

AN ABSTRACT OF THE THESIS OF

George J. Kleinknecht for the degree of Master of Science in Geography presented on September 17, 2014.

Title: Introducing and Validating a High-Throughput Bud Break Sensor

Abstract approved:

Heather E. Lintz

Bud break is a key adaptive trait that can help us understand how plants respond to a changing climate from the molecular to landscape scale. Despite this, acquisition of bud break data is currently constrained by cost, scale, and a lack of information at the plant scale on the environmental stimuli that precede and cause bud dormancy to release.

Additionally, to date, little effort has been devoted to phenotyping plants in nature owing to the challenge of controlling for environmental variation to isolate genomic effects on plant phenotype. Notwithstanding, natural selection operates on natural populations, and investigation of adaptive phenotypes *in situ* is warranted and can validate results from controlled laboratory experiments. In order to identify genomic effects on individual plant phenotypes in nature, environmental drivers must be concurrently measured and characterized. Here, we innovated and evaluated a sensor to meet these requirements for temperate woody plants. It was designed for use on a tree branch to measure the timing of bud break together with its key environmental drivers, temperature and day length.

Specifically, we evaluated the sensor through independent corroboration with time-lapse photography and a suite of environmental sampling instruments. We also tested whether the presence of the device itself on a branch influenced the timing of bud break. Our results indicated the following: the bud break sensor's digital thermometer closely approximated a thermocouple touching plant tissue; the photoperiod detector measured ambient light with the same accuracy as time lapse photography; the phenology sensor accurately detected the timing of bud break; and the sensor itself did not influence bud phenology. Future use of the sensor can help galvanize a new field of study, landscape phenomics, or high-throughput phenotyping of organisms at the landscape level for integration with landscape genomics among other potential applications and disciplines.

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Introducing and Validating a High-Throughput Bud Break Sensor

by
George J. Kleinknecht

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

George J. Kleinknecht, Author

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1. INTRODUCTION

Bud break is when plants initiate tissue growth from their buds, signaling the end of winter dormancy and the beginning of the growing season. The timing of bud break in plants influences biomass accumulation and carbon sequestration, and informs us about the responses of genes and ecosystems to a changing climate (Menzel et al., 2006, Aitken et al., 2008). Climate change may alter the timing of bud break with potentially serious implications (Chaine, 2010). Climate change could alter the amounts of chilling and forcing units sensed by vegetative buds, and may cause bud break to occur early, late, or not at all (Pope et al., 2013). The advance of the date of bud break over recent decades has been documented for a number of species and across a range of biomes (Chaine, 2010). Phenological shifts in agricultural crops can alter not only the beginning and length of growing seasons, but can also cause crop failures (Chmielewski et al., 2004). A deeper understanding of plant response to climate change is imperative for addressing the effects of future climate change on agriculture and forest management (Badeck et al., 2004).

The timing of bud break in populations of temperate trees is determined largely by air temperature and genetics (Campbell and Sorenson, 1973, Chaine and Cour, 1999, Harrington et al., 2010, St. Clair et al., 2005). For some species, photoperiod and plant water status are additional cues to the timing of bud break (Yakovlev et al., 2006, Lagercrantz, 2009, Linares et al., 2012). Air temperature, however, is perhaps the most widely studied and easily measured environmental cue. Trees in temperate regions typically have a chilling requirement that deepens bud dormancy in order to prevent an early bud break from occurring and exposing the young foliage to frost damage (Bailey

and Harrington, 2006). After the chilling requirement has been met, dormancy is then gradually released by warming temperatures in the spring (Figure 1).

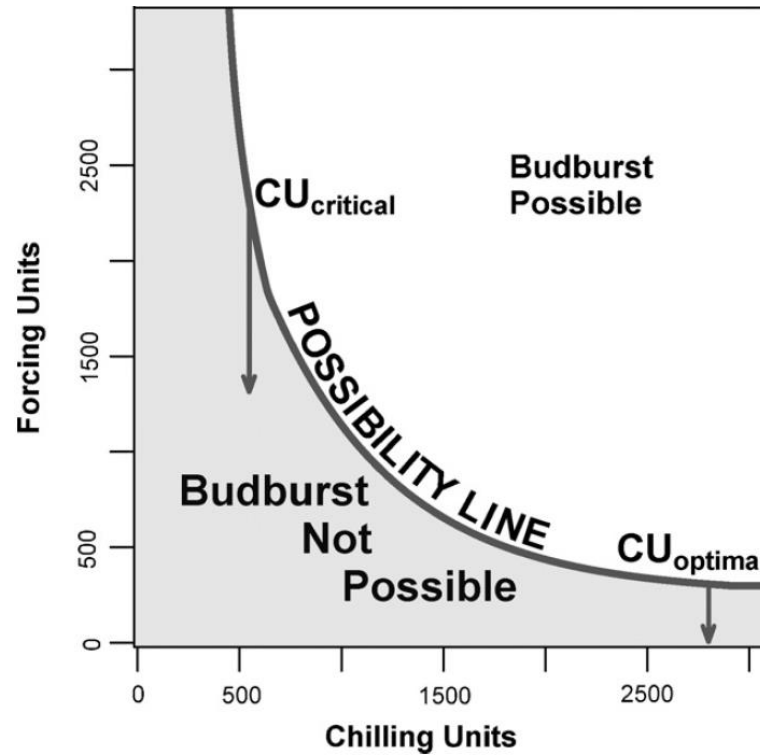


Figure 1: Conceptualization of when it is possible for bud break to occur, from Harrington et al. 2010.

Despite the increasing evidence for epigenomic and genomic bases of bud break phenology (Gould et al., 2011, Yakovlev et al., 2011, Yordanov et al., 2014), our understanding of bud break processes remains limited. Although the molecular basis of bud break is widely studied for model tree species (Hsu et al., 2011, Yordanov et al., 2014, McKown et al., 2013), studies for non-model species including conifers are limited to coarse quantitative trait associations (Jermstad et al., 2001), low coverage screens of candidate genes (Eckert et al., 2009), differential gene expressions before and after bud break (Yakovlev et al., 2006, 2008, Mathiason et al., 2009), and some recent work in epigenetics of spruce (Yakovlev et al., 2011).

One challenge we face in deciphering the nature of adaptive processes like bud break in woody plants is the development of new methods in high-throughput phenotyping to relate genes to traits. Molecular data in genomics and systems biology are now being generated in high quantity, which necessitates phenotyping methods that are inexpensive, practical, and can accommodate large numbers of individual plants. Additionally, emulating natural conditions can be difficult in laboratory, green house, or common garden settings (Granier et al., 2006, Poorter et al., 2012). Consequently, it is not surprising to find studies that identify adaptive markers and genes for plants in laboratories that either do not extrapolate to other laboratories (Massonnet et al., 2010) or to natural plant populations (Mishra et al., 2012). Furthermore, to date there have been very few efforts to overcome the challenge of identifying adaptive genes in natural populations because the environment in most ecosystems is impossible to control and thus establish traditional cause-and-effect relationships. These difficulties, in addition to others, hinder advancement in our understanding of the non-linear interactions between a plant's genes, the environment, life stage, and growth stage (Hänninen and Tanino, 2011).

Here, we advocate a new conceptual framework for identifying the genomic and molecular basis behind adaptive processes in natural woody plant populations. Since it is difficult to control the environment in forests, we argue that our next best option is to measure and account for the effect of environmental drivers on phenotypes in nature, and to then assess the genomic basis of phenotypes in nature. This can occur through co-measurement of pertinent environmental drivers alongside phenotypes in nature. Specifically, here, we present results that contribute technology innovation to facilitate the future implementation of this conceptual framework. Our sensor allows for high-

throughput phenotyping of plants in nature with simultaneous measurements of bud break, temperature, and photoperiod on vegetative buds. The measurement of adaptive traits like bud break and their environmental drivers in nature can allow us to step closer to decoding complex processes like the genomic basis of natural phenotypes and how temperate trees will adapt to changing climate (Houle et al., 2010). Such measurements have the potential to galvanize a new field of study, landscape phenomics, by providing researchers with a measured phenotype and continuous environmental data collected at the individual plant scale across a landscape to dovetail with studies in landscape genomics.

Data acquisition for the timing of bud-break itself is currently limited in scale. Remotely sensed data from satellites are difficult to resolve to a single species, and often bud break occurs during cloudy weather, obscuring the actual date of the event (Schwartz et al., 2002). Human observation data are cost-prohibitive and difficult to collect in remote areas. Near-surface digital time lapse cameras are not calibrated scientific instruments (Sonnentag et al., 2012), and suffer from issues of battery life, file storage, and impracticality of distribution across a landscape. The eddy covariance method for measuring carbon fluxes can infer aspects of phenology like bud break from increased photosynthetic rates; however, these results do not correlate well with the actual date of bud break, they are not resolvable to individual trees or species, and they can be difficult to interpret in sub-optimal weather conditions (Chiang and Brown, 2007, Niemand et al., 2005).

Current methodologies for measuring or interpreting foliar temperatures also leave room for further innovation. Infrared gas analyzers control leaf temperature to better measure variables like respiration (Bolstad et al., 1999, Xu and Griffin, 2006), but are

expensive and are not designed to measure ambient foliar temperatures of organisms in the field. Thermocouples placed against underside of needles (Martin et al. 1999) or on the inside of plant tissues (Michaletz and Johnson, 2006) are fragile and run the risk of being moved or broken during long-term deployments. Iteratively calculating leaf temperatures to balance an energy budget (De Boeck et al., 2012) requires collecting other micrometeorological data, which can be an expensive investment in equipment and time. Placing leaves in water baths of known temperatures is useful for assessing heat tolerance, as is the use freezers to assess cold tolerance (Cunningham and Read, 2006), but these techniques are not suitable for field study. Although thermal cameras are non-invasive and provide greater spatial coverage and temporal sampling than most other approaches (Meron et al., 2013, Prashar and Jones, 2014), they are costly and the image processing is not trivial.

Though air temperature alone has proven successful for many phenological models, we know that leaf, bud, and meristem temperatures differ from air (Grace, 2006, Michaletz and Johnson, 2006) even in moderate environments (Savvides et al., 2013) and that foliar temperatures are a likely more physiologically-relevant (Still et al., 2014). The digital thermometer we describe here is durable, ready for long-term field deployment, and designed to provide an indirect metric of foliar temperature. To our knowledge, it is the only existing thermometer designed with a clear acrylic coating to more closely approximate foliar temperature compared to air temperature for cost-effective yet biologically-relevant information. This biological relevance is of even higher significance when considering the highly localized nature of the temperature data and that temperatures can vary up to 10°C throughout a single tree (Stockfors, 2000, Leuzinger and Körner, 2007).

Here, we report results from several sensor validation experiments. Our goal was to innovate and validate the sensor in order to improve our capacity to measure bud break and related environmental drivers for high-throughput phenotyping in nature. To ensure the data collected by this sensor are accurate, reliable, and non-invasive, we asked the following questions for this work:

- Does the sensor accurately record temperature, photoperiod, and the timing of bud break?
- Does the presence of the sensor itself influence the timing of bud break?

2. MATERIALS AND METHODS

The Bud Break Sensor

Principle of Operation

The bud break sensor used a pair of plastic optical fibers to detect bud break. After being attached to a branch below a bud, one of these fibers guided light from a green LED outwards to illuminate the bud while the other fiber received light reflected from the bud and guided the signal to a photodetector and signal amplifier (Figure 2). Light pulses were emitted from the illuminating fiber at 320Hz, a frequency not harmonically related to common man-made light sources, for approximately two seconds (Li et al., 2013). An analog switch routed the light received from the LED through amplifiers with gain +1 (LED illuminated) and -1 (LED dark). This formed the multiplier action of a lock-in detection scheme to rectify the signal (Horowitz and Hill, 1989, Sydenham and Thorn, 2005, Li et al., 2013). Additionally, by averaging the photodetector output when the LED is dark, the sensor provided ambient light information which we used to determine day

length. The data were then transmitted to a flash drive and stored with a timestamp (Figure 3). The sensor's integrated circuit (IC) thermometer was protected by a coating of clear, waterproof acrylic (Figure 2). The electronics and batteries were housed in a plastic case designed to be impervious to water.

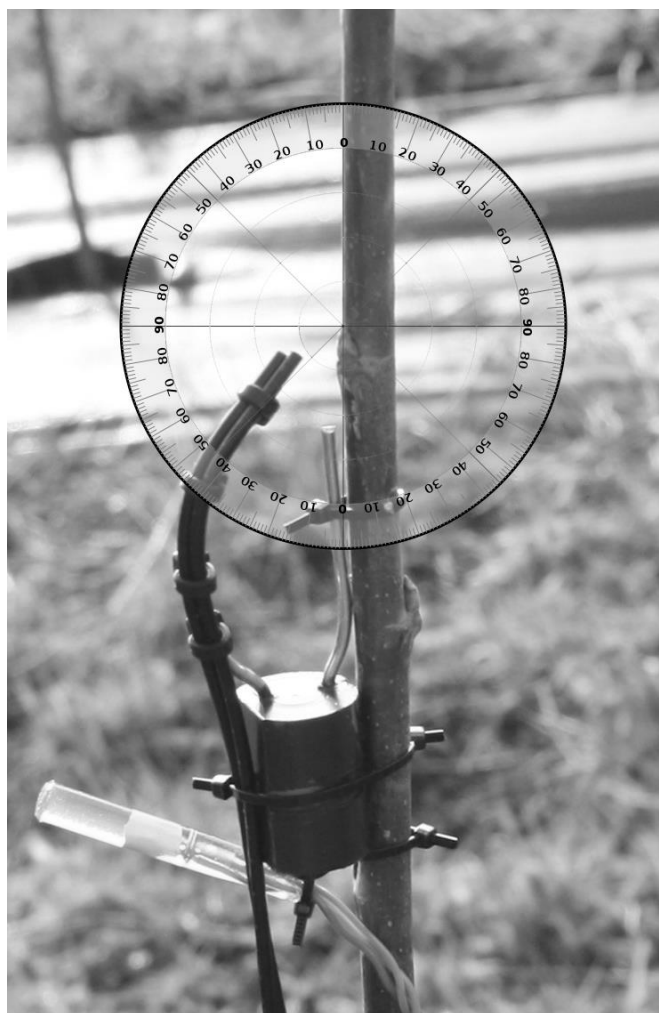


Figure 2: The bud break sensor's fiber optic cables targeting a dormant bud, with digital thermometer below.

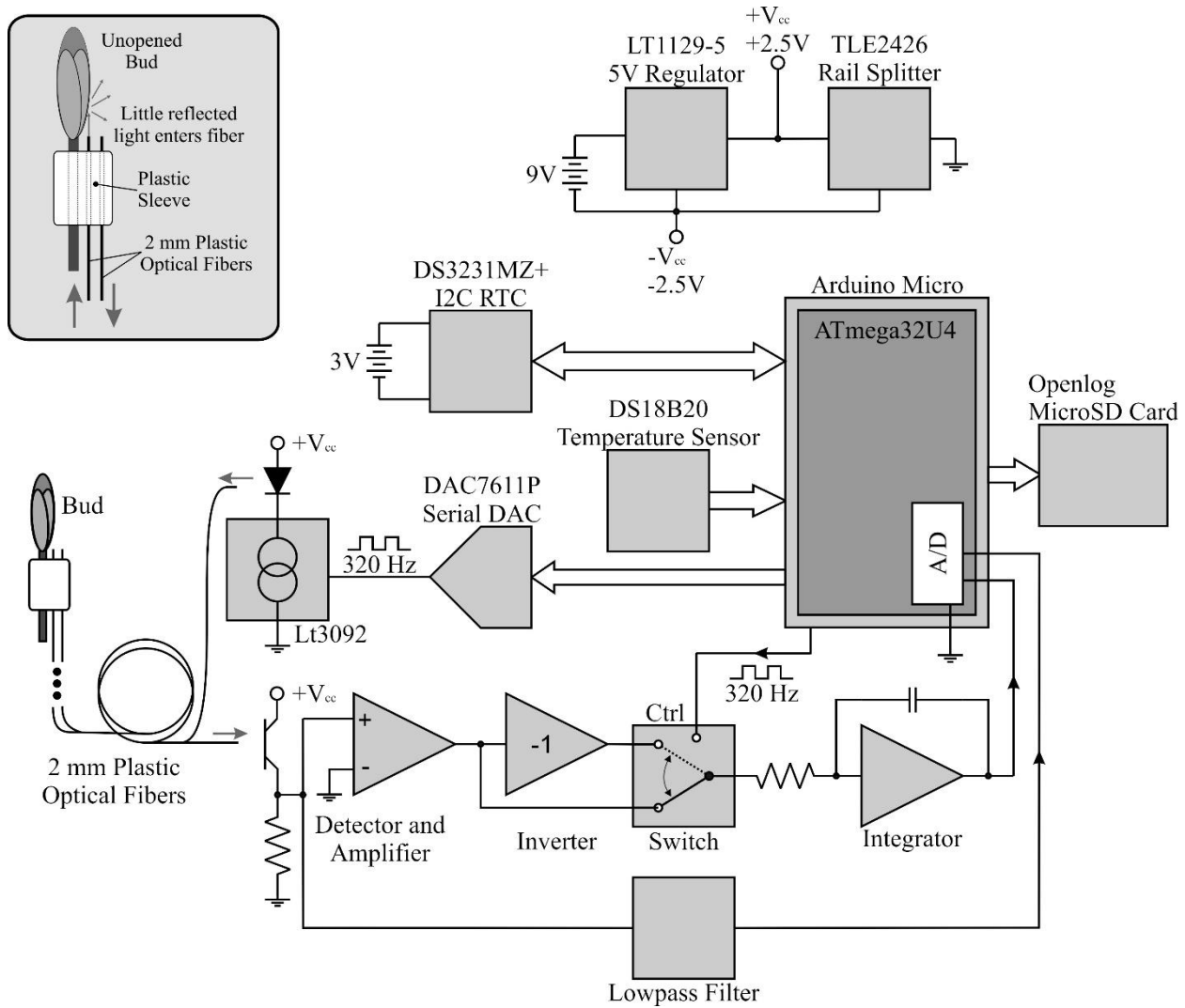


Figure 3: Schematic diagram of the bud break sensor.

Using the Bud Break Sensor

The sensor was designed to be relatively simple to use. By connecting the sensor to a terminal emulator on a computer via USB port, one can set the real-time clock, sampling intervals, device identification, and instrument calibration. Sampling intervals can range from once every ten minutes to once per six hours, allowing users to balance battery life with sampling resolution. During calibration, the sensor's LED brightens and dims until the device finds a proper signal to noise ratio for the object it is pointing at. This calibration

can be done in the field as well with the press of a single button, once the sensor is in place and targeting an object of interest. After disconnecting the sensor from the computer and shutting the housing, it is ready for deployment. The main body of the sensor can be strapped to trunks or large branches, and the wire-mounting system attaches just below the bud. The final positioning of the sensor wires can then be adjusted by moving the bent aluminum wire on the plastic attachment system (Figure 2).

Validation Experiments

Temperature

To assess the bud break sensor's digital thermometer, comparisons were made from two data collection periods. The digital thermometer was compared with air and thermocouples temperatures during the winter, and compared with air, foliar, and thermocouple temperatures during spring for different trees. Thermocouples and bud break sensor digital thermometers were nestled on test buds. The instruments used for these validation experiments provided a suite of co-occurring measurements to corroborate the digital thermometer's temperature measurements. Each experiment consisted of bud break sensors, Type-T thermocouples, one thermal hygrometer (Campbell Scientific® HMP45C) aspirated as per Thomas and Smoot (2013). The spring experimental period also included one thermal imager (FLIR® SC305), a net radiometer (Hukseflux® NR-01), and a three-dimensional sonic anemometer (Campbell Scientific® CSAT3). The first test evaluated the thermometer's performance on a potted *Pinus pinea* tree (0.5 meters in height) during warm spring weather at Oregon State University's Botany & Plant Pathology farm just east of Corvallis, Oregon, for seven days in April and May 2013. The thermal imager was

situated 0.5 meters above the ground, and 2 meters away from the plant foliage, pointing 20° east of north to encompass the entire tree. The second test investigated the thermometer's measurements on a taller *Pseudotsuga menziesii* tree during wintry conditions on the Oregon State University campus for fourteen days in January 2014. Due to the cost of instrumentation, complete replication of the spring data collection period was not possible for the winter experiment.

Photoperiod Detector

Bud break sensor day length measurements were corroborated with time lapse cameras (Wingscapes® Timelapse Cam) during January 2014 on the Oregon State University campus and during April 2014 in a common garden north of Corvallis, Oregon. The cameras operated from 4 am to 9 pm during each sampling event, which provided several hours of dark images before sunrise and after sunset. Day length calculated from brightness values (BVs) of time lapse image pixels was compared to day length values from the sensor with simple Wilcoxon signed-rank tests.

Sensor Effect on Bud Break

Bud break sensor placement onto plant tissue may influence the timing of bud break. This could occur due to changes in the microclimate immediately surrounding a bud due to the sensor collecting heat energy from the sun during the daytime, or producing its own heat from turning the energy stored in its batteries to electricity. Additionally, thigmotropic responses in plants can occur from moderate, subtle stimuli, and often they occur rapidly (Chehab et al., 2009), raising the question of whether bud break may be

affected by the sensor touching branches. Furthermore, since conifers can have systemic responses to localized perturbations (Bonello and Blodgett, 2003), a thigmotropic response from a specific bud may influence the entire tree. To address this, we tested to see if the presence of the sensor could affect bud break on trees with sensors attached to their branches. Specifically, we tested for a difference in median bud break and a difference in days between phenological phases, with test units consisting of trees with sensors present and trees with sensors absent. We examined the timing of bud break on thirty-six trees in a common garden north of Corvallis, Oregon, during March and April of 2014. The trees tested were two years old, with trees spaced 1.5 meters apart within rows and 3 meters apart between rows, and a maximum distance of 49 meters between sampled trees. To reduce the influence of genetic variation, all trees studied were clones of the *Populus tremula* x *Populus alba* hybrid genotype 717-1B4.

Twenty bud break sensors were placed on sixteen poplars at the common garden study site on March 1st, and the condition and temperature of the buds were monitored for two months. Sixteen pairs of juvenile trees were used, consisting of one tree with a sensor attached and one without, which allowed us to test whether phenological differences between control and sensor trees could be due to random chance alone. One vegetative bud per tree was examined on all pairs. Bud condition was recorded during these site visits using five discrete classes (Table 1) simplified from Turok *et al.* (1996). The time-lapse images were compared with sensor output to aid in interpreting the effect of bud swelling and shoot elongation on the sensor output.

Table 1. The five spring phenology classes used in this study, modified from Turok et al. (1996)

0	Dormant bud; no sign of any activity; brown color.
1	Bud swollen and more rounded; scales turn reddish; no breakage.
2	Bud starts breaking; tip of shoot appears.
3	Bud break; red shoot turns green; very young leaves observed.
4	Green leaves separated and growing; venation observable.

Data Analysis

Temperature

There were several steps taken to process the thermal infrared (TIR) images after the time-lapse image regions of interest (ROIs) containing bud break sensors and thermocouples were identified (12 pixels by 12 pixels in size). After calculating means for the thermal ROI's raw emittances, the data were radiometrically calibrated in MATLAB (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States) to correct for emissivity effects in accordance with the Stefan-Boltzmann Law. The thermal images were then corrected for reflected sky temperatures from the foliage surface, as defined by Kirchhoff's Law. Emissivity values for the foliage were calculated from the MODIS emissivity libraries for new and old pine needles (MODIS UCSB Emissivity Libraries). The emissivity values within these libraries that fell within the thermal imager's spectral bands were averaged in order to define our target's emissivity. We assumed our study trees' transmissivity to be zero. After the data processing was completed, root mean squared errors (RMSEs) and correlation coefficients were calculated pair-wise between the temperature recorded by the bud break sensor and

the calculated foliar temperatures, air temperatures, and thermocouple temperatures respectively. This was performed on the whole time series, as well as smaller components representing day, night, clear, and overcast conditions. Mean temperatures for each hour of the day were also calculated for the bud break sensor's digital thermometer, air temperature, and foliar temperature. To better assess the biological relevance in the difference between these sensors, chilling and forcing units were calculated for each instrument as per Harrington et al., 2010.

Photoperiod

Threshold values were used to define daytime periods for BVs from the time lapse images and for digital numbers (DNs) from the bud break sensors' digital numbers. Visual examination of the data indicated that sensible threshold values for the bud break sensors ranged from 40 to 70 DNs, while a BV of 20 discriminated light-versus-dark for the time lapse camera. Daytime occurrence was determined using hourly values that exceeded threshold values. Correlation coefficients were then calculated from sensor DN and camera BV data collected during the winter temperature experiment and the spring phenology experiment.

Bud Break Sensing

To eliminate high-frequency noise present within the time series of sensor output, the data produced by the bud break sensors were filtered with a frequency based low-pass filter. The use of a zero-phase fifth-order Butterworth filter eliminated the diurnal noise in the sensor's output signal. The time series were then visually compared with time lapse movies of buds opening and visual observations of phenological phase.

Sensor Effect on Phenology

The visually-scored phenology phases were transformed to first differences, where if $y(t)$ is the bud score at time t , the first difference is equal to $y(t) - y(t-1)$. The resulting time series for each of the forty study buds informed the number of days between any given bud score, which we used to develop statistics for the two study groups, controls and sensors. We used a Wilcoxon signed-rank test to assess if the number of days between phenological phases two (tip of bud breaking, visible tip of reddish shoot) and three (bud break; red shoot turns green and young leaves are visible) differed between test and control groups. We also used the same signed-rank test to determine if there was a statistically significant difference in the date of bud break between sample groups as the test was robust to our non-normally distributed data (Ott & Longnecker, 2010). For the eight trees with two study buds a piece, one bud per tree was randomly selected for inclusion in the analysis to maintain independence among analyzed sample units.

3. RESULTS

Temperature

In general, the temperatures measured by the bud break sensor's digital thermometer were a better approximation of thermocouple temperatures than were air and foliar temperatures. In the spring when downwelling shortwave radiation was below 150 W m^{-2} (over twelve hours a day), digital thermometer values fell between air and TIR foliar temperatures; during higher irradiance conditions, the digital thermometers measured higher values than the air and TIR foliar temperatures (Figure 4). In cold weather, chilling units calculated from the digital thermometer are similar to units calculated from other

devices, and the same finding holds for forcing units calculated in warm weather. Across seasons, the bud break sensor's thermometer best approximates a thermocouple, and air temperature secondarily.

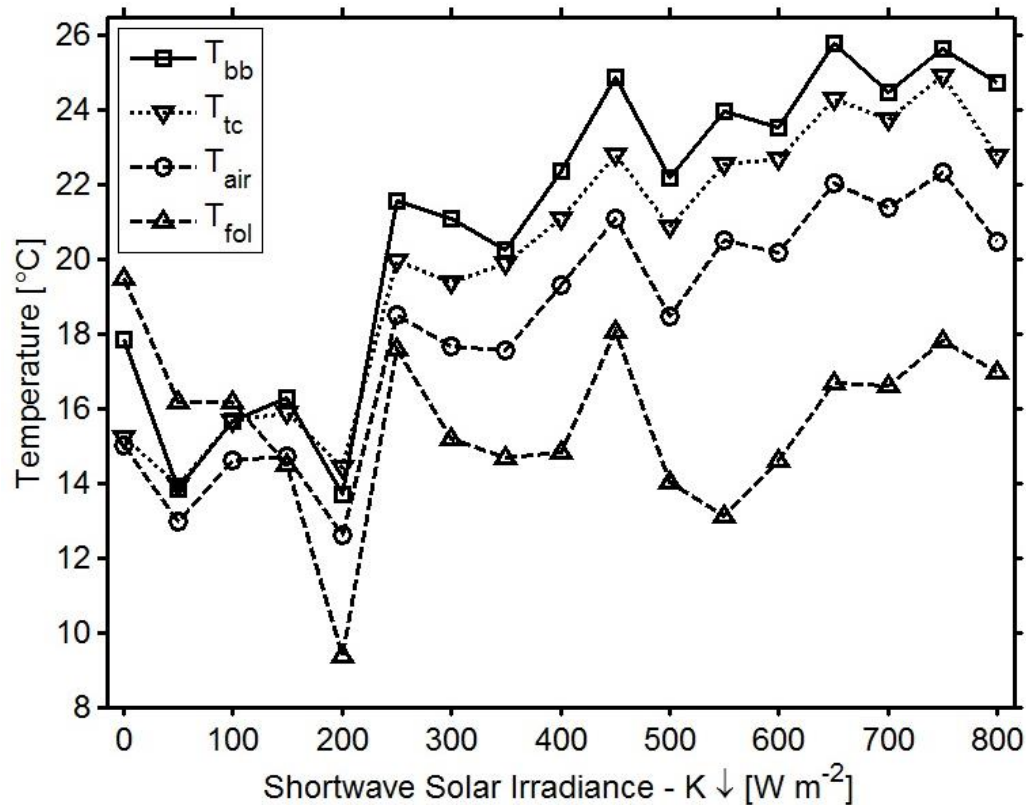


Figure 4: Mean temperatures by irradiance class for the bud break sensor's digital thermometer (squares), air temperature (circles), thermocouple (squares), and foliar temperature (triangles).

In the spring measurement period, mean temperature differences of the bud break sensor's digital thermometer were consistently less than 5°C from air and thermocouple temperatures (Figure 4). These differences nearly always resulting from the digital thermometer measuring higher temperatures than air or thermocouples; the two exceptions occurred when thermocouples measured warmer temperatures at incoming solar radiation levels of 200 and 850 $W m^{-2}$. Differences between digital thermometer and TIR foliar

temperatures were greatest when there was less than 250 W m^{-2} of downwelling radiation, although even at higher irradiances these mean differences were never less than 3°C .

Calculations of chilling and forcing units (per Harrington et al., 2010) helped to illustrate some biological relevance to the differences between measurement types. During the winter data collection period, the difference in chilling units between the digital thermometer, thermocouple, and air temperature varied by less than thirty-one units, although there was a larger difference in accrued forcing units. Conversely, during the spring data collection period, the difference in forcing units accrued by the digital thermometer, thermocouple, air, and TIR foliar temperatures varied by fewer than twenty-five units, but there was a larger variation in accrued chilling units (Table 2).

Table 2: Chilling and forcing units calculated from different temperatures, calculated as specified by Harrington et al. 2010

	Time Period	Chilling Units	Forcing Units
BB Sensor Digital Thermometer	Spring	111.05	505.12
	Winter	1268.78	183.44
Thermocouple	Spring	116.76	501.06
	Winter	1237.50	200.37
Air	Spring	136.71	483.84
	Winter	1265.04	283.60
Foliar	Spring	128.30	480.94

Data from both the winter and spring measurement periods showed that temperature values between the bud break sensor's digital thermometer and a type-T thermocouple had a RMSE of less than 3°C (Table 3, Figures 5 and 6). All correlation coefficients between measurements made by the digital thermometer and the thermocouple were greater than 0.89, and error between measurements was lower in the winter than the spring. The variability between the two instruments' measurements was higher ($r^2 = 0.76$) when temperatures dropped below 5°C in the winter (Figure 6).

Table 3: RMSE values and correlation coefficients for temperature measurements concurrent with bud break sensor's thermometer measurements.

Bud Break Thermometer Temperature Comparison to:	Data Collection Period	Time Segment	RMSE (°C)	r²
Air Temperature	Spring	whole experiment	3.18	0.94
Air Temperature	Spring	day	3.81	0.93
Air Temperature	Spring	night	2.05	0.92
Air Temperature	Winter	whole experiment	1.62	0.88
Air Temperature	Winter	day	1.56	0.87
Air Temperature	Winter	night	1.70	0.90
TIR Foliar Temperature	Spring	whole experiment	7.12	0.44
TIR Foliar Temperature	Spring	day	7.79	0.55
TIR Foliar Temperature	Spring	night	6.12	0.59
Thermocouple	Spring	whole experiment	2.67	0.93
Thermocouple	Spring	day	2.43	0.90
Thermocouple	Spring	night	2.29	0.90
Thermocouple	Winter	whole experiment	1.25	0.91
Thermocouple	Winter	day	1.28	0.89
Thermocouple	Winter	night	1.13	0.93

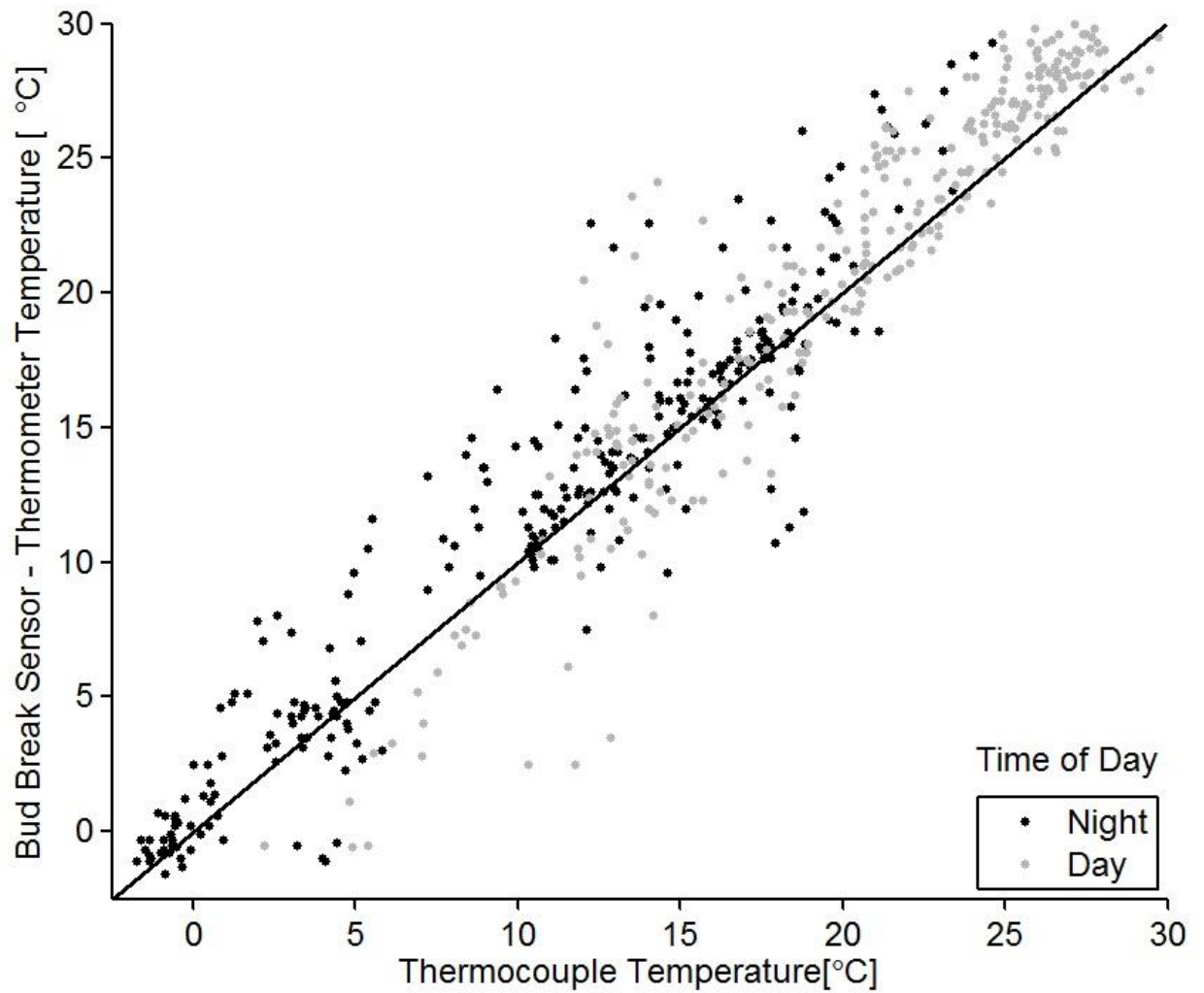


Figure 5: Co-occurring sensor and thermocouple temperatures during the spring data collection period, color-coded by time of day. The black 1:1 line indicates perfect agreement in measured temperatures.

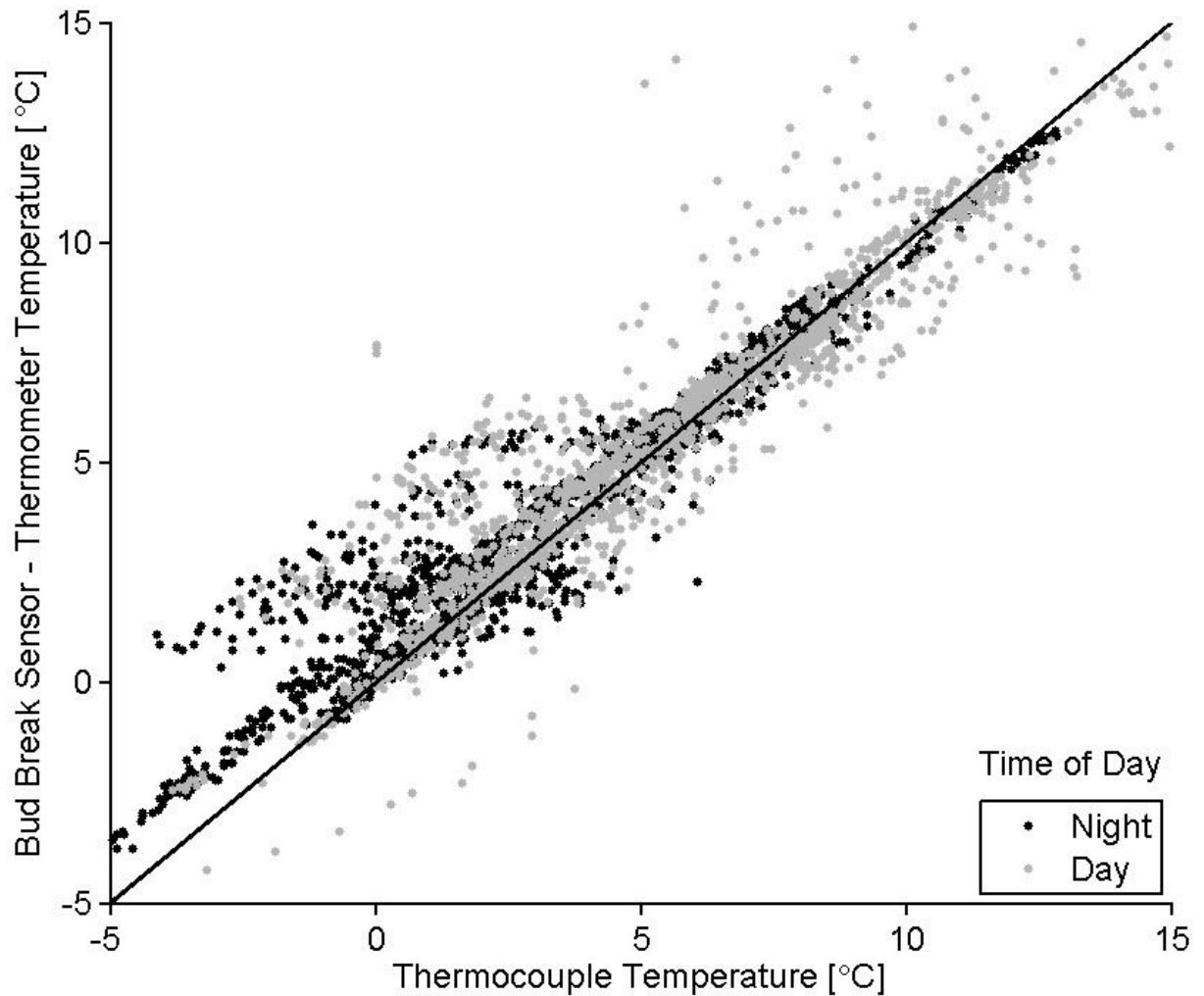


Figure 6: Co-occurring sensor and thermocouple temperatures during the winter data collection period, color-coded by time of day. The black 1:1 line indicates perfect agreement between measurements.

The bud break sensor's thermometer approximated air temperature during both data collection periods (Table 3; Figures 7 and 8). In the spring, thermometer temperatures were consistently warmer than air temperature, although with increased variance at night. Daytime measurements made in the springtime from the digital thermometer had increased error relative to air temperatures, although this trend reversed at night (Figures 7 and 8). During the winter data collection period, measurements made from the digital thermometer estimated air temperature less accurately compared to springtime measurements

(correlation coefficient of 0.88 vs. 0.94), but the measurements were more precise (1.62°C vs. 3.18°C). The correlation coefficient in the springtime (0.94) was higher than in the wintertime (0.88), and had a greater RMSE (3.18 and 1.62 degrees, respectively).

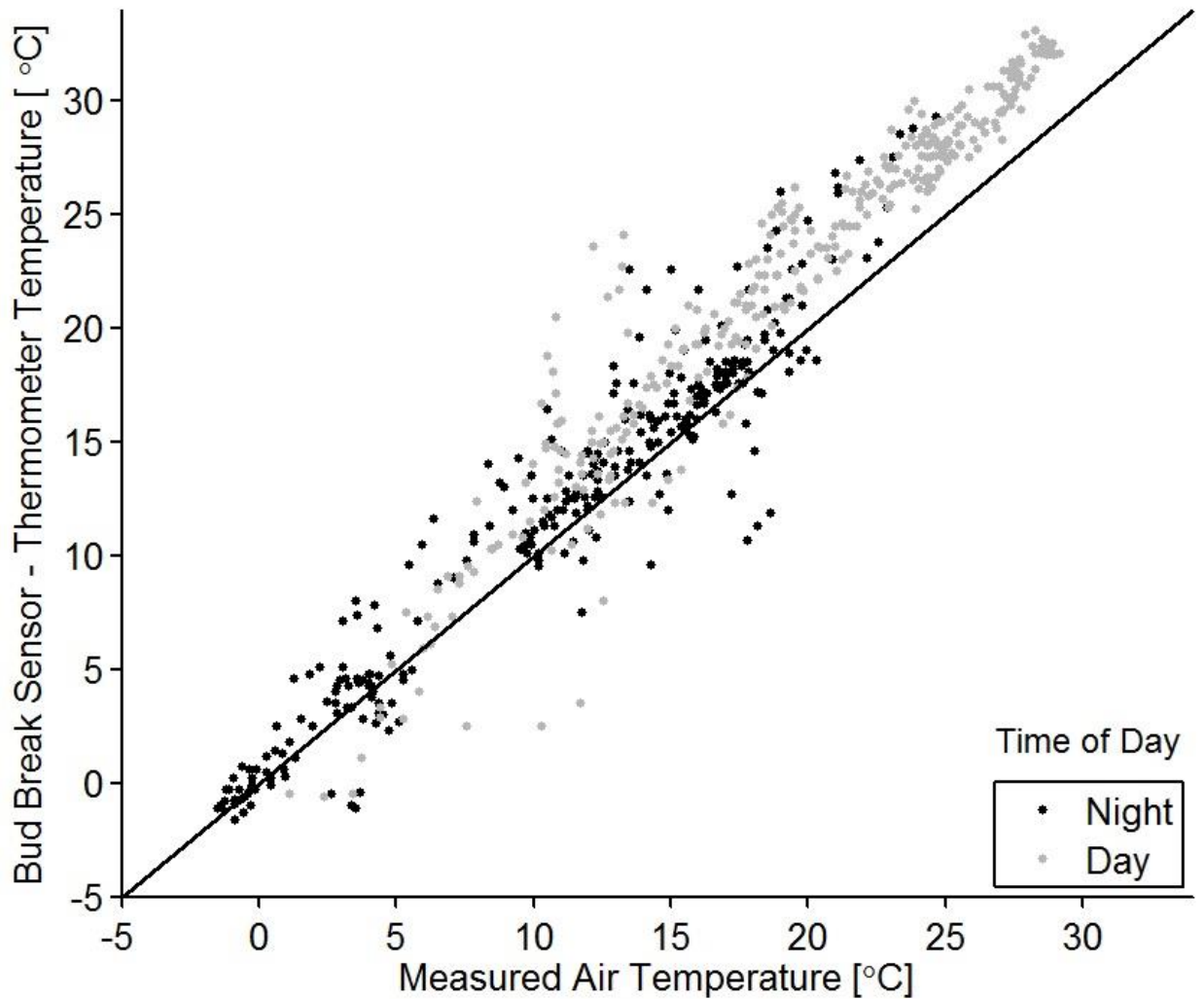


Figure 7: Co-occurring sensor and air temperatures during the spring data collection period, color coded by time of day. The black 1:1 line indicates perfect agreement in measured temperatures.

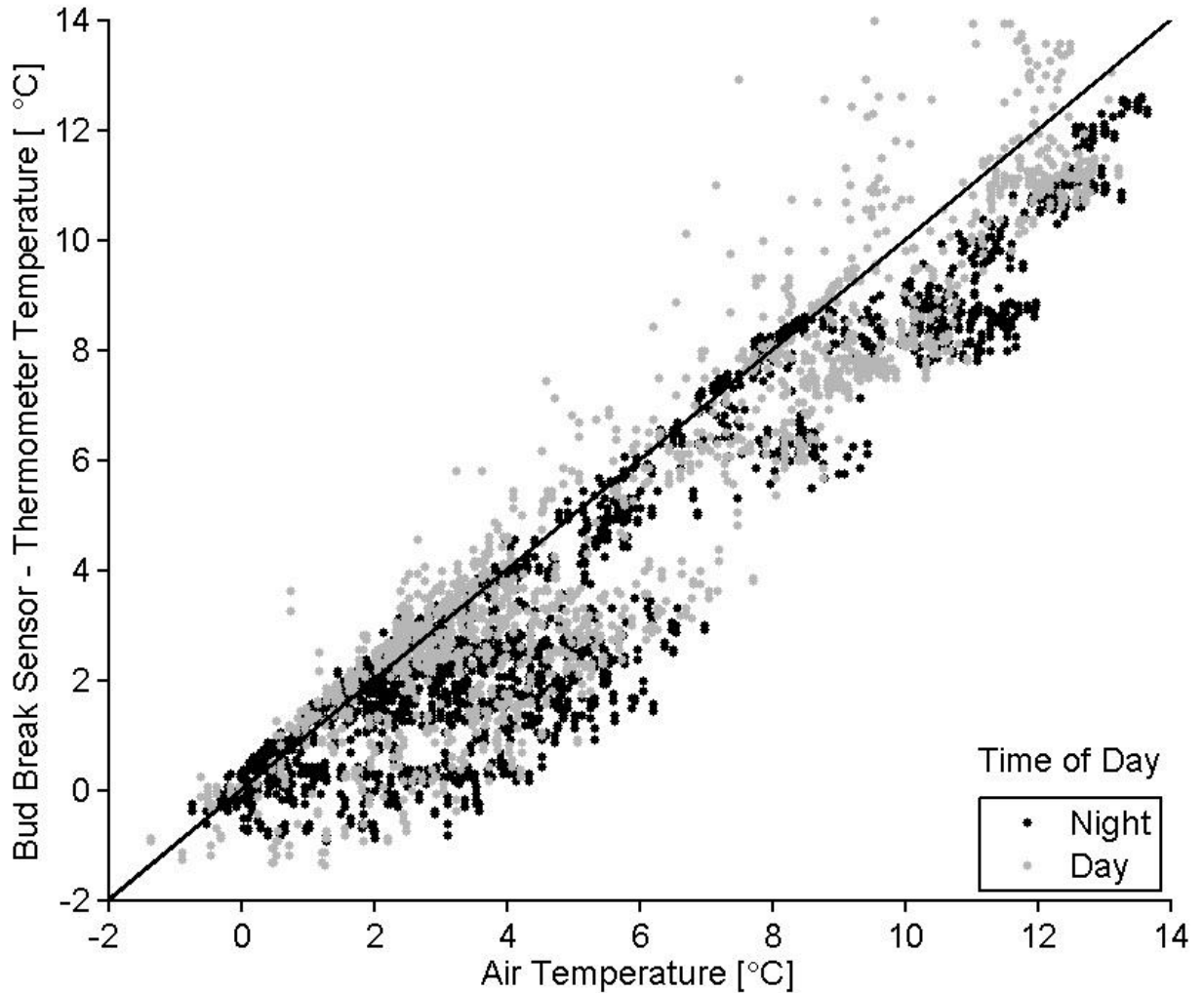


Figure 8: Co-occurring sensor and air temperatures during the winter data collection period, color-coded by time of day. The black 1:1 line indicates perfect agreement in measured temperatures.

TIR Foliar temperatures during the springtime data collection period tended to be cooler than the bud break sensor's thermometer measurements in the daytime, and warmer than the thermometer measurements during the night (Table 3, Figure 9). The overall variation was quite large, with a correlation coefficient of 0.44 and a RMSE of 9.58°C (Table 3). Isolating the nighttime data yielded a slightly lower RMSE of 7.58°C; however, this segregation increased the error for the daytime. Maximum TIR foliar temperatures were higher than maximum temperatures from any other method of measurement.

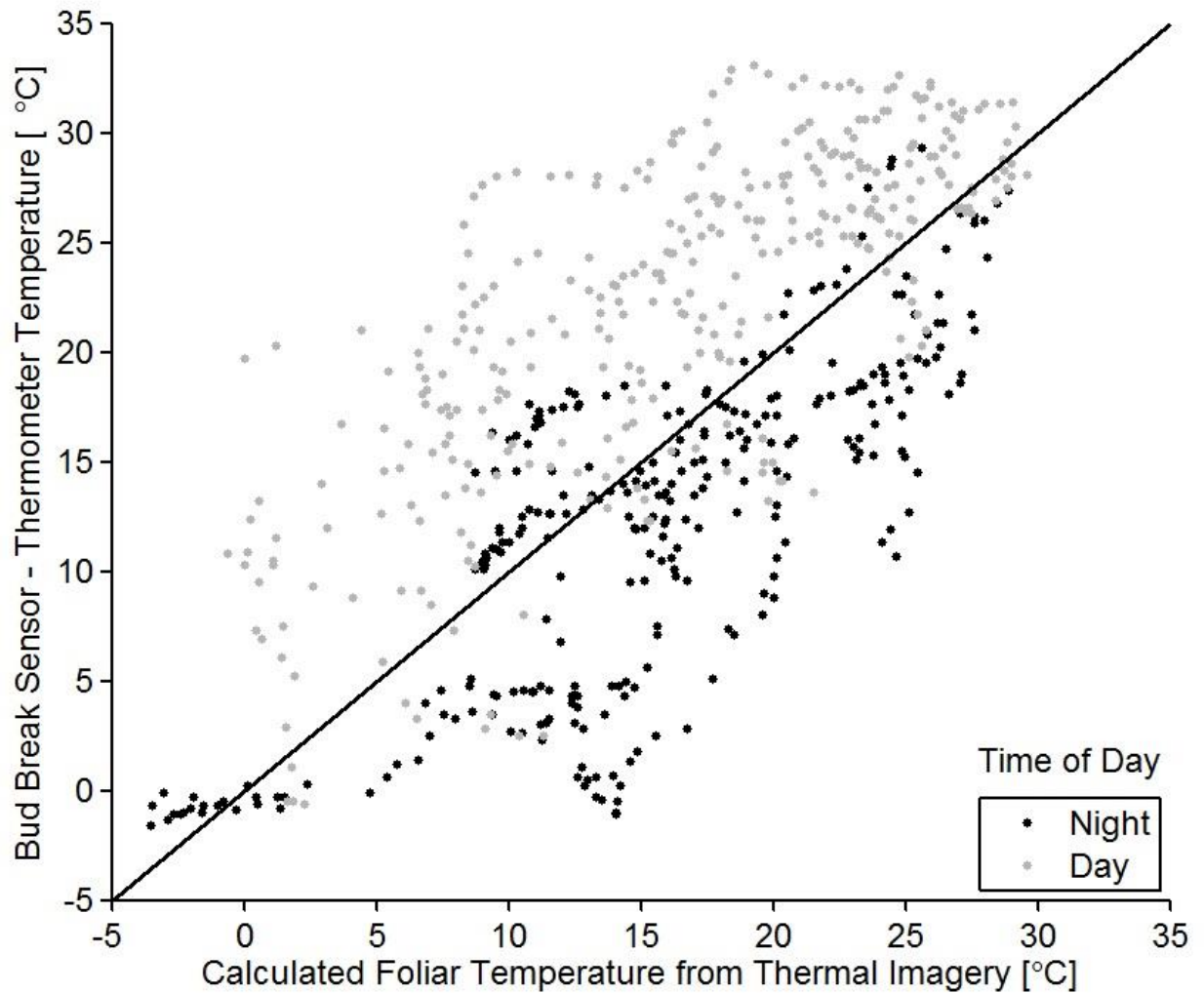


Figure 9: Co-occurring sensor and foliar temperatures during the spring data collection period, color-coded by time of day. The black 1:1 line indicates perfect agreement in measured temperatures.

Photoperiod

The bud break sensor's photoperiod detector was able to determine ambient light conditions as well as time lapse imagery (Figure 10). Each sensor's day length values correlated strongly with day lengths calculated from the imagery ($r^2_{\text{winter}} > 0.99$, $r^2_{\text{spring}} > 0.98$).

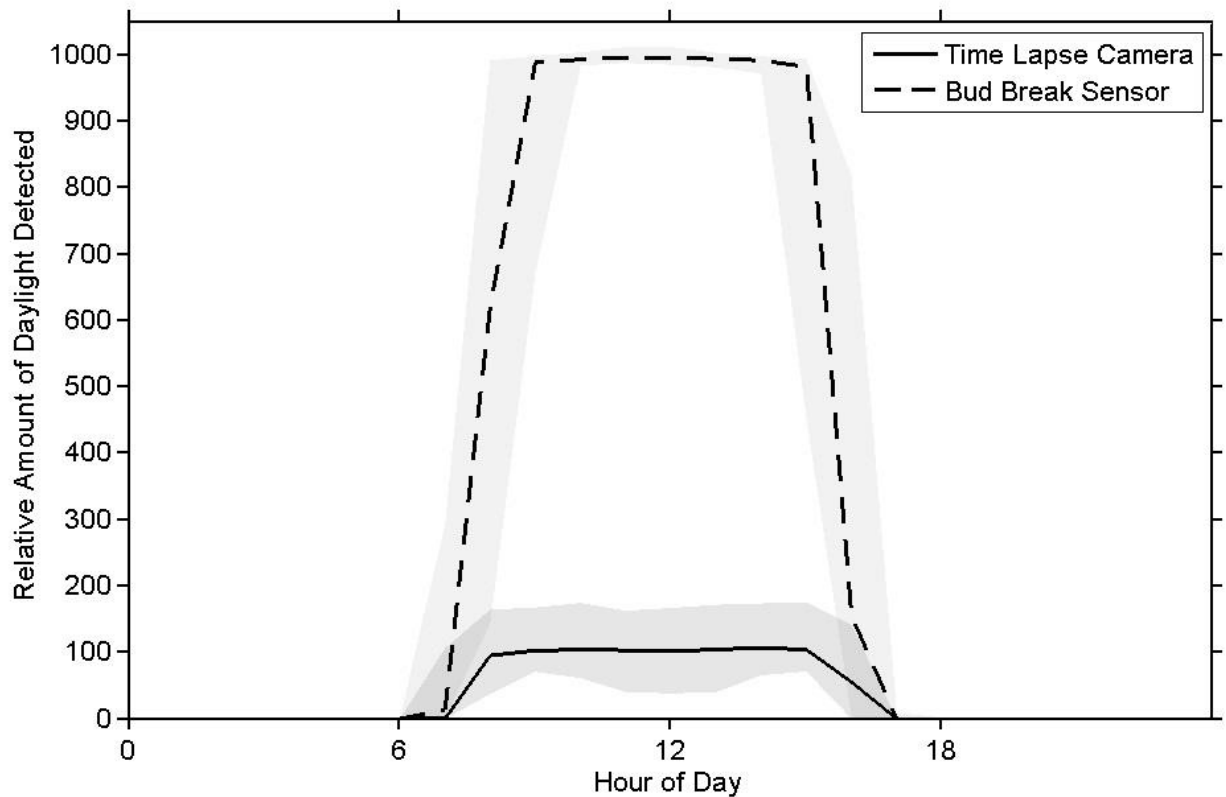


Figure 10: Mean measurements of daylight from the bud break sensor's day length detector (in DN units) and time lapse images (in BV units) with 5% and 95% quantiles shaded.

Bud Break Sensing

By comparing sensor output with confirmed bud break dates derived from site checks and time lapse images, eleven of the twenty sensors (55%) deployed for this study detected bud break as confirmed by site checks and time lapse images (Figure 11). For each of these time series, there was a noticeable and abrupt increase in reflected light for the time period measured. The reflected light changed from a low, flat line before bud break to a higher, flat line after bud break. Eight of the failing sensors (40%) succumbed to water damage prior to the date of bud break, and thus were unable to detect phenological changes, and one sensor's (5%) signal did not show an increased signal at bud break. Overall, of the twelve operational sensors, 91.6% of them successfully detected bud break.

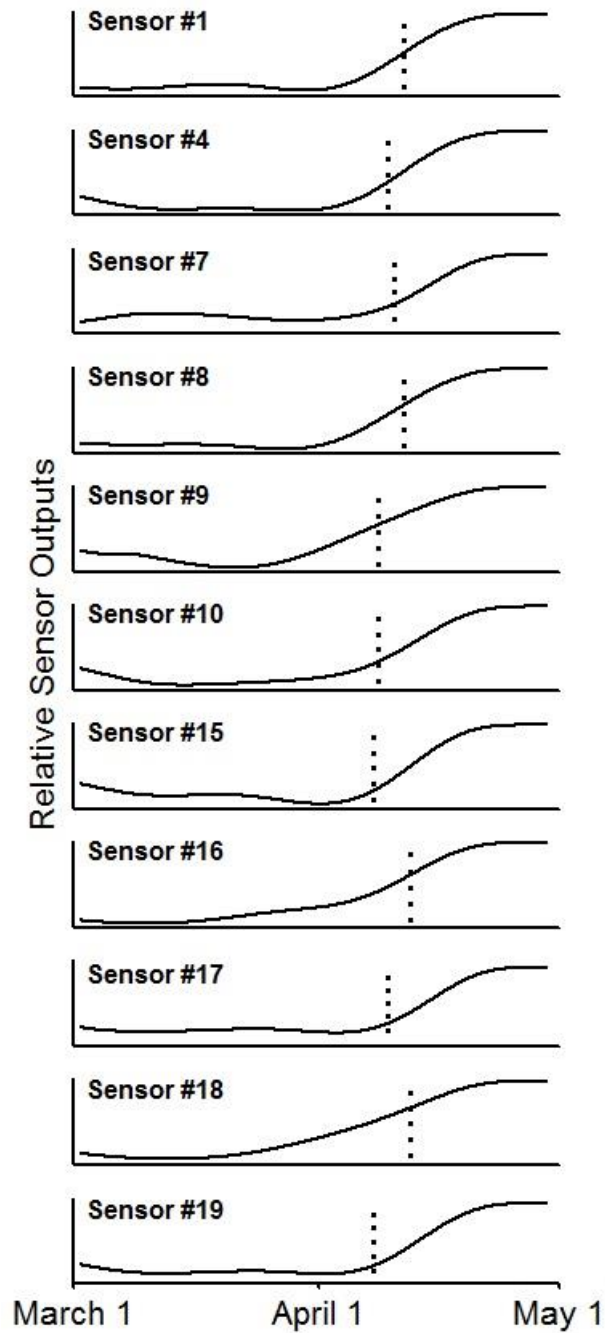


Figure 11: Smoothed time series from the eleven sensor outputs showing an increased signal near the date of bud break, indicated by the vertical dotted line, using a zero-phase 5th order Butterworth filter.

Sensor Effect on Phenology

The mean number of days between phenological phases two and three was 5.3 for buds with sensors attached and 6.1 for control buds; the median number of days between

these phenological phases was 6 for both groups. No significant difference was found for the number of days between phenological phases two and three amongst control and test groups ($p = 0.24$). We found no significant difference between median bud break dates for buds with sensors and buds without sensors ($p = 0.82$).

4. DISCUSSION

Overall, for the bud break sensors unaffected by water damage, the field tests of each sensing component matched or exceeded our expectations. The signal from the day length detector detected the beginnings and endings of daylight. The acrylic-coated digital thermometer measured temperatures very similarly to thermocouples placed against foliage, and that the digital thermometer's measurements fell between air and TIR foliar temperatures when solar irradiance was low. The bud break sensor did not influence the timing of bud break for poplar trees, and successfully detected the timing of bud break.

Temperature

The close correspondence of measurements from the bud break sensor's digital thermometer and those from thermocouples provide support for digital thermometer deployments due to their durability, ease of deployment, and affordability relative to thermohygrometers and thermal cameras. The digital thermometer on the bud break sensor is much more robust and hardy in comparison to thermocouples, which tend to be fragile. When four sensors were deployed on the limbs of a large conifer tree and paired with thermocouples, all of the thermometers were still functioning at the end of data collection, whereas three of the four thermocouples had broken due to their thin and fragile nature. The self-contained setup also made deployment within a tree crown much quicker for the

bud break sensor than for thermocouples, as it does not require external wiring to an energy source and data logger.

The convenience and cost-effectiveness of the bud break sensor relative to thermal cameras and thermohygrometers makes it an excellent option for field and laboratory research on plants. Thermal cameras can cost in the tens of thousands of dollars, and require extensive processing to record accurate temperatures. The need for a constant power source and computer to time measurements and log data complicates field deployment. These problems of power sourcing and additional required equipment remain for data logging setups for measuring air temperature. Furthermore, the highly localized measurements made by the digital thermometer near a bud provide useful information about the conditions near the bud sit, even if it is not measuring bud temperature.

The digital thermometer provided consistently warmer temperature than air and TIR foliar temperature. In warm spring weather, latent heat exchange from transpiration caused the TIR foliar temperature signal to lag behind the digital thermometer and air temperature until mid-morning when irradiance saturated the tree, providing evidence of the leaf-to-air temperature equality around 33°C (Linacre, 1964). Then, TIR foliar temperature increased and eventually surpassed air temperature (Figure 3). This threshold also concurs with theoretical models of leaf-air temperature differences at different temperatures and humidities (Jones, 1992).

An examination of Table 3 shows that in cold weather, there was better agreement in calculated chilling units than forcing units between measurement methods. In warm weather, better agreement was shown between calculated forcing units than chilling units.

We conclude that chilling and forcing units calculated from data collected by the bud break sensor's digital thermometer tend to approximate units calculated from other instruments during relevant periods (chilling units in cold weather, forcing units in warm weather).

Comparisons of precision and accuracy (as indicated by correlation and error, respectively) between the digital thermometer and other devices were generally similar between day and night across seasons. In spring comparisons with thermocouples, TIR foliar temperatures, and air temperatures, accuracy was greatest at night. Accuracy of the digital thermometer in the winter was greater in the daytime for comparisons with thermocouples, and greater at night for comparisons with air temperature. Differences in accuracy and precision, however, should be expected, considering that the digital thermometer is inherently different from the other measurements. The acrylic coating on the digital thermometer changes the device's energy balance to differ from that of the thermocouple, thermohygrometer, and foliage. Thus, a true one-to-one plot between the bud break sensor's digital thermometer and another measurement should not be expected due to the inherent differences between what the devices measure. Further testing will be needed to see if the thermometer's measurements provide greater predictability of bud break compared to air temperature near a plant. Future research implementing the bud break sensor could help to determine if the data from the bud break sensor can distinguish microclimate sufficiently to explain differences in bud break timing.

There were numerous potential sources of error for the measurements of TIR foliar temperature. Systematic deviation between the bud break sensor's digital thermometer and TIR foliar temperature in the spring was likely a result of increased latent heat exchange between the foliage and air from transpiration. Increased solar influence, the small size of

the tree, and a larger variance between day and night temperatures could explain the higher measurement error in the spring for comparisons with TIR foliar temperature. Accuracy of the thermal camera is reported to be up to $\pm 2^{\circ}\text{C}$. Four assumptions made while recording and processing thermal images could have led to additional error: uniform emissivity across pixels, uniform reflectance across pixels, ignored boundary-layer resistance, and a full transmissivity.

Evaluations of the thermohygrometers and thermocouples indicated that these instruments functioned normally throughout the experimental periods. Air temperatures tended to be slightly warmer than temperatures recorded by thermocouples embedded within the trees, which could be due to shielding from the thermocouples' placement within the leaf boundary layer. Correlation coefficients higher than 0.90 and errors below 1.75°C in both seasons indicated harmony between data from each sampling instrument. In agreement with theory (Jones, 1992), TIR foliar temperatures tended to be warmer than the air during cold weather, and cooler than the air during warm weather.

Photoperiod

The bud break sensor's photoperiod detector has been shown to work as well as a time-lapse camera at determining day length. Knowing that microsite light availability in forest understories can be highly heterogeneous (Parent and Messier, 1996), the primary advantage of using this photoperiod detector instead of a time-lapse camera is the improved resolution of having a direct measure of day length at the bud. This could yield subtle differences of the light environment at the bud that would not be learned from time-lapse photography or by calculating the solar track from a geographic coordinate.

Bud Break Sensing

The 40% rate of damaged sensors from the spring data collection was unusually high compared with the thermal validation experiments, which had a 75% sensor survival rate or higher. The plastic housings for the sensors' electronics were stored on the ground at the base of the tree for the duration of this test because the saplings were not large enough to strap the boxes to their trunks. We believe that this positioning caused additional exposure during several heavy rain storms that occurred during the data collection period and thus increased the rate of water damage to the sensors. We suspect that the number of sensors damaged could have been reduced if the devices were either kept off the ground or if they had better waterproofing. Additional waterproofing modifications will be made to the housing in future models. The sensor is in the final innovation phase, and fine-tuned modifications will further optimize the instrument for future commercialization. In addition to improved waterproofing, the fiber optic attachment requires further testing and refinement. The varying quantities and ranges of sensor response indicated the sensor's sensitivity to millimeter-scale differences in distance to bud may influence sensor response. Future investigation is necessary to ascertain optimal distances and angles to the bud required for optimal sensor output, as well as testing on differing bud types.

Our experiment confirmed that the sensor presence did not affect the phenomenon under investigation for the time period studied. However, in order to be absolutely certain that the sensors themselves have no effect on spring phenology, testing through longer-term deployment is necessary as the sensor batteries can accommodate placement in the fall and retrieval in the spring after bud break. This test will also confirm how well the sensor wires stay in place through a winter. Proof of successful operation throughout a

winter prior to bud break will establish the sensor's viability for studies necessitating such deployment lengths. Additionally, testing across a variety of species is necessary as the physiological response to sensor placement may vary across species or populations (Braam 2005). The transition from phenological phase two to three averaged near six days in length for both experimental units, whereas the length of time shown between two steady states from the sensors' time series averaged around seventeen days. A differing analysis method may improve this. This work experimented with several analyses, including double-mass plots and other filter designs, before determining that this form of the Butterworth filter worked best. Further investigation into analysis methods may improve results over the Butterworth filter. Changes in the analysis of the bud break sensor's data, in addition to refinements to the device itself, could refine this transition to less than six days, the resolution may be sufficient for genomic work.

This sensor was designed to sense bud break at the scale of a single bud. Since not all buds on woody plants break dormancy at the same time, the bud selected may not represent the mean bud break of a whole tree. Recent work suggests that within-tree temperature variation may help to explain differences in bud break dates at the scale of individual trees (Miller and Lintz, unpublished data). Ideally, it would be possible to model bud break at the scale of a tree as a function of temperature to enable sensor of a single bud to approximate the trait at the level of a whole tree.

This sensor enables a new caliber of data and insight that can expand our ability to predict the response of natural and agricultural plant populations to a changing climate. Such data can provide the precision, throughput, and standardization necessary for genome-wide association studies to find the genes underlying adaptive traits, and to better

understand molecular, genomic, and ecological mechanisms of phenology and related processes (Neale and Kremer, 2011). Such ground-based quantification also reduce bias from human observations of phenology. The sensor's co-occurring measures of day length and temperature make it a compelling alternative to digital cameras and other temperature devices. Investigations using the bud break sensor in large-scale analyses could help determine if universal response functions can predict bud break in natural populations for evergreen and deciduous species, or determine the degree of correlation between vegetative and reproductive bud break (Wang et al., 2010). Furthermore, since variation in the date of bud break, if measured precisely and continuously, quantifies biological effects of climatic variation (Cleland et al., 2007, Keeling et al., 1996), the sensor's data can validate satellite measurements and components of land surface models to understand and predict biogeophysical interactions (Studer et al., 2007). Applied research applications in orchards, vineyards, and other agricultural crops further extend the utility of the bud break sensor.

5. CONCLUSIONS

We innovated a sensor for use in landscape phenomics of woody plants. The device is one of the first instruments to measure an adaptive phenotype in nature, bud break, along with environmental factors that influence the phenotype. The device can facilitate the accounting of environmental effects on phenotype to derive genomic effects on phenotype in natural populations. After several minor modifications it will be ready for commercialization at a price that is cost-effective for high-throughput phenotyping. The device measures bud break, bud temperature, and photoperiod. The sensor's thermometer provides an alternative to thermocouples (for temperature), time lapse cameras (for photoperiod), and other methods of phenological measurements. We envision this tool will

have interdisciplinary application while facilitating progress in the fields of landscape phenomics and bud break phenology. Future work utilizing many bud break sensors across tree populations at a landscape level can provide new insights into genomes, ecosystems, and interactions between vegetation and atmosphere.

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