ABSTRACT

RIAR, MANDEEP KAUR. Benghal Dayflower (*Commelina benghalensis* L.) in Agronomic Systems. (Under the direction of Dr. Thomas Rufty).

Benghal dayflower is an invasive, federal noxious weed and a serious threat to agronomic crop production in the southeastern United States. This weed is a native of tropical Asia, Africa and pacific islands and was introduced into Florida in early 1930s. Benghal dayflower possesses several physiological characteristics that aid in its survival and invasiveness. Extensive aerial and subterranean seed production, tolerance to commonly used herbicides including glyphosate, and ability to regenerate from stem fragments make Benghal dayflower extremely difficult to control. The present research was conducted to characterize Benghal dayflower responses to various environmental conditions. The responses allow prediction of Benghal dayflower invasiveness into various agronomic systems.

In the first series of experiments, Benghal dayflower seed viability was examined after exposure to high temperatures commonly found in hay bales and to simulated ruminant digestion. Ability of stem fragments to regenerate under different temperatures and soil depths was also tested. Measurement of temperatures in hay bales indicated that temperatures could reach 70 °C in the weeks following bailing. Seeds were non-viable within 1 day at 65 °C and within 14 days at 50 and 45 °C, indicating sensitivity to extremely high temperatures. It thus seemed unlikely that seeds captured in hay bales would remain viable and be spread during animal feeding. Seed viability might be expected with animal consumption of unbaled hay or with foraging.

Benghal dayflower seeds were more tolerant to ruminant digestion of 12 to 96 hours than seeds of other weed species and retained their viability. If viable seed were consumed,

subsequent dispersal in manure would probably occur. In the case of stem fragments, regeneration occurred at 2 cm and 6 cm when aerial temperatures were about 25 °C. New roots developed at both soil depths, but leaf development was restricted at 6 cm and subterranean spathe development at 2 cm.

In the second series of experiments, long-term soil burial studies were conducted to examine longevity of Benghal dayflower seeds at different sites in North Carolina, Florida, and Georgia. Seed remained viable for a couple of years, and then declined. Viability was reduced to $< 10\%$ at all locations after 36 months. This indicates that management programs must prevent seed production for at least 3 growing seasons to appreciably reduce the Benghal dayflower soil seedbank.

In the last series of experiments, Benghal dayflower growth and reproductive responses to differing environmental conditions were examined over a 56 d period. Plants produced greatest vegetative biomass at 35/28 °C day/night temperature. Flowering was suppressed by 3 days at 35/28 °C and by 7 days at 30/22 °C with the night interruption. However, aerial and below ground reproduction was influenced very little by changes in photoperiod. In the second part of this experiment, reduced nutrient and shading markedly reduced vegetative biomass of plants. Aerial and subterranean spathe production expressed different degrees of adjustment to suboptimal conditions. Aerial reproduction was reduced at sub-optimal conditions, but belowground reproduction remained relatively stable. The results therefore indicate that Benghal dayflower would persist in agronomic systems even under reduced resource availability.

Benghal Dayflower (*Commelina benghalensis* L.) in Agronomic Systems

by
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DEDICATION

This dissertation is dedicated to my brother Dr. Ranjit Singh Riar; the lighthouse guiding my education.

BIOGRAPHY

Mandeep K. Riar grew up in a small village of Punjab, a predominantly agricultural state of northern India. Since early childhood, she watched and later helped her mother tend their kitchen and flower garden. Due to her interest in plants and a lot of guidance from her brother, she pursued her Bachelors in Agriculture (Honors) at Khalsa College, Amritsar, India and was taken from the very first class. After four years of a comprehensive education she graduated with distinction. Then she earned a Master's degree in Agricultural Meteorology from Punjab Agricultural University with merit. Immediately after completing her Master's degree she was accepted for a Ph.D. program in Crop Science at North Carolina State University. During her Ph.D. she worked on invasive weed physiology and ecology. Mandeep has always been fascinated by weeds and the crops they infest and she looks forward to keep on working with these.

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CHAPTER I

Persistence of Benghal Dayflower (*Commelina benghalensis*) in Sustainable Agronomic Systems: Potential Impacts of Hay Bale Storage, Animal Digestion, and Cultivation

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ABSTRACT

Experiments were conducted to evaluate factors affecting persistence of the invasive, federal noxious weed Benghal dayflower in sustainable agronomic systems. Seeds were exposed to a range of temperatures simulating those found in hay bales in the field and periodically tested for viability over 21 days. Seeds were non-viable after one day at 65 °C and after 14 days at 50 or 45 °C. A second series of experiments examined the effects of simulated rumen digestion on germination and viability of Benghal dayflower seeds and seeds of five common weed species. Time courses revealed that seeds from the other weeds were acutely damaged by digestion and viability depressed after 48 and 96 hours, but germination of Benghal dayflower seeds was increased at 48 hours, and only a slight decrease occurred after 96 hours. In the third experimental series, stem fragments of Benghal dayflower were buried in soil at 2 and 6 cm depths and exposed to aerial temperatures of 20, 25, 30, and 35 °C for 30

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d. Root development occurred at both depths, but leaf development was restricted at 6 cm and subterranean spathe development at 2 cm. Temperatures greater than 25 °C favor regeneration at both depths. The results, collectively, show the difficulty encountered when trying to control or eradicate Benghal dayflower in sustainable farming systems. Farmers must avoid using fresh hay as animal feed when Benghal dayflower is present in hay fields, as little restraint on seed viability will be exerted during digestion and generation of manure. Cultivation during summer months is unlikely to be an effective control strategy for Benghal dayflower, because soil temperatures are optimal for vegetative regeneration and its growth is most aggressive.

Nomenclature: Benghal dayflower, *Commelina benghalensis* L. COMBE.

Key words: Invasive weed, ruminant digestion, seed viability, seed dispersal, vegetative regeneration.

INTRODUCTION

Benghal dayflower is an invasive, noxious weed that is a serious threat to agriculture in the southeastern United States. Originating in tropical Asia and Africa (Budd et al., 1979; Holm et al., 1977), Benghal dayflower became established in Florida in the early 1930s, but was not identified as a serious pest in crop production until the past decade (Culpepper, 2006). Survival and spread of the weed has been aided by its tolerance to the widely used herbicide glyphosate (Culpepper et al., 2004; Culpepper, 2006) and its multiple types of reproduction. Benghal dayflower produces aerial and subterranean seeds in dimorphic flowers. Seed size varies, depending on position on the plant and within the fruit (Maheshwari and Singh, 1934; Maheshwari and Maheshwari, 1955), and differences in seed morphology lead to different dormancy characteristics (Kim et al., 1990; Walker and Evenson, 1985). Furthermore, new plants can be generated vegetatively from stem fragments (Budd et al., 1979; Chivinge and Kawisi, 1989).

Relatively little information is available on how Benghal dayflower survives and spreads within farming systems, particularly those operating in a sustainable or organic framework. It is generally recognized that fauna can facilitate rapid dispersal of small seeds within or between ecological systems (Richardson et al., 2000). Goddard et al. (2009) found that Benghal dayflower seed can be carried in the crop and gizzard of mourning doves (*Zenaida macroura* L.), which may be involved in dispersal over longer distances. A more likely means of local dispersal would involve livestock. Seeds and stem fragments from infested landscapes could be consumed directly (Lanyasunya et al., 2008) or captured in hay that is consumed by livestock and then dispersed in manure. Considerable evidence with weeds other than Benghal dayflower indicates that movement of seed by livestock can occur, but with large variation in seed viability occurring among weed species when exposed to animal digestion (Burton and Andrews, 1948; Conn et al., 2010; Dastgheib, 1989; Harmon and Keim, 1934; Hogan and Phillips, 2011; Lacey et al., 1992; Mt. Pleasant and Schlather, 1994; Rupende et al., 1998; Russi et al., 1992; Schoenbaum et al., 2009; Simao Neto and Jones, 1987).

Temperature plays an important role in weed growth and competitiveness (Patterson et al., 1999), and that certainly would be true within an agricultural operation. Optimum germination and growth of Benghal dayflower occurs at approximately 30 °C which is characteristic of many tropical weeds (Sermons et al., 2008). It is unclear, however, how viability of Benghal dayflower seeds is impacted by very high temperatures. This is an important issue, because seed contained in hay bales can be exposed to extremely high temperatures during spontaneous heating generated by microbial respiration (Coblentz and Hoffman, 2009; Martinson et al., 2011). Temperature also may play a key role in determining the likelihood of vegetative reproduction. Depending on the crop, cultivation can occur at different times during the year, and under very different temperatures.

An understanding of seed ecology and modes of dispersal is an important component for success of sustainable farming systems, where cultural weed control tactics are heavily utilized. To minimize or avoid the use of pesticides, it is essential to know the potential flow of viable seed and recognizing possible control points where infestations can be avoided or contained. The objectives of this research were to determine seed viability of Benghal dayflower at temperatures that occur in hay bales and when exposed to rumen fluid from fistulated cattle. Research was also conducted to determine the influence of temperature on regeneration of vegetative propagules.

MATERIALS AND METHODS

Hay bale temperature simulations

Sorghum–Sudangrass [*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan] was cut and round baled at approximately 40% moisture with a bale diameter of approximately 122 cm between August and October, 2009 at The Center for Environmental Farming Systems (CEFS), Goldsboro, NC. Temperatures were recorded continuously at 5 min intervals for 2 months at 20, 40, and 60 cm depths from the outer edge of a typical uncovered hay bale using thermocouple temperature probes (Type K 12" Probe

Thermocouple Sensor - TCP6-K12, Onset Computer Corporation, Bourne, MA, 02532) and loggers (HOBO U12 J,K,S,T Thermocouple-U12-014, Onset Computer Corporation, Bourne, MA, 02532) . Based on the hay bale temperature measurements and other similar measurements in the published literature, large and small aerial and subterranean seeds of Benghal dayflower were exposed to 45, 50, or 65 °C in controlled environment chambers. Fifty ml of saturated $MgCl_2$ solution was added to plastic Tupperware containers to maintain a relative humidity of approximately 40% inside containers. Fifty seeds of each category were placed on small mesh screens inside the containers but had no physical contact with the $MgCl_2$ solution. After 1, 3, 7, 14, and 21 days, seeds were removed from the containers and viability determined by sectioning across the embryo and soaking in 1% 2, 3, 5-triphenyltetrazolium chloride (2, 3, 5-triphenyltetrazolium chloride solution, Sigma-Aldrich, St. Louis, MO, 63103) (TZ) solution at 35 °C for 18 to 24 hours (Peters, 2000). The experimental design was a completely randomized design and the experiment was repeated. Seed viability data were analyzed using PROC Mixed model in SAS version 9.1. (SAS Institute, Inc., Cary, NC).

Seed viability under simulated rumen digestion

Simulated rumen digestion involved exposure of seeds to rumen fluid containing micro-organisms with a pH of about 6.5, followed by acidic conditions (pH 3.5) as found in the abomasum (true stomach). This was achieved by obtaining rumen fluid from a steer with rumen fistula fitted with a cannula and maintained on an all forage diet. At sampling the cannula was opened, the rumen contents stirred and rumen fluid transferred into an insulated jug previously flushed with warm water to retain fluid temperature. The jug was completely filled with rumen fluid to exclude oxygen and transported to the laboratory. Fermentation was carried out in the laboratory using a Daisy II incubator (Daisy II incubators, Ankom Technology, Macedon, NY, 14502) according to the general procedure of Tilley and Terry (1963). The incubator unit consisted of 4 glass fermentation vessels with a capacity of 2000 ml each. The rumen fluid was filtered through 4 layers of cheesecloth to remove particulate matter and diluted (400 ml of rumen fluid: 1600 ml buffer solution) before transferring into vessels. Buffer solution contained 0.116 M $NaHCO_3$, 0.026 M Na_2HPO_4, 0.008 M NaCl, 0.007 M KCl, 0.0009 M $MgSO_4$, 0.0004 M $CaCl_2$, and 0.008 M Urea. Seed for evaluation were placed into filter bags (F57, Ankom Technology, Macedon, NY, 14502) and the bags placed into the rumen fluid media. Standard forage samples were included representing high and low dry matter disappearance to verify microbial activity of the initial rumen fluid. In addition, 10 filter bags containing alfalfa (*Medicago sativa* L.) (0.25 g each) were also added to each vessel to provide soluble nutrients for the inoculum. The fermentation vessels were placed into the Daisy II incubator, preset to 39.5 °C and continuously agitated (Ocumpaugh and Swakon, 1993; Tilley and Terry, 1963).

In addition to Benghal dayflower, seeds of five common weed species - redroot pigweed (*Amaranthus retroflexus* L.), crimson clover (*Trifolium incarnatum* L.), white clover (*T. repens* L.), Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot) and doveweed (*Murdannia nudiflora* L.) were also included in the study. Twenty five seeds of each species were added in individual filter bags. Thus each glass vessel contained 1600 ml of buffer solution, 400 ml of rumen fluid media, and 17 filter bags. The experiment was replicated and was repeated three times.

A subsample of each species was removed from the rumen solution at 0, 12, 24, 48 and 96 hours. The digestion periods were selected based on mean retention time in ruminants, which usually does not extend beyond 48 hours (Gardener et al., 1993a; Schaefer et al., 1978; Schoenbaum et al., 2009; Simao Neto and Jones, 1987).

Fermentation was terminated by draining the rumen fluid from the vessels, then the samples were thoroughly washed with distilled water, and the acid phase was initiated. Acid pepsin solution (0.1 M HCl and 0.2% pepsin) was added (1600 ml) to each vessel to stop further microbial activity. The vessels were returned to the incubator and removed after 12 hours of acid pepsin treatment, and then seeds were thoroughly cleaned with distilled water and blotted dry. The standard forage samples were dried and their in vitro dry matter disappearance was calculated.

The number of damaged and intact seeds was recorded by visual observation and undamaged seeds germinated in Petri dishes for 8 days at 25 °C day/night temperature in the dark. Germination counts were taken every day for 8 days and reflected appearance of a radicle. After 8 days, non-germinated seeds were tested for viability with TZ as described earlier. Data were subjected to analysis of variance using PROC Mixed model in SAS version 9.1. (SAS Institute, Inc., Cary, NC) in which incubation durations were fixed effects and replications were random effects.

Vegetative regeneration

Benghal dayflower plants were grown in growth chambers at the North Carolina State University Southeastern Plant Environment Laboratory. Three node stem fragments (<15 cm) were cut from 8 week old plants and the leaves removed. Four stem fragments were buried in plastic pots containing steam sterilized Norfolk sandy loam soil (kaolinitic, thermic Typic Kandiudults, pH 6.1, humic matter 0.32) at depths of 2 cm and 6 cm and pots were placed in four growth chambers with aerial temperatures of 20, 25, 30, and 35 °C. These temperatures were selected as representative aerial temperatures in North Carolina during a growing season (refer to Sermons et al., 2008). Soil temperatures at both depths were also monitored continuously using soil temperature sensors (WatchDog A-Series Data Loggers, Spectrum Technologies, Illinois, 60585). Soil temperatures were within 2 °C of aerial temperatures at both soil depths. The pots were watered daily with de-ionized water and once every other day with complete Hoagland nutrient solution (Thomas and Downs, 1991). Soil surface in the pots was observed daily for stem regeneration. Stem fragments from three pots were harvested at each of the six intervals: 7, 10, 15, 20, 25, and 30 d after burial. Stem fragments were washed with water and observed for root, shoot, leaf, aerial and subterranean flower and fruit regeneration at each harvest. The experiment was repeated twice. Data were subjected to analysis of variance using PROC Mixed model in SAS version 9.1. (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Seed Survival at Hay Bale Temperatures

Temperatures were measured in typical hay bales *in situ* in eastern North Carolina to confirm a relevant temperature range for seed testing. Although there was some variation within the bale in the first week after harvest, temperatures in the bale rose to almost 70 °C and then slowly declined over an eight-week period to about 40 to 45 °C (Figure1).

Temperature ranges of 40 to 77 °C have been reported for orchardgrass (*Dactylis glomerata* L.) hay (Martinson et al., 2011), alfalfa-orchardgrass hay (Coblentz and Hoffman, 2009) and bermudagrass (*Cynodon dactylon* L.) hay (Coblentz et al., 2000) in the weeks after harvest. Considering the reported values together with our own measurements, seed viability tests were conducted at 45, 50, and 65 °C.

Benghal dayflower seeds were rendered non-viable after 14 days at all treatments (Figure 2). Rapid loss of seed viability occurred within 24 h at 65 °C for all large and small aerial and subterranean seeds. Viability diminished more slowly at lower temperatures and differed slightly by seed type; some seeds remained viable after a week at 45 °C and 50 °C, but all were killed by 14 days of exposure.

In addition to uncovered hay bales, periodic temperature measurements were made inside a covered bale at 20, 40, and 60 cm depths at the same field location. The bale was wrapped with white plastic, but otherwise in the same environmental conditions as the uncovered bale. Temperature differences at different depths within the bale were negligible and average temperature recorded for the two-month period was 32 °C (data not shown). Lower temperatures inside covered hay bales compared to uncovered bales also were reported in a study with orchardgrass hay (Martinson et al., 2011). Based on previous temperature experiments (Sermons et al., 2008), there is no reason to think that Benghal dayflower seed viability would be damaged by temperatures in the 30 to 35 °C range.

The results indicate that Benghal dayflower seeds harvested in hay and baled in large rolls are unlikely to remain viable when passed to animals in feed as long as hay is not wrapped and not immediately consumed. The exact time required for microbial processes to be initiated and reach the maximal activity levels that generate high temperatures is difficult to pinpoint and would likely vary in different conditions. Microbial processes are highly dependent on temperature and moisture (Coblentz and Hoffman, 2009; Coblentz et al., 2000), which could be quite variable in different farming situations.

Animal Digestion

The impact of ruminant digestion on seed survival and germination has been examined in several studies with different grass and legume species (Blackshaw and Rode, 1991; Gardener et al., 1993a; Kneuper et al., 2003; Lyon et al., 1992; Michael et al., 2006). Seeds ingested by a ruminant undergo chewing, grinding and mastication followed by exposure to anaerobic conditions and microbial activity in the rumen and large intestine, as well as highly acidic conditions in the abomasum and small intestine (Gardener et al., 1993a; Hofmann, 1988). Due to physiological differences in digestive tracts, the rate of seed viability loss varies among ruminants. Compared to sheep and goats, the larger reticulo-omasal orifice and omasum of cattle can allow passage of larger particles without great damage (Hofmann, 1988, Cox et al., 1993). Greater passage of undamaged mesquite seeds (*Prosopis glandulosa* Torr. var. *glandulosa*) occurred through cattle, for example, than during passage through sheep and goat digestive tracts (Kneuper et al., 2003). A greater percent of viable Italian ryegrass seeds was found in cattle feces than sheep feces after similar digestion durations (Stanton et al., 2002).

Invitro dry matter disappearance values for standard forage samples (Figure 3) confirm the microbial activity of the initial rumen fluid. In our study, rumen digestion had limited negative effects on the germination of large and small aerial seeds of Benghal dayflower (Figure 4a). With large seed, germination remained similar to the non-digested control for 24 or 48 hours of digestion and only decreased to about 40% of the maxima after 96 hours of digestion. Digestion increased germination of the small seeds from 15% to about 40%. Different dormancy characteristics of large and small seeds (Maheshwari and Maheshwari, 1955) could be responsible for the different digestion responses. Considering that the process of digestion in cattle rumen *in situ* generally lasts for 12-48 hours (Gardener et al., 1993a), it is unlikely that the viability of either size class of Benghal dayflower seeds would be negated by ruminant digestion.

With the comparison of other weed species, seeds of white and crimson clovers were least tolerant to rumen digestion at all durations (Figure 4b). The decline in Italian ryegrass followed a similar pattern. Seeds of these sensitive species were completely disintegrated after 96 h of digestion. Doveweed and redroot pigweed seeds were more tolerant to rumen digestion, but germination of both declined noticeably after 48h of digestion, and thus both were less tolerant than Benghal dayflower (Figure 4c). Further, viability of non-germinated seeds at the end of 8d germination reveals a greater tolerance of Benghal dayflower seeds to rumen digestion compared with other weed species (Figure 5).

The weed seed responses to digestion resemble those reported previously in the literature. Seed survival of all species typically declines with increased time spent in the digestive tract of animals (Atkenson et al., 1934; Blackshaw and Rode, 1991; Janzen, 1984). Prior reports on digestion of two of the species included in our study demonstrated similar responses. Viability of Italian ryegrass seeds was found to be very low (0 to 12%) when exposed to cattle and sheep rumens (Stanton et al., 2002; Haidar et al., 2010). Also, redroot pigweed seeds had enhanced germination after 24 hours of digestion in sheep rumen and, although germination declined with longer exposures, >50% survival was found after as long as 96 hours of digestion (Haidar et al., 2010). The relatively small amount of damage done to Benghal dayflower seed during ruminant digestion is consistent with results in a previous study by Goddard et al. (2009) examining seeds held in the intestinal tracts of mourning doves. They concluded that the likely key to Benghal dayflower seed survival is its structural make up. The seed coat, made up of a reinforced thick inner layer coupled with a thin outer one provides structural integrity and is likely responsible for the enhanced dormancy characteristics. Others have also suggested that 'hardseededness' is one of the main factors responsible for seed survival during digestion (Gardener et al., 1993b; Haidar et al., 2010; Olson and Wallander, 2002).

The hay bale and digestion experiments identify circumstances that would provide situations that aid Benghal dayflower spread. If cattle were fed fresh hay containing Benghal dayflower seed or were consuming Benghal dayflower seed while grazing in pastures, then seed could be disseminated readily in manure. Control over Benghal dayflower spread in these latter situations would rely on agronomic practices that exclude this weed from cropping systems.

It should be added that manure composting is likely to help alleviate the problem of Benghal dayflower spread. Composted manure has higher temperature during curing and contains fewer viable weed seeds than fresh manure (Larney and Blackshaw, 2003). Temperatures in the range of 60 °C can be reached during the initial 20 days of composting (Eghball and Lesoing, 2000; Larney and Blackshaw, 2003; Wiese et al., 1998; Zaller, 2007).

Based on our temperature experiments (Figure 2), 60 °C should be sufficient to kill any viable Benghal dayflower seeds within short-exposure duration.

Vegetative Regeneration

Regeneration experiments with Benghal dayflower stem tissues planted at 2 or 6 cm beneath the soil surface indicated that planting depth markedly influenced regrowth. When stem fragments were buried at 2 cm depth, leaves and roots developed from nodes over the 30 d experiment (Figure 6), but no subterranean spathes were observed. With stems buried at 6 cm, no leaves were initiated, but roots and subterranean spathes were numerous (Figure 7). Vegetative regeneration was always initiated from nodes and not from internode tissue.

The number of leaves that emerged from soil with stems at 2 cm increased with increasing temperatures. While some leaves emerged at 20 °C, four times the number of leaves was found at 35 °C after 30 days (Figure 6a). Root development exhibited a different temperature response pattern at both planting depths. Development was always least at 20 °C, but the number of roots at the greatest temperature of 35 °C was either clustered with or slightly below those at 30 °C and 25 °C (Figures 6b and 7b). The results indicate that development of leaves (when it occurs) and roots is restricted but not eliminated at cool temperatures of 20 °C and below. Sermons et al., (2008) reported similar vegetative growth and reproduction responses at this temperature range. Enhanced growth at greater temperatures would be the expected response of a species originating from tropical regions (Holm et al., 1977; Tungate et al., 2007).

Development of underground spathes, when it occurred at the 6 cm depth, was most prolific at 30 °C (Figure 7c). There was little separation of spathe number at the other temperatures, with perhaps a tendency for spathe number to be slightly elevated at 25 °C. The observation that leaf emergence of Benghal dayflower was restricted at a soil depth of 6 cm is similar to previous observations by others. Little leaf initiation on stem cuttings occurred at 10 to 14 days after burial at a 4 cm depth at temperatures of 20 to 27 °C (Chivinge and Kawisi, 1989). And, plant regeneration did not occur at depths greater than 2 cm when stem cuttings were buried in soil held in a greenhouse without temperature control (Budd et al., 1979).

The physiological mechanism responsible for the restriction of leaf development when fragments are buried below 2 cm is unknown. In our experiments, temperature and moisture at 6 cm were similar to that nearer the soil surface and seemed appropriate for development to occur. Thus, the main difference in the two depths was proximity to the soil surface and to light, and it is conceivable that some light penetrated to a depth of 2 cm. Very little light would be necessary to initiate a phytochrome response, which one might presume to stimulate the leaf development process. We also have no explanation for the lack of spathe development at the shallow depth, although it would not be surprising if partitioning and utilization of the limited energy and nutritional reserves in the buried tissues were prioritized for leaf and root growth.

The results imply that cultivation will not be an effective control measure for Benghal dayflower during summer months when its growth is most active. Soil temperatures approach 25 and 30 °C (depending on depth below the surface) and much regeneration likely would occur. On the other hand, spring cultivation for a winter cover crop should be fairly safe. Even in that case, however, no one knows how long stem fragments would remain viable. If viability extended out 60-90 days, until soil temperatures increased into a more favorable range, then regeneration may still be a problem.

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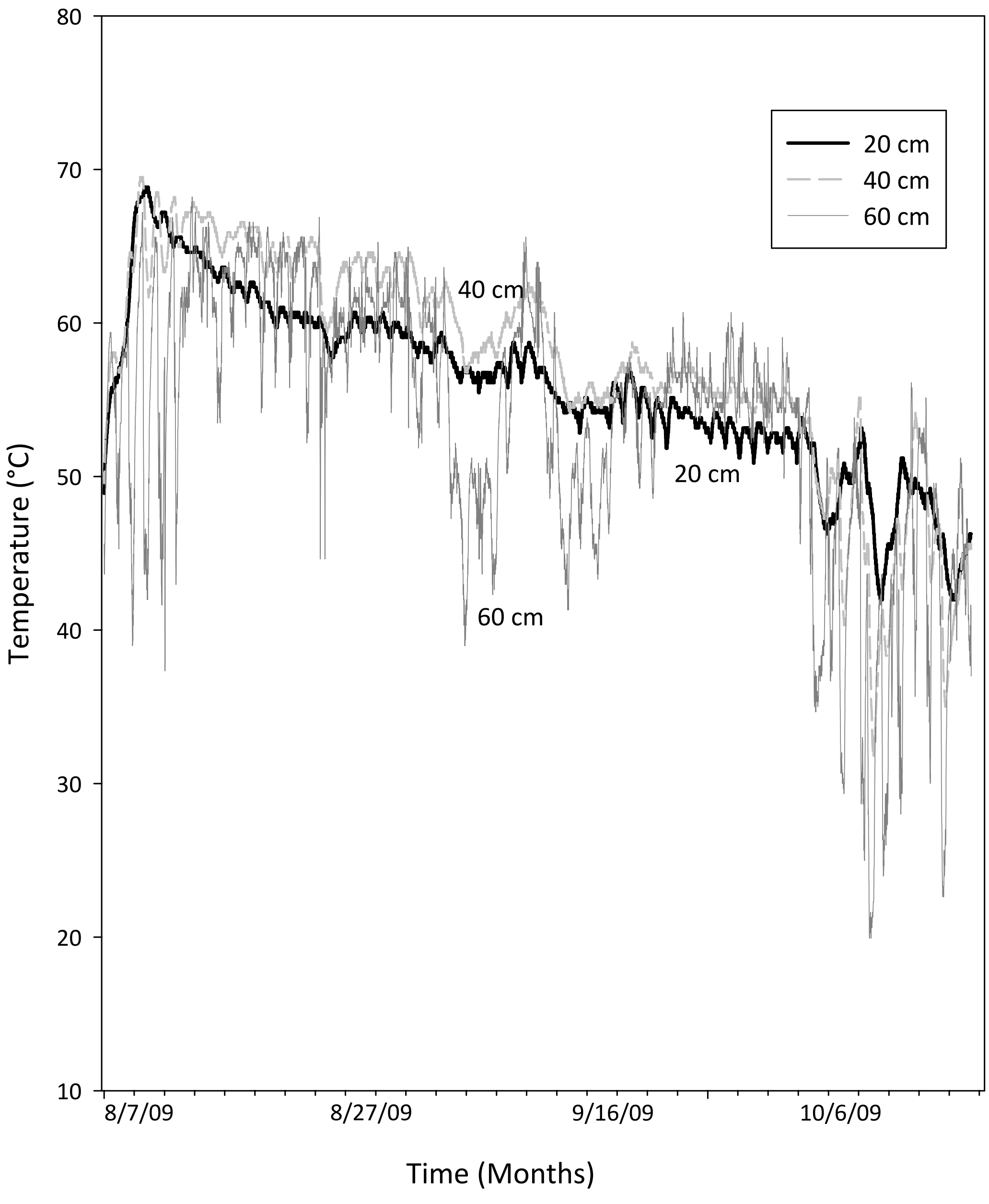

Figure 1. Continuous temperature measurements at three different depths from the outer edge of an uncovered hay bale from 08/07/09 to 10/06/09.

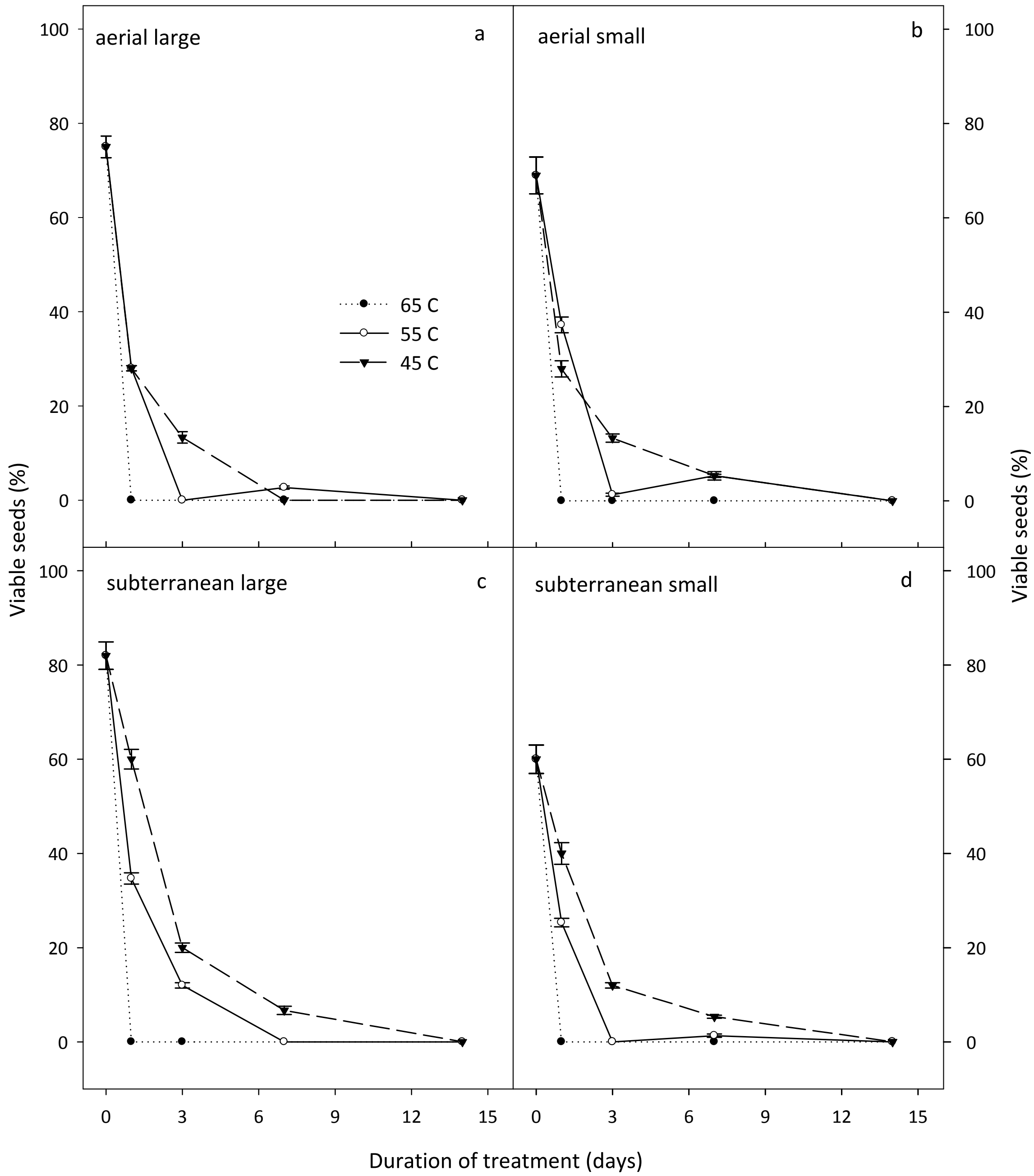

Figure 2. Benghal dayflower seed viability for (a) aerial large, (b) aerial small, (c) subterranean large, and (d) subterranean small for different simulated hay bale temperatures. Error bars represent standard errors of the mean.

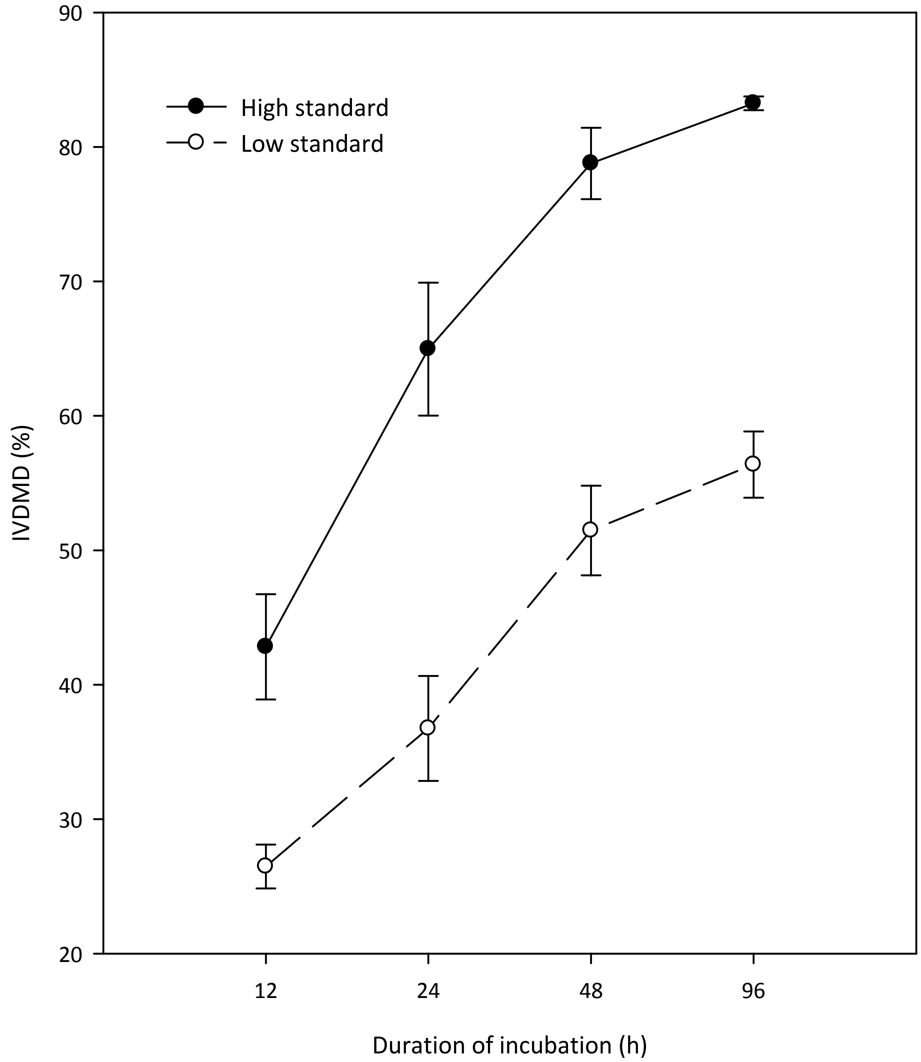

Figure 3. High and low standard invitro dry matter disappearance (IVDMD%) for different durations of rumen incubation. Error bars represent standard error of the mean.

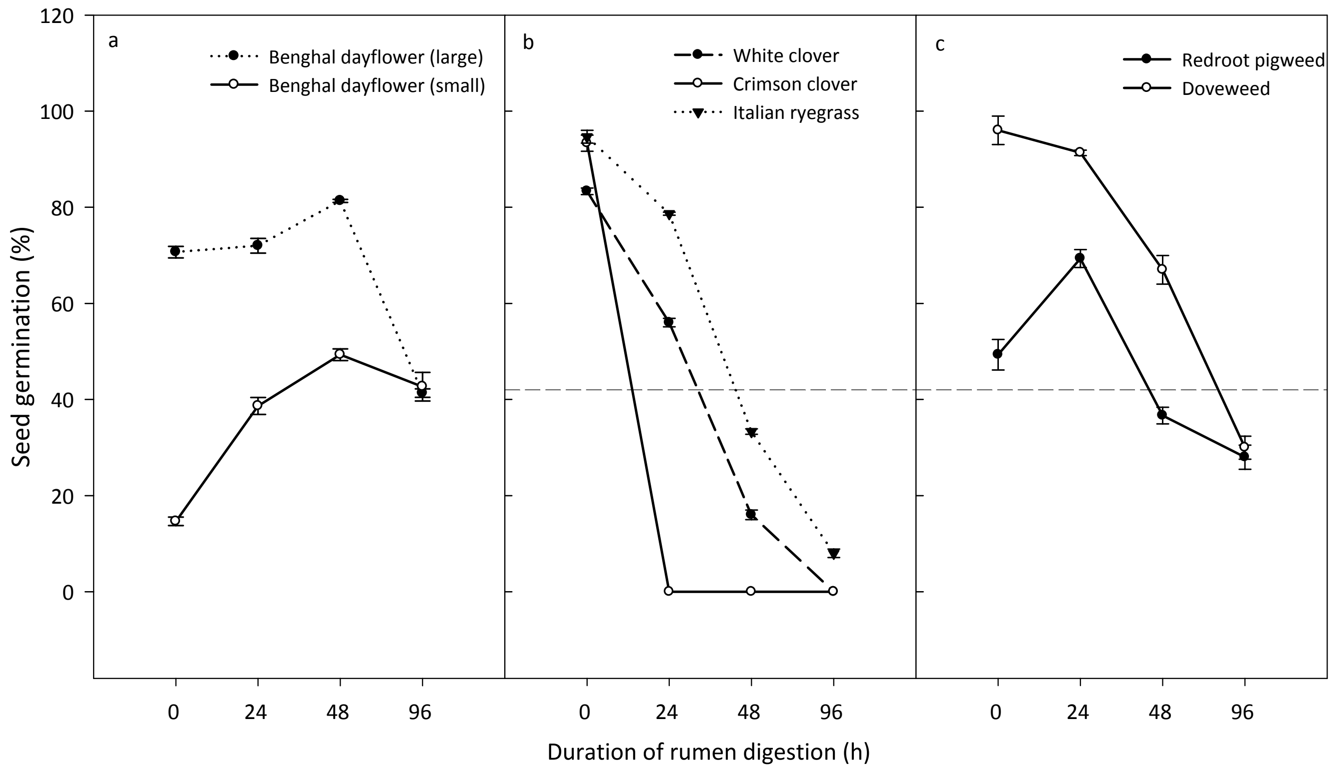

Figure 4. Response of duration of rumen digestion on percent seed germination of (a) Benghal dayflower seeds, (b) sensitive, and (c) tolerant weed seeds after 8 days of germination. Error bars represent standard error of the mean (averaged over three runs).

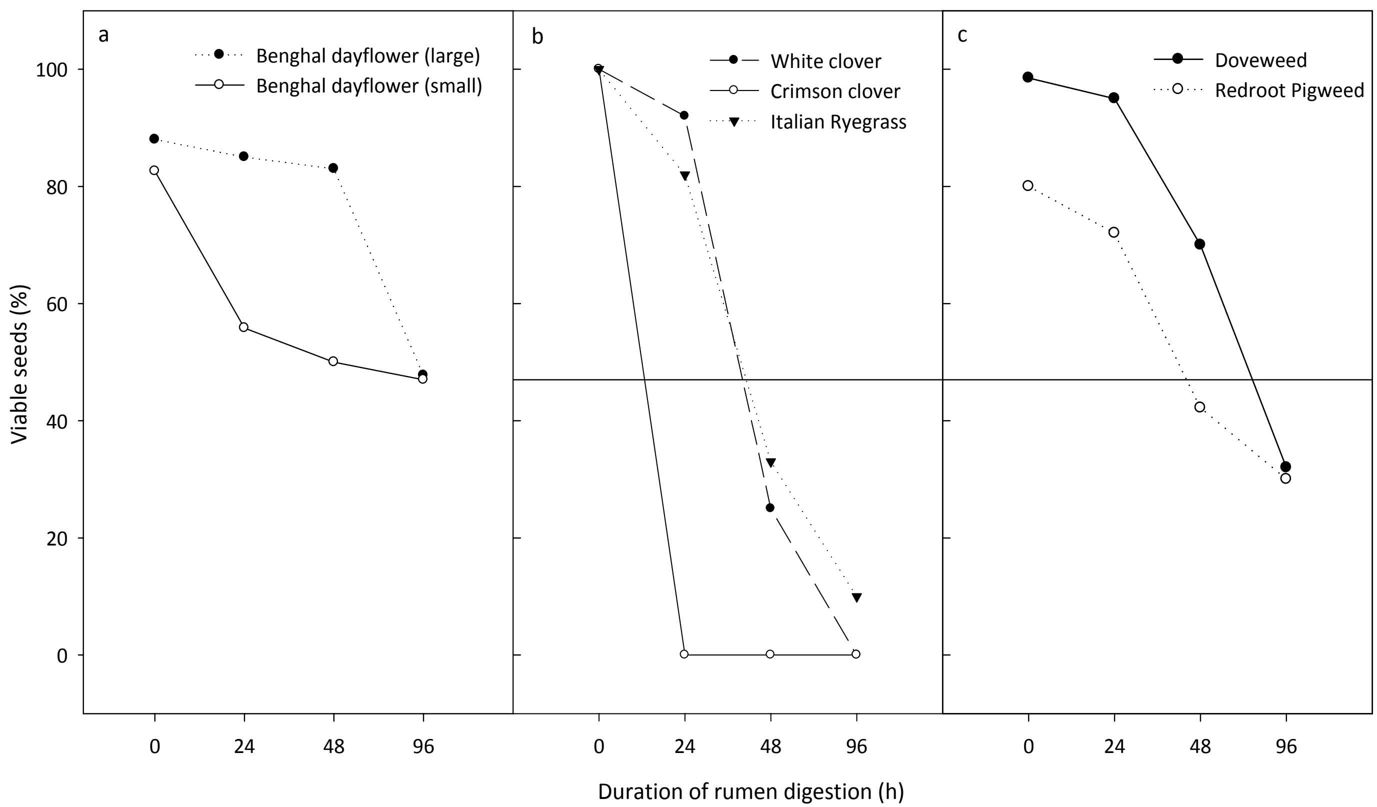

Figure 5. Response of duration of rumen digestion on percent seed viability of (a) Benghal dayflower seeds, (b) sensitive, and (c) tolerant weed seeds.

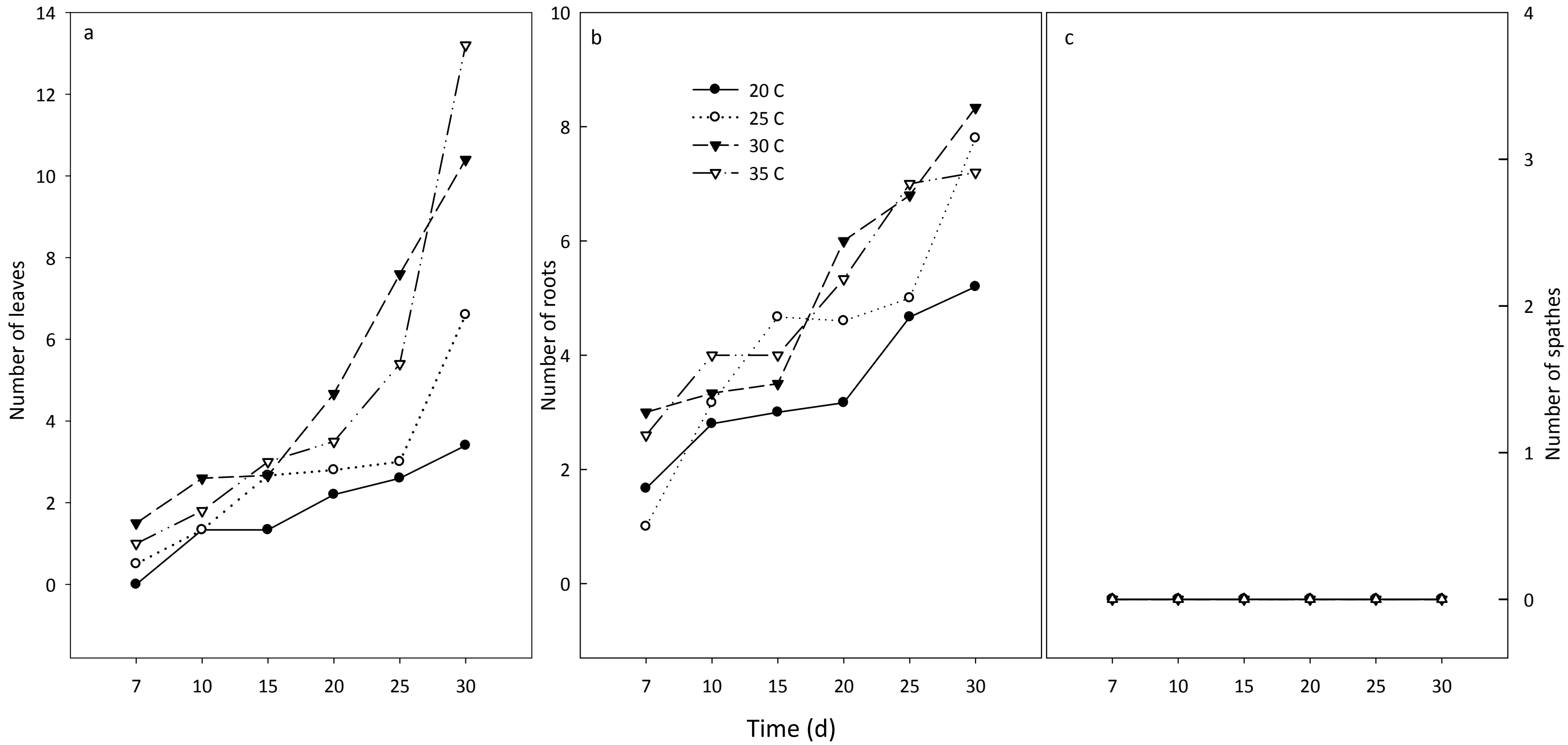

Figure 6. Number of Benghal dayflower (a) leaves, (b) roots, and (c) subterranean spathes per stem fragment at 2 cm soil depth.

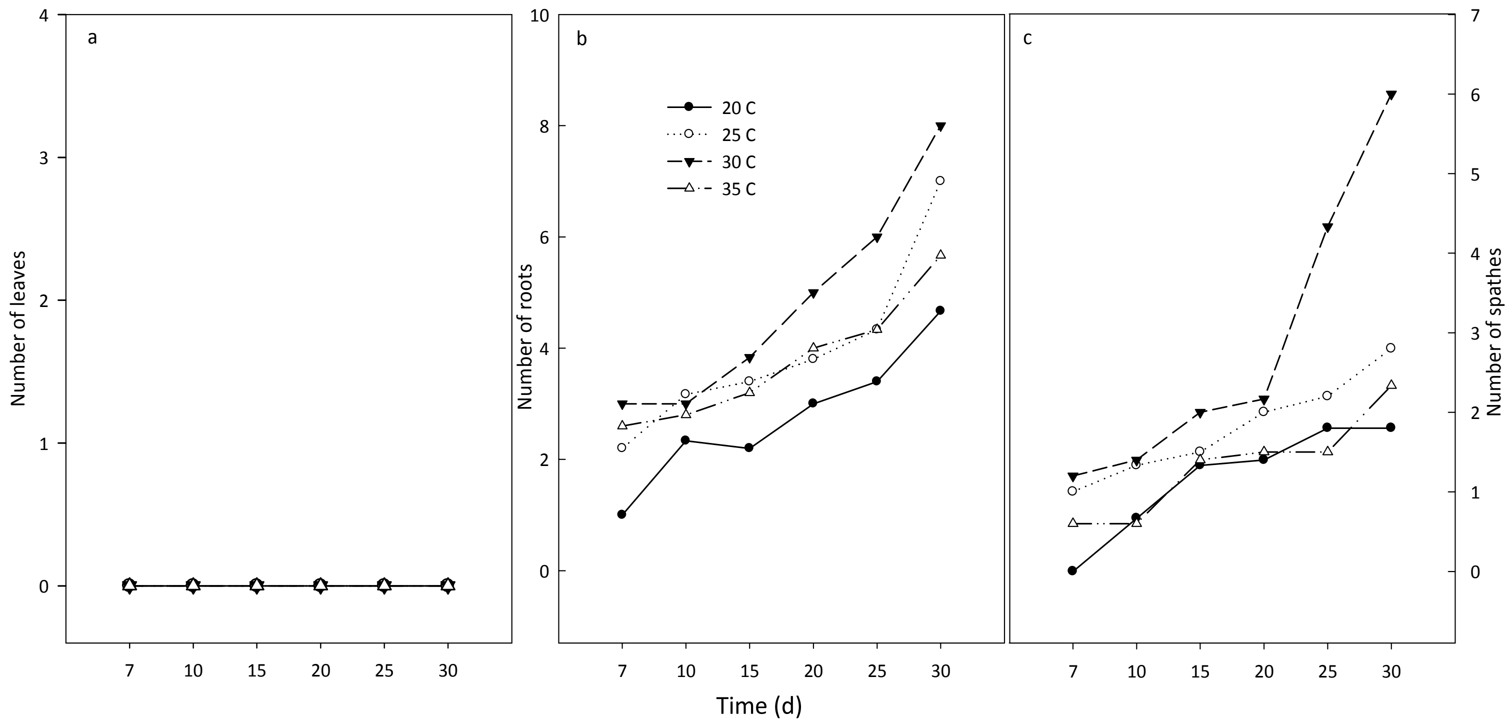

Figure 7. Number of Benghal dayflower (a) leaves, (b) roots, and (c) subterranean spathes per stem fragment at 6 cm soil depth.

CHAPTER II

Benghal Dayflower (*Commelina benghalensis*) Seed Viability in Soil

Mandeep K. Riar, Theodore M. Webster, Barry J. Brecke, David L. Jordan, Michael G. Burton, and Thomas W. Rufty[1]

ABSTRACT

Benghal dayflower is a challenging weed to manage in agricultural settings. Research was conducted in North Carolina, Georgia, and Florida to evaluate the longevity of buried Benghal dayflower seeds. Seeds were buried for 6 to 60 months at a depth of 20 cm in mesh bags containing soil native to each area. In North Carolina, the decline in Benghal dayflower seed viability was described by a sigmoidal regression model, with seed size having no effect on viability. Seed viability at the initiation of this study was 81%. After burial, viability declined to 51% after 24 months, 27% after 36 months and less than 1% after 42 months. In Georgia, initial seed viability averaged 86% and after burial declined to 63 and 33% at 12

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and 24 months, respectively. Burial of 36 months, or longer, reduced seed viability to less than 2%. The relationship between Benghal dayflower seed viability and burial time was described by a sigmoidal regression model. In Florida, there was greater variability in Benghal dayflower seed viability than at the other locations. Seed viability at the first sampling date after two months of burial was 63%. Though there were fluctuations over the first 24 months, the regression model indicated approximately 60% of seed remained viable. After 34 months of burial, seed viability was reduced to 46% and then rapidly fell to 7% by 39 months, which was consistent with the decrease in seed viability at the other locations. While there is a physical dormancy imposed by the seed coat of Benghal dayflower, it appears that a decline in buried seed viability to minimal levels occurs within 39 to 48 months in the southeastern U.S. This indicates that management programs must prevent seed production for at least four growing seasons to appreciably reduce the Benghal dayflower soil seedbank.

Nomenclature: Benghal dayflower, *Commelina benghalensis* L. COMBE.

Key Words: Federal noxious weed, invasive species, seed burial, soil seedbank, tropical spiderwort

INTRODUCTION

Benghal dayflower is one of the most troublesome weeds of cotton (*Gossypium hirsutum* L.) in the southeastern Unites States, and one of the first new weeds to appear in glyphosate-resistant cotton production (Webster and Sosnoskie, 2010). A native of tropical Asia and Africa, Benghal dayflower was introduced into Florida in the 1920’s and was first reported in Georgia in 1967 (Duncan, 1967; Faden, 1993; Webster et al., 2005). Following the widespread adoption of glyphosate-resistant cotton cultivars in the late 1990's, Benghal dayflower has become problematic in corn (*Zea mays* L.), cotton, and peanut (*Arachis hypogaea* L.) production in southern Georgia and Florida (Culpepper, 2006). By 2000, Benghal dayflower was also detected in southeast North Carolina (Krings et al., 2002).

Benghal dayflower has a natural tolerance to many of the commonly used herbicides in the production of corn, peanut, and cotton, including glyphosate (Culpepper et al., 2004; Webster et al., 2006). Studies have shown that Benghal dayflower control in the field is difficult once seedlings exceed a height of 10 cm (Culpepper et al., 2004). Effective weed control is necessary as season-long interference from Benghal dayflower has the potential to reduce cotton and peanut yields 50 and 100%, respectively (Webster et al., 2007; Webster et al., 2009).

A perennial weed in much of its native range, Benghal dayflower grows as an annual in the invaded temperate regions. While this weed species readily regenerates vegetatively from broken stem pieces (Budd et al., 1979), its only means of overwintering in the southeast US is through seed formation. Benghal dayflower is an amphicarpic species, with dimorphic seeds in both aerial and subterranean flowers. The seed are believed to serve different roles, with the limited number of subterranean seed ensuring some reproduction under stress, such as drought, while abundant aerial seeds are produced under more optimal growth conditions (Webster and Grey, 2008). This diversified reproduction strategy reflects inconsistency of rainfall in the native environment of Benghal dayflower (Kaul et al., 2002).

To assist in the development of effective multiple-season Benghal dayflower management strategies, a greater understanding of the longevity of Benghal dayflower in the soil seedbank is needed. Seedbank viability in soil is highly variable. Previous studies have documented seed viability of other weed species after approximately 20 years of soil burial in porous packets (Conn et al., 2006; Lewis, 1973) Further, seed viability was detected in two species after 120 yrs in the Beal burial study, which was initiated in 1879 (Telewski and Zeevaart, 2002). However, many species will rapidly lose seed viability within the first few years of burial (Chantre et al., 2009; Conn et al., 2006; Egley and Chandler, 1983; Lewis, 1973; Lutman et al., 2002). To date, there have been no studies published on the duration of Bengal dayflower seed viability in the soil, nor of any member of the Commelinaceae family. Therefore, seed burial studies were initiated at three locations in the southeast US to evaluate potential Benghal dayflower seed longevity.

MATERIALS AND METHODS

Burial studies were initiated in June 2004 and May 2005 in Goldsboro, NC (35.38194,-77.97805); April 2005 in Tifton, GA (31.513941, -83.544967); and in November 2007 and 2008 in Jay, FL (30.77669, -87.14224) to evaluate the potential longevity of buried Benghal dayflower seeds. Seeds used in each state originated from naturalized populations in that state, with the exception of the Georgia location, which also included packets of seed from the North Carolina population. Seed packets were buried to a soil depth of 20 cm in nylon mesh bags containing unsterilized soil native to each area and exhumed during 60 months in North Carolina and Georgia. Packets were exhumed after 6, 12, 18, 24, 30, 36, 42, 48, and 60 months in NC and 6, 12, 24, 36, 48, and 60 months in GA. In Florida, packets were exhumed over 39 months, with sampling occurring every other month during the first year, and then every four months for the second and third years. Packets in North Carolina consisted of 100 subterranean seeds, which included 25 large and 75 small seeds. In Georgia and Florida, packets contained only large aerial seeds (100).

Once packets were exhumed, seeds were evaluated for viability. Seed viability was initially determined by putting gentle pressure on the seed coat to test for firmness (Forcella et al., 2003); decayed seeds were marked as nonviable and discarded. Intact seeds were placed on germination paper in Petri dishes for 14 d at a 30/20 °C day/night temperature. All non-germinated seeds, after this time period, were analyzed for viability by bisecting across the embryo using a razor blade. The seeds were then dipped into 1% 2, 3, 5-triphenyltetrazolium chloride solution (Sigma-Aldrich, St. Louis, MO, 63103) at 32.5 °C for 12 hours (Goddard et al., 2009). Total number of viable seeds was the sum of the germinated seeds and seeds showing pink coloration of the embryo after exposure to tetrazolium chloride.

While these studies were coordinated among the locations, there were subtle differences in preparation and processing of samples. Therefore, data were analyzed by location. Data were analyzed using a mixed model in SAS version 9.1. (SAS Institute, Inc., Cary, NC) with burial time as a fixed effect and replications of the study as random effects. Data on viability of buried Benghal dayflower seed over time were fit by a sigmoidal regression model.

RESULTS AND DISCUSSION

In North Carolina, neither Benghal dayflower seed size nor burial year had any detectable effect on viability, so data were combined for analysis (Figure 1). Decline of Benghal dayflower seed viability was described by a sigmoidal regression model (R^2 = 0.87,

$p<0.0001$). Seed viability at the initiation of the study was 84%, declining to 81% after 6 months and to 51% after 24 months of burial. By 36 months, seed viability was 27%, with viability less than 1% after 42 months.

There were no detectable differences between the populations originating in Georgia or North Carolina at the Georgia location; therefore data were combined for analysis (Figure 2). At initiation of the study, seed viability averaged 86%, declining to 63 and 33% at 12 and 24 months, respectively. Burial of 36 months or longer reduced seed viability to less than 2%. The relationship between Benghal dayflower seed viability and burial time was described by a sigmoidal regression model (R^2 = 0.89, $p<0.0001$).

In Florida, there were no detectable differences between burial years, therefore data were combined for analysis. There was greater variability in Benghal dayflower seed viability in Florida than at the other locations (Figure 3; R^2 = 0.19, p = 0.0007). Seed viability at the first sampling date after two months of burial was 63%. Though there were fluctuations over the first 24 months, the regression model indicated approximately 60% seed viability over this time period. After 34 months of burial, seed viability was reduced to 46% and then rapidly fell to 7% at 39 months, which was consistent with the findings of seed viability at the other locations.

A burial study in Mississippi with agronomic weeds revealed that only five of the 20 tested species had >50% viability after 42 months, while only four species had at least 30% after 66 months of burial (Egley and Chandler, 1983). Of the 41 weed species buried in a Nebraska study, 12 and 17 species had more than 30% seed viability after 36 months of burial at two locations, while six and eight species had greater than 30% seed viability after

72 months (Burnside et al. 1996). However, there was great variability for several species among sites or years (Burnside et al., 1996; Donald, 1993).

Previous studies on weed seed longevity have classified seeds by ephemeral seedbanks and persistent seedbanks, with the first four to five years as the critical separation point (Lewis, 1973; Thompson, 1992). Across numerous species, persistence in the seedbank was correlated with seed coat thickness (Gardarin et al., 2010). Benghal dayflower seeds have a rigid honeycomb-pattern seed coat that often requires some abrasion to facilitate germination (Goddard et al., 2009; Kim et al., 1990; Sermons et al., 2008). While physical dormancy is imposed by the seed coat of Benghal dayflower, decline in buried seed viability to less than 7 and 2% within 39 and 48 months indicates that the soil seedbank of Benghal dayflower is more ephemeral than persistent. Management programs in the southeast must prevent seed production for at least four growing seasons to reduce the soil seedbank population. It must be cautioned that the duration of the current study did not exhaust seed viability, and there is the potential for a re-colonization of Benghal dayflower as long as some viable seed remain in the soil seedbank, as has been previously noted with other species (Burnside et al., 1986).

In the current study, Benghal dayflower seeds were buried at the deepest portion of the plow layer, thereby lengthening the seed survival period in soil. Extended persistence of weed seeds buried at deeper soil depths, compared with shallow depths have been reported (Kannangara and Field, 1985; Mennan and Zandstra, 2006; Omami et al., 1999; Taylorson, 1970). Reduced temperature fluctuations, reduced pathogen and insect activity, as well as restricted air flow, have been considered as responsible factors for prolonged seed survival at deeper soil depths (Stoller and Wax, 1974; Taylorson, 1970; Thomson et al., 1977).

Benghal dayflower seeds can emerge from a soil depth of 12 to 15 cm, but a field survey indicated that 42 and 94% emerged from the top 1 and 4 cm, respectively (Sabila et al., 2012; Walker and Evenson, 1985). It has been suggested that tillage, which brings buried seed closer to the soil surface, could reintroduce a weed even though the shallow seed bank had been depleted (Froud-Williams et al., 1984; Harrison et al., 2007; Roberts and Feast, 1972; 1973).

Benghal dayflower is a tropical species, but has invaded and become established in temperate regions, including the southern US. Estimates of the range of Benghal dayflower modeled using occurrence data indicate that this species will be limited by cold temperatures (Webster, T.M., unpublished research). However, in spite of the tropical origin of Benghal dayflower, Sermons et al. (2008) demonstrated that temperatures during the growing season in North Carolina were sufficient to support vegetative growth and reproduction, at temperatures below those previously reported in the literature (Burton et al., 2003). Winter temperatures and their effects on Benghal dayflower seeds in the soil were suspected to be the limiting factor in regulating the northern range of this species. While seeds in our trial were buried at 20 cm, long-term soil temperature data are available at only 10 cm depths (Florida-Automated-Weather-Network, 2011; Hoogenboom, 2011; NC-CRONOS, 2011), but these data provide a relative comparison among the locations. Average annual soil temperatures were 14, 20, and 21 °C for Raleigh, NC, Tifton, GA, and Jay, FL, respectively (Figure 4). Average soil temperatures at 10 cm depths in Raleigh were 4.46 to 8.44 °C cooler than those in Tifton and Jay. Benghal dayflower seed viability was less than 2% after

42 months of burial in North Carolina and Georgia, and less than 7% after 39 months, the maximum burial duration at the Florida location. Despite cooler temperatures, Benghal dayflower seeds have similar viability in NC as in the warmer GA and FL environments.

Additionally, the four seed types of Benghal dayflower have different degrees of dormancy and show different response to germination temperature (Walker and Evenson, 1985). However, the similarity in seed longevity of different seed types among the tested locations indicates that cold tolerance at this soil depth was not a limiting factor that contributed to Benghal dayflower seed longevity.

Additional research is needed to characterize the climatic limits of Benghal dayflower, especially cold tolerance of vegetative growth and propagules in the soil seedbank. Thus far, it is known to tolerate drought stress, able to capitalize on excess soil nutrients, and in the upper range for C3 plants in response to elevated carbon dioxide concentrations, but it is not tolerant of saline conditions found in coastal estuaries (Burns, 2004; Price et al., 2009; Sabila et al., 2012; Webster and Grey, 2008). Factors which regulate the potential and realized niches of Benghal dayflower will help guide outreach programs with a focus on plant identification and management of this exotic and economically important species.

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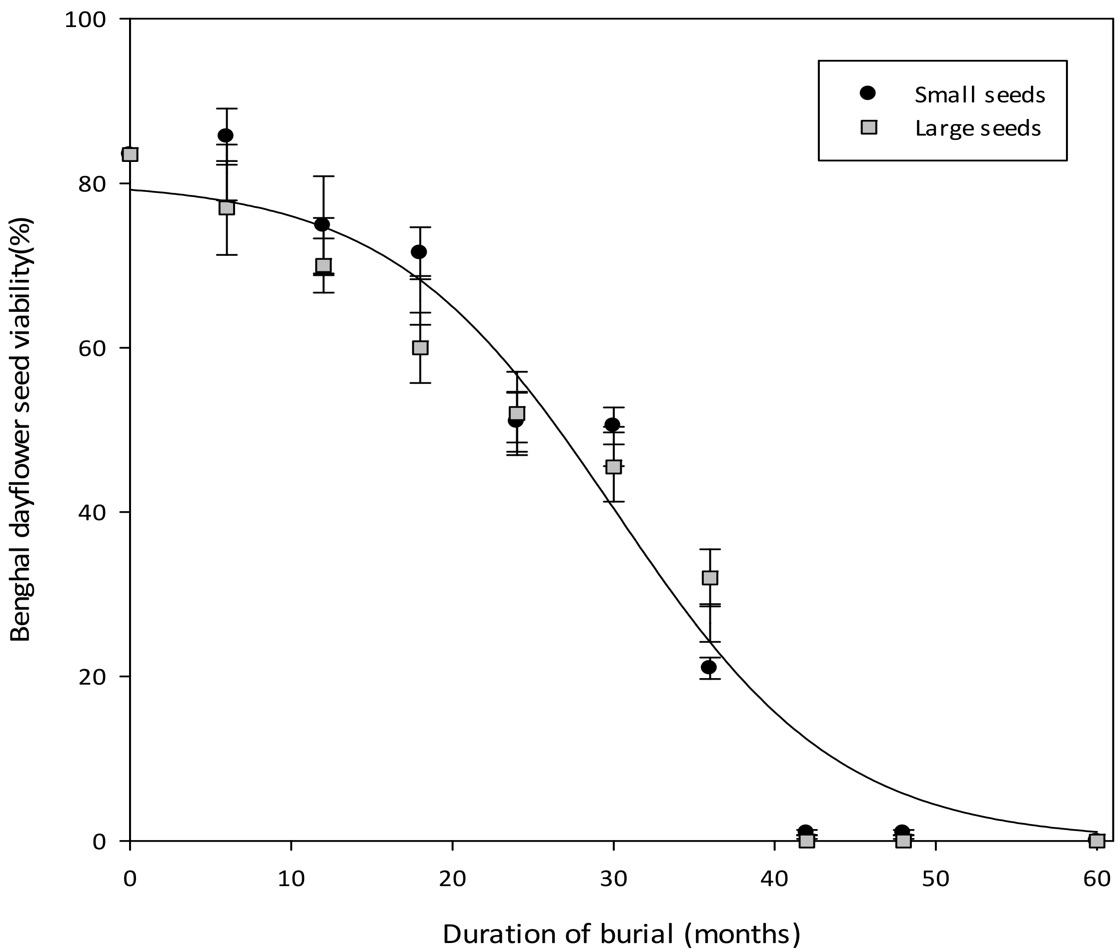

Figure 1. The influence of seed burial duration at Goldsboro, NC on viability of large and small subterranean Benghal dayflower seeds.

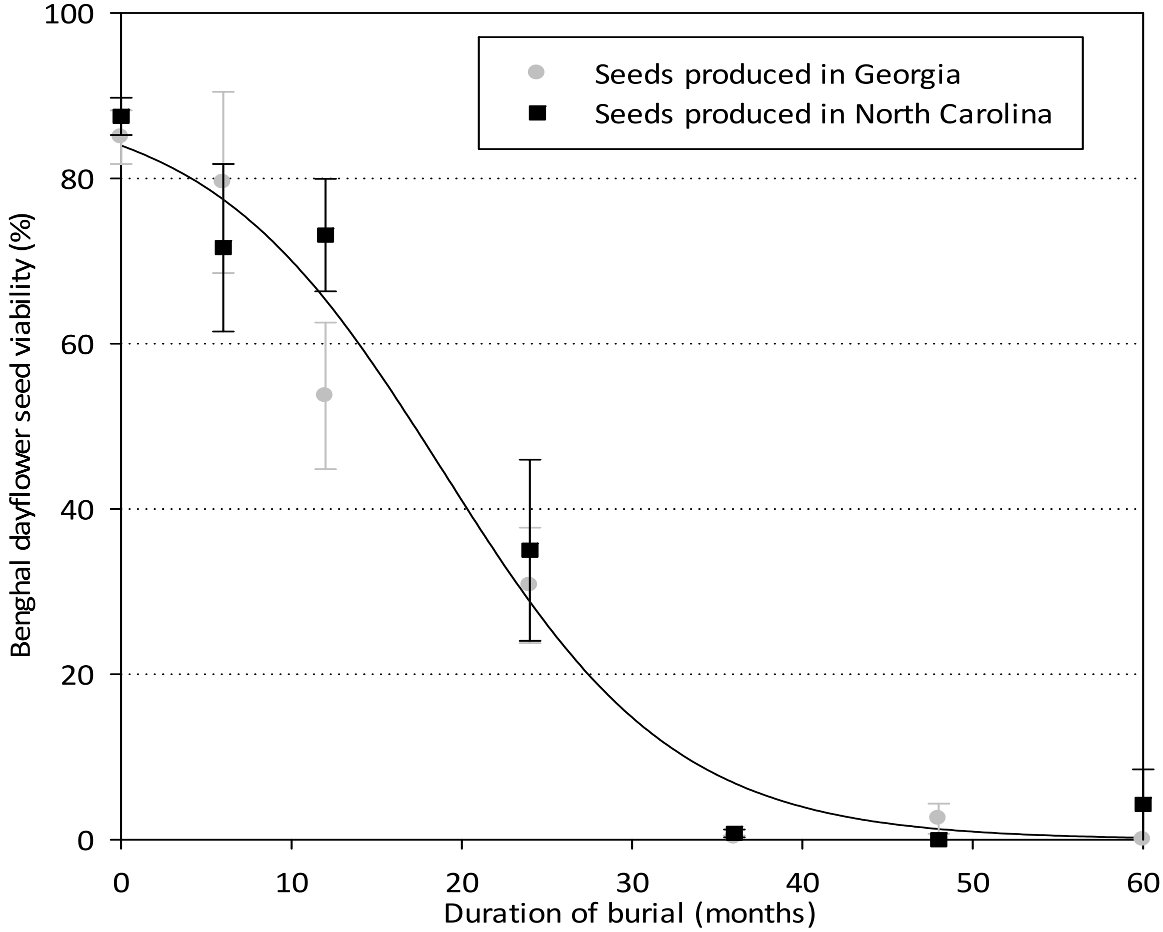

Figure 2. The influence of seed burial duration at Tifton, GA on viability of large aerial Benghal dayflower seed produced in Georgia and North Carolina.

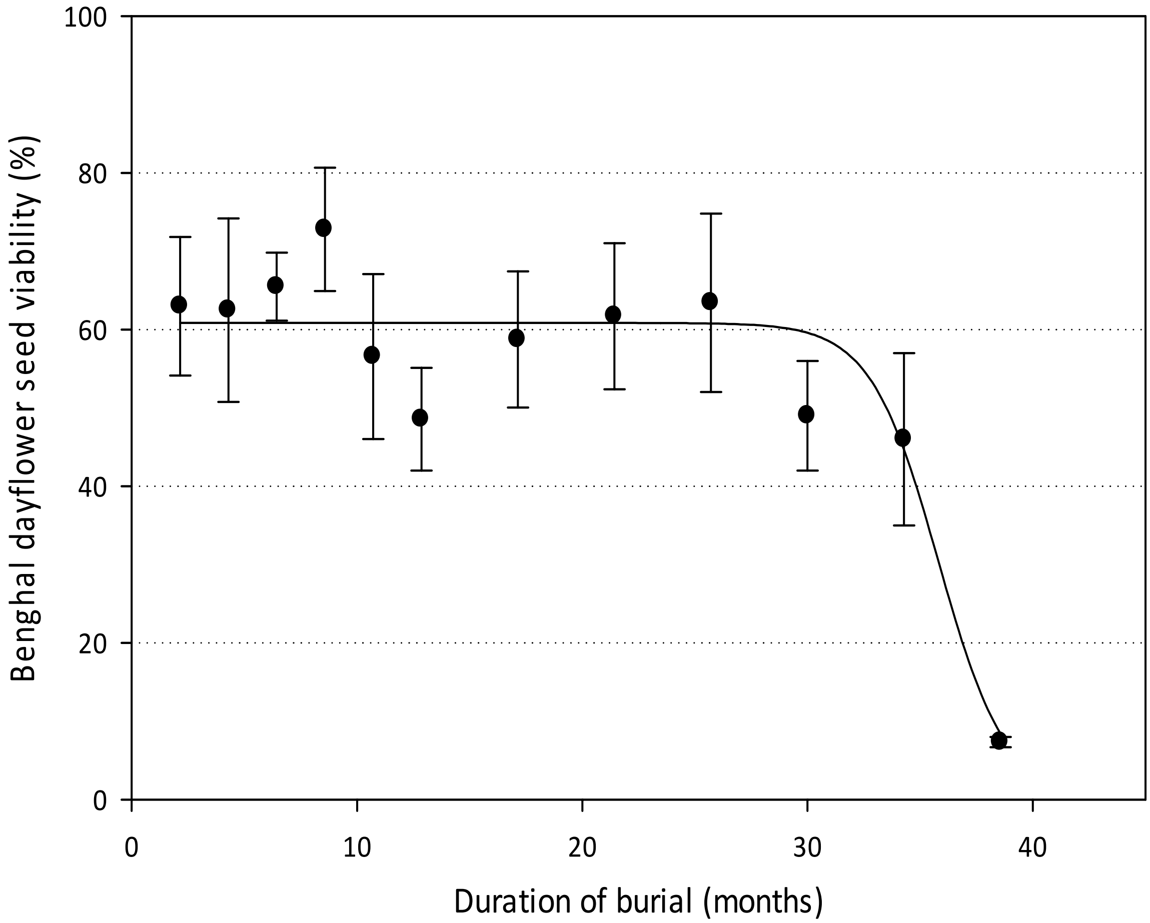

Figure 3. The influence of seed burial duration at Jay, Florida on viability of large aerial Benghal dayflower seeds.

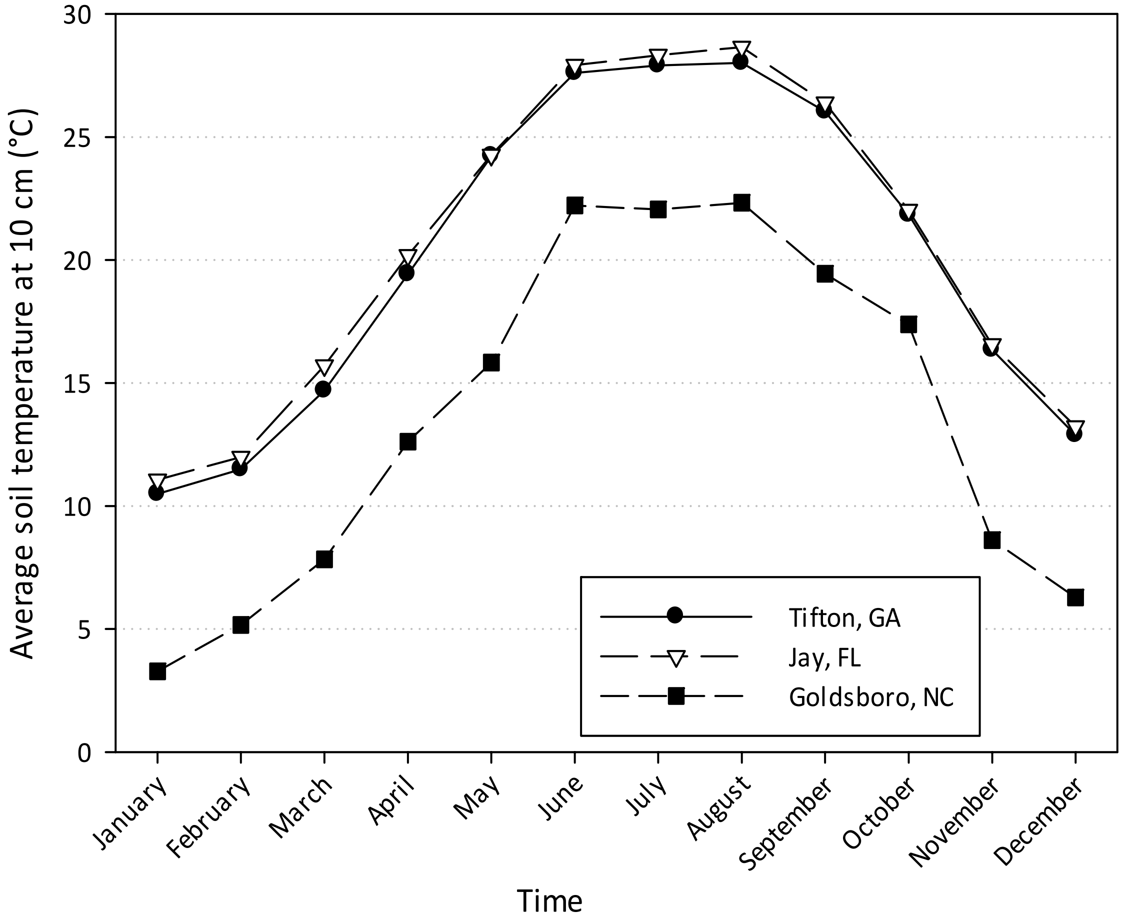

Figure 4. Average monthly soil temperatures at a depth of 10 cm over a four-year period at Jay, FL (Florida-Automated-Weather-Network 2011); Tifton, GA (Hoogenboom 2011) and Raleigh, NC (NC-CRONOS 2011).

CHAPTER III

Growth and Reproduction of Benghal Dayflower (*Commelina benghalensis*) in Response to Temperature, Photoperiod, Nutrition and Shade

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ABSTRACT

Benghal dayflower is an invasive weed in the southeastern United States. Experiments were conducted under controlled conditions to determine the growth and development of Benghal dayflower in response to various environmental conditions. In the first series of experiments, Benghal dayflower plants were exposed to five treatment combinations consisting of 3 day/night temperatures with and without a 3 h night interruption (+NI) for a period of 56 days. Plants had greatest vegetative growth and biomass at 35/28 °C, apparently reflecting its tropical origins. The lowest growth occurred at 30/22 °C. Night interruption delayed flowering by 3 to 7 days and led to increased whole plant and root growth, and larger leaves. Aerial spathe production was unaffected by temperature and night interruption and subterranean spathes were slightly affected by temperature ($P \leq 0.10$). This was true even at

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30/22 °C when whole plant growth was relatively reduced. In the second experimental series, Benghal dayflower plants were grown for a 56 day period under high and reduced soil nutrition and full and reduced light levels. Light and nutrient stress reduced plant biomass at all stages compared to high nutrition and full light supply. Aerial spathe production was affected by both nutrients and light while subterranean spathe production was only affected by light reduction. Benghal dayflower responses fit the profile commonly observed with successful invasive plants, with rapid growth occurring when resources were plentiful. The results also demonstrate the large phenotypic plasticity of Benghal dayflower, even when growth and reproduction are decreased by suboptimal conditions. Benghal dayflower appears to have a considerable reproductive output even under limited resources.

Nomenclature: Benghal dayflower, *Commelina benghalensis* L. COMBE.

Key words: Invasive weed, temperature, night interruption (NI), nutrition, light

INTRODUCTION

Environmental conditions and resource availability influence the establishment and performance of non-native, invasive species in new habitats. Temperature and photoperiod are two of the most important environmental factors that affect phenological development and geographic distribution of plants (Huang et al., 2001; Patterson, 1995; Woodward, 1988). Moisture, light, and nutrition also alter plant growth and are significant components in determining crop-weed competition (Patterson, 1995; Rahlao et al., 2010).

Benghal dayflower is an invasive, noxious weed, native to tropical Asia, Africa and the Pacific Islands, and it has been established in Florida since the early1930s (Faden, 1993). It is among the world's worst weeds, infesting 25 crops in 29 countries, and is now the most troublesome weed in cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.) production in Georgia (Webster and Sosnoskie, 2010). Season-long competition of Benghal dayflower commonly causes 60% to 100% yield reductions in the two crops (Webster et al., 2007, 2009).

Benghal dayflower grows as a perennial in the tropics but can survive as an annual in temperate regions (Holm et al., 1977). Tolerance to glyphosate (Culpepper et al., 2004), aerial and subterranean seed production in dimorphic flowers (Maheshwari and Maheshwari, 1955), and ability to regenerate from stem fragments (Budd et al., 1979) make Benghal dayflower extremely difficult to control in agronomic systems. It is fast growing and a prolific seed producer (Walker and Evenson, 1985a). The seeds have variable dormancy and germination characteristics (Kim et al., 1990; Walker and Evenson, 1985b) depending on seed size and position on the plant.

In addition to competition, Benghal dayflower can cause several other types of negative effects in agronomic crops. It has been found to serve as a host of plant parasitic nematodes (Davis et al., 2006), a monocot-infecting bacterial spot pathovar (*Xanthomonas campestris* pv. vesicatoria) in tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.), and cucumber mosaic virus (CMV) in tobacco (*Nicotiana tabacum* L.) and pepper (Gibbs, 2002; Kucharek et al., 1998). Further, aqueous extracts of Benghal dayflower severely reduce seed germination of corn (*Zea mays* L.) and seedling vigor of soybean and corn (Singh et al., 1989), indicating allelopathic effects.

Little information is available on the conditions where Benghal dayflower is likely to be most competitive in the field. Recent evidence suggests that it grows and reproduces in higher temperature ranges (Sermons et al., 2008), which would be expected with a plant species originating in the tropical regions (Tungate et al., 2007). But the influences of photoperiod, nitrogen fertilization, and shading remain largely obscure. We were particularly interested in responses to the environment that occurred in the high temperature ranges, because they represent those likely to be present in current temperate areas during summer months when crop interference most often occurs. The objective of this study was to examine growth and reproductive responses of Benghal dayflower exposed to a number of environmental conditions.

MATERIALS AND METHODS

Temperature and Photoperiod

Experiments were conducted at the Southeastern Plant Environment Laboratory at North Carolina State University, Raleigh, NC. Large aerial seeds of Benghal dayflower were germinated in 6000 ml (25.4 cm diam) pots containing Norfolk sandy loam soil (kaolinitic, thermic Typic Kandiudults, pH 6.1, humic matter 0.32). Prior to sowing, seeds were disinfected by soaking in 5% bleach solution (0.25% NaOCl) for 5 min, rinsed with water and then scarified with a blade to break physical dormancy and enhance germination (Goddard et al., 2009). Seeds were germinated at constant day/night temperature of 30/30 °C, with a 9 h photoperiod. Seedlings were thinned to 2 per pot at the one-leaf stage (approximately 10 days after planting) and then exposed to various temperature-photoperiod regimes established in 5 reach-in environmental chambers (growing space 1.11 m^2). Temperature and photoperiod treatment combinations were 30/22 °C day/night temperature with and without a night interruption of 3h, 35/28 °C day/night temperature with and without a night interruption of 3h and 30/30 °C constant temperature without night interruption. Day and night temperatures were maintained from 0800 to 1700 h and 1700 to 0800 h respectively and the night interruption was implemented after 7 hours of dark period at midnight. Pots were randomly placed within the chambers.

Illumination was provided by incandescent and fluorescent lamps with a photosynthetic photon flux density (PPFD) of 500-600 µmol m^{-2} s^{-1}. The non-photosynthetic 3 h night interruption was 40 µmol m^{-2} s^{-1} and was provided through incandescent lamps. Plants were watered twice daily with 150 ml de-ionized water and once every other day with 200 ml complete Hoagland nutrient solution (Thomas and Downs, 1991). Soil temperatures were monitored continuously at 2 cm depth in the pots using temperature probes (WatchDog A-Series Data Loggers, Spectrum Technologies). Soil temperatures were within a 3 to 5 °C range of aerial temperatures under all treatments. Every other week for 8 wk, 3 pots from each chamber were selected randomly and harvested. Plants were separated into aerial and subterranean tissue. The tissues were further separated into aerial vegetative (shoots and leaves), aerial reproductive (aerial spathes), subterranean vegetative (roots) and subterranean reproductive (rhizomes and spathes). All tissues were dried to a constant mass at 60°C in a drying oven and weighed. To avoid seed dissemination, aerial fruits were collected prior to dehiscence throughout the treatment period. Plant height, number of leaves and leaf area were also measured. A standard Li-Cor model LI-3100C Area Meter (LI-COR Biosciences, Lincoln, NE) was used to measure leaf area per plant. The experiment was replicated 3 times within each treatment and was repeated twice. Experimental design was a completely randomized design and data were analyzed using PROC Mixed model of SAS version 9.1.

(SAS Institute, Inc., Cary, NC). Temperature and photoperiod treatment combinations were treated as fixed effects and replications of the study as random effects. Means were separated using Fisher's Protected $LSD_{0.10}$.

Nutrition and Shade

Experiments were conducted in walk-in environmental chambers (growing space 9 m^2) at the Southeastern Plant Environment Laboratory. Seed treatment, germination, seedling establishment conditions and soil type were the same as described previously. Plants were thinned to two seedlings per pot at the one-leaf stage and treatments were implemented. Pots were assigned at random to a combination of treatments including 2 light levels and 2 nutrient levels. Light treatments consisted of full light (600 µmol m^{-2} s^{-1} PPFD) and reduced light (324 µmol m^{-2} s^{-1} PPFD) provided by a combination of incandescent and fluorescent lamps. The reduced light treatment involved covering the plant area with shade cloth. The PPFD was measured with a LI-191 Line Quantum Sensor (LI-COR Biosciences, Lincoln, NE). A 9 hour day and 15 hour night cycle was maintained, and temperature was held constant at 30 °C to minimize interactions with very high temperatures.

The experiment was a split-plot design with light levels as main plots and nutrient levels as subplots. Each light treatment was subdivided into 2 nutrient treatments consisting of a reduced level of 200 ml complete Hoagland nutrient solution added once every week and a high level of 200 ml nutrient solution added every day. Each pot was flushed with de-ionised water prior to nutrient additions to minimize residual nutrient accumulation. Each treatment combination was replicated 3 times and the experiment was repeated twice. Three pots from each treatment combination were randomly selected and harvested at 14, 28, 35, 42, 49, and 56 d after implementing the treatments. Plant measurements and procedures were similar to those described previously. Data were analyzed using PROC Mixed model of SAS version 9.1. (SAS Institute, Inc., Cary, NC) and means were separated using Fisher's Protected $LSD_{0.05}$.

RESULTS AND DISCUSSION

Temperature and Photoperiod Responses

Summer temperatures in central North Carolina were used as a template for the initial series of experiments. Average monthly temperatures measured at the National Weather Service Station located at the Raleigh-Durham International Airport were obtained from the National Climatic Data Center (Figure 1, NCDC, 2011). Our experiments were conducted with daytime temperatures between 30 and 35 °C, which typically occur from the first week of June to about the first week of September, a time frame when most summer annual agronomic crops are grown. Previous studies had indicated that maximal vegetative growth and production of seeds by Benghal dayflower occur at 30 °C or greater (Sermons et al., 2008; Sabila et al., 2012).

The experiments revealed that vegetative growth within the 30 to 35 °C temperature range was influenced by both temperature and photoperiod (Figure 2). The greatest aerial and subterranean mass accumulated in plants with a night interruption to suppress flowering. By the final harvest date, dry mass accumulation varied widely among treatments. Plants in the 35/28 °C +NI treatments were ~ 3.5 times (20g) larger than those at 30/22 °C and a continuous dark period. A comparison of mass at 35/28 °C with 30/22 °C, both without the night interruption, revealed that that temperature caused about 45% of the difference.

A photoperiod effect could be seen in two types of Benghal dayflower responses. One was the timing of reproductive maturity, where the night interruption caused a 3-day delay in flowering at the highest temperature (35/28 °C) and a 7-day delay at 30/22 °C (Figure 3). The other response was in overall plant size and morphology. Comparisons of 35/28 °C +NI with 35/28 °C or 30/22 °C +NI with 30/22 °C show the considerable increase in root mass accumulation due to night interruption (Figure 4a). Also, plants with the night interruption were consistently taller than those without at both the 35/28 °C and 30/22 °C temperatures (Figure 4b). Furthermore, canopy morphology was altered by photoperiod. Plants growing at 35/28 °C without the night interruption had a similar leaf number as 35/28 °C +NI (Figure 4c), reflecting very rapid leaf production early on and continued leaf emergence after flowering at 28 days. But, their relatively slow increase in total canopy leaf area after day 28 (Figure 4d) indicated that leaf size was reduced compared to the +NI treatment.

One of the biggest management challenges with Benghal dayflower is to limit its seed load in agronomic fields. Studies have shown that as many as 8,000 to 12,000 seeds m^{-2} can be produced (Walker and Evenson, 1985a). As expected, production of aerial and subterranean spathes per plant increased over the study period (Table 1). Differences in aerial spathe number could not be distinguished, statistically, among the treatments even at a P value of 0.10. The most striking observation is the spathe generation level in the cooler 30/22 °C environment. Plants tended to be smaller but produced a very high number of reproductive structures. Thus, over this high temperature range, reproductive performance by Benghal dayflower appears to have a high degree of elasticity and can be sustained at a high level regardless of whole plant growth rate.

These experiments were not designed to be an intensive examination of photoperiod response. The 3h interruption was selected because it is sufficient to strongly suppress flowering of crop plants such as soybean [*Glycine max* (L.) Merr.] and tobacco (Thomas et al., 1975; Thomas and Raper, 1977). We are aware of no comparative studies that can be used for direct confirmation of our observations or additional insights. However, one set of authors concluded that photoperiod effects would not greatly affect growth of Benghal dayflower (Gonzalez and Haddad, 1995).

A foreboding factor in modern agriculture is the progression of global warming even as demands for food security increase to accommodate expanding human populations (Gregory et al., 2005; Tilman et al., 2001). It is logical to expect that tropical weeds like Benghal dayflower will present an increasing challenge in the southeastern U.S. and more northern areas as temperatures rise. Rapid growth at high temperature suggests that Benghal dayflower will develop more like a perennial with increasing temperatures, growing vigorously and reproducing at greater rates for longer periods and becoming even more difficult to control. A similar concern will exist with other tropical weeds. Experiments with tropical soda apple (*Solanum viarum* Dunal), for example, an exotic weed originating from South America, indicated that temperature and photoperiod responses were unlikely to limit spread into more northern areas at latitudes similar to those for North Carolina (Patterson et al., 1997). Similar threats might be expected from others like sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] and prickly sida (*Sida spinosa* L.), which also originate from tropical areas and have relatively high temperature optima (Patterson, 1993; Tungate et al., 2007; Wright et al., 1999).

Nutrition and Shade Responses

The second series of experiments was initiated to determine the impacts of lowered resource availability on vegetative growth and reproduction of Benghal dayflower. The two resources examined were fertility and light. Fertility can be very different in agricultural fields with different cropping systems. Corn is heavily fertilized with nitrogen, for example, whereas legumes like soybean might be fertilized very little. Light becomes an issue when Benghal dayflower is growing within a developing crop leaf canopy.

The nutritional treatments were successful in producing very different growth rates. Higher nutrition greatly increased plant mass accumulation (Figure 5). The increases in total mass were associated with increased root mass, plant height, number of leaves, and leaf area all of which were increased at greater nutrition compared with reduced nutrition at a given light level (Figure 6a-d). The impacts on leaf number and area were particularly large. The physiological changes are typical for higher plants growing faster under higher nutrition, where shoot growth is stimulated more than root growth and shoot to root growth ratios increase (Chapin, 1991; Rufty, 1997). Reproductive performance also was increased by high nutrition, with the number of aerial spathes being 3 times more than under reduced nutrition (Table 2). In contrast, while the number of subterranean spathes was increased by nutrition, the magnitude was much less.

Based on results of experiments with other invasive species, it has been proposed that invasiveness is strongly linked with increased growth rates under improved resource conditions, and invasive species may not out-perform native or non-invasive species when resource availability is limited (Maillet and Lopez-Garcia, 2000; Kolb and Alpert, 2003;

Burns, 2004; Burns and Winn, 2006). These results indicate that would be true for Benghal dayflower. It should be mentioned that there are circumstances where weed species might acquire nitrogen from alternative sources. Experiments using ^{15}N natural abundance, for example, found that weeds could obtain large amounts of nitrogen transferred from N_2-fixing soybean (Moyer-Henry et al., 2006). However, because transfer occurred through and was dependent on mycorrhizae, this type of alternative nitrogen acquisition would not be available to Benghal dayflower. Our recent analyses have indicated that it is not a mycorrhizal host species (data not shown).

Shading treatments were initiated to examine Benghal dayflower growth and reproductive output under reduced light, simulating conditions that might exist beneath a crop leaf canopy. Reduced PPFD had a noticeable impact on Benghal dayflower growth within the elevated and reduced nutritional treatments (Figure 6). However, the response showed definite physiological acclimations. As expected, plant height was increased by shade, reflecting the typical etiolation (Figure 6b).

Leaf number and leaf area also were somewhat elevated, however. Leaf canopy mass is not shown, but it was decreased substantially, reflecting production of much thinner leaves. This response indicates compensation for reduced irradiation by increasing the available photosynthetically active area. Greater specific leaf area and leaf area ratio under reduced light conditions have been reported in jimsonweed (*Datura stramonium* L.), velvetleaf (*Abutilon theophrasti* Medik.), and soybean (Regnier et al., 1988). Thinner, less dense leaves also are considered to be an important adaptive characteristic of plants growing under shaded conditions that permits light penetration to deeper leaves and their chloroplasts lower in the plant canopy (Bjorkman, 1981; Stoller and Myers, 1989).

In this series of experiments, floral initiation commenced 38 days after seed germination and was not affected by the nutrition and shading treatments (data not shown). Reduced light led to large decreases in aerial spathe production (Table 2). Subterranean spathe production also declined but the effect was relatively minor. The different degrees of adjustment in spathe production above and below ground in the shaded plants, as seen before at reduced fertility, indicates that Benghal dayflower shifts to a ‘survival’ strategy. Even with severely reduced growth and restriction of above ground spathe production, below ground reproduction remains much more intact. On an individual plant basis, this increases the likelihood of genetic persistence.

From these results, it is quite clear that Benghal dayflower would be more competitive in cropping systems with high rates of fertilization like those with corn and much less so in crops grown with lower fertilizer additions like soybean. Growth rate is primarily a function of N uptake and assimilation (Thornley, 1976; Wann and Raper, 1979; Lemaire et al., 2008), and corn crops typically receive as much as 70 kg N ac^{-1} (ISU, 1997). Nitrogen fixation allows soybean to be grown with little if any nitrogen fertilizer addition (ISU, 2007) even in sandy soils that have little native N fertility (Tucker, 1997).

Our observations indicate that resource limitations are unlikely to inhibit Benghal dayflower survival and reproduction in North Carolina agricultural fields. Vegetative regeneration and production of large amounts of aerial and subterranean seed with different dormancy characteristics make containment strategies difficult for this weed. With the 30-35

°C temperatures commonly experienced in North Carolina summers and the length of time they persist, it seems unlikely that environmental factors will limit the invasion of Benghal dayflower.

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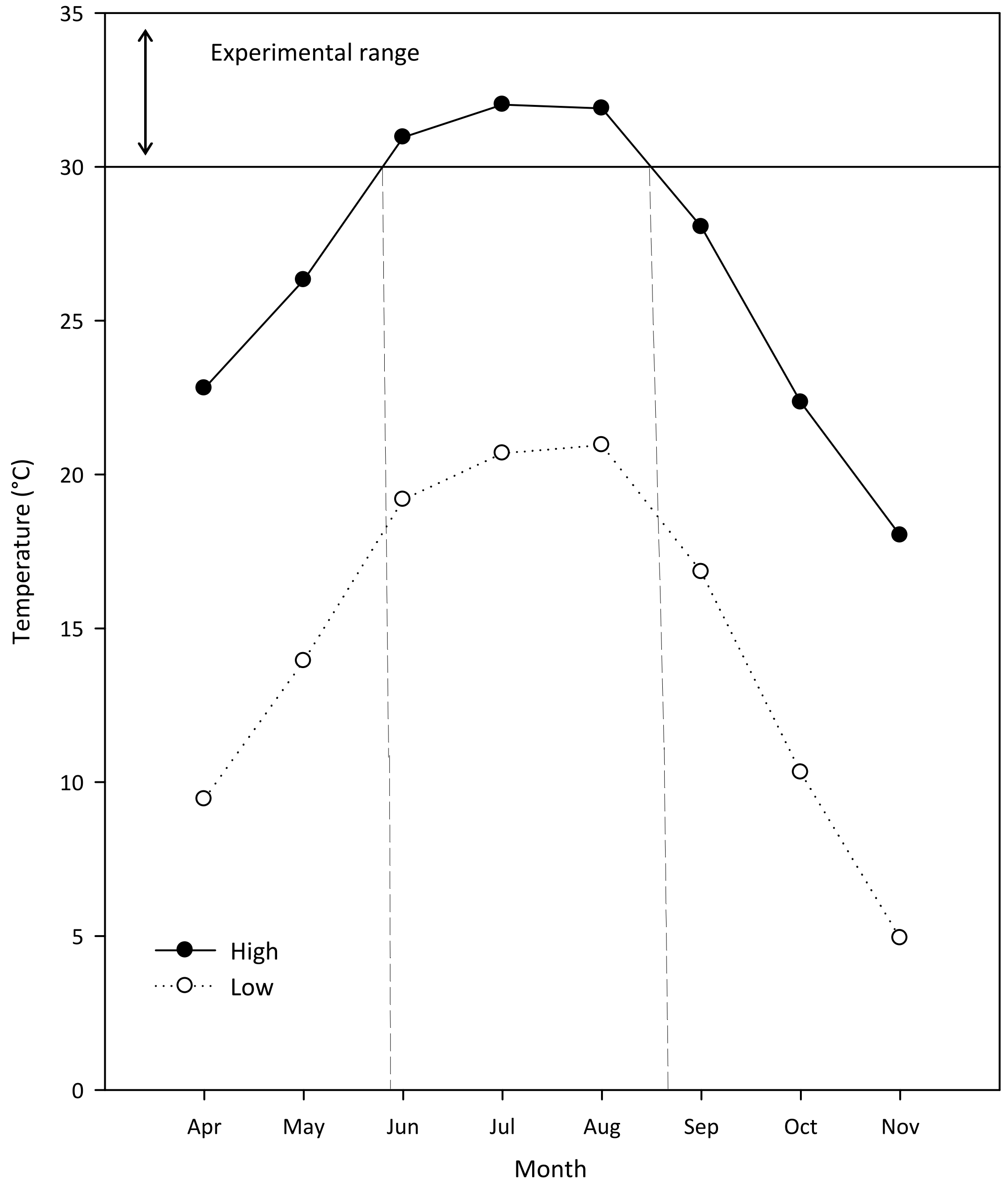

Figure 1. Average regional monthly high and low air temperature for 2001-2011.

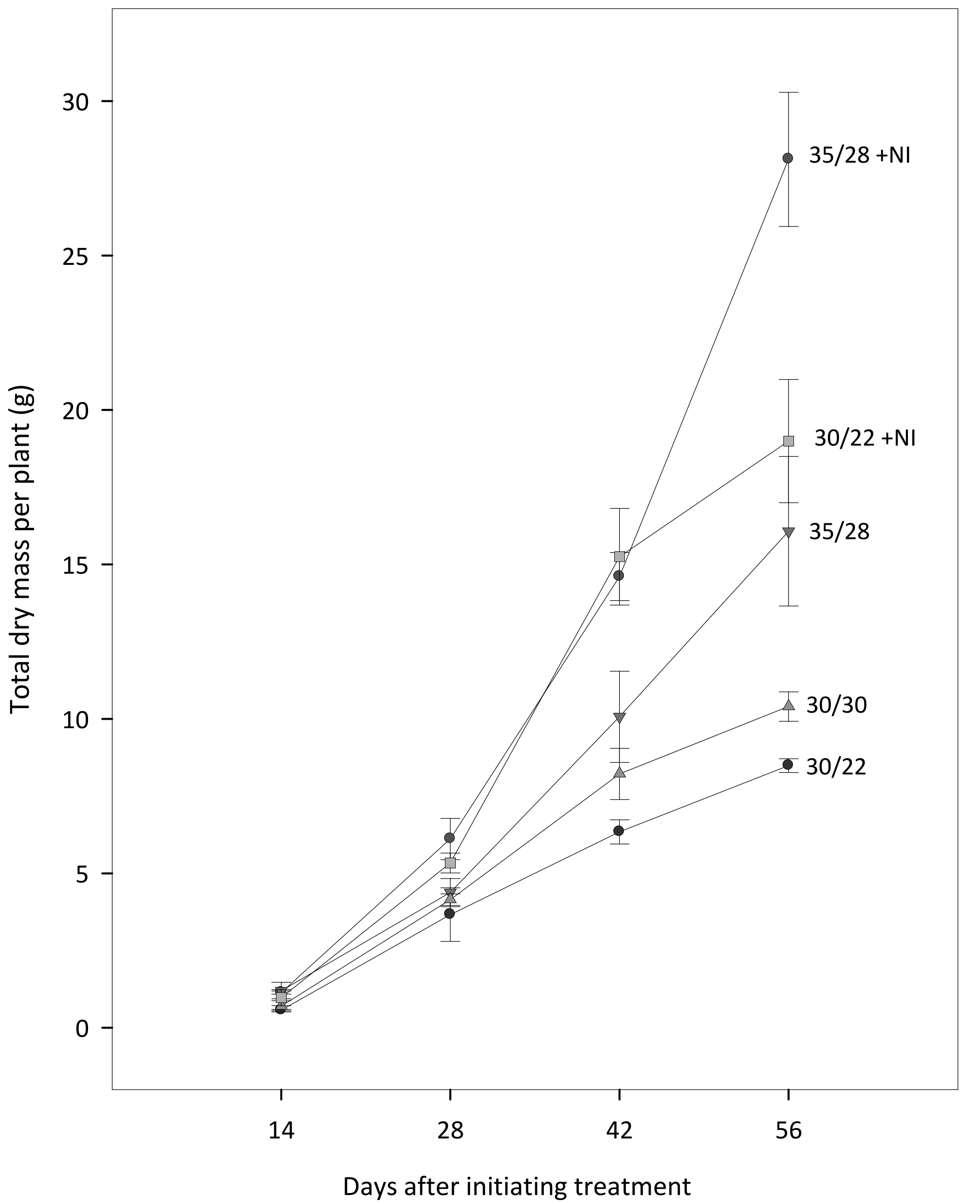

Figure 2. Benghal dayflower total dry mass per plant at different day/night temperatures without and with night interruption (+NI). Vertical bars indicate standard error of mean.

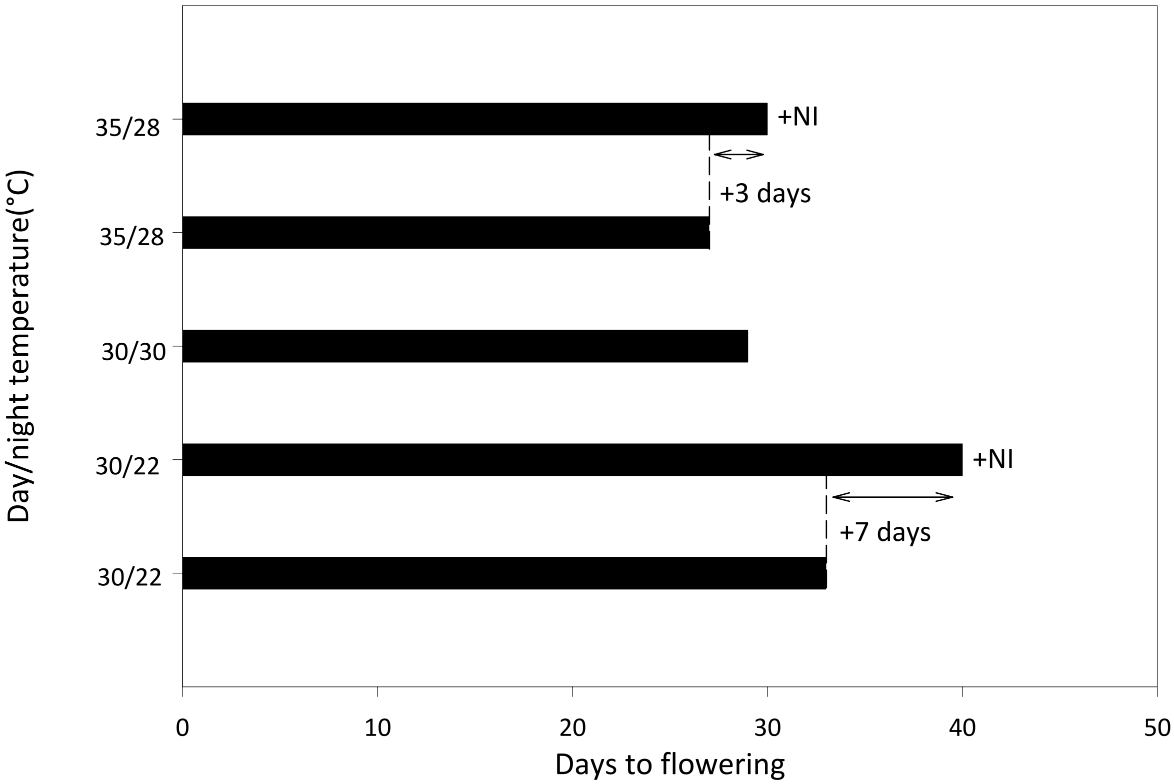

Figure 3. Days to flowering at three temperature regimes without and with night interruption (+NI)

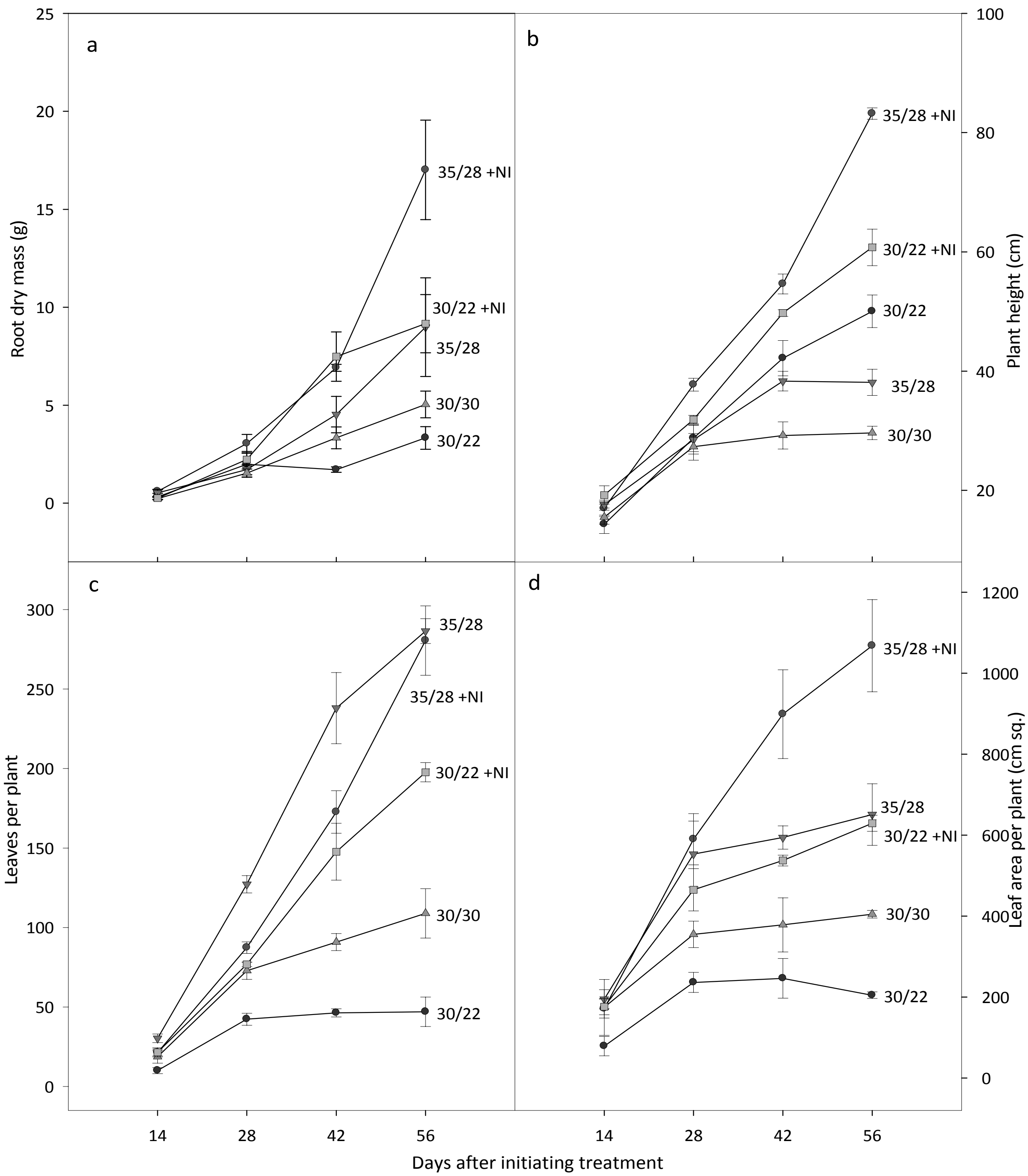

Figure 4. Benghal dayflower (a) root dry mass, (b) plant height, (c) number of leaves, and (d) leaf area per plant at different day/night temperatures without and with night interruption (+NI). Vertical bars indicate standard error of mean.

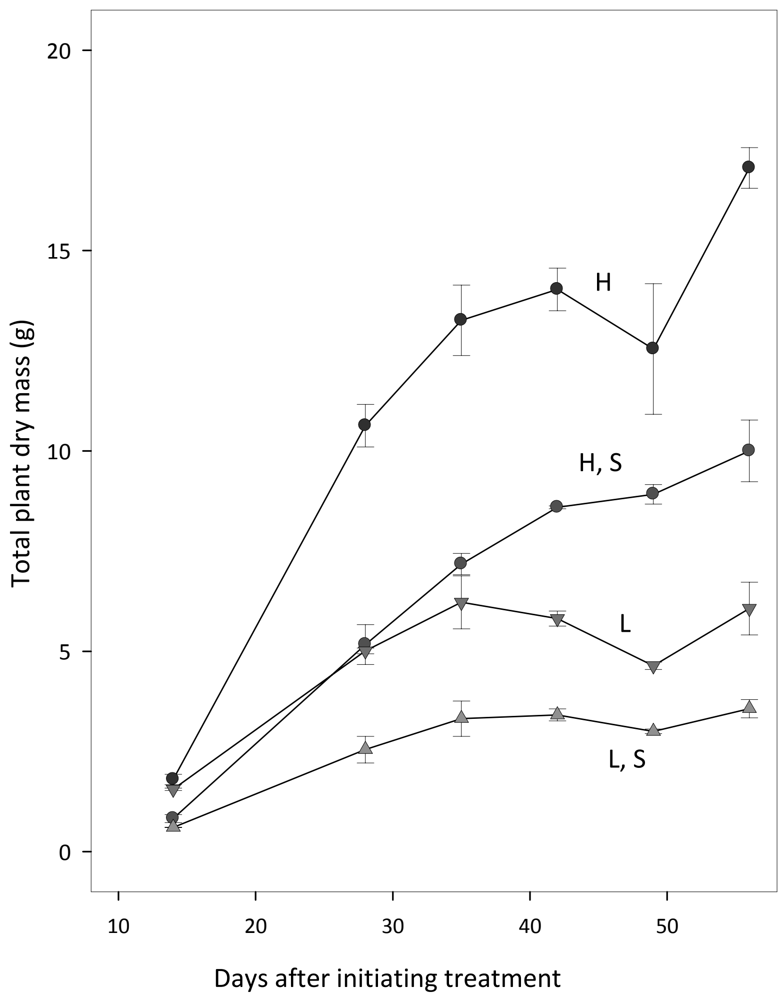

Figure 5. Benghal dayflower total dry mass per plant under different nutrient and light regimes: Greater nutrients and full light (H), greater nutrients and reduced light (H, S), reduced nutrients and full light (L), and reduced nutrients and reduced light (L, S).Vertical bars indicate standard error of the mean.

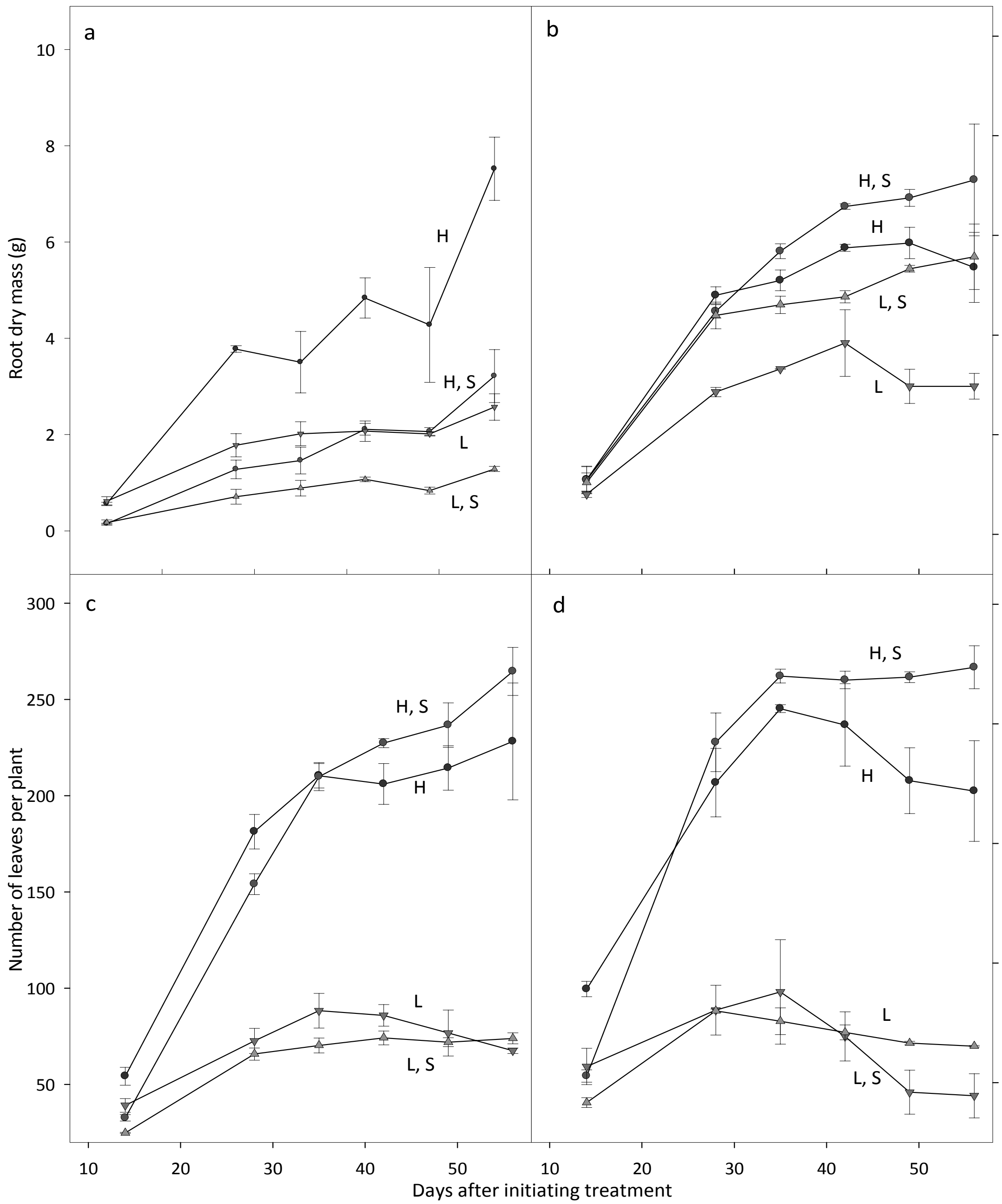

Figure 6. Benghal dayflower (a) root dry mass, (b) plant height, (c) number of leaves, and (d) leaf area per plant under different nutrient and light regimes: Greater nutrients and full light (H), greater nutrients and reduced light (H, S), reduced nutrients and full light (L), and reduced nutrients and reduced light (L, S).Vertical bars indicate standard error of the mean.

Table 1. Influence of three temperatures regimes on Benghal dayflower aerial and subterranean spathe production (number of spathes per plant) [a]

	Aerial spathes			Subterranean spathes		
	Day 28	Day 42	Day 56	Day 28	Day 42	Day 56
Temperature(°C)[b]	No. of spathes plant^{-1}					
30/22	22±0.8*	94±1.5*	120±4.0*	1±0.3^{b}	15±2.0^{c}	22±1.1^{c}
35/28	18±1.5*	68±6.0*	87±7.0*	4±0.1^{a}	29±0.8^{a}	31±1.4^{b}
30/30	42±0.5*	84±3.1*	98±3.2*	5±1.0^{a}	25±1.0^{b}	37±2.3^{a}

a Because the 3h night interruption did not differ significantly from continuous night treatment at a significance level of $P \leq 0.10$, the results were averaged over photoperiod regimes at a given temperature treatment. Means followed by * within a column are not significantly different ($P \leq 0.10$), means with the same letters indicate no significant differences at the $P \leq 0.10$ probability level.

b Values are means, and numbers following ± represent standard errors of mean.

Table 2. Influence of four nutrient and light treatment combinations: greater nutrients and full light (H), greater nutrients and reduced light (H, S), reduced nutrients and full light (L), and reduced nutrients and reduced light (L, S) on Benghal dayflower aerial and subterranean spathe production (number of spathes per plant)

	No. of aerial spathes plant^{-1}					No. of subterranean spathes plant^{-1}				
	Days after treatment									
Treatment [a]	28	35	42	49	56	28	35	42	49	56
H	132 ±34	251 ±22	286 ±18	379 ±23	558 ±16	15.8 ±5	22.5 ±6	34 ±7	55 ±13	80 ±11
H, S	68 ±18	150 ±16	212 ±24	284 ±30	398 ±4	7.25 ±3	13 ±3	26 ±6	37 ±6	54 ±10
L	65 ±10	115 ±5	126 ±2	124 ±3	150 ±1	11 ±3	20 ±3	30 ±4	35 ±1	52 ±4
L, S	38 ±12	66 ±3	92 ±5	90 ±4	124 ±7	5 ±3	13 ±3	22 ±5	29 ±1	41 ±2

a Values are means, and numbers following ± indicate standard errors of mean.