ABSTRACT

SERMONS, SHANNON MICHELLE. Weed ecological interactions with environment: An investigation of temperature response of *Commelina benghalensis* and a method for description of seed shape. (Under the direction of Dr. Thomas Rufty and Dr. Michael Burton.)

One of the most important challenges in agriculture is the threat posed by weeds. In recent years, the federal noxious weed *Commelina benghalensis* L. has become troublesome in Georgia and there are some indications that it is moving northward. Its tolerance of herbicides, including glyphosate, which is a primary management tool, as well as its reproductive elasticity are of particular concern. Temperature is an important determinant of plant range. Therefore, to help determine the potential for survival of *C. benghalensis* in North Carolina, a series of experiments examined its growth and reproduction over a range of temperatures. The results were then compared with historical temperature data and temperature responses of other weeds that grow and compete successfully in the North Carolina climate. These comparisons indicated that temperature would not pose a restraint to survival of *C. benghalensis* in North Carolina and further northward.

In addition to these studies, a method was developed for describing seed shape. Seed characteristics can be affected by several factors and can have important impacts on germination and vigor of offspring. Although many studies have addressed seed characteristics, few quantitative tools exist for its study. The method that we have developed provides a more comprehensive, quantitative description of seed shape that can be utilized to evaluate reproductive characteristics of problem weed species.

WEED ECOLOGICAL INTERACTIONS WITH ENVIRONMENT: AN INVESTIGATION OF TEMPERATURE RESPONSE OF *COMMELINA BENGHALENSIS* AND A METHOD FOR DESCRIPTION OF SEED SHAPE

by

SHANNON MICHELLE SERMONS

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APPROVED BY:

Dr. Thomas Rufty Advisory committee co-chair	Dr. Michael Burton Advisory committee co-chair
Dr. Nancy Creamer	

BIOGRAPHY

Shannon Michelle Sermons was born in Plymouth, North Carolina. She lived there with her parents, Linda and Bill, and sister Diana through completion of the tenth grade, at which time she entered the North Carolina School of Science and Mathematics in Durham. After completion of high school, she attended The Florida State University, graduating *cum laude* with a Bachelor of Science in Biological Science in 2000. During her time at FSU, she worked as an assistant in the ecology lab of Dr. Thomas Miller, which was a tremendously valuable experience. Her desire to pursue ecology research that would yield practical results led her into graduate studies in the Department of Crop Science at North Carolina State University.

ACKNOWLEDGEMENTS

For their unfailing support, I thank my parents, Linda Parker Sermons and William Joseph Sermons Jr., and also my sister, Diana. I also appreciate the encouragement provided by my extended family, of whom there are many. Thanks to Brent McCraven, and to our "babies" Madeline and Stanley for always being there.

I also wish to extend thanks to those who more directly contributed to my research: to my advisors, Tom Rufty and Mike Burton; to my fellow graduate students for their mentorship, especially Erin Naegle and Joe Chiera; to Melissa Brake Plummer and Mike Jennette; to faculty members who provided advice and assistance, most notably Dr. Cavell Brownie; to the Phytotron staff and to our hourly helpers. Thank you.

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INTRODUCTION

One of the most important challenges in agriculture is the threat posed by weeds. The presence of extraneous plants in a field deprives the crop of water, nutrients, and light, and can interfere with harvest and contaminate the product. Costs of control measures and lost productivity probably exceed eight billion dollars annually in the United States (Zimdahl 1999). In recent years, new technologies have brought control for previously troublesome pests, but new problems have arisen. One weed that recently has become a concern is tropical spiderwort (*Commelina benghalensis*), which is listed as a federal noxious weed. It is tolerant of glyphosate, which is a primary weed control tool in cotton and soybean. Tropical spiderwort has become troublesome in several crops in Georgia and recently has been found in other states, including North Carolina.

This thesis examines the potential for tropical spiderwort proliferation in new habitats. Our focus was to determine the potential for tropical spiderwort survival and reproduction at various temperatures; temperature is known to be an important determinant of plant range (Patterson 1995; Patterson et al. 1999). The study included analysis of vegetative and reproductive growth across a range of temperatures, as well as comparison with other weeds with known ranges. A method for describing seed shape was also developed. This technique was tested on two species having distinct seed shapes, sicklepod and corn. Such a technique may be valuable in evaluating characteristics of tropical spiderwort, which has significant reproductive elasticity and produces seeds of four distinctive types (large and small seeds, produced both aboveground and belowground). The

types are known to differ in germination dynamics, and plants grown from the different seed classes have quantitatively different growth and reproduction.

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CHAPTER 1. TEMPERATURE RESPONSE OF TROPICAL SPIDERWORT: COMPARISON TO WEEDS OF VARIOUS GEOGRAPHIC RANGES

INTRODUCTION

Tropical spiderwort (*Commelina benghalensis* L.) is an invasive plant in the southeastern United States with the potential to adversely affect many crops. As has been detailed by Webster et al. (2005), tropical spiderwort is a troublesome weed in cotton and peanut in Georgia, and it has recently been found in other states, including North Carolina (Krings et al., 2002). Thus, there are indications that it is spreading throughout the Southeast.

Tropical spiderwort has several physiological characteristics that could contribute to its potential as a successful invader. Perhaps the most important is its reproductive elasticity. It can exist as an annual or perennial, which may be dependent on climate (Holm et al., 1977) or on genetic factors (R. Faden, pers. comm.) More importantly, it can produce large numbers of aboveground and belowground seeds (Walker and Evenson, 1985a). Seed size varies with position within the fruit, and is associated with different dormancy and germination characteristics (Maheshwari and Maheshwari, 1955; Walker and Evenson, 1985b). Furthermore, tropical spiderwort can reproduce vegetatively, with plants regenerating from stem fragments (Budd et al., 1979). Another major factor contributing to tropical spiderwort's invasiveness its tolerance of many herbicides, making control difficult in agronomic settings. In particular, it has a high degree of tolerance to glyphosate, which is the primary management tool used for weed control in Roundup-Ready cotton and soybean systems throughout the Southeast (Culpepper et al., 2004).

We are examining factors that will help predict the potential geographic range of tropical spiderwort invasion. The focus of these experiments is on responses to aerial and

root temperatures. It has been proposed previously that temperature is an important determinant of the potential for geographic spread (Patterson et al., 1999). Tropical spiderwort appears to have originated in tropical areas (Fernald, 1950), which might lead to the expectation that it is tolerant of high temperatures. Conversely, a lack of tolerance of cool temperatures would limit its potential to spread northward into North Carolina and nearby states. To assist with interpretation of the applicability of results to the field, experiments included examination of root temperature responses of a number of weed species that are known to successfully inhabit agronomic fields in the Mid-Atlantic states.

MATERIALS AND METHODS

To provide a background for understanding temperature patterns in the upper Southeast, the aerial temperature history of the geographic area was obtained from the National Climatic Data Center (NCDC, 2003). The data set included monthly averages of the daily high and low air temperatures recorded at the regional National Weather Service station at the Raleigh-Durham International Airport from 1948 to 2001.

Soil temperature was measured at a central agronomic location. Measurements were made using temperature data loggers equipped with stainless-steel probes[1] placed horizontally at depths of 5, 10, and 18 cm below the surface of bare-soil plots in Goldsboro, NC during 2000 and 2001. The soil type was a Tarboro loamy sand (mixed, thermic Typic Udipsamment).

Controlled environment studies

[1] StowAway TidbiT XT, Onset Computer, Bourne, MA

Experiments were conducted in the Southeastern Plant Environment Laboratory (Phytotron) on the campus of North Carolina State University. Tropical spiderwort was grown in sand at various ambient temperatures and in solution culture with constant aerial temperature and various root temperatures. Growth of sicklepod (*Senna obtusifolia* (L.) H.S. Irwin & Barneby), velvetleaf (*Abutilon theophrasti* Medik.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill), and jimsonweed (*Datura stramonium* L.) were also determined at different root temperatures in hydroponic culture.

Aerial temperature. Tropical spiderwort produces seeds that can be classified into four groups: large and small, produced both aboveground and belowground (Maheshwari and Maheshwari, 1955). Seeds of each class were sown separately 1.5 cm deep in flats of sand, to determine effect of seed type on germination. The flats were placed in growth chambers, which were set at 12-hour day/night cycles with a three hour night interruption. Light during the day period was provided by fluorescent and incandescent lamps with a photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1}. The growth chambers were programmed for constant temperatures described in the results section.

Temperature effects on germination were determined by the appearance of shoots above the soil surface in the five growth chambers. The time to germination was recorded and, when uniform groups of five seedlings from the same seed type reached two to three cm in height, they were transplanted into 21-cm diameter pots containing equal parts sand and gravel. The seedling group was then distributed randomly among five growth chambers and used as a replicate in the longer-term growth study. Plants were watered with a complete nutrient solution (Thomas and Downs, 1991) and deionized water daily throughout the growth experiment.

Each replicate group was allowed to develop for 28 days after transfer, and then were harvested. At harvest, each plant was divided into aboveground vegetative, aboveground reproductive, root, rhizome, and belowground reproductive biomass. The tissues were dried to constant mass at 70 C and then weighed.

Root temperature. Small aerial seeds of tropical spiderwort were soaked in a 5% bleach solution (0.25% NaOCl) for 5 minutes, then rinsed with water and each seed nicked with a razorblade. They were planted approximately 3 mm deep in 10-cm square pots filled with sand, which was moistened with 0.9 mM gibberellic acid (GA_3) solution[2]. The pots were kept in a germinator with temperatures of 30 C during the day and 26 C at night, 12-hour light/dark cycles, and 90% relative humidity. After 27 days in the germinator, seedlings (approximately 5 to 7 cm total length) were removed from the sand, rinsed in 70% ethanol and then in water, and placed into hydroponics units. Seeds of sicklepod, velvetleaf, hemp sesbania, and jimsonweed were placed in rolls of germination paper saturated with 100 µM $CaSO_4$, and the rolls were placed upright in 4 L beakers and kept moist by capillary action from 200 mL of the same $CaSO_4$ solution. Velvetleaf seeds were boiled for 10 seconds for the purpose of breaking dormancy prior to germination in rolled paper, while the other species required no pre-treatment. The rolls were placed into a dark germination chamber at 27 C and 100% humidity until roots were of sufficient size (approximately 3 cm length) to be placed into hydroponic units.

The newly germinated seedlings were held by sponge supports in the tops of eight 53-liter continuous flow hydroponic units located in a walk-in growth chamber. The day/night aerial temperatures were 30/26 C, with a 9-hour day period and a 3-hour night interruption.

[2] Item G7645, Sigma, St. Louis, MO 63178

Light during the day was provided by a combination of incandescent and fluorescent lamps with a total PPFD of 500 to 600 µmol m^{-2} s^{-1} and the night interruption was provided by incandescent lamps with a PPFD of 40 µmol m^{-2} s^{-1}. Solution temperature treatments ranged from 18 to 39 C, as specified in the results section. Solution pH was held between 5.6 and 6.0 by automated monitoring and additions of H_2SO_4 or KOH. The solutions contained: 1000 µM KNO_3, 600 µM KH_2PO_4, 800 µM $CaSO_4$, 300 µM $MgSO_4$, 19 µM H_3BO_3, 3.7 µM $MnCl_2$, 317 nM $ZnSO_4$, 132 nM $CuSO_4$, 50 nM $NaMoO_4$, and 17.9 µM iron as Sequestrene 330[3]. Concentrations of NO_3^-, PO_4^-, and SO_4^- were monitored during experiments and used as indicators of nutrient depletion. When significant depletion was observed (~ 50%), all nutrients were supplemented proportionately to return levels to original concentrations.

The weed species grew at different rates, thus they were harvested at different times to allow for adequate dry matter accumulation. Tropical spiderwort was harvested 32 days after transplant (DAT), and sample size was between one and six plants per treatment (n (temperature) = 3 (20), 3 (23), 4 (26), 6 (29), 4 (32), 5 (35), 3 (38), 1 (41)) due to mortality, possibly caused by a pre-treatment of seedlings by immersion in 70% ethanol, which was required as a phytosanitary procedure for introduction into the Phytotron. Jimsonweed (n=9 per treatment) was harvested at 100 DAT, hemp sesbania (n=5) at 43 DAT, sicklepod (n=4) at 43 DAT, and velvetleaf (n=5) at 36 DAT. At harvest, tropical spiderwort plants were divided as previously described, while other weeds were separated into shoot and root. The tissue was dried to constant mass at 70 C and then weighed.

Before statistical analysis, shoot and root biomass values for each species were divided by the mean shoot and root biomass for that species at 30 or 31 C. This temperature

[3] Sprint 330, Becker Underwood, Inc., Ames, IA 50010

range was chosen because it was near the temperature at which each species exhibited maximal growth. This normalized the data so that the maximum of each growth parameter for each species was near 1. Shoot and root biomass data were fit with broken-stick and quadratic models using the NLIN and REG procedures. Values for R^2 for the NLIN procedure were calculated as 1-(residual sum of squares / corrected total sum of squares), while R^2 for the REG procedure was calculated as 1-(error sum of squares / corrected total sum of squares).

RESULTS

Aerial and root temperatures in the field. The general pattern of changes in aerial temperatures in central North Carolina is shown in Figure 1 (NCDC, 2003). In hot summer months of July and August, high temperatures are in the 30 to 32 C range, and highs of 25 C or greater occur from May until September. Soil temperatures from a typical field site in the central part of the state follow a similar trend during the year. As might be expected, temperatures vary with depth from the soil surface, and the differences between high and low temperatures are smaller with greater depth.

Seed germination. Seed germination of tropical spiderwort was maximized at 30 to 35 C (Fig. 2). Many more large seeds than small seeds germinated; of the large seeds, those formed aboveground and belowground had similar germination. Of the small seeds, those produced belowground had higher germination percentages than those from aboveground. An interesting aspect of the germination responses was the steepness of the germination curves at lower temperatures. At 30 C, for example, germination of large seeds ranged from 70 to 90% compared to only 10 to 30% at 25 C, and none at 20 C.

Growth and reproductive response to aerial temperature. Because of the low germination numbers of small seeds, only seedlings from large seeds were used for the whole plant development experiment. Regardless of whether seedlings were from above- or below-ground seeds, the temperature response curves were similar (Fig. 3). The optimal temperature for growth was about 30 C. This was true for shoot, root, and rhizome growth (Figs. 3a, 3b, and 3c), as well as the production of above- and below-ground spathes (Figs. 3d and 3e). Response of spathe mass demonstrated the same pattern as spathe number (data not shown). For the purposes of this study, it is notable that sharp decreases occurred in shoot and root masses and spathe number at temperatures above and below the optimum. Shoot growth, for example, was suppressed by about 70% at 25 C compared to 30 C.

Response to root temperature. When exposed to various root temperatures in hydroponic culture, tropical spiderwort shoot biomass accumulation was greatest at 32 C (Fig. 4). Shoot growth was strongly affected by root temperature treatments, dropping off markedly at lower and higher root temperatures. The root growth response was less obvious, but also demonstrated inhibition at the lowest and highest temperature treatments. These results should be interpreted with caution because the alcohol wash resulted in injury and death of some seedlings. The alcohol injury and the more stressful temperature treatments, together, may have contributed to the high mortality levels.

The other weed species displayed very different root temperature responses. The most similar to tropical spiderwort was jimsonweed, which had optimal shoot growth at about 33 C (Fig. 4e), and hemp sesbania with optimal growth at about 30 C (Fig. 4d). The optimal root temperature for sicklepod growth was lower, at about 25 C, and velvetleaf showed minimal response to increasing root temperature until about 35 C. Related studies conducted on

velvetleaf and sicklepod indicate that time of harvest had few effects on temperature response (see Appendix).

The shapes of the response curves were dissimilar in some cases. Broken-stick and quadratic models were fit to biomass data for all species (Table 1), but R^2 values indicate that tropical spiderwort was better fit by the quadratic model, indicating that its growth was optimized at a moderate temperature (T_{max}, the peak temperature of the model, was 29) and inhibited at higher and lower temperatures. The same was true of hemp sesbania and sicklepod. In contrast, velvetleaf was more closely fit by the broken-stick model, so its temperature response was characterized by a plateau at lower temperatures with a drop-off at higher temperatures. The break point (Th), beyond which growth was inhibited, was 36. Jimsonweed shoots exhibited a more quadratic response and roots were better fit by the broken-stick model, but neither gave a particularly good fit.

The reproductive output of tropical spiderwort, comprising rhizome mass and aboveground and belowground spathe number, was optimized at a root temperature of 29 C (Fig. 5). As with plant growth parameters, spathe number decreased at higher and lower temperatures, and reproductive output was severely inhibited at the lowest and highest temperatures.

DISCUSSION

These experiments defined the temperature response of the invasive species tropical spiderwort and several other weeds. Effects exerted by air and soil temperatures on plant growth and competition are thought to be key elements of predicting a species' potential geographical range (Patterson et al., 1999).

The response profiles of tropical spiderwort indicate that germination was maximized in the range of 30 to 35 C, and vegetative and reproductive biomass at about 30 to 32 C. The growth and reproductive responses occurred whether plants were exposed to different aerial or different root temperatures. Considerable sensitivity to cooler temperatures was observed. Shoot biomass production, for example, was suppressed by about 60 to 70% at an aerial temperature of 25 C; reproductive output was also suppressed at this temperature.

Our hypothesis was that tropical spiderwort would express maximal growth at a relatively high temperature because of its proposed tropical origin. Temperature responses were not as high as were expected, however, considering previously reported temperature responses of other tropical species. Two agronomically important weeds, sicklepod and prickly sida, for example, were found to have maximal growth at an aerial temperature of 36 C in these same growth chambers (Tungate et al., 2006). A previous study with Palmer amaranth observed rapid growth at even higher temperatures (Wright et al., 1999). Further, 32 C is the same temperature where maximal growth is observed in new soybean lines being developed for the southeastern U.S., which contain genes from exotic germplasm (Carter and Rufty, 1993; Tungate et al., 2006).

Tropical spiderwort germination and whole plant growth did exhibit sensitivity to cooler temperatures, with reductions of 60 to 80% at 25 C. Nonetheless, there is no reason to suspect that this invasive species would have substantial difficulties becoming established in the state of North Carolina or even further northward. Visual evaluation of the aerial and soil temperature plots (Fig. 1) suggests that tropical spiderwort would have favorable temperatures for rapid germination, growth, and reproduction from June through August, which is the case with many of the problem agronomic weeds. Moreover, while few studies

in the published literature have detailed temperature responses of weed species, the growth responses to different root temperatures (Fig. 4) demonstrate that several problem weed species in the southeast region have similar characteristics. Jimsonweed and hemp sesbania had growth response profiles similar to that of tropical spiderwort as temperatures were decreased below the optimum. Both weed species are suspected of being native to tropical America (Parsons and Cuthbertson, 1992; USDA and NRCS, 2002; Weaver and Warwick, 1984), and both are certainly troublesome in North Carolina (Webster, 2001) and further north in cooler climates (USDA and NRCS, 2002).

In considering the potential success of tropical spiderwort, we should also consider that models of global warming predict an increase in temperature of the southeastern U.S. by as much as 5 degrees C during the next century (IPCC, 2001). Warmer temperatures will mean an expanded season for growth and development of tropical spiderwort as well as many other problem weed species, and also may increase competitiveness with many of the major crop species.

Thus, whether based on comparisons of growth response in controlled environment chambers and actual field temperatures, or on comparisons with temperature response curves of successful weeds, we see no reason to believe that temperature, at current or at predicted future levels, would be a restraint for tropical spiderwort successfully invading northward from current infestations in Georgia.

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Table 1. Parameters, F, and P values of broken-stick and quadratic models for normalized shoot and root biomass of each species. Broken-stick model parameters include **p**, the value of the y intercept of the plateau; **Th**, the temperature threshold above which growth response is negative; and **b**, the slope of the line at temperatures greater than Th. Quadratic model parameters are of the form $Y = \mathbf{a} + \mathbf{b}T + \mathbf{c}T^2$, where T is temperature; $\mathbf{T_{max}}$ is the temperature at which normalized biomass was greatest.

	Broken-stick model						Quadratic model						
Species	p	Th	b	R^2	F	P	a	b	c	T_{max}	R^2	F	P
	----------Shoot----------												
Velvetleaf	0.99	36	-0.19	0.90	23.00	0.003	-0.33	0.25	-0.005	23	0.71	6.11	0.045
Sicklepod	1.10	32	-0.09	0.72	6.58	0.040	-4.83	0.66	-0.013	26	0.77	8.51	0.025
Hemp sesbania	0.70	38	-0.28	0.36	3.32	0.119	-4.51	0.42	-0.007	29	0.72	6.29	0.043
Tropical spiderwort	0.96	38	-0.29	0.41	1.71	0.272	-20.60	1.58	-0.025	31	0.73	6.89	0.037
Jimsonweed	0.92	37	-0.07	0.14	0.97	0.362	-5.36	0.80	-0.013	30	0.76	7.96	0.028
	----------Root----------												
Velvetleaf	0.86	36	-0.31	0.92	72.39	<0.001	-1.35	0.17	-0.003	26	0.55	3.00	0.140
Sicklepod	1.10	33	-0.12	0.71	6.14	0.045	-3.16	0.35	-0.007	26	0.80	9.93	0.018
Hemp sesbania	0.64	31	-0.06	0.40	1.64	0.283	-3.06	0.27	-0.005	28	0.71	6.18	0.045
Tropical spiderwort	0.89	36	-0.18	0.58	3.38	0.118	-2.15	0.17	-0.003	30	0.78	9.03	0.022
Jimsonweed	1.10	37	-0.15	0.58	8.17	0.020	1.29	0.12	-0.002	26	0.16	0.48	0.645

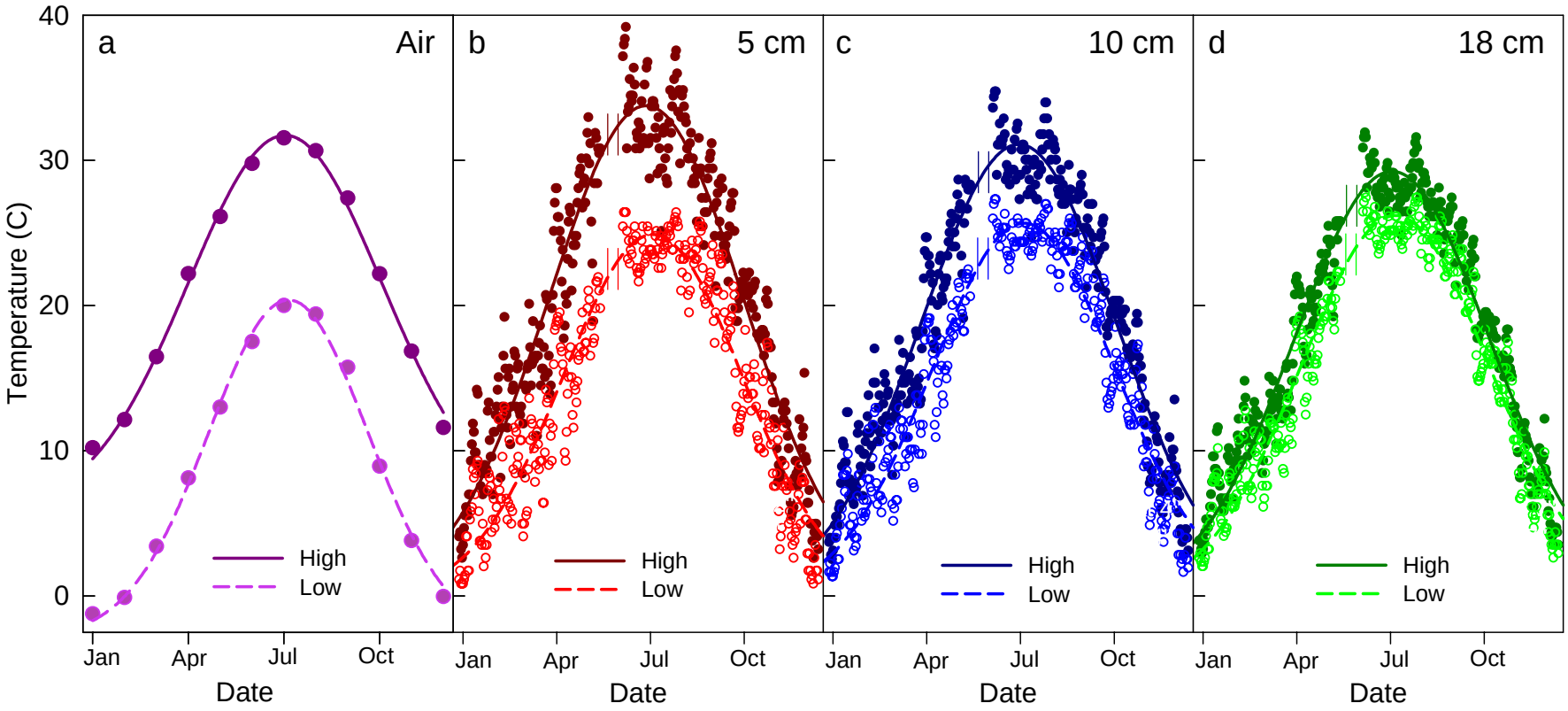


Figure 1. Average regional monthly high and low air temperature from 1948 to 2001 (a), and daily soil temperatures at depths of (b) 5 cm, (c) 10 cm, and (d) 18 cm from June 2000 to May 2001.

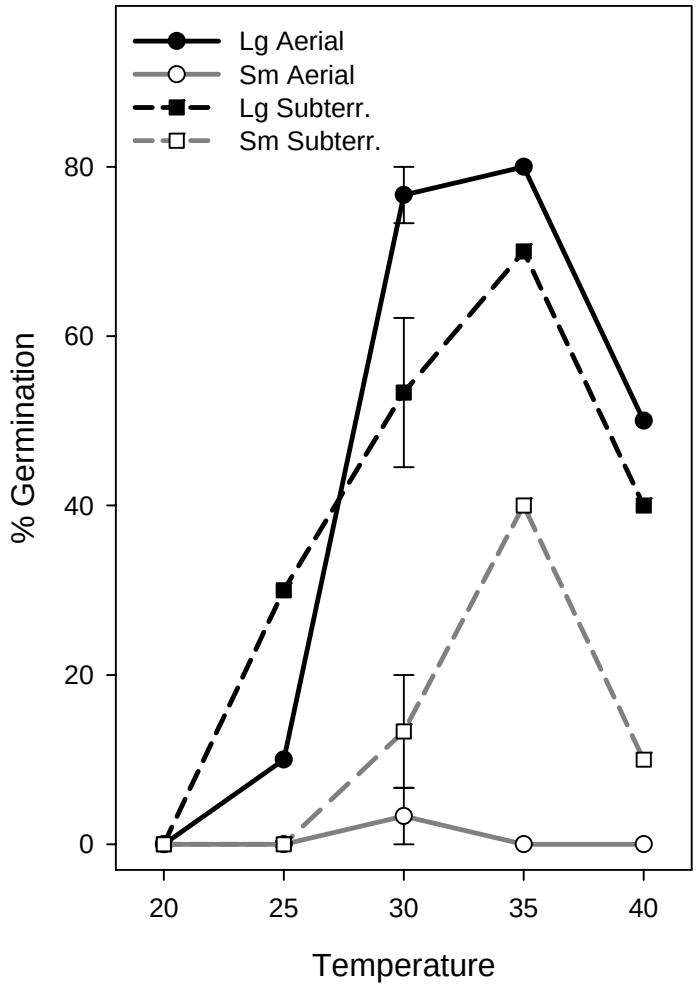


Figure 2. Germination of tropical spiderwort seeds representing each of the four classes at different temperatures.

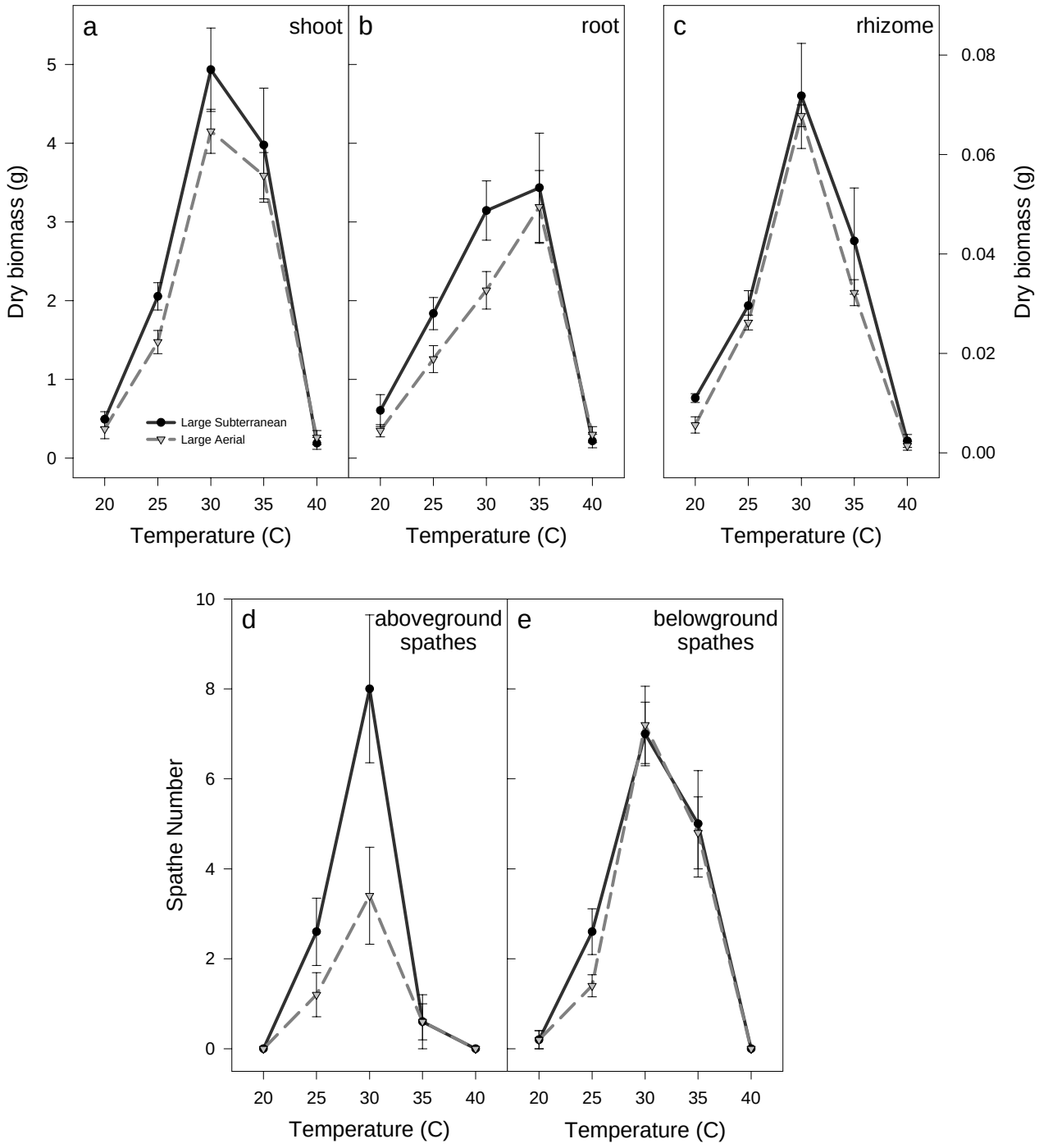


Figure 3. Tropical spiderwort (a) shoot dry mass, (b) root dry mass, (c) rhizome dry mass, (d) aboveground spathe number, and (e) belowground spathe number of plants grown at different aerial temperatures. Plants were grown from large subterranean and large aerial seeds. Error bars represent standard error.

Figure 4. Shoot and root dry mass of plants grown at different root temperatures: (a) tropical spiderwort, (b) sicklepod, (c) velvetleaf, (d) hemp sesbania, and (e) jimsonweed. Error bars represent standard error.

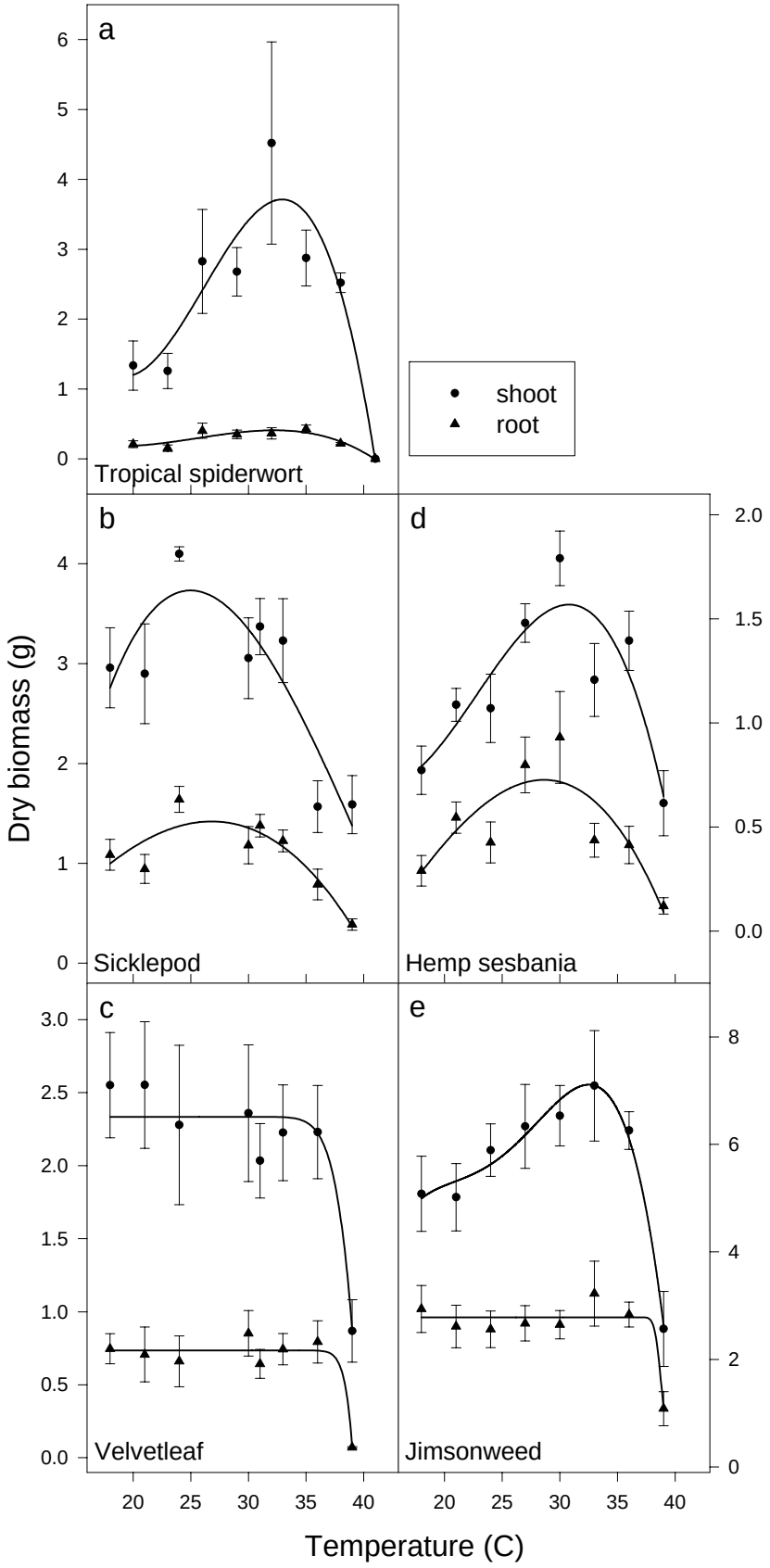
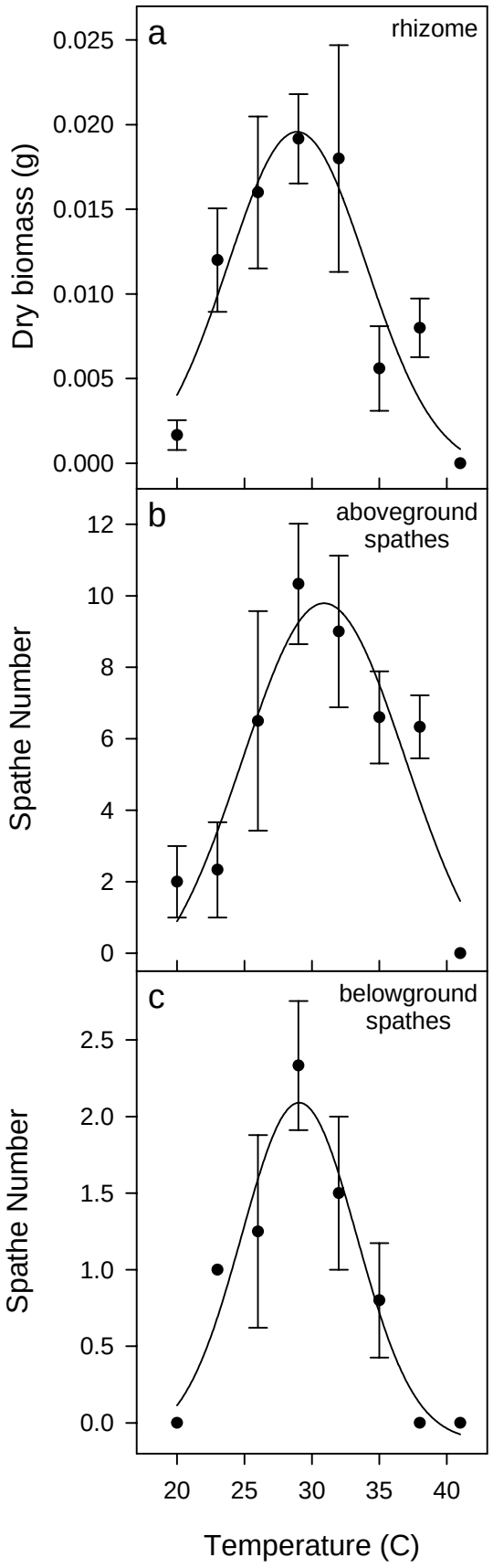

a
b
c
d
e
shoot
root
Tropical spiderwort
Sicklepod
Velvetleaf
Hemp sesbania
Jimsonweed
Dry biomass (g)
Temperature (C)

Figure 5. Tropical spiderwort (a) rhizome dry mass, (b) aboveground spathe number, and (c) belowground spathe number of plants grown at different root temperatures. Error bars represent standard error.


a
rhizome
Dry biomass (g)
0.025
0.020
0.015
0.010
0.005
0.000
b
aboveground
spathes
Spathe Number
12
10
8
6
4
2
0
c
belowground
spathes
Spathe Number
2.5
2.0
1.5
1.0
0.5
0.0
20
25
30
35
40
Temperature (C)

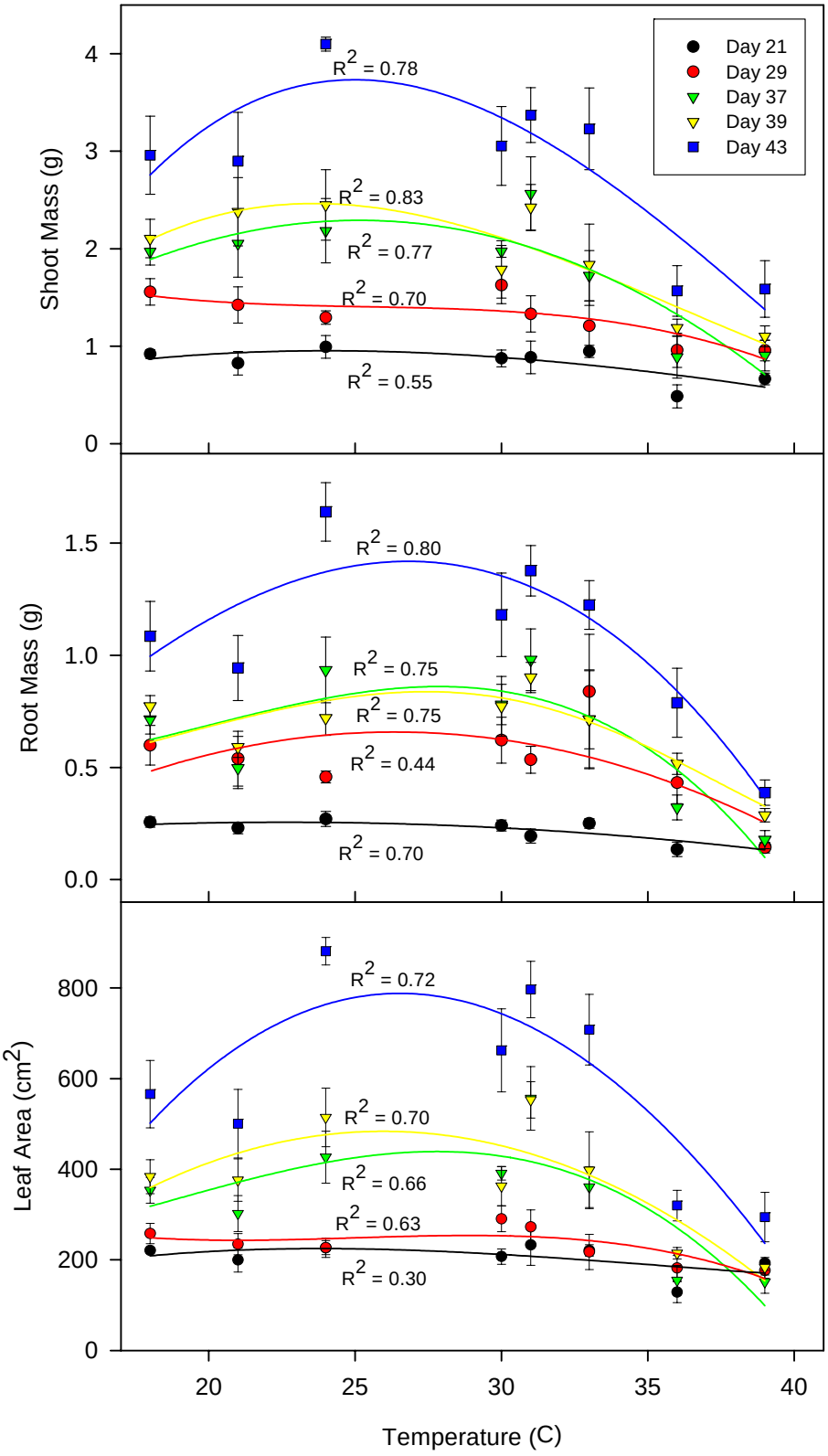
APPENDIX

In the root temperature hydroponics experiment, sicklepod was harvested at five dates (21, 29, 37, 39, and 43 days after transplant), with four plants per temperature per harvest. Velvetleaf was harvested at six dates (17, 22, 29, 32, 36, and 40 days after transplant), with five plants per temperature per harvest. At sicklepod and velvetleaf harvests, leaf area was measured using a LI-3100 leaf area meter (LI-COR, Lincoln, NE); the plants were then divided into shoot and root material, which was dried to constant mass at 70 C and weighed. Biomass data were log transformed to reduce variance heterogeneity, then subjected to analysis of variance within each species using the GLM procedure (SAS, Cary, NC). Helmert contrasts (Ruberg, 1989) were used within each species to determine at which temperature biomass was significantly reduced. Helmert contrasts are a set of orthogonal contrasts that, in this case, were used to compare the biomass harvested at the highest temperature with the mean at all lower temperatures. Then the process was repeated to compare the second and third highest temperatures against the average of all lower values.

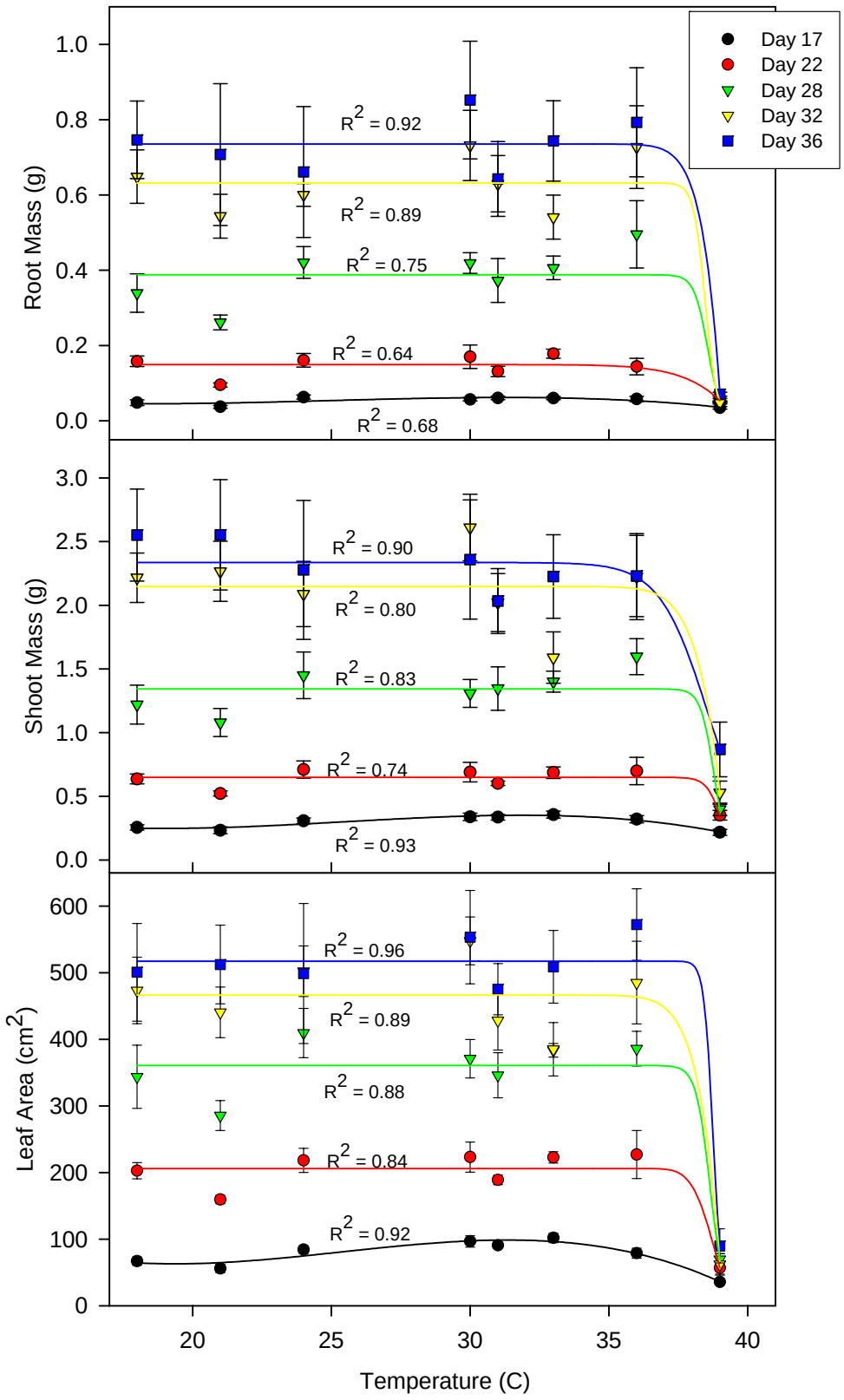
Data from these studies were plotted and no differences in response by harvest date were obvious, although contrasts did indicate a significant interaction of harvest date and the 39 C temperature on the sicklepod log shoot biomass and velvetleaf log shoot and log root biomass (Appendix Table 1). Temperature effects on leaf area (Appendix Fig 1c and 2c) were very similar to effects on shoot mass. Therefore, we determined that the multiple harvests and leaf area measurement did not provide significantly more information than biomass measurement at a single harvest, and they were omitted from all later runs. Helmert contrasts for both species indicate that, across harvests, shoot and root biomass at root temperatures of 39 and 36 C were lower than the mean of biomass produced at lower temperatures (Appendix Table 1).

Appendix Table 1. F and P values of Helmert contrasts evaluating temperature response of shoot and root biomass of sicklepod and velvetleaf across harvest dates (day). A significant 'Temp' value indicates a difference between biomass at that temperature and the average of biomass at all lower temperatures.

	Sicklepod		**Velvetleaf**	
Source	F	P	F	P
	----------log(Shoot)----------			
day	279.5	**<0.001**	966.8	**<0.001**
day*day	1.8	0.196	21.2	**<0.001**
Temp39	46.2	**<0.001**	178.0	**<0.001**
day*Temp39	6.3	**0.017**	19.0	**<0.001**
Temp36	85.2	**<0.001**	4.2	**0.046**
day*Temp36	0.4	0.511	2.3	0.136
Temp33	3.3	0.081	0.1	0.761
day*Temp33	0.6	0.455	3.2	0.080
	----------log(Root)----------			
day	142.2	**<0.001**	898.2	**<0.001**
day*day	0.2	0.690	38.8	**<0.001**
Temp39	65.4	**<0.001**	389.5	**<0.001**
day*Temp39	2.2	0.148	65.1	**<0.001**
Temp36	17.7	**<0.001**	5.3	**0.027**
day*Temp36	0.1	0.760	3.5	**0.070**
Temp33	0.0	0.972	0.6	0.446
day*Temp33	0.7	0.419	1.1	0.304



Appendix Fig 1. Sicklepod (a) shoot dry mass, (b) root dry mass, and (c) leaf area vs. root temperature from multiple harvest dates. Error bars represent standard error.



Appendix Fig 2. Velvetleaf (a) shoot dry mass, (b) root dry mass, and (c) leaf area vs. root temperature from multiple harvest dates. Error bars represent standard error.

CHAPTER 2. A METHOD FOR DESCRIPTION OF SEED SHAPE

INTRODUCTION

Numerous studies have shown that seed characteristics can have important impacts on germination and vigor of offspring. The characteristics most often considered are seed mass and nutrient content, which have been found to be positively correlated with germination rate, as well as seedling growth rate, height, and leaf area (Wulff and Bazzaz 1992, Parrish and Bazzaz 1985, Aarssen and Burton 1990, Tungate et al. 2002).

Perhaps the least examined seed characteristics are seed shape and volume. The challenges associated with obtaining these data have limited their availability (Harper et al. 1970). Quantification of other parameters, such as density, relies on an estimate of volume, which has usually been estimated only as an average for a large number of seeds. Surface area and shape characteristics may help to provide a more thorough system for comparing seeds.

There have, however, been many attempts to quantify shape. These attempts fall into several groups: verbal descriptions, numerical descriptions based on seed measurements, and classification by use of Fourier coefficients.

Verbal descriptions have been used to classify seeds into shape categories, such as flat, pear-shaped, oval, round, elliptic, oblong, triangular, or irregular; or as resembling one of several widely recognized races (Varier et al. 1999, Moreno-Martinez et al. 1998, Wilson et al. 1990, Chang et al. 2000).

Numerical descriptions have characterized seed shape using one or more values that are calculated from measurements. Some examples of this approach include calculation of

seed “thinness”, “flatness”, or “roundness” through use of measurements that can include area and perimeter of a seed silhouette, largest and smallest seed diameters, or variance of seed length, width, and height (Travis and Draper 1985, Szentesi and Jermy 1995, Cober et al. 1997a, 1997b, Bekker et al. 1998). A novel approach describes representative shapes using elliptic Fourier coefficients, which can be used to classify shapes into similar groups, such as seeds from particular species or populations (Selin 2000, Oide and Ninomiya 2000).

Despite the importance of seed shape and the many techniques that have been used to describe it, most methods can be used only in specific applications. For example, they may be useful for classifying seeds into similar groups (e.g. species, cultivars) and distinguishing among such groups, or for correlating a specific shape factor, such as roundness or width, with an ecological characteristic (e.g. infestation by seed predators, probability of germination). This paper outlines a new method for quantifying seed shape; a method which will have the flexibility to be used for a wider range of purposes. It involves digital imaging and polynomial functions, which can be used to calculate a variety of seed characteristics such as volume, surface area, and density. The method allows a more comprehensive description of seed shape and requires only a minimal amount of equipment.

MATERIALS AND METHODS

The method involves two main steps. First, digital images of each seed are captured using a camera mounted on a dissecting microscope. Then, polynomial functions are used to define the perimeters of the seed. These functions represent information about the seed shape, which can produce numerical definitions of aspects of seed shape.

Image Capturing

Ten sicklepod (*Senna obtusifolia*) and ten maize (*Zea mays*) seeds were aligned one at a time under a dissecting microscope. The sicklepod seeds were aligned such that the longest axis of the seed ("length"), was horizontal and the hilum was on the left-hand side, facing the camera (Fig. 1). The vertical dimension of this projection was called "height". Images were captured using a Spot digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI). Each seed was then rotated ninety degrees around its length axis, so the hilum was facing away from the x axis, and imaged again. Adhesive tape was used under the seeds to hold them in position, if necessary. Maize seeds were oriented in a similar fashion and imaged in the height-vs.-length and width-vs.-length views. Image Pro-Plus version 2.0 computer software (Media Cybernetics, Silver Spring, MD) was used to trace outlines of the seeds.

Polynomial functions

Advanced Grapher (SerpikSoft, www.serpik.com) was used to fit polynomial functions, up to the ninth degree, to the seed outlines. Functions were fitted to each of the two views of each seed. The function representing the upper portion of the height vs. length view of a seed was designated as A(x); another function, B(x), represents the lower portion of this view (Fig.2). C(x) represents the upper portion of the width vs. length view of a seed, and D(x) represents the lower portion of this view (Fig.3).

Subtraction of B(x) from A(x) yields the height of the seed at any point x along the length of the seed. Subtraction of D(x) from C(x) yields the width of the seed at any point along the length of the seed.

The function F(x) relates A(x), B(x), C(x), and D(x) and represents the circumference of the seed at any point x along its length (Fig. 4). Integration between F(x) and y=0, from the x value at which the seed's image begins to the x value at which the seed's image ends, yields the surface area of the seed.

The function E(x) relates A(x), B(x), C(x), and D(x) and represents the area of a cross-section of a seed at any point x along the length of the seed (Fig. 5). Integration between E(x) and y=0 yields the volume of the seed. The function E(x) assumes that the shape of a cross-section of the seed is a smooth oval.

Comparison of Volumes

To test the accuracy of the method, calculated volumes of seeds of sicklepod (*Senna obtusifolia*) and maize (*Zea mays*) obtained through imaging were compared to measured volumes that were obtained through water displacement (Fig. 6). A test tube just large enough to accommodate the seed (for sicklepod, a 6mm x 50 mm test tube; for maize, a 12mm x 75 mm test tube) was placed in a foam support in the bottom of a large amber plastic vial. A hole was drilled in the side of the vial and a magnifying glass was taped over the hole. Pieces of tape marked with horizontal lines were placed in front of the magnifying glass and on the opposite side of the vial, behind the test tube. A piece of tape with a vertical line was placed on the side of the vial behind the test tube, for alignment of the test tube.

Because of the difficulty in measuring volumes of small seeds, sicklepod seeds were grouped into pairs, while maize seeds were large enough to be measured individually. Distilled water was introduced into the test tube using a pipet so that the meniscus was aligned with the horizontal lines in front of and behind the test tube. One pair of sicklepod seeds or a single maize seed was placed into the tube. Water was removed using a Hamilton

syringe (for sicklepod, a 50 μL syringe; for maize, a 500 μL syringe) until the meniscus was once again aligned with the calibration marks. The water from the syringe was then weighed on a microbalance (Model AT20, Mettler-Toledo Inc., Columbus, OH). Each milligram of displaced water equaled one cubic millimeter of seed volume.

After the ten sicklepod seeds, grouped into five pairs, were measured, then they were re-grouped into five different pairs and measured a second time. The calculated and measured volumes were graphed using SigmaPlot (SPSS Inc., Chicago, IL). The individually calculated volumes of the two sicklepod seeds in each pair were added together for comparison to the measured volumes.

Evaluation of Method

Correlations between calculated and measured volumes demonstrate that volumes of sicklepod seeds can be accurately calculated by the digital imaging method (Fig. 7, r^2=0.75). In the comparison of calculated vs. measured volumes of sicklepod seeds, there are two outlying data points. Each of these two points represents a pair of seeds. If the two pairs are deleted from the graph, the r^2 becomes 0.98. It is apparent that one or both of the seeds in each of these pairs are not good candidates for the measurement method. Each outlying pair contains the same particular seed that has an atypical shape and, therefore, varies more extremely from the shape requirements than do the other sicklepod seeds.

Recall that each seed was measured in two dimensions, and its parameters were calculated as though the two perpendicular measurements were connected by a smooth oval shape. The further that a seed varies from this standard, the less acceptable that seed is as a candidate for the digital imaging method. Any concavities or other deviations from an oval cross-sectional shape will cause inaccuracies in the calculations. Seeds that are ideal for use

in this shape quantification method are those having at least one axis about which the seed is a smooth oval.

Consideration of the shape requirements reveals why maize seeds were not well modeled by this method (Fig. 8, r^2=0.19). The maize seeds that were used in this experiment were more trapezoidal than oval. Each seed also had a concave indentation on one side that could not be seen in either of the two-dimensional pictures. Thus, as demonstrated by the lack of correlation between calculated and measured volumes, maize seeds are not appropriate for use in this digital imaging method.

If a seed is of a suitable shape, then the digital imaging method can be used to calculate many of its parameters. Volume and surface area can be calculated; from those, density and surface area-to-volume ratio can be found. The volume contained within segments of the seed can be calculated. The method can easily be modified to calculate parameters that may be of interest in different situations.

The ability to calculate many parameters could provide more comprehensive answers to questions that have been presented in previous studies. For example, Travis and Draper (1985) represented seed shape by a comparison of the area of a two-dimensional picture to its perimeter. In addition to two-dimensional thinness, other important factors might have been identified through utilizing measures of volume, density, and surface area-to-volume ratio. Similarly, Szentesi and Jermy (1995) may have found that factors other than seed flatness are important in predicting seed infestation by predators. Studies similar to Nelson and Wang's (1989) could be made more precise by using the digital-imaging method to characterize seeds in a more quantitative way than the visual classification scheme. The digital imaging method

can be a useful tool for allowing researchers to better study many aspects of seed morphology.

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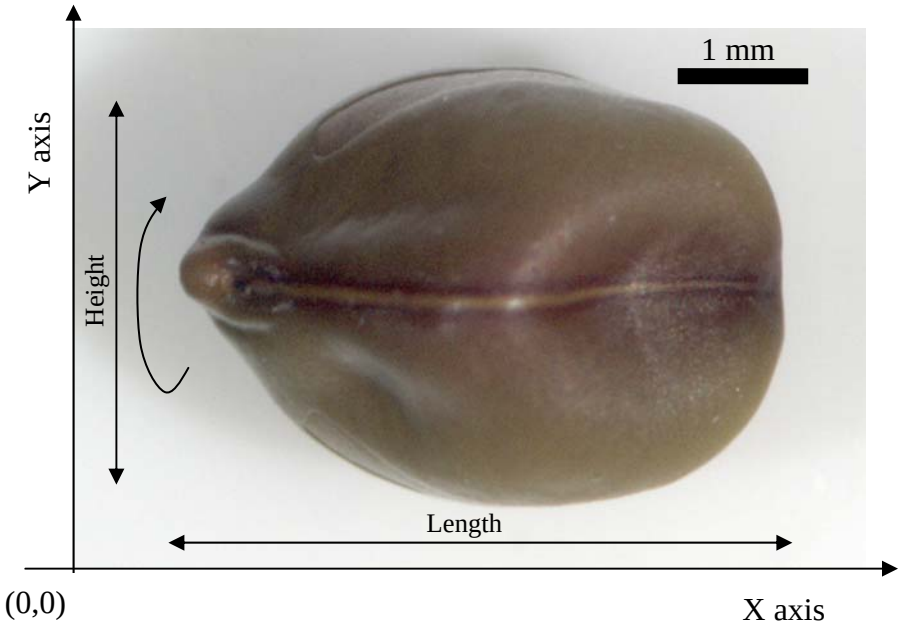

Figure 1. Orientation of sicklepod seed in height vs. length view. Seed was rotated 90 degrees on the length axis, as shown by curved arrow, for width vs. length view.

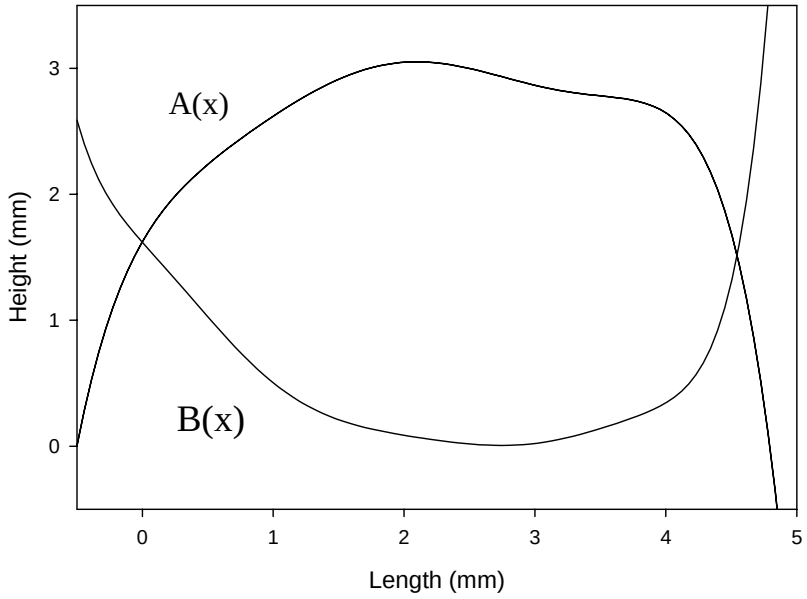

Figure 2. Functions fitted to height vs. length view of sicklepod seed. A(x) represents the outline of the upper part of the seed while oriented in height vs. length view, and B(x) represents the lower part. A(x) minus B(x) at any point along the length of the seed represents the height of the seed at that point.

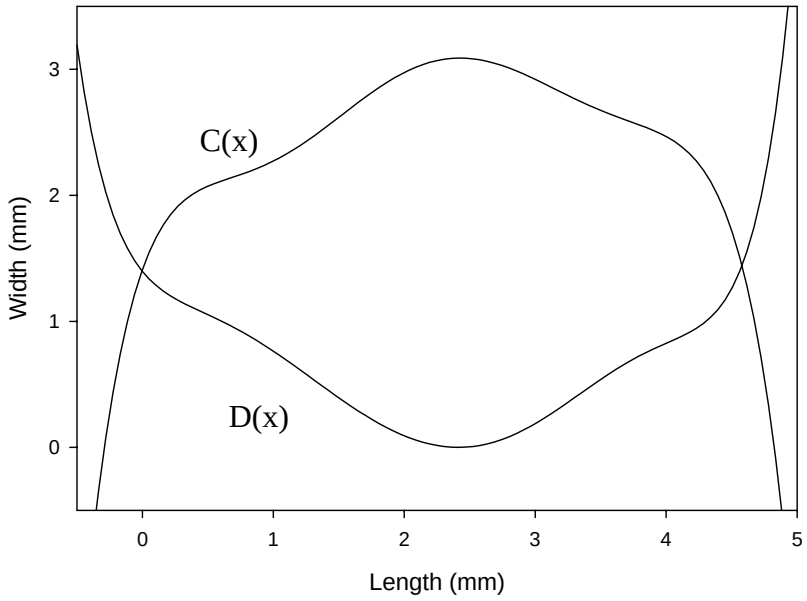

Figure 3. Functions fitted to width vs. length view of sicklepod seed. C(x) represents the outline of the upper part of the seed while oriented in width vs. length view, and D(x) represents the lower part. C(x) minus D(x) at any point along the length of the seed represents the width of the seed at that point.

$$F(x)= \pi * \left(\left(\frac{A(x)-B(x)}{2}\right) * \left(\frac{C(x)-D(x)}{2}\right)\right) * \left\{1 + \frac{3 * \left(\left(\frac{A(x)-B(x)}{2}\right) - \left(\frac{C(x)-D(x)}{2}\right)\right)^2}{10 + \sqrt{4 - 3 * \left(\frac{\left(\frac{A(x)-B(x)}{2}\right) - \left(\frac{C(x)-D(x)}{2}\right)}{\left(\frac{A(x)-B(x)}{2}\right) + \left(\frac{C(x)-D(x)}{2}\right)}\right)^2}}\right\}$$

Figure 4. Function F(x) represents the circumference of the seed at any point along the length of the seed. Integration of this function along the length of the seed yields the surface area of the seed.

$$E(x)=\{A(x)-B(x)\}*\{C(x)-D(x)\}*0.7854$$

Figure 5. Function E(x) represents the area of a cross-section of the seed at any point along the length of the seed. Integration of this function along the length of the seed yields the volume of the seed.

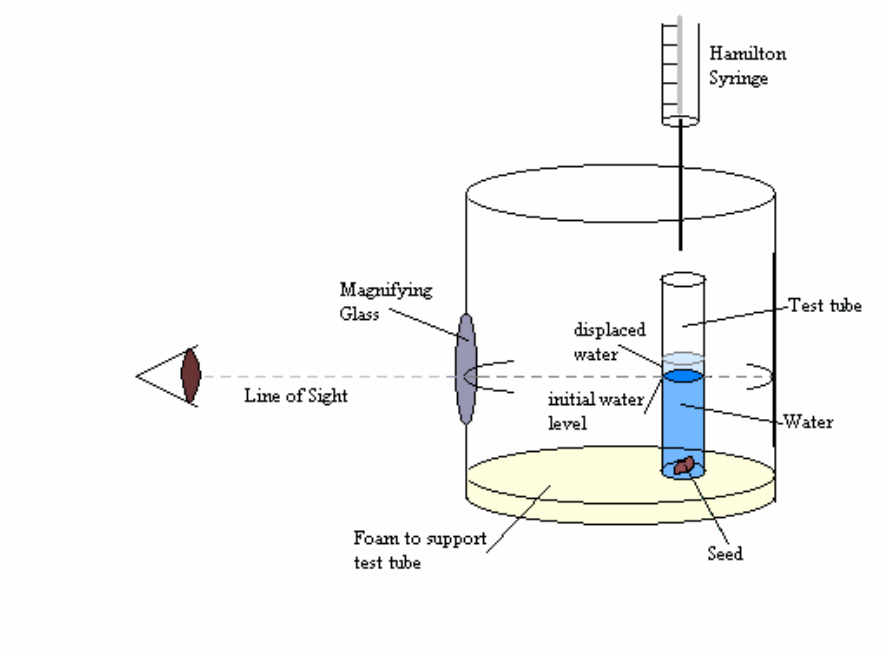

Figure 6. Device for measuring volume of seeds by water displacement.

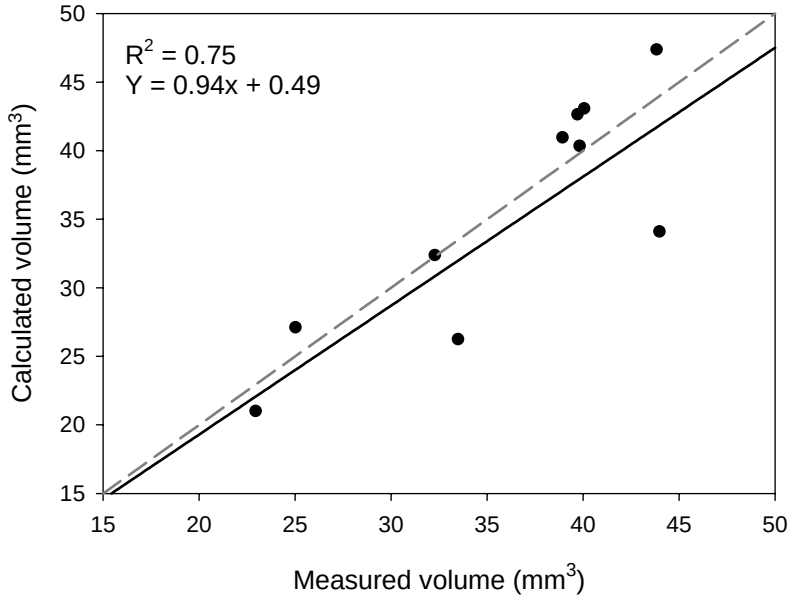

Figure 7. Summed calculated vs. measured volumes of paired sicklepod seeds. Dashed line represents Y=X.

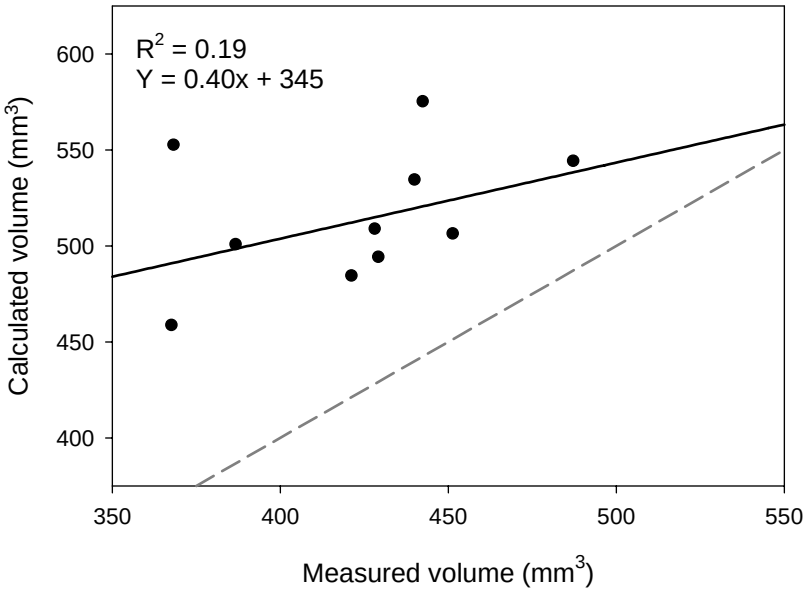

Figure 8. Calculated vs. measured volumes of individual maize seeds. Dashed line represents Y=X.