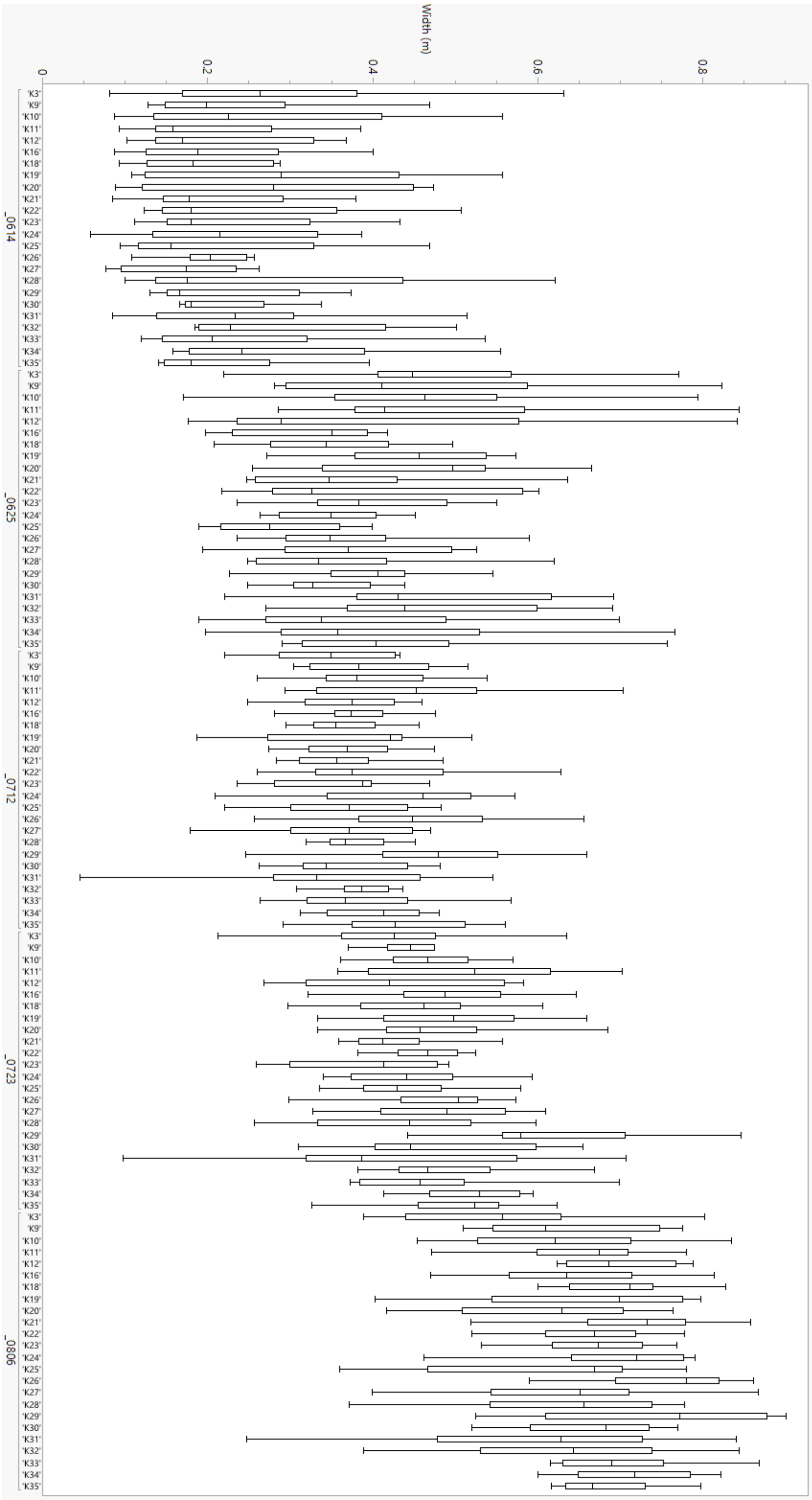


Figure 29. 2019 Automatically generated width phenotypes of pepper plants calculated from the 3D Kinect data across the 2019 season by genotype and date.

7.2.2. Tomatoes

Tomato genotype widths were calculated across the season for 2018 and 2019 as seen in Figure 26 and Figure 30

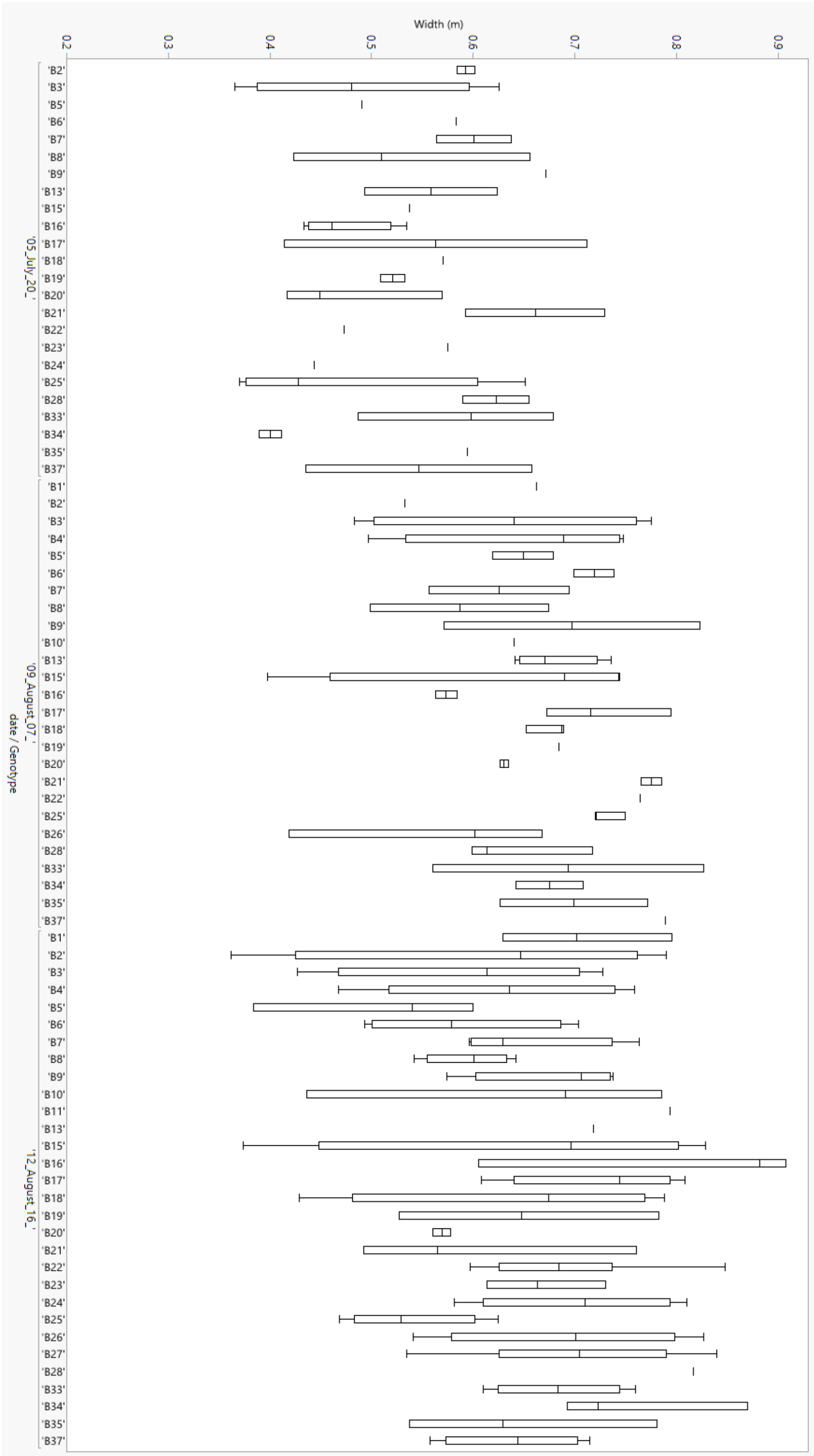


Figure 30 2018 Automatically generated width phenotypes of tomato plants calculated from the 3D Kinect data across the 2018 season by genotype and date.

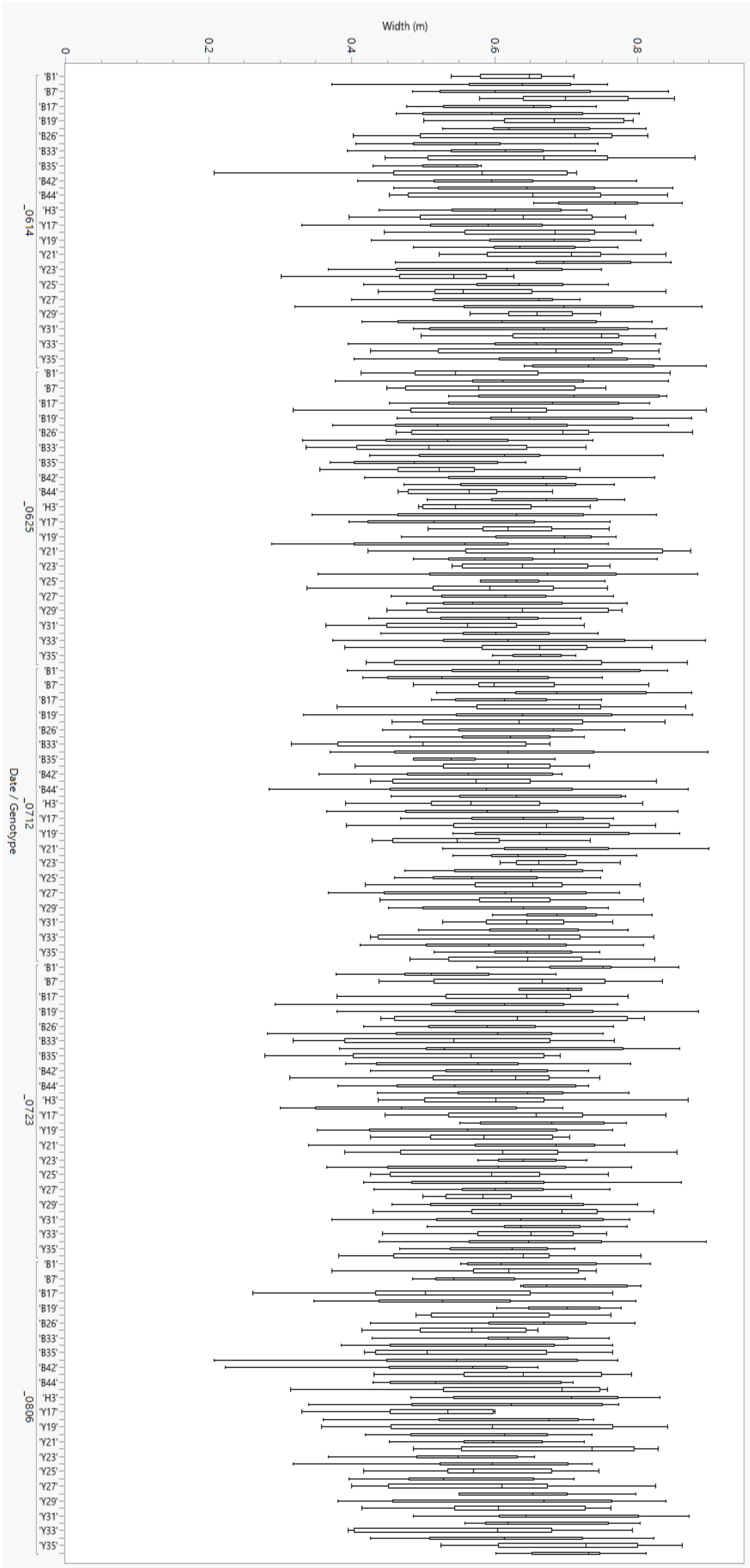


Figure 31 2019 Automatically generated width phenotypes of tomato plants calculated from the 3D data x

In 2019, the tomatoes were at a stage in their growth where they spanned the entire width of the furrow the first time (June 14, 2019) the Spider drove through the rows that season as seen in Figure 32. In 2018 there was a heat wave about two weeks after the transplant date which shocked the plants into early flowering and resulted in breeder intervention. Due to this unexpected weather and intervention the plants took longer to mature because the plants put more energy into developing flowers and replenishing the removed flowers instead of foliage growth.

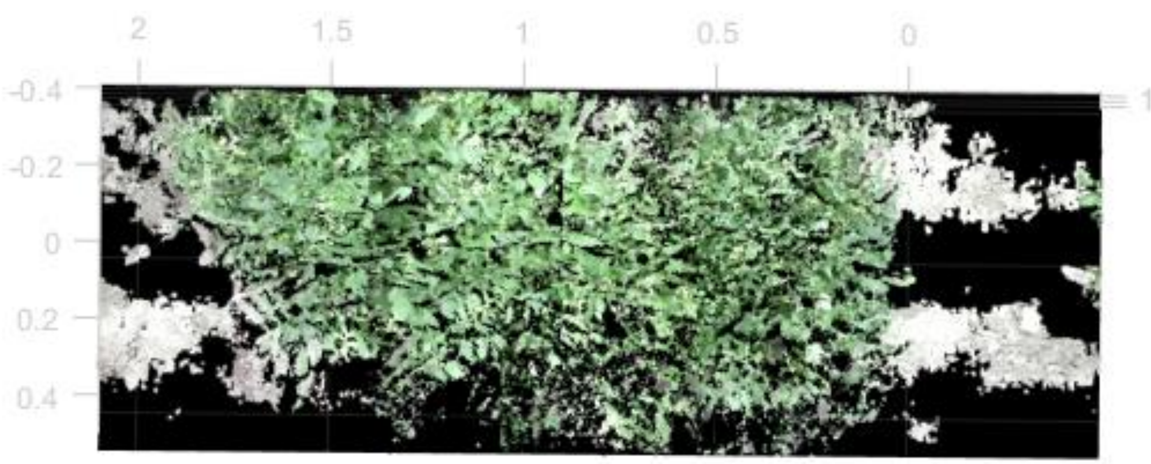


Figure 32 Tomato plot on June 14, 2019

7.3. Automatic Volume Phenotypes

Calculating volume presented several challenges. The plant breeders were interested in a remote sensing volumetric equivalent to a plant biomass phenotype. This requires an estimate of the open cell volume where the interior void space is not included in the plant volume. The

Kinect was able to capture detailed 3D models of the outer surface of the foliage in the plots but lacked information about the interior void spaces of the plant. This means the Kinect measurements provided a lot of information about the shape and size of the plant but not how dense the plant was. Some genotypes (of both peppers and tomatoes) are spread out and have long stems with lots of space between the fruit and leaves in the interior of the plant whereas others are more densely packed with very little space within the interior of the plant.

7.3.1. Peppers

As seen in Figure 33, in 2018 the pepper plants increase in volume over the course of the season. The last date, the volume decreases because the plant breeders conducted a destructive yield and biomass measurement resulting in the loss of volume for the Kinect to measure. In 2019, the Figure 34 shows the increase in pepper volume over the course of the season. For this year the last date is a lot earlier due to the breeder taking destructive manual measurements over the course of numerous dates which for safety reasons prevented the platform from running.

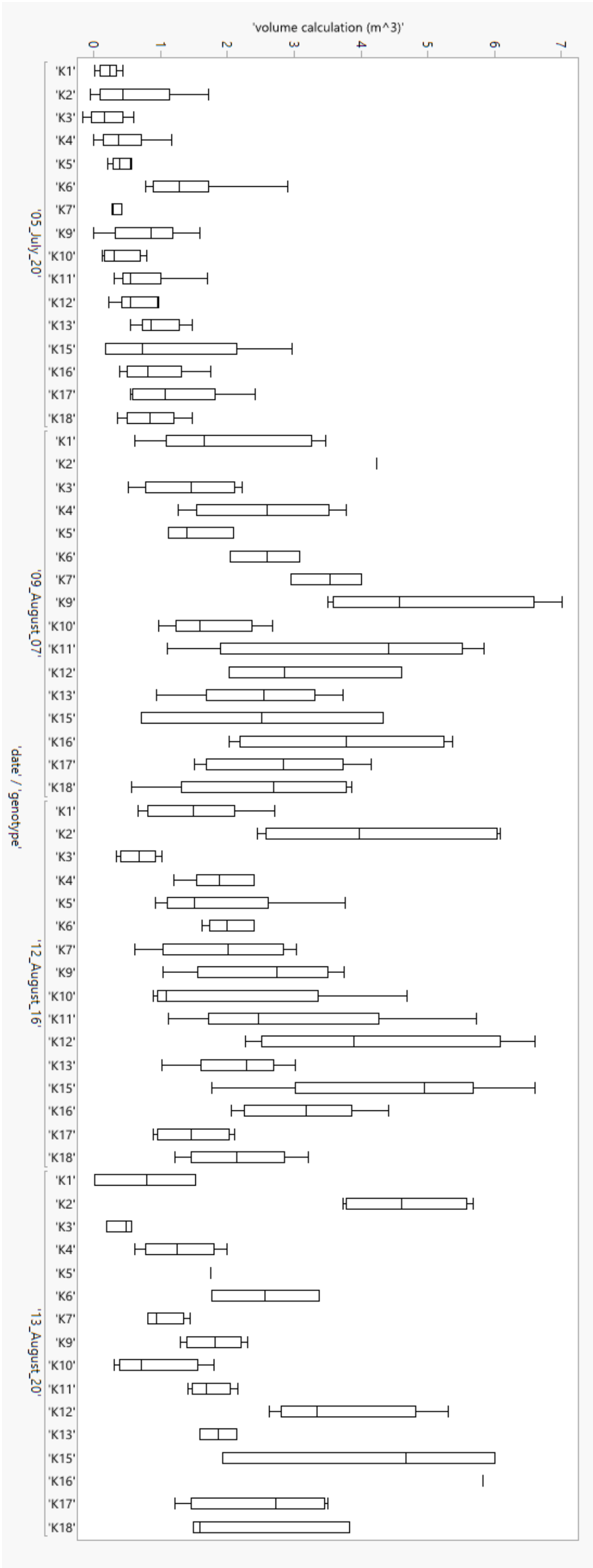


Figure 33. 2018 Automatically generated volume phenotypes of pepper plants calculated from the 3D Kinect data across the 2018 season by genotype and date.

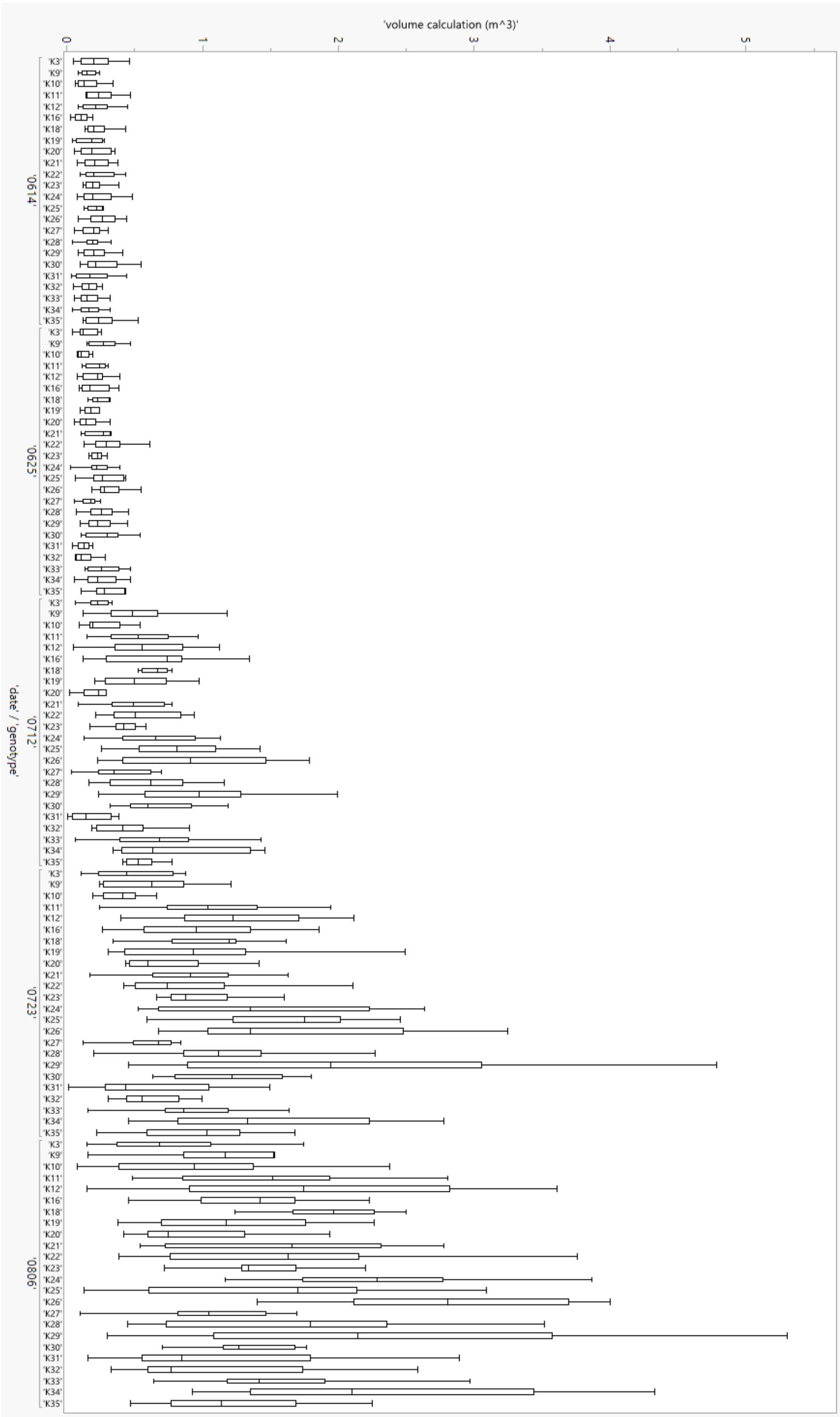


Figure 34. 2019 Automatically generated volume phenotypes of pepper plants calculated from the 3D Kinect data across the 2019 season by genotype and date.

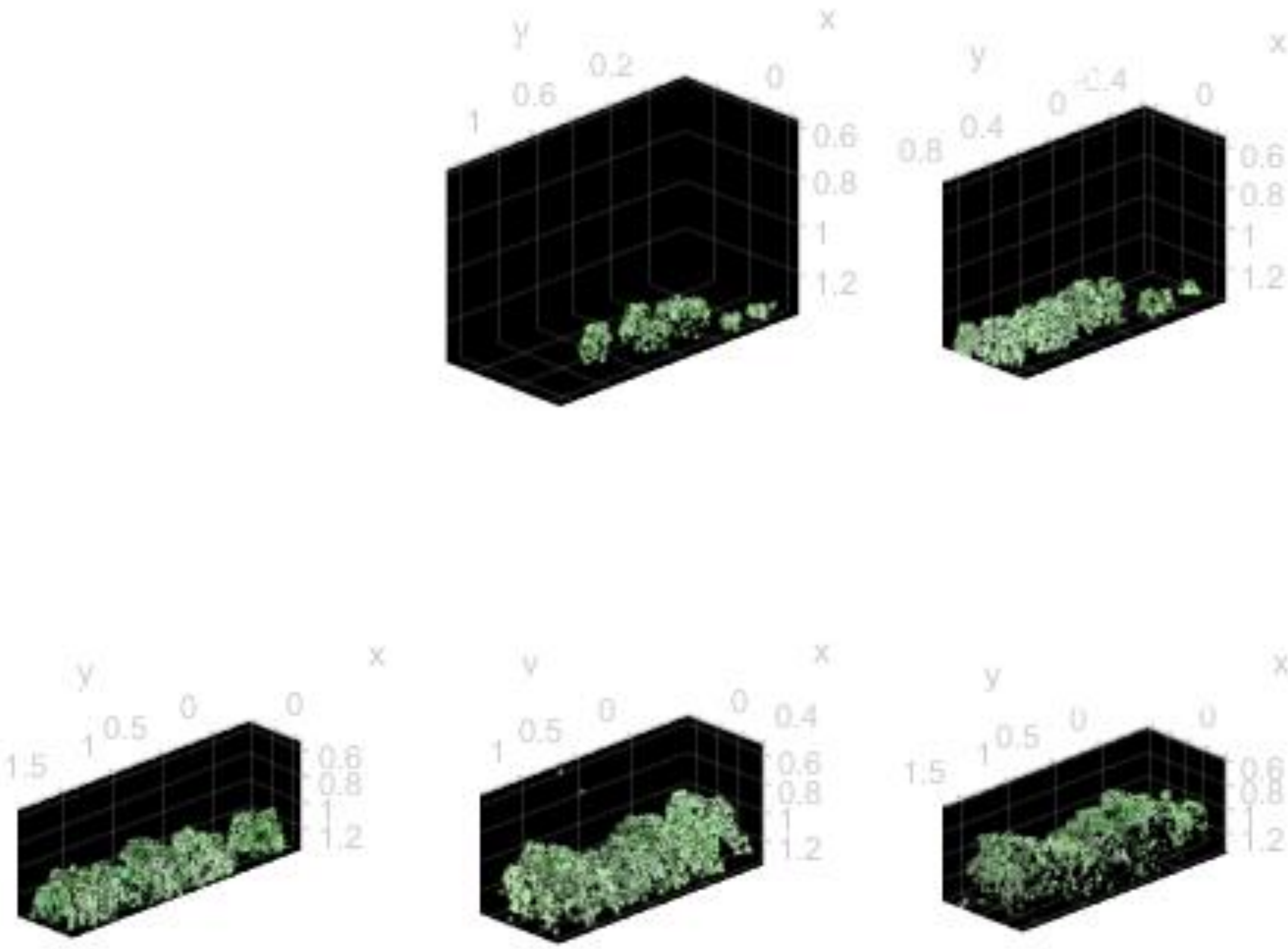


Figure 35. 2019 Pepper point clouds over the course of the season. (Starting from top middle, June 14, Top right June 25, Bottom left July 12, Bottom middle July 23, Bottom right August 6)

7.3.2. Tomatoes

Tomato volume varied greatly across the dates collected. In 2018, the last date (August 16, 2018) was taken after a destructive harvest and therefore has a much lower volume as seen in Figure 36. In 2019 there is more dates as well as data per date due to the implementation of the RTK-GPS and fine tuning of the irrigation system from the previous year, as seen in Figure 34.

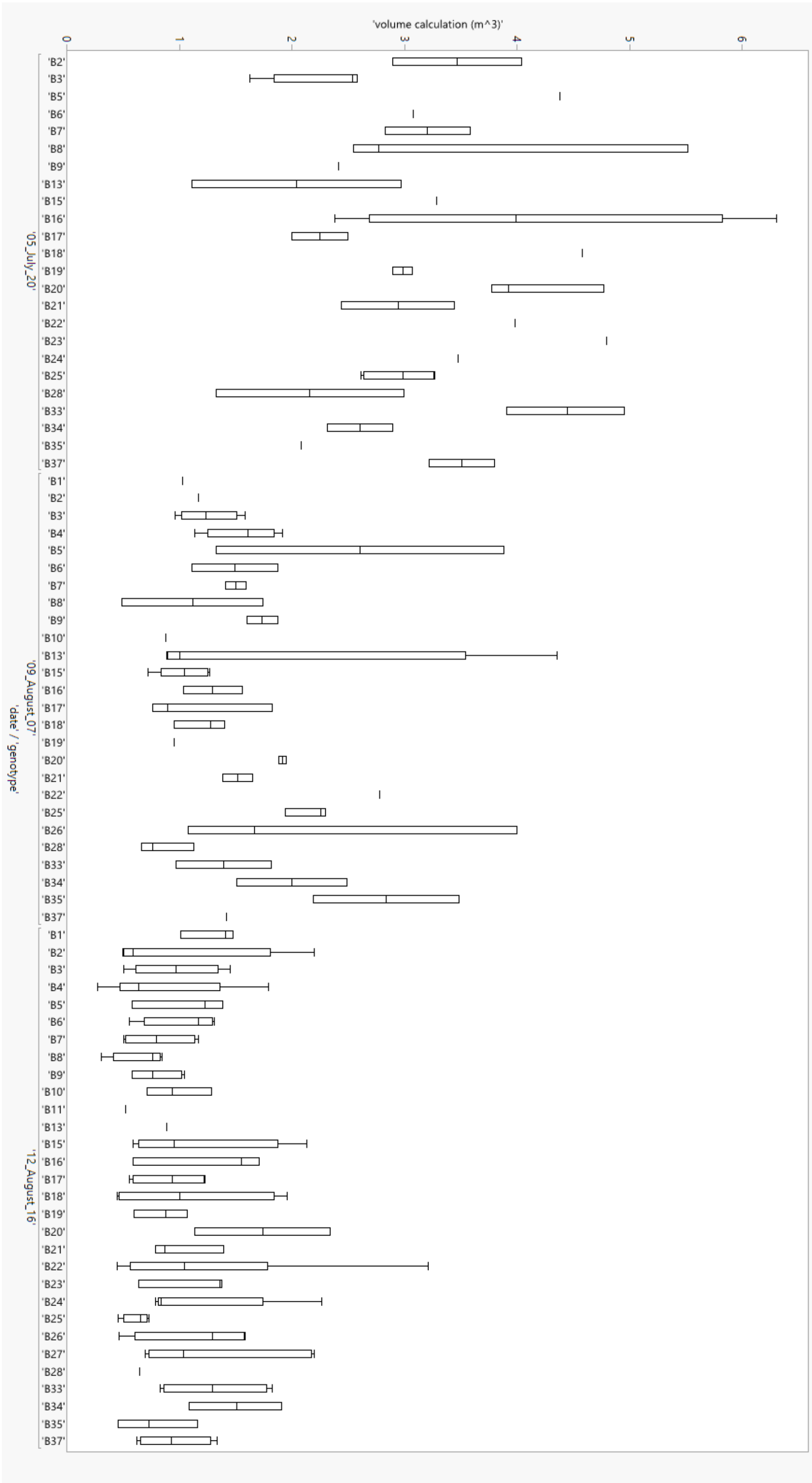


Figure 36. 2018 Automatically generated volume phenotypes of tomato plants calculated from the 3D Kinect data across the 2018 season by genotype and date.

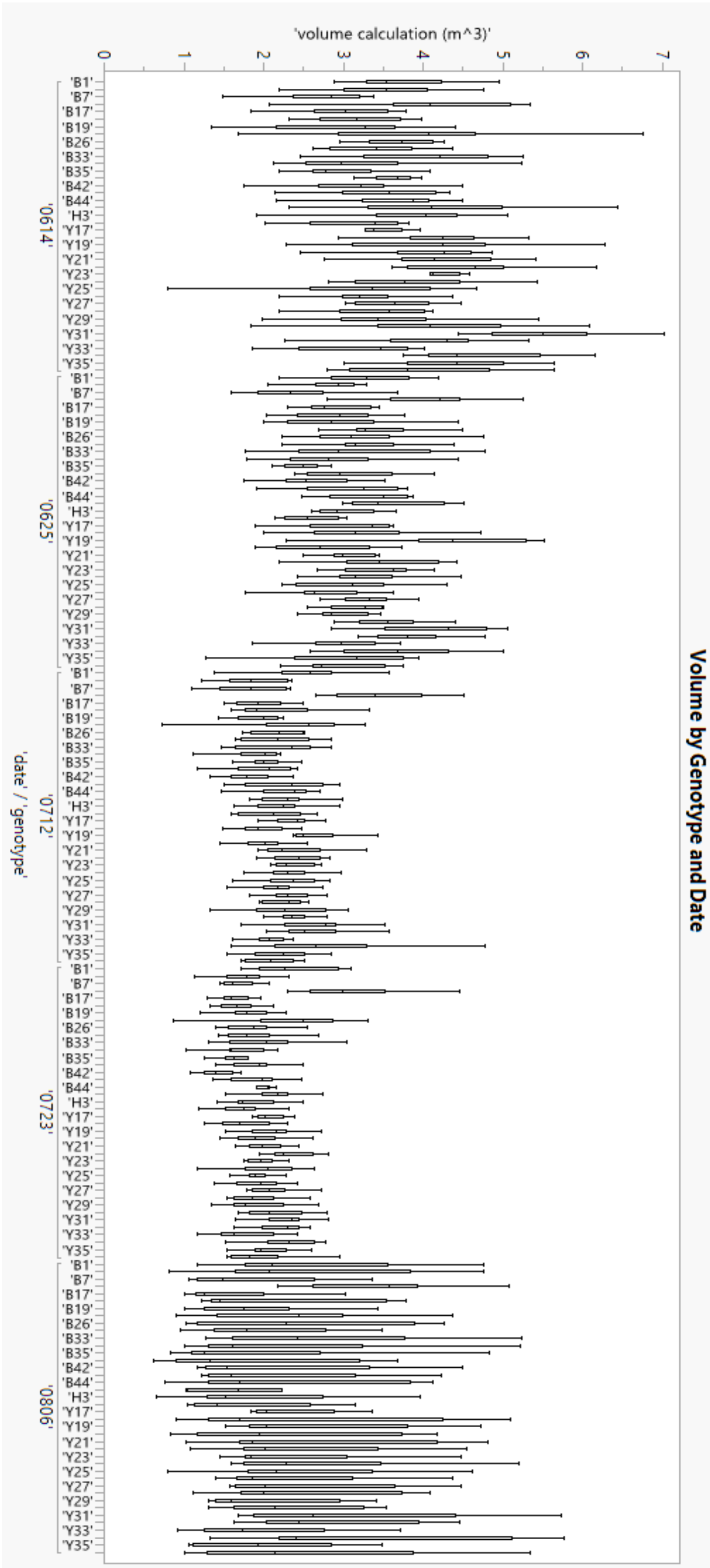


Figure 30. Automatically generated volume phenotypes of tomato plants calculated from the 3D Kinect data across the 2019 season by genotype and date.

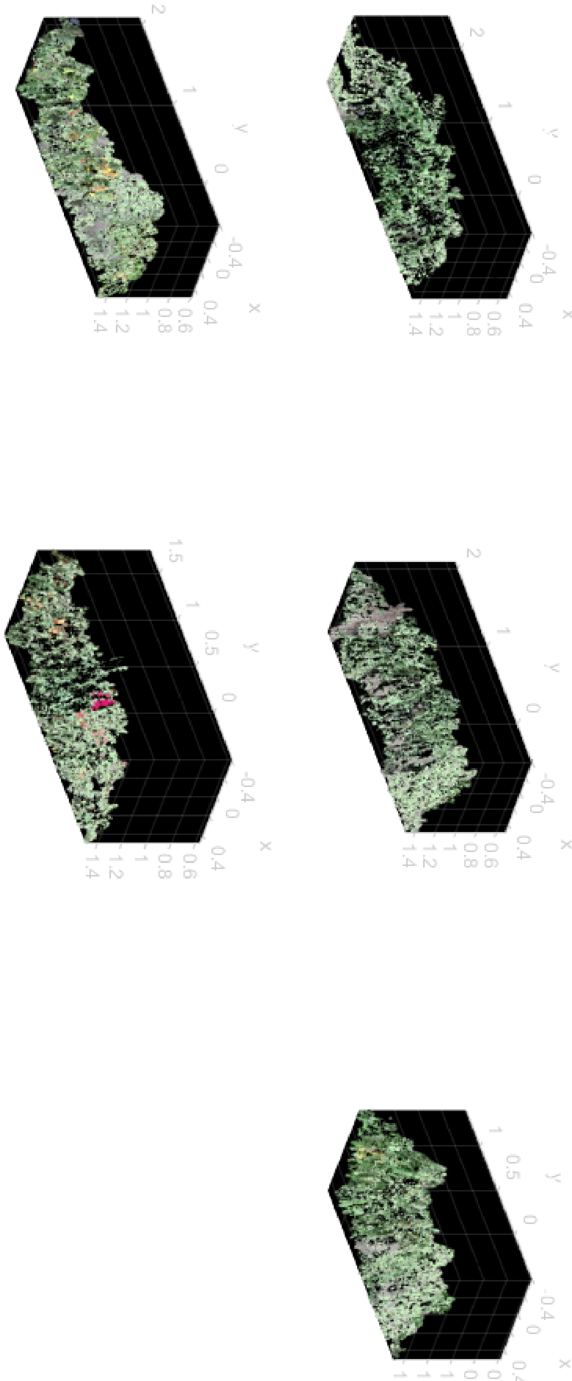


Figure 31, Tomato plots over time (Starting from top left, June 14, Top middle June 25, Top right July 12, Bottom left July 23, Bottom middle August 6)

8. DISCUSSION

Over the course of three years the Spider was taken out to the field every summer to collect data of Solanaceae crop varieties. There were obstacles during the data collection process that limited what data could be collected, however despite the issues there is a substantial database of three dimensional and RGB data for a variety of plants.

8.1. Challenges

This project was large and intensive which understandably led to challenges and obstacles along the way. Data collection was not a trivial portion of the project. The irrigation for these fields were converted to Softline irrigation to allow the Spider to drive over irrigation lines without damaging the irrigation systems. Due to inexperience using Softline irrigation methods, there were significant irrigation issues that resulted in numerous flooded rows during the season. The Spider was not able to run through rows with muddy soil, essentially excluding those plants from the study. Due to the inconsistency of the irrigation issue and resulting flooding, data was collected of some plants for a portion of the dates but not all, which is why 2018 has less data than 2019. In 2018 there was also an unexpected heatwave in May. To preserve the integrity of the study, the flower buds for the tomatoes and peppers were removed prior to phenotyping because the heat wave induced early flowers and fruiting which would not have been representative of how a genotype would perform under normal conditions.

In 2019, Davis received a rather unprecedented amount of rainfall in May that flooded the field and delayed the start date for the phenotyping platform.

Figure 37 2019 Field after unexpected rain early in the trial

Because this rain occurred quickly after the initial transplanting from the greenhouse to the field, many of the pepper plants were stunted for several weeks due to decreased soil temperature, resulting in root temperature stress and delayed growth. While the rain did not seem to affect the tomato varieties in terms of growth because the tomatoes were planted earlier than the peppers, the platform was unable to traverse the field until later in the growth cycle, so early-stage tomato growth was not available for that year.

8.2. High Throughput Phenotyping Scale and Pipeline

The goal of High Throughput Phenotyping is to provide phenotypic data for various genotypes on a large scale quickly. With any high throughput phenotyping project comes the challenge of handling and processing large amounts of data quickly. A single field run generated about one terabyte of data from the sensors, all of which needed to be sorted, processed, and analyzed into meaningful results. After the point cloud creation, one day of data from a single Kinect was just over 100 gigabytes of data. When compared to traditional methods of architectural phenotype measurement, which generally consisted of an excel sheet with one to two measurements per plot, the Kinect provides significantly more information, but that information is not useful unless processed and output in a meaningful way.

To ensure that the data collected was secure and not susceptible to hardware failure, all the data collected was uploaded onto a cloud-based server as well as saved on external hard drives. Because the platform traversed through the fields twice a week, twice a week a terabyte of data had to be checked (ensure no obvious errors or equipment malfunctions) and backed up twice a week.

After the raw data was saved came the task of establishing automated methods of sorting, processing, and analyzing the data. In 2018, the RTK GPS had not yet been integrated with the Time-of-Flight Camera (Kinect) and therefore was manually turned on at the start of each row and turned off. While this time difference between rows allowed the row sorting algorithm to be relatively straight forward, understanding where the plots started and stopped was not a trivial automated task. Dividing the number of point clouds per row by the number of plots and sorting it was successful but required many hours of manually checking that the plots were sorted properly. In 2019, the integration of the RTK GPS allowed for much more rapid sorting with less human intervention because the time in between plots was consistent. In the end, over 4,000 point clouds of full plots were created over the course of the three years.

Now that this process has been developed and automated, a single person could run the phenotyping platform through a set of fields of similar size and have automated results of height, width and volume returned in less than 5 days. This would include multiple height measurements per plant (as well as averages), a width estimation per plant, and a volume estimation of the plot. In addition, there would be 3D models of each plot which could be used for future analysis of features not measured in this study. Traditional phenotyping methods cannot provide this quality of data using a single person over five days.

8.3. High Throughput Phenotyping Methodology Challenges

Developing automated methods for the measurement of height, width and volume proved to be a difficult problem. In Figure 38, it is not difficult for a human to pick several points where they would take a height from.

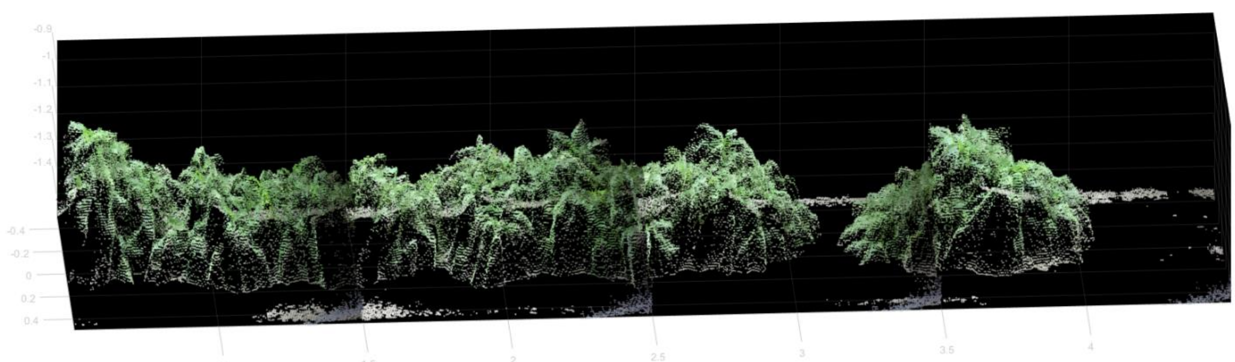


Figure 38 Automated 3D model generated from Kinect Data of a pepper plot

If the figure could be rotated in a text document like this, it would also be easy to decide where to take the width measurements also. But it is not a trivial problem to automate those measurements. Heights taken at the same location for every plot do not give an accurate comparison to how a person would take a manual measurement in the field, or even from a 3D model like in Figure 38. Plants, like any biological specimen, have variations from individual to individual and require adaption in the measurement process that are difficult to automate.

Volume was not easy to phenotype to measure or validate. There is no obvious way for a human to estimate volume by hand which made automating the process tricky. Numerous methods like Convex Hull which was utilized in line scans by del Campo et al, was tested but concluded not to be viable for this data set. Once settling on the method used in this paper, validating, or comparing the data was not like height or width. The breeders understandably did not have a similar method of measurement because volume is not a straightforward field measurement.

8.4. Data Validation

Even so, phenotyping via remote sensing has shown promising results. This project's objective of creating an autonomous method to process 3D data to measure architectural phenotypes was successful. This method of phenotyping captured high spatial resolution data in all three fields twice a week (2-3 hours per run including set up) across the entire season with only one person whereas traditional methods require a large time investment and large amount of manual labor to find similar measurements. As seen with height measurements, the Kinect's results were not proven to be statistically different compared to the manually measured heights. All p values when compared were greater than 0.01 with the exception of one genotype (K34, 2019). But the Kinect results had more samples per plot as well a relatively high temporal resolution. The traditional method resulted in only two measurements taken per plot whereas the Kinect point cloud had millions of points in the point clouds of the plot.

While not all types of measurements can be replicated with remote sensing with the methods in this project (mass), comparable types of measurements that are more efficient to collect could potentially change how plant characteristics are measured and analyzed. For example, mass or weight is traditionally the metric used to measure the size of plants because it is relatively easy to bring a scale to the field or collect samples and bring them back to a laboratory to measure mass. A measurement like volume is not easy to collect by hand for plants and therefore has not been utilized in phenotyping.

Figure 39 Phenotypes measured by hand by researchers for tomato varieties

However, overall volume is a more feasible measurement to collect via remote sensing whereas mass is much more difficult due to the unknown density of an object. Volume measurements via remote sensing are also nondestructive in nature allowing for temporal data on the same plant across the growing season. Measurements like volume that have not widely been used in describing plant architecture due to ease of measurement are now much more feasible and have the potential to provide a more comprehensive picture of plant growth over the season.

9. CONCLUSION

The objective of this study was to identify and measure architectural plant phenotypes (height, width, and volume) of the Solanaceae family by utilizing remote sensing and a fully automated post experiment processing system. Data for those phenotypes were successfully collected and measured over the course of three years. Of the data that could be validated against traditional phenotyping methods, there was not a significant statistical difference between the data measured via remote sensing and the data measured manually. The data collected and processed using automated methods also create a more comprehensive picture of the growth of a genotype over the course of a season with many more sample points, higher temporal resolution and an entire 3D model. The phenotyping platform and automated analysis methods could allow a single person to collect architectural phenotypes of Solanaceae varieties in less than five days which is not possible with traditional measurement methods. There is potential for this high-speed phenotyping to improve the efficiency of breeding crop varieties in California and the rest of the world.

10. FUTURE WORK

Based on observations made during this study, the following suggestions for future work are proposed below:

i) A similar project as the one conducted but with fewer genotypes and more replications of each genotype should be conducted. These genotypes should be specifically chosen for their architectural differences to allow a wide range of phenotype values to be present and better facilitate comparison across methods.

a. In this project, standardize the way manual measurements are collected (where to measure height and width) and take these measurements for every plant instead of a very small sample of the plot

ii) Utilizing the data from this project or through repeating a similar project, merge all three Kinect views together in a single point cloud to have more comprehensive models of the plants with less obstruction. Side views would provide more detail and a higher density of points in views that are not possible from the top Kinect view.

iii) Utilizing the data from this project or through repeating a similar project, estimate the visible fruit volume to understand size of yield

iv) Utilizing the data from this project explore more thorough and robust filtering methods for noise reduction

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12. APPENDIX – CODE

Access to the code for this project can be found here:

https://github.com/csroo/architecturalphenotypes_solonaceae.git