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Phenology, sexual reproduction, and the factors affecting sexual reproduction of the marine angiosperm, Thalassia testudinum, in the Florida Keys National Marine Sanctuary (FKNMS)

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PHENOLOGY, SEXUAL REPRODUCTION, AND THE FACTORS AFFECTING SEXUAL REPRODUCTION OF THE MARINE ANGIOSPERM, *THALASSIA TESTUDINUM*, IN THE FLORIDA KEYS NATIONAL MARINE SANCTUARY (FKNMS)

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Kevin M. Cunniff

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To: Interim Dean Mark D. Szuchman
College of Arts and Sciences

This thesis, written by Kevin M. Cunniff, and entitled Phenology, Sexual Reproduction, and the Factors Affecting Sexual Reproduction of the Marine Angiosperm, *Thalassia testudinum*, in the Florida Keys National Marine Sanctuary (FKNMS), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Joel Trexler

James W. Fourqurean, Major Professor

Date of Defense: July 18, 2005

The thesis of Kevin M. Cunniff is approved.

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Interim Dean Stephan L. Mintz
University Graduate School

Florida International University, 2006

DEDICATION

I dedicate this thesis to the memories of my father, Kevin R. Cunniff, who introduced me to the wonders of Nature, to my grandfather, Donald Christ, who allowed me to love Florida, and to my uncle, James Christ, who helped me to discover the wonders of ocean. While you are no longer on this Earth, you are always in my heart and in my mind.

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As an addendum, I would like to thank all of the faculty at Florida International University who instructed me in my curriculum and evaluated my progress in this program. Collectively, you all have helped me to become a scientist.

ABSTRACT OF THE THESIS

PHENOLOGY, SEXUAL REPRODUCTION, AND THE FACTORS AFFECTING SEXUAL REPRODUCTION OF THE MARINE ANGIOSPERM, *THALASSIA TESTUDINUM*, IN THE FLORIDA KEYS NATIONAL MARINE SANCTUARY (FKNMS)

by

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Florida International University, 2006

Miami, Florida

Professor James W. Fourqurean, Major Professor

This study investigated phenology and the factors affecting sexual reproduction of *Thalassia testudinum* in the FKNMS. Flowering was assessed at 30 permanent monitoring sites via direct observation and age reconstruction techniques of seagrass cores in 2002. The mean flowering frequency was 1.49%, was spatially variable, and exhibited sex-specific timing in floral anthesis. Historical flowering reconstruction demonstrated that flowering frequencies are not temporally variable. Floral sex ratios were female-biased, spatially variable, and likely temporally variable. Relative nitrogen availability was most important in influencing flowering and was negatively correlated with flowering. Higher flowering occurred with low N availability and lower flowering occurred with high N availability. A 15 month *in situ* nutrient addition experiment conducted at 10 sites in the upper Florida Keys, where N + P were added at ecologically significant loading rates, significantly reduced flowering in the N + P treatment plots at all 10 sites.

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Chapter I. PREFACE

This manuscript details the research history, methodology, data analysis, and discussion of my investigation into the phenology and factors affecting the phenology of *Thalassia testudinum*, a seagrass, in the Florida Keys National Marine Sanctuary (FKNMS), U.S.A. This work was conducted and completed as part of the requirements necessary to earn a Master of Science degree. Before I present the formal sections of this manuscript, I would like to take some time to discuss my motivation for choosing this particular facet of marine ecology to explore as well as the research questions I developed. Following this brief preface, I will present an abbreviated, yet informative Introduction, that describes certain life history characteristics of *T. testudinum* and contains a literature review highlighting pertinent previous research regarding this species needed to provide my audience with the background necessary to consider my data in the proper context. The review will act as a segue into outlining the research questions I addressed. The remainder of this manuscript is structured after the manner of most scientific journals, with Materials and Methods, Results, Discussion, and Conclusion sections, respectively.

I joined the Seagrass Ecosystems Research Lab (SERL) at Florida International University as a graduate student in January of 1999 looking to work in the seagrass environments of south Florida. While completing my course curriculum, I wanted to become more familiar with the ecosystem I was about to investigate as well as start to formulate research questions for my thesis. I was given the opportunity to be a research assistant on a long term monitoring project in the Florida Keys National Marine Sanctuary charged, in part, with the collection of data relating to such aspects of seagrass

ecology as population demography, benthic flora species diversity and frequency, primary productivity, and regional trends in relative nutrient availability. Since the project I was working on was aimed at "documenting and determining the status and trends of seagrass environments of the Florida Keys National Marine Sanctuary, " I was able to observe seagrass throughout all of the waters within the sanctuary. I was immediately struck by the diversity of these environments from region to region and site to site. Everything I saw and did was new, and every dive allowed me to question and learn something different about seagrass environments. I tried to make qualitative conclusions about phenomena I observed that were common to all seagrass environments throughout the sanctuary, however there were few to conclude upon. The only certainty seemed to lie in the fact that the seagrass environments of the FKNMS could be wholly characterized only by their complex differences and lack of uniformity.

While in the field during the summer of 1999, I took notice of the *T. testudinum* flowering that had been occurring across the Sanctuary. What became evident to me was the irregular spatial pattern in where *T. testudinum* was flowering and where it was not flowering. Certain sites maintained very high numbers of flowers and fruits, while other sites had very few or none. Furthermore, when I returned to those sites in the following summer of 2000, flowering patterns were very different. I began to question why there was such variability in flowering patterns not just from site to site, but from year to year at common sites. For example, was it normal for flowering patterns to be spatially and/or temporally variable? What was the degree of that variability? Were there areas that were perennial "hot spots" of flowering and fruiting that acted as propagule sources? Were there corresponding propagule "sinks" as well? What were the factors (physiological,

environmental, etc.) responsible for controlling flowering patterns at small spatial scales (local seagrass beds) as well as at regional scales? Were any of these factors able to be quantified for the purpose of modeling or predicting sexual reproduction in *T. testudinum* within the FKNMS? I searched the literature to learn what previous investigations of *T. testudinum* reproductive ecology had been conducted. My search showed me that there was relatively little investigation into this subject, with most of the published information related to describing flowering events from various locations of *T. testudinum* across its range, however, none of these studies had been conducted within the FKNMS. Additionally, there were very few experimental studies. I concluded that this was a relatively wide open area to investigate for my thesis, and the potential to conduct meaningful research and contribute to the scientific community was great.

As I became more familiar with the ecological functioning of seagrass environments in the FKNMS through the literature and from the research coming out of my lab, I started to focus my research interests. Specifically, I was interested in population dynamics and reproduction of *T. testudinum*. Nutrient availability, water quality, and physical environmental trends have been previously indicated as being important determinants of *T. testudinum* population structure in the FKNMS. Therefore, I wanted to direct my research to investigate how these factors may influence and determine patterns in *T. testudinum* sexual reproduction in the FKNMS. Additionally, I would be able to utilize large related data sets from my lab as well as from a concurrent water quality monitoring project, each dating back to 1995, respectively, to aid in my investigation of factors that may influence *T. testudinum* sexual reproduction in the FKNMS. With the insight and direction of my advisor and my committee, my research

was to be focused on describing the phenology of *T. testudinum* in the FKNMS, describing spatial and temporal trends in flowering, describing sex ratios, and investigating the relationship of flowering with environmental and demographic factors.

Chapter II. INTRODUCTION

Marine angiosperms, seagrasses, are an ancient albeit relatively small group of vascular plants that inhabit temperate and tropical coastal waters all over the world. Their longevity as a group, no doubt, owes in part to the relative temporal stability of the nearshore oceanic and estuarine environments they have evolved to exploit throughout geologic history. These plants have developed strategies for reproduction not much unlike those of their terrestrial and freshwater allies, yet they are specialized enough to warrant distinction. For most seagrass species, the dominant mode of reproduction is through asexual clonal growth of vertical ramets (short shoots) along a submerged horizontal rhizome. Correspondingly, sexual reproduction is often highly reduced. Previous investigators of seagrass reproduction have noted the elaborate ways that these plants reproduce sexually with completely submerged flowers (except *Enhalus*) and hydrophilous pollination, yet there has often been failure in drawing concise conclusions regarding the factors responsible for the cuing and expression of sexual reproduction (see Les 1988). In many species, sexual reproduction is quite variable and unpredictable in space and time, and for some species, there still has been no qualitative or quantitative documentation of observed flowers or fruits. In lieu of the emphasized importance that seagrass environments pose toward the overall health and ecological functioning of coastal marine environments and estuaries around the world, much effort has been expended on researching, managing, and in many cases restoring these ecosystems. An intimate understanding of seagrass reproductive ecology is paramount to insuring the overall protection of these marine resources.

On *Thalassia testudinum.* *Thalassia testudinum* is the dominant seagrass species in the Caribbean basin of the Atlantic Ocean. In Florida, its range includes both the Atlantic and Gulf coasts, Florida Bay, and the nearshore waters of the Florida Keys. The depth zonation of *T. testudinum* is dependent upon the amount of ambient light reaching the bottom and typically lies within the subtidal zone of <1 m to 12 m (Zieman 1985). *T. testudinum* exists across a broad range of physical and environmental conditions which affect such population properties as density, phenotype, productivity, reproduction, and demography. The most notable and highly documented of factors affecting *T. testudinum* growth and health include salinity (Zieman 1975), water temperature (McMillan 1979), photoperiod (Marmelstein et al. 1968), sediment type (Fourqurean and Rutten 2003), and nutrient availability (Patriquin 1972; Powell et al. 1989; Fourqurean and Zieman 1991; Fourqurean and Zieman 1992a; Fourqurean et al. 1995; Lee and Dunton 1999; Fourqurean and Zieman 2002).

Historically, *T. testudinum* sexual reproduction has received very little attention from investigators. Orpurt and Boral (1964) were among the first to document, in detail, the flowers, fruits, and seeds of *T. testudinum*. In their observations, they noted that *T. testudinum* was a dioecious plant with short shoots producing unisexual inflorescences. Tomlinson (1969) went on to formally describe the anatomy of *T. testudinum* flowers and fruits. Floral structures are reduced and may be colored white or purple. Male flowers possess anywhere from eight to thirteen whorls of stamens surrounding two or more central stamens with each anther containing four pollen sacs (Plate 1). Female flowers are shorter than males and reside at the base of a short shoot just above the sediment (Plate 2). Female flowers contain one ovary and typically have even numbers of twelve

to eighteen paired stigmas. Genets are either wholly male or female, and will not contain both male and female short shoots (Grey and Moffler 1978).

Pollination in seagrasses is hydrophilous and has been highly described (Faegri and Pijl 1971; Ducker and Knox 1976; McMillan 1976; Frankel and Galun 1977; Pettitt 1980; Pettitt et al. 1981; Phillips et al. 1981; Cox 1983; Cox and Sethian 1985; Cox 1988; Cox and Tomlinson 1988; Cox 1993; Ackerman 1997 a; Ackerman 1997 b), and pollination in *T. testudinum* is hyphydrophilous (pollen is transported exclusively under water). Male flowers bloom slightly earlier than female flowers (Durako and Moffler 1987; van Tussenbrock 1994) and typically dehisce within two weeks of initial development (pers. obs.). Anthesis and anther dehiscence in staminate flowers occur during full moon spring tides. This manner of anther dehiscence is very similar to that of *Thalassia hemprichii* reported from Kenya (Pettitt 1980), a species closely related to *T. testudinum*. Pollen is dispersed at low tide in negatively buoyant rafts of pollen grains which are bound by a slime of thecal origin (Cox and Tomlinson 1988), and pollen dispersal is two-dimensional along the plane of the substratum. The stigmas on the pistillate plants are linear, stiff, and densely papillate. The probability of successful pollination is increased due to the lower relative water flow velocities inherent with the positioning of the female flowers under the seagrass canopy (Cox and Tomlinson 1988).

A *T. testudinum* fruit may be described as an elliptical to globose capsule (Plate 3). As a fruit matures it completely fills and splits the enclosing spathe. The surface of the fruit is, at first, echinate, becoming tuberculate at maturity. Fruits typically take eight weeks to reach maturity. As the fruit ripens, it softens and changes from a bright green to a yellow-green color, and mature fruits may occasionally be red. *T. testudinum* seed

germination is viviparous, and dehiscence of the fruit may occur while still being attached to the short shoot, or it may break free during heavy wave or current activity. Free fruits remain afloat until dehiscence, thus, affording an excellent means of dispersal. Fruits normally contain three seedlings, occasionally one or two, and rarely up to six (Tomlinson 1969). Seedling success is highly variable spatially and temporally and is generally considered to be low (Thorhaug 1979; Lewis and Phillips 1980; Durako and Moffler 1981; Williams and Adey 1983; Zieman 1985; Kaldy and Dunton 1999).

The flowering season of *T. testudinum* typically begins in March with fruits persisting into October. The appearance of the first visible floral structures is largely controlled by water temperature (Phillips et al. 1981). Flowering is controlled by the yearly progression of warming and cooling that the waters of the Gulf of Mexico, the Florida coast, and the Caribbean experience. Specifically, the intensity and duration of winter minimum water temperatures determines the timing of initial floral expression and the subsequent rate of floral development in the early Spring. Studies performed over four years on *T. testudinum* in St. Croix, Mexico, and the Bahamas indicated that *T. testudinum* progressed slowly toward anthesis after water temperature stabilization of 24-26° C for one month (Phillips et al. 1981). Their data further suggest that the nearly synchronous flowering of *T. testudinum* at different latitudes may be related to genotypical adaptation of populations to regional warming water temperature trends following the winter minimum water temperature of each region, respectively. Additionally, floral induction of *T. testudinum* in tropical habitats may be genotypically responsive to higher water temperatures following the winter minimum than that required by plants in temperate habitats (Phillips et al. 1981).

Reports of *T. testudinum* flowering frequencies demonstrate regional and local variability as well as temporal variability. Based on reports from the literature, a readily accepted mean percentage of 1-5% of short shoots in a given population of *T. testudinum* may flower in a season. However, small scale observations of specific beds may be highly variable about this mean. Most reported observations of flowering frequencies have been made on relatively small spatial scales, therefore, reports of unusually low and high flowering frequencies are common (Table 1).

The discovery of *T. testudinum* floral structures in Tampa Bay, FL in January, 1981 led to high reported percentages of short shoots with reproductive bud primordia that ranged from 29-75%, and the mean number of buds per reproductive short shoot ranged from 1.4-2.7 (Durako and Moffler 1985b). The percentage of short shoots containing floral structures from this report is significantly higher compared to the 1-15% range estimated for easily observable reproductive structures in south and west-central Florida populations during the normal flowering season (Orpurt and Boral 1964; Zieman 1975; Grey and Moffler 1978; Thorhaug 1979). These results suggest that early bud development in January may indicate that initial *T. testudinum* floral induction is a short-day phenomenon (i.e. November-December) (Moffler et al. 1981). Clear visible evidence of floral development (resulting from increased cell elongation and division) occurs later when water temperatures increase (i.e. April-June). These high percentages of early bud primordia may mean high rates of environmentally induced mortality during the winter when compared with the lower percentage of flowering shoots observed in the spring and summer.

Based on reports of floral sex ratios, *T. testudinum* may operate as a pollen-limited breeding system (Les 1988). This theory details that the successful pollination and subsequent development of fruits and seedlings is a function of the relative amount of male flowers (pollen) to female flowers (ovules) produced in a given flowering population. Reports of *T. testudinum* floral sex ratios demonstrate spatial and temporal variability (Table 2), and this theory has yet to be substantiated with rigorous phenological or experimental data. Based on these reports, it is difficult to first, conclude as to whether or not *T. testudinum* floral sex ratios are primarily male or female-biased, and second, to describe pattens of spatial or temporal variability. Furthermore, it is difficult to infer upon the importance and ecological role of floral sex ratios in determining successful pollination and fruit set of *T. testudinum* in local and regional populations.

Research objectives. I investigated and described the phenology of *T. testudinum*, spatial and temporal patterns in flowering, fruiting, and sex ratios, and the factors that may influence *T. testudinum* flowering in the FKNMS. I developed several research questions to consider during my investigation that were targeted at describing and comparing patterns in *T. testudinum* sexual reproduction from both direct observation of flowering and through historical reconstruction of past flowering events.

Question 1: Does *T. testudinum* exhibit spatial and/or temporal variability in flowering frequency patterns in the FKNMS over the course of a flowering season?

Question 2: Is there a difference in the timing of male and female floral anthesis in populations of *T. testudinum* in the FKNMS, and if so, what is the timing

observed in each sex?

Question 3: What is the male:female floral sex ratio of *T. testudinum* in the FKNMS, and is this ratio spatially variable among separate populations?

Question 4: What is the fruiting success of *T. testudinum*?

In order to describe *T. testudinum* phenology, assess patterns in flowering frequency, fruiting, and sex ratios, I made repeated *in situ* observations of *T. testudinum* populations at 30 permanent seagrass monitoring stations located throughout the FKNMS over the course of one full year during 2002.

Previous reports of *T. testudinum* flowering have largely been restricted to single sampling events (Grey and Moffler 1978; Thorhaug 1979; Durako and Moffler 1987; Gallegos et al. 1992; van Tussenbrock 1994; Witz and Dawes 1995). These studies likely underestimated flowering frequencies and sex ratios due to the differential timing in *T. testudinum* floral anthesis. Male flowers bloom earlier than females and are short-lived, while female flowers persist throughout the flowering season as developing fruits. Additionally, *T. testudinum* flowering intensity, both sex-specific and inclusive of both sexes, is staggered over the duration of the flowering season and is a further source of variability to consider when assessing flowering frequency and sex ratios. Fruiting success, which is ultimately dependent upon pollination as dictated by the sex-specific floral timing, is spatially and temporally variable as well. I proposed that more frequent and intense sampling would allow me to: 1.) quantify flowering and derive more accurate *T. testudinum* flowering frequencies in the FKNMS, 2.) assess spatial and temporal variability of flowering in separate *T. testudinum* populations, 3.) describe sex-specific

timing in floral anthesis, 3.) determine a more accurate floral sex ratio, and, 4.) assess fruiting.

In order to assess historical trends of spatial and temporal *T. testudinum* flowering variability in the FKNMS, I utilized short shoot age-reconstruction techniques of *T. testudinum* short shoots collected from the same populations where *in situ* observations were made. These techniques have previously been used to estimate *T. testudinum* growth rate, production, and age (Patriquin 1973), reconstruct population demographics and detect population growth trends (Duarte et al. 1994), and for assessing reproduction (Kaldy 1997). These methods rely on the ability to age individual short shoots by counting the leaf scars and extant leaves, yielding a total number of leaves, and then multiplying by a Plastochrone interval to derive an age (in days) (Erickson and Michelini 1957). The age-frequency distribution of short shoots is a reflection of recruitment and mortality of individual short shoots in a specific seagrass population. Previous examinations of seagrass population dynamics have been mostly restricted to censuses conducted on small spatial scales (0.1-1 km) (Duarte et al. 1994; Durako 1994; Gallegos et al. 1994; Jensen et al. 1996). However, more recently Peterson and Fourqurean (2001) utilized these techniques on a significantly larger spatial scale of *T. testudinum* populations spanning the FKNMS (~9,500 km^2, n=146). Specifically, I utilized these techniques to describe historical patterns of flowering frequency and sex ratios in the FKNMS, as evidenced by the sex-specific floral scars left behind on a flowering *T. testudinum* short shoot between leaf scars that are created from the regular sloughing off of old leaves. The potential advantage to using these techniques is that estimates of previous flowering events, based on the relative ages and ages at flowering of short

shoots in a given population of *T. testudinum*, may be obtained from a single sampling event.

Question 5: Does *T. testudinum* historically exhibit spatial and temporal variability in flowering patterns in the FKNMS?

Question 6: What is the historical ratio of male:female *T. testudinum* short shoots based on evidence from floral scars in the FKNMS?

Question 7: How well does observational flowering data compare with historical reconstructive flowering data in assessing *T. testudinum* flowering patterns?

The application of age-reconstructive techniques to populations of short shoots for the purpose of exploring trends in growth, recruitment, and mortality has been criticized (Jensen et al. 1996; Durako and Duarte 1997; Jensen et al. 1997). These authors argue that the required assumptions of constant age-specific mortality and recruitment rates in a given population are untenable. They also raise the concern that durations in Plastochrone intervals often exhibit considerable spatial and temporal variability. Recently, this concern was reiterated specifically regarding *T. testudinum* where the authors further discouraged the use of the Plastochrone interval as a viable method for constructing age-frequency distributions in this species due to its violating the assumption of equal time between the formation of successive leaves (Kaldy 1997). Additionally, Kaldy et al. (1999) proposed that this bias in leaf formation measurements leads to the commonly reported *T. testudinum* age frequency distributions with too few young shoots to account for the older shoots in the population.

I had considered the potential problems and criticisms raised by previous investigators that may affect the validity or applicability of population demographic data

obtained from age-reconstruction techniques. The focus of my study did not specifically address certain aspects of short shoot demography (i.e. recruitment and mortality) that may be estimated by these techniques and that have been most contested. Rather, I employed age-reconstruction of individual short shoots using the plastochrone interval in conjunction with floral scars for the purpose of, in part, exploring regional patterns of historical *T. testudinum* flowering events in the FKNMS. Additionally, these reconstructive flowering data were compared with observational flowering data in the attempt to explore the validity and applicability of data from both techniques respectively and adjunctively. Data on Plastochrone intervals was taken from a regional, multi-year, quarterly sampling program. Peterson and Fourqurean (2002) that demonstrate a clear seasonal sinusoidal pattern in *T. testudinum* Plastochrone intervals from each of the monitoring stations (n = 30) used in my investigation. Plastochrone intervals are longest during the winter months when productivity and growth are highly reduced and shortest during the summer months when productivity and growth are at the maximum. To account for this seasonal variability, I calculated a mean Plastochrone interval, respective of each site, which incorporated several years of quarterly estimates to yield one single Plastochrone interval estimate and applied it to age *T. testudinum* short shoots from each site. I believe these methods may be used with caution to describe historical trends of *T. testudinum* flowering in the FKNMS.

The influence of environmental factors on sexual reproduction in *T. testudinum* has largely been unexplored. Given the manner in which the literature documents how environmental factors affect a broad range of biological activities in seagrasses, the lack of investigations relating environmental factors with reproductive

ecology becomes even more evident, and therefore, necessary. Thus, I sought to investigate how environmental factors may influence observed flowering trends in *T. testudinum*.

> Question 7: How do environmental parameters affect patterns in *T. testudinum* sexual reproduction in the FKNMS, and which environmental parameters may be inferred to exact the greatest influence?

I utilized data relating to water quality variables, seagrass vitality (i.e. short shoot morphometrics, productivity), and nutrient availability to explore correlative relationships with *T. testudinum* flowering trends in the FKNMS. Data on all parameters were obtained from two concurrent long-term monitoring projects taking place at the permanent sites where my flowering investigations were targeted.

I was particularly interested in exploring the relationship between nutrient availability and flowering. Based on stoichiometric elemental analysis of *T. testudinum* leaf nutrient content, there is strong evidence to suggest a gradient of nitrogen limitation offshore and phosphorus limitation nearshore in the FKNMS (Fourqurean and Zieman 2002). This trend is particularly pronounced in the waters oceanside of the upper Florida Keys. I hypothesized that *T. testudinum* flowering may be influenced by nutrient limitation, and to test this, I conducted an *in situ* nutrient addition experiment in the upper Florida Keys. Specifically, I was interested in whether or not I could illicit a positive or negative flowering response by effectively eliminating the nutrient limitation through sediment addition of nitrogen and phosphorus. The goal was to experimentally investigate the relationship between nutrient availability and *T. testudinum* flowering and compare these results to the observational and historical flowering data that I collected.

Chapter III. MATERIALS AND METHODS

Study sites. There are >15,000 km^2 of seagrass beds in the south Florida region (Fourqurean et al. 2002). The Florida Keys National Marine Sanctuary (FKNMS) consists of ~9,500 km^2 of coastal and oceanic waters extending from the southern tip of Key Biscayne, encompassing the islands of the Florida Keys, and includes the Dry Tortugas. Much of the water within the FKNMS supports seagrass communities of varying density and diversity. Sites were selected to coincide with thirty (30) permanent seagrass monitoring stations located within the FKNMS (Fig. 1). These are Level 1 sites from an ongoing Seagrass Status and Trends in the FKNMS monitoring project (J. W. Fourqurean, P. I.., NOAA contract NA16OP2553, EPA contract X97468102-0) that has been documenting and evaluating seagrass resources in the FKNMS since 1995. The sites were originally located using a stratified-random approach (EMAP), with distance offshore and broad geographic regions as the strata. *T. testudinum* is present in low to high density at each of the 30 sites (Fig. 2).

Observation of *T. testudinum* flowering. To assess trends in *T. testudinum* flowering, a rapid visual assessment technique was employed. Sampling for flowering was conducted on a quarterly basis from March 2002 through January 2003 with two additional sampling efforts being conducted in February and May 2002 to yield a total of six separate efforts. At each site a target number of 700 short shoots was visually and manually inspected via SCUBA for evidence of flowering and fruiting. This target number was selected to coincide with the amount of short shoots generally observed in ten 0.25 m^2 sample quadrats as utilized in the modified Braun-Blanquet rapid visual assessment techniques described in Fourqurean, et al. (2001). Short shoots were counted

along a transect, the direction of which was determined by a random compass bearing from a fixed underwater site marker. A 0.25 m^2 quadrat was placed along the transect at random intervals until the target number of short shoots was observed. Short shoots containing floral structures or fruits were sexed, and the number of inflorescences and/or fruits was recorded. For my purposes, a flower was recorded whether it was in a developing stage, in active anthesis, or dehisced. A fruit was counted as a single female inflorescence. The flowering frequency of each site was calculated as the percentage of flowering short shoots {(total number of flowers and fruits/total number short shoots observed)*100}. The corresponding sex ratio for each site was calculated as total male:female inflorescences. These data were subsequently pooled to yield the overall flowering frequency and sex ratio of all sampled short shoots for the 2002 flowering season. Total flowering frequency, male flowering frequency, total female flowering frequency (including fruits), fruit frequency, and sex ratio data were mapped for the sampling months in which flowering was observed using ArcView GIS 3.2 for Windows software.

Seagrass short shoot collection. A target of ~110 *T. testudinum* short shoots (herein referred to as "cores") was collected via SCUBA from the 30 monitoring stations in September 2001. Alternate replacement cores at sites 214, 220, and 273 were collected in September 2002 due to the original cores being degraded by freezer rot earlier that year. In an effort to help minimize any bias from the effects of seasonality, these three alternate cores were collected during the same month as the original sampling. I made the assumption that population demography had not changed significantly enough in one year as to render the data incomparable or unusable. The cores were collected in a

randomly determined area of homogeneous short shoot density, and all short shorts were removed from the sampling area by hand and garden hand shovel (when needed). The cores were gently cleaned of all sediment and debris, and the short shoots were separated while under water. The relative size of the collection area at each site varied with *T. testudinum* density, but was generally refined to a 0.25 m^2 section . Care was taken to extract short shoots with rhizomes intact in order to insure a proper subsequent analysis of shoot demography and phenology. Short shoots without intact rhizomes were not discarded in the effort to minimize biasing the collection against older or longer shoots which often exist in a deeper connected rhizosphere relative to younger short shoots. There existed the inherent possibility that including broken short shoots may have led to an underestimation of the age of those respective short shoots. However, when a short shoot was broken, it tended to break at the base of the shoot where it was attached to the rhizome, and was therefore included in the age frequency and phenology estimations. Furthermore, the short shoots taken in these cores were assumed to be a representative sample of the larger population at each site, respectively. Upon returning to the boat, the short shoots were placed in a plastic bag, labeled, and frozen until further analysis in the lab.

Short shoot morphometrics, age reconstruction, and phenology. Upon thawing a core, short shoot morphometrics were assessed. The horizontal rhizome was cut from the base of the short shoot (if attached) using a razor blade, and the rhizome diameter was recorded. Additionally, if the horizontal rhizome fragment contained an apex, it was recorded, and loose apices in the core were counted for the purpose of assessing asexual reproduction. The remaining short shoot was stripped of its protective

sheath material to reveal the bare shoot with leaf scars and floral scars (if present). The extant leaves were cut at the top of the shoot, and the length and width of all leaves (in mm) was recorded in ascending order from youngest to oldest. The shoot length was measured and any branching apex present on the shoot was recorded. Leaf scars and floral scars were counted from the base of the shoot using a dissection magnifying lens and dissection pointer. Floral scars are sex-specific (Fig. 3), and any floral scars present were sexed and recorded at their relative positions among the leaf scars on the shoot. All short shoots were assessed in this manner.

Short shoot age reconstruction techniques have been used by investigators to assess such aspects of *T. testudinum* population dynamics as demography, recruitment, mortality, growth and production, phenology, and reproductive ecology (Durako 1994; Kaldy 1997; Kaldy et al. 1999; Peterson and Fourqurean 2001). In order to reconstruct and assess historical flowering events of *T. testudinum* in the FKNMS, short shoot age reconstruction techniques were employed. In particular, this technique takes advantage of the inherent nature in which individual *T. testudinum* ramets systematically record their age and reproductive history on their shoots with leaf scars and floral scars. A *T. testudinum* short shoot continuously produces new leaves over the course of its lifetime. Correspondingly, older leaves are sloughed off at a rate which roughly matches the rate that new leaves are produced. When a leaf is sloughed off, it produces a scar on the shoot. Counting the total number of leaf scars and extant leaves on a short shoot produces a record of age in a manner analogous to the growth rings produced by deciduous and coniferous trees. In order to effectively estimate the age of an individual short shoot, the estimated rate at which leaves are produced must be determined. The

Plastochrone interval measures the rate at which two successive leaves are produced (for a full description see Patriquin 1973). Estimates of the Plastochrone interval for *T. testudinum* exhibit variability through time due to the seasonality of growth rates and primary production (Fourqurean et al. 2001). For my study, I used a site-specific mean Plastochrone interval estimate for each site calculated from quarterly primary productivity estimates (1995-2003), respectively, taken as part of the FKNMS seagrass status and trends monitoring project formerly described above. The age (in days) of any individual short shoot may be represented by the following equation:

$$Short\ shoot\ age\ (days) = (s + l)PI$$

where s is the number of leaf scars counted on a shoot, l is the number of extant leaves, and PI is the calculated mean Plastochrone interval from the respective site. The ages in days were then converted into real-time dates (based on when the cores were collected) in order to estimate the date of birth and date(s) of flowering. All short shoots from the 30 sites were aged in this manner. Age frequency histograms were constructed for each site, and these data were further pooled to yield an age frequency distribution of all short shoots sampled in the FKNMS.

Phenology of *T. testudinum* was assessed by pooling all short shoots and separating them into cohort classes by shoot age calculated from the discreet estimates. Past flowering events were assessed by pooling all flowering short shoots and calculating the relative flowering frequency for each year where flowering shoots existed in a cohort class. Calculation of the flowering frequency may be represented by this equation:

$$Flowering\ frequency\ (\%) = (f/t)100$$

where f = the number of flowering shoots in a given cohort class and t = the total number

of eligible shoots sampled in a older cohort classes up to the present cohort, yielding flowering estimates for a given year in which floral scars were observed, respective of that year. A site-specific absolute relative flowering frequency was calculated from all sampled short shoots. The male:female sex ratio of flowering shoots was calculated by pooling all flowering short shoots assessed. Site-specific short shoot sex ratios were calculated in the same manner.

Water quality data. Water quality data sets from the seagrass monitoring project and from a concurrent water quality monitoring project (Water Quality Monitoring Network, Southeast Environmental Research Center, Joe Boyer, P. I., EPA contract #X994621-94-0) were used to assess the relationship between environmental factors and *T. testudinum* flowering. These data, commonly sited in various marine ecological investigations, were compiled and combined from quarterly sampling, in each respective project, of the 30 permanent monitoring stations from 1995-2003. The water quality variables (n = 25), included inorganic N and P variables: nitrogen oxides (NO_x, surface), nitrate (NO_3^-, surface), nitrite (NO_2^-, surface), dissolved inorganic nitrogen (DIN, surface), ammonium (NH_4^+, surface), and soluble reactive phosphorus (SRP, surface); organic variables: total organic carbon (TOC, surface), total nitrogen (TN, surface), total organic nitrogen (TON, surface - defined as the difference between TN and DIN), total phosphorus (TP, surface), alkaline phosphatase (APA, surface), chlorophyll *a* (Chl *a*, surface), turbidity (NTU, nephlometric turbidity units, surface), and silica (SI, surface). Elemental ratios were calculated on a mole:mole basis and included: TN:TP, N:P, and DIN:TP. Physical water properties measured included: water temperature (surface), salinity (surface), and dissolved oxygen (DO, surface and bottom). Light variables were

directly measured in the water column using a 4π PAR sensor (LI-COR, Lincoln, Nebraska). Readings were taken at the surface and at each subsequent meter of depth until the sensor reached the bottom or until 8 m. Readings were taken both on the way down and on the way up through the water column. Ambient light (%Io), the diffuse light attenuation coefficient (Kd), and % saturation (%Sat, surface and bottom) were estimated from the profile data according to the Lambert-Beer law. A full description of analytical methods is described in Boyer et al. (1999).

Seagrass vitality. Data on *T. testudinum* relative growth rates (primary production) and shoot morphology were taken from quarterly measures of above-ground productivity from1995-2003 in the FKNMS monitoring project to investigate the relationship of growth and morphology with patterns in flowering at the thirty monitoring sites. Site-specific means of each category (n = 13) were calculated and include: leaf mass ($mgSS^{-1}$), leaf area (cm^2SS^{-1}), leaf length (mm), leaf width (mm), leaf number (SS^{-1}), short shoot density (SSm^{-2}), standing crop (gm^{-2}), short shoot productivity ($mgSS^{-1}d^{-1}$), specific productivity (mgg^{-1}), areal productivity ($gm^{-2}d^{-1}$), leaf area productivity ($cm^2SS^{-1}d^{-1}$), leaf area productivity ($cm^2m^{-2}d^{-1}$), and Plastochrone interval. For a full description of methods, see Fourqurean, et al. (2001).

Nutrient availability and ratios. *T. testudinum* leaf tissue nutrient content and stoichiometric elemental ratios from the FKNMS monitoring project were used to investigate the relationship between relative nutrient availability and *T. testudinum* flowering patterns. Nutrient content of seagrasses, including *T. testudinum*, are good proxies of relative nutrient availability because they often grow in oligotrophic waters, are anchored in the sediment, and are able to uptake nutrients (mostly from the sediment)

that are incorporated into their tissues in a manner that is consistent with the local availability of the nutrients (Atkinson 1983; Duarte 1990; Fourqurean and Zieman 2002; Ferdie and Fourqurean 2004). Therefore, the % content and ratios of elements in *T. testudinum* leaf tissues are able to provide a spatial representation of sediment nutrient dynamics and availability in a particular area of the FKNMS. Data were obtained from quarterly measurements of leaf tissue nutrient content of *T. testudinum* collected at the 30 monitoring stations (1995-2003). At each sampling site, six intact *T. testudinum* short shoots were collected haphazardly from a 10 m^2 area. Within a 24 hour period, all attached green leaves of each short shoot were cut at the basal meristem and cleaned of their epiphytes by gentle scraping with a razor blade. All leaves were pooled and dried to a constant weight at 70°C. Dried leaves were ground to a fine powder with a ceramic mortar and pestle to insure sample homogeneity. Powdered samples were analyzed in duplicate for carbon and nitrogen content using a CHN analyzer (Fisons NA1500) and phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean and Zieman 1992a). Elemental content was calculated on a dry weight basis. For my study, the mean *T. testudinum* leaf tissue %C, %N, and %P was calculated for each site, respectively, and the mean elemental ratios C:N, C:P, and N:P were calculated on a mole:mole basis for each site, respectively.

Nutrient addition experiment. I conducted a nutrient addition experiment to further investigate the relationship between nutrient availability and *T. testudinum* flowering. Ten sites were selected in the upper Florida Keys, where a natural nitrogen and phosphorus limitation gradient and distance offshore were the proxies for site

selection (Fig. 4). Nearshore sites are characterized as P-limited, and contain fine mud sediments with relatively high organic content and detritus. Offshore sites are characterized as N-limited, and contain coarse carbonate sand sediments low in organic content and detritus. At each site, two 6m x 6m plots made of PVC plastic were randomly laid out, anchored at the four corners by rebar, and further stabilized by anchoring in the sediment with coated copper wire clips. One plot was randomly designated the control and the other the experimental. Within each treatment plot, ten 0.25 m^2 PVC flowering observation plots were randomly set and anchored to the sediment by coated copper wire clips. Flags were placed in the sediment at the corners of the observation plots to facilitate their relocation during sampling.

Nutrient loading rates in nutrient addition experiments vary greatly in the literature, often not being characterized as ecologically relevant or biogeochemically feasible (for a review, see Worm et al. 2000). For my experiment, two conditions for calculating the loading rates of each element had to be satisfied: first, they had to be ecologically significant, and second, they had to exceed the amount of limitation that the plants experience naturally. To address the ecological significance issue, I consulted Florida Keys land use and stormwater runoff models to obtain a real-life proxy of the natural eutrophication that the surrounding nearshore coastal waters of the Florida Keys and seagrass environments may be experiencing. Land use practices and stormwater runoff are considered two of the largest threats to eutrophication of nearshore waters in the Florida Keys (EPA 1999). Based on regulatory estimates of maximum potential wastewater discharge from land use and stormwater runoff in the Florida Keys (MCSM 2001), and correcting for geochemical (e.g., sorption) and biological (e.g., denitrification)

processes, I calculated a final loading rate of 0.77 g N m^{-2} d^{-1} and 0.12 g P m^{-2} d^{-1}. This rate exceeds the N and P demand of peak *T. testudinum* summer growth rates as assessed by multiplying the peak growth rates (productivity measurements) by nutrient content (%N and %P leaf tissue content, respectively) (Fourqurean et al. 1992b).

The experimental treatment plot at each site received monthly doses of N and P from May 2001 through July 2002 for a total of 15 doses. N was delivered via a urea-coated slow release N fertilizer (Poly-on, Pursell Technologies; 38-0-0, 94% N). P was added as finely ground deflourinated phosphate rock (Multifos, IMC Phosphates; $CA_3(PO_4)_2$, 18% P). Based on the calculated loading rate, the amount of fertilizer needed to fertilize a single experimental treatment plot each month equaled 2079 g N and 528 g P. Each fertilizer type was weighed out and placed in separate plastic bags. Via SCUBA, I swam back and forth over the plot while slowly delivering each fertilizer type onto the sediment and under the seagrass canopy. Care was taken to insure that the fertilizer was applied evenly throughout the whole treatment plot.

Flowering was assessed monthly at all ten sites from April through July 2002, and January 2003. A total of 90-180 short shoots (depending on site) was visually and manually examined for evidence of flowering in each of the flowering observation plots in both treatment plots. Short shoots were counted, flowers were sexed and counted, and fruits were counted. Fruits were considered as a single female inflorescence. Flowering frequencies were calculated as the % of flowering short shoots. Sex ratios were reported as male:female flowers.

Statistical analysis. All water quality, seagrass vitality, and nutrient availability data were compared to *T. testudinum* observational and historical flowering data by

constructing correlation matrices to explore data for significant relationships. Significant cases were subsequently linearly regressed to explore model fit. All correlation matrices and linear regressions were calculated using SPSS 8.0 for Windows. Descriptive statistics and figures were generated using SigmaPlot 2001. Unless otherwise stated, all relationships are significant at $p \leq 0.05$.

Chapter IV. RESULTS

FKNMS observational flowering data. A total of 115,400 *T. testudinum* short shoots were observed for evidence of flowering at 30 permanent monitoring stations in the FKNMS over the course of six separate sampling events from February 2002 - January 2003 (Table 3). Only 13 sites were visited in February due to persistent unfavorable weather and ocean conditions for the majority of the month. The May sampling saw only 23 sites visited due to poor weather in the lower Florida Keys oceanside in the middle of the month. One site (309) was missed during the January sampling due to hazardous seas nearshore gulfside of the lower Florida Keys that prevented sampling of the site. All 30 sites were visited in March, June, and September. With respect to raw numbers, a total of 387 male flowers, 195 female flowers, and 368 fruits were observed at all sites from May 2003 - January 2003 (Table 4). One male flower was observed in March. The majority of the male flowers were observed during the May sampling (272) and tapered off in the June sampling (114). No male flowers were observed during the February, September, or January samplings. Female flowers numbered 142 (82.6%) and fruits 30 (17.4%), respectively, of the total female floral bodies (172) during the May sampling. Female floral bodies reached their peak (249) in the June sampling, with the majority being fruits that numbered 196 (78.7%) while flowers numbered 53 (21.3%). Fruits were observed exclusively (142, 100%) during the September sampling. No female floral bodies were observed during the February, March, or January samplings.

Flowering frequencies were calculated for the March, May, June, and September samplings (Table 5). The mean pooled FKNMS flowering frequency (% of observed

short shoots) was 1.49%, and was calculated from the May (2.51%), June (1.53%), and September (0.64%) samplings (only one male flower was observed in March). Site-specific flowering frequencies were highly variable spatially and temporally, ranging from 0 to $1.26e^{-3}$% in March 0 to 21.86% in May (Fig. 5), 0 to 24.21% in June (Fig. 6), and 0 to 4.92% (Fig. 7) in September. Flowering and/or fruiting was observed at 1 of 30 (3.33%) sites sampled in March, 21 of 23 (91.3%) sites sampled in May, 18 of 30 (60%) sites sampled in June, and 5 of 30 (16.67%) sites in September. With respect to sex-specific floral anthesis, male flowering frequency (1.54%) was higher than total female flowering frequency (0.97%), consisting of female flowering (0.80%) and fruiting frequency (0.17%), in May (Fig. 8). Male flowering frequency declined in June (0.48%) while total female flowering frequency (1.05%) increased (flowers = 0.22%, fruits = 0.83%, respectively). Male flowering frequency was zero in September while total female flowering frequency (0.64%) consisted wholly of fruits. When fruits were considered separately from flowers, fruit frequencies were generally low at sites where fruits were observed during May and June yet exhibited higher site-specific variability, ranging from 0 to 2.19% in May and 0 to 20.79% in June. While fewer overall sites were observed to have fruits in September, fruiting frequencies exhibited lower site-specific variability (where fruits were observed), ranging from 0 to 4.92%. The mean pooled fruiting frequency for May, June, and September was 0.57%.

Male:female floral sex ratios exhibited spatial and temporal variability (Table 6). No ratio was calculated for the March sampling because only one male flower was observed. The floral sex ratio was 1.58 and 0.46 during the May and June samplings, respectively. No ratio was calculated for the September sampling because only fruits

were observed. No ratios were calculated in February or January samplings due to no observed flowers. The overall floral sex ratio that was calculated from the three months where flowers were observed in significant numbers (May, June, and September) was 0.69. Site-specific floral sex ratios were highly variable spatially and temporally, ranging from 0 to 7.75 in May and 0 to 2.33 in June. No site-specific ratios could be calculated for March (all males), September (all fruits), or February and January (no flowers). Interestingly, sites demonstrated flowering patterns of either only male flowers (i.e. 214, 235, 241, 243, 284, 287, 307, and 314), only female flowers (i.e. 239, 267, and 276), both sexes (i.e. 215, 216, 220, 223, 225, 227, 237, 248, 255, 269, 285, 291, 294, and 305), or no flowers (i.e. 260, 271, 273, 296, and 309). These patterns account for much of the observed spatial and temporal variation in sex ratios as well as the subsequent difficulty in calculating ratios for many of the sites at a given time. Sites characterized as exclusively male accounted for 8 of 30 (26.67%) sites, as exclusively female accounted for 3 of 30 (10%) sites, as both male and female accounted for 14 of 30 (46.67%) sites, and where no flowers were observed accounted for 5 of 30 (16.67%) sites. Generally, the early part of the flowering season demonstrated male dominance and the latter part demonstrated female dominance. However, the overall floral sex ratio was female-biased and may also reflect the longevity of female floral bodies as developing and maturing fruits.

FKNMS demographic core data. A total of 3758 short shoots was assessed for age structure, sexual reproduction (flowering), and asexual reproduction (as estimated by apex frequency). The mean number of leaf scars per short shoot for all sites was 33.48, ranging from 16.97 to 64.27, the mean number of leaves per short shoot for all sites was

3.29, ranging from 2.61 to 4.10, and the mean total leaves (extant leaves + leaf scars) for all sites was 36.77, ranging from 20.15 to 67.98 (Table 7). The mean Plastochrone interval for all sites was 42.66 days, ranging from 31.94 to 63.73 days. Total leaves was multiplied by the site specific calculated mean Plastochrone interval to estimate the ages of all short shoots yielding a mean short shoot age for all sites of 1471 days (4.03 years), with site-specific means ranging from 697 (1.91 years) to 2474 days (6.78 years).

T. testudinum flowering shoot totals and calculated shoot flowering frequencies were variable among sites. Additionally, sex-specific flowering shoot totals and shoot flowering frequencies were equally variable. A total of 261 flowering shoots were counted with site totals ranging from 0 to 42 flowering shoots (Table 8). A total of 27 sites (90%) contained short shoots with observed floral scars, and 3 sites (10%) contained no short shoots with floral scars. A total of 64 male flowering shoots was observed, with a range of 0 to 10 flowering shoots per core. A total of 22 sites (73.33%) contained male flowering shoots, and 8 sites (26.67%) contained no male flowering shoots. A total of 197 female flowering shoots was observed, with a range of 0 to 34 flowering shoots per core. A total of 27 sites (90%) contained female flowering shoots, and 3 sites (10%) contained no female flowering shoots. The mean calculated shoot flowering frequency for all pooled short shoots among the 30 sites was 6.95%, with a site-specific range of 0 to 35.29%, the mean pooled male shoot flowering frequency was 1.70%, with a range of 0 to 8.85%, and the mean pooled female shoot flowering frequency was 5.24%, with a range of 0 to 18.57%. In general, there appeared to be a notable spatial flowering trend, as evidenced from the floral scar data.. *T. testudinum* core total shoot flowering frequencies were higher at the upper Keys sites (214, 215, 216, 220, 223, 227, 235, 237,

239), with correspondingly higher sex-specific shoot flowering frequencies, relative to other regions of the FKNMS (Fig. 9). Although two of these sites (235 and 237) had low frequencies (0.78 and 0%, respectively), the overall trend is higher shoot flowering frequencies in this broad geographic region of the FKNMS.

Age reconstruction techniques allowed for a more detailed examination of *T. testudinum* phenology with respect to minimum short shoot age at first flowering, average short shoot age at first flowering, and short shoot reflowering . Through the estimation of short shoot ages and the estimation of dates of flowering, the minimum age at first flowering for all flowering shoots averaged 911 days across sites with a range of 252 to 2668 days (Table 9). Site-specific data on minimum flowering ages are not reported as site means, rather, they represent the lowest calculated age at first flowering of a single flowering shoot (regardless of sex) observed among the total number of flowering shoots observed from a site, respectively. The mean male minimum age at first flowering for all sites was 1425 days, with a range of 483 to 3286 days and the mean female minimum age at first flowering for all sites was 962 days with a range of 252 to 2668. Based on the sex-specific minimum flowering age data, males generally flower for the first time at an older age relative to females. Additionally, males were observed to have the highest single age at first flowering (3268 days) and females were observed to have the lowest single age at first flowering (252 days). The mean average short shoot age at first flowering for all flowering shoots observed at the 30 sites was 1658 days, with a range of 684 to 3175 days, the mean average male short shoot age at first flowering for all sites was 1763 days, with a range of 789 to 3286 days, and the mean average female short shoot age at first flowering for all sites was 1611 days, with a range

of 684 to 3175 days (Table 10). As was observed in the minimum shoot age at first flowering data, these average shoot age at first flowering data suggest that male flowering shoots are generally older at first flowering relative to female flowering shoots. Additionally, male flowering shoots were observed to have the highest average age at first flowering (3286 days) and female flowering shoots were observed to have the lowest average age at first flowering (684 days), respective of sites, demonstrating the same trend as observed in the sex-specific minimum shoot age at first flowering data. To assess the level that *T. testudinum* reflowered, it was necessary to consider raw floral scar numbers from all flowering shoots observed among the 30 sites. For all shoots assessed, 340 total floral scars were observed, with sex-specific totals of 71 males and 269 females. Short shoots that contained more than one floral scar were counted, respective of site and sex, and subsequently pooled. Individual shorts shoots were observed to reflower up to 7 times, based on floral scars, but generally reflowered only once or twice (data not shown). A total of 52 reflowering short shoots was observed among the 30 sites, with 7 male reflowering short shoots (13.46% of total reflowering shoots) and 45 female reflowering short shoots (86.54% of total reflowering shoots). Reflowering short shoots occurred at 13 of 27 (48.15%) sites where flowering shoots were observed. The mean reflowering frequency for all sites was 19.19%, with a site-specific range of 0 to 42.86%; mean male reflowering frequency was 10.94%, with a site-specific range of 0 to 33.33% and reflowering short shoots occurring at 6 of 22 (27.27%) sites where male flowering shoots were observed; the mean female reflowering frequency was 22.84%, with a site-specific range of 0 to 46.15% and reflowering short shoots occurring at 12 of 28 (44.44%) where female flowering shoots were observed (Table 11). Female short shoots

constituted the majority of all observed reflowering short shoots with respect to numbers. Additionally, female short shoots reflowered at a higher frequency relative to male short shoots.

The male:female sex ratios of flowering short shoots was examined. The mean male:female sex ratio of flowering shoots (n_{pooled} = 261, n_{male} = 64, n_{female} = 197) for all sites was 0.32, with a site-specific range of 0.12 to 1.50 (Table 12). A total of 5 out of 27 (18.52%) sites was observed to contain only female short shoots, based on floral scars (216, 235, 260, 276 and 284, respectively). No sites were observed to contain only male short shoots. Generally speaking, the demographic flowering data suggests a female-biased floral sex ratio for the FKNMS.

Asexual reproduction was assessed by counting the number of rhizome apices in a core. Apices were observed to be attached at the leading end of a rhizome or branching directly from a short shoot. Live rhizomes that were found loose in a core were counted and included in the total for each site, respectively. The rhizome frequency (% of short shoots) was calculated for each site, and it is the relative estimation of the amount of asexual reproduction of that site, respectively. A total of 585 apices were observed among the 30 sites, corresponding to a mean apical frequency of 15.57%, with spatially variable site-specific apical frequencies ranging from 0 to 34.13% (Table 13).

Historical reconstruction of flowering events. All aged short shoots were pooled together to produce an age-frequency distribution of *T. testudinum* in the FKNMS. Short shoots were separated into bins corresponding to 365 days (1 year), and based on the ages of the youngest and oldest shoots, a total of 22 bins was necessary to categorize all short shoots into age cohorts. The mean short shoot age was 1471 days

(4.03 years) (Fig. 10). Age-frequencies are skewed to the left which is indicative of relatively higher totals of younger short shoots (<1 to 4 years old) being observed among the total population. The histogram suggests higher mortality for short shoots that are 1 year or younger. Once short shoots reach an age of two years, the slope of the mortality curve appears to decrease to a more stable slope among cohort groups. Short shoots were observed in all age cohort classes from 1 year to 21 years. By counting back days from the dates of core collections, the birth dates for all short shoots were estimated (data not shown). From these estimates, the birth of oldest short shoot collected dates back to 1981.

Historical trends in *T. testudinum* flowering frequencies were assessed by estimating the date at flower for all floral scars based on their relative positions between leaf scars on a short shoot. Flowering dates were calculated as a discreet date in time (day/month/year) and subsequently pooled according to year. Flowering frequencies were calculated as the % of flowering shoots in the eligible pooled short shoot total, respective of year. For example, the calculated flowering frequency for 1990 was based on floral scars observed from the total number of short shoots that were alive from 1981-1990. The flowering frequency for all years (1990-2001) was calculated in this manner by incrementally increasing the pool of eligible shorts, respective of year and relative short shoot ages. The flowering frequency for 2002 was calculated from the cores at sites 214, 220, and 273 because these alternate cores were collected in September of 2002. Therefore, the flowering frequency for 2002 could only be estimated from the pool of short shoots from these sites, solely. Historical flowering frequencies estimated from 1990-2002 ranged from 0.70 to 3.60% (Table 14). In general, flowering frequencies

were relatively invariable over these 13 years based on the collection of shoots measured.

Comparison of observational and demographic flowering data. Flowering data was compared to assess the relative agreement and consistency between direct observation and age reconstruction of *T. testudinum* flowering trends and sex ratios in the FKNMS. Demographic age reconstruction site-specific flowering frequencies were higher in 29 of 30 sites (305 being the exception) compared to observational estimates, and the mean demographic flowering frequency exceeded the mean observational estimate by nearly five times. Mean annual historical flowering reconstruction estimates, while not site-specific, had an overall agreement with the mean observational estimate, as the mean observational flowering frequency estimate (1.49%) fell within the range of annual historical mean flowering frequency estimates (0.70-3.60%) (Table 15). There was not a high degree of consistency between site-specific observed and demographic core floral sex ratios. Observed floral sex ratios constituted a higher amount of male-biased ratios (15 of 30 sites, 50%) than did demographic floral sex ratios (5 of 30 sites, 16.67%), and the range of estimates was considerably greater in the observed versus demographic data. However, the mean sex ratio for both observed and demographic data was consistent in being female-biased (Table 16).

The relationship of water quality, seagrass vitality, and nutrient availability with *T. testudinum* flowering trends in the FKNMS. A total of 25 water quality variables, 13 seagrass vitality variables, 6 nutrient availability variables, and *T. testudinum* apex frequency (measured from the demographic cores) was correlated to 14 flowering variables (observed total flowering frequency (OTFF), observed total male flowering frequency (OMFF), observed total female flowering frequency (OFFF),

observed fruit frequency (OFrF), demographic shoot flower frequency (DSFF), demographic male shoot flower frequency (DMFF), demographic female shoot flower frequency (DFFF), minimum shoot age at first flowering (MAF1), minimum male shoot age at first flowering (MMF1), minimum female shoot age at first flowering (MFF1), average shoot age at first flowering (AAF1), average male shoot age at first flowering (AMF1), average female shoot age at first flowering (AFF1), and apex frequency (AF). There were 350 cases in the water quality group with 9 significant correlations (Table 17). DSFF was negatively correlated with NO_2^-, DMFF was negatively correlated with NO_2^-, TOC, TN, and TON, AAF1 was negatively correlated with TON and Kd, AFF1 was negatively correlated with Kd, and AF was positively correlated with NO_3^-. There were 182 cases in the seagrass vitality group with 7 significant correlations (Table 18). OFrF was negatively correlated with leaf number, DMFF was negatively correlated with productivity, AAF1 was negatively correlated with leaf number, and AMF1 was correlated with density, areal productivity, leaf area productivity ($cm^2m^2d^{-1}$), and Plastochrone interval. There were 84 cases in the nutrient availability group with 10 significant correlations (Table 19). OTFF was negatively correlated with %N and positively correlated with C:N, OTFFF was negatively correlated with %N and positively correlated with C:N, OFrF was negatively correlated with %N and positively correlated with C:N, AAF1 was negatively correlated with %N and positively correlated with C:N, and AMF1 was negatively correlated with %N and positively correlated with C:N. When AF was used as a predictor variable, there were 13 cases yielding one significant negative correlation with DFFF (Fig. 11).

N + P nutrient addition experiment. At all sites, mean calculated flowering frequencies were higher in control versus N + P plots. The mean flowering frequency for inshore control and N + P plots was 2.11 and 0.46%, respectively, and the mean flowering frequency for offshore control and N + P plots was 2.63 and 0.37%, respectively (Table 19). Flowering at inshore control plots was 4.58 times greater than inshore N + P plots, and flowering at offshore control plots was 7.11 times greater than offshore N + P plots. Site-specific mean flowering frequencies were variable over the course of the flowering season in both inshore and offshore control and N + P plots. At inshore sites, mean control and N + P flowering frequencies at 213 were 3.36% (0-11.84%) and 0%, respectively (Fig. 12), mean control and N + P flowering frequencies at 214 were 3.10% (0-9.49%) and 0.71% (0-1.60%), respectively (Fig. 13), mean control and N + P flowering frequencies at 220 were 1.45% (0-3.73%) and 0.18% (0-0.65%), respectively (Fig. 14), mean control and N + P flowering frequencies at 223 were 2.02% (0-5.62%) and 1.12% (0-2.64%), respectively (Fig. 15), and mean control and N + P flowering frequencies at 227 were 0.25% (0-0.97%) and 0%, respectively (Fig. 16). At offshore sites, mean control and N + P flowering frequencies at 215 were 1.31% (0-2.41%) and 0.06% (0-0.16%), respectively (Fig. 17), mean control and N + P flowering frequencies at 216 were 0.77% (0-2.30%) and 0.61% (0-1.43%), respectively (Fig. 18), mean control and N + P flowering frequencies at 217 were 2.26% (0-6.89%) and 0.15% (0-0.37%), respectively (Fig. 19), mean control and N + P flowering frequencies at 224 were 7.23% (0-23.25%) and 0.59% (0-1.98%), respectively (Fig. 20), and mean control and N + P flowering frequencies at 225 were 1.39% (0-3.66%) and 0.73% (0-2.18%), respectively (Fig. 21).

Floral numbers were compiled and sex ratios were calculated for all control and N + P treatment plots at all ten sites. The mean inshore control sex ratio was 1.24 with a site-specific range of 4.55-0.18, the mean inshore N + P sex ratio was 2.17 with a site-specific range of 5 to 0 to 1.27, the mean offshore control sex ratio was 3.58 with a site-specific range of 95 to 0 to 0 to 30, and the mean offshore N + P sex ratio was 10 with a site-specific range of 15 to 0 to 5.33 (Table 20). The total inshore + offshore control and N + P sex ratio was 2.29 and 4.13, respectively. Generally, sites are characterized as being male biased, with 8 of 10 control sites (80%) and 8 of 8 (100%, sites 213 and 227 had no observed flowers) N + P sites having male ratios or only male flowers observed. Control sites had higher numbers of observed flowers and fruits ($n_{male} = 702$; $n_{female(total)} = 306$; $n_{fruit} = 86$) relative to N + P sites ($n_{male} = 132$; $n_{female(total)} = 32$; $n_{fruit} = 11$).

Chapter V. DISCUSSION

On *T. testudinum* phenology through observation and demographic age reconstruction. *T. testudinum* demonstrated variable spatial and temporal patterns in flowering across the FKNMS. Indeed, among the 30 sites, a range of 0% to 24.21% of short shoots were observed to be flowering at a particular time. In general, the relatively low mean flowering frequency calculated for all sites suggests that *T. testudinum* flowering is a rare phenomenon when considered among the vast number of individual short shoots present in the FKNMS. The timing of sampling is very important to consider when attempting to describe *T. testudinum* flowering due the patchiness of flowering effort observed among separate populations. Therefore, it is possible to either underestimate or overestimate the flowering frequency depending on location and the relative stage of floral anthesis. Previous investigations of *T. testudinum* flowering have demonstrated this "hit or miss" timing with respect to floral sampling. Reports from single samplings of *T. testudinum* flowering in Tampa Bay were as high as 38%, and from 3% to 28% at three sites in the lower Florida Keys in the same year (1979) (Durako and Moffler 1985a). Observations of *T. testudinum* flowering from the Mexican Caribbean (1991) ranged from 1.8 to 3.5% (van Tussenbrock 1994). While it may be difficult to compare highly variable mean flowering trends on smaller spatial scales from one region to the next, it is noteworthy that the degree of variability I observed in the FKNMS populations fell within the ranges reported from other geographic regions. Thus, it may be inferred that *T. testudinum* exhibits similar variability in flowering patterns across its range, and future attempts at quantifying *T. testudinum* flowering, for the purpose of describing or modeling flowering trends, may enjoy a wider functional

applicability of investigations ranging from the local to regional scales. Based on my observations, it is important to institute a more temporally rigorous sampling schedule to insure an increased accuracy of flowering estimates in lieu of a long and staggered flowering season. I sought to obtain a more accurate estimate of *T. testudinum* flowering, on a far greater geographic scale than previously attempted, through multiple observations of specific populations over the entire duration of a flowering season. While I expected floral expression to vary over the several months, it was interesting to observe a pronounced sex-specific timing to floral anthesis. Males accounted for the majority of flowers observed at the beginning of the flowering season, with fewer female flowers and some immature fruits observed as well. By June, an opposite trend was observed with a greater number of females observed relative to males. Additionally, >50% of the females observed were fruits. By September, the only remaining observed flowering activity was maturing fruits. Based on this observed flowering pattern, males appear in large numbers and pollinate scarcer females very shortly after anthesis, which in turn immediately begin to set fruit early in the season. However, immature fruits were observed to be developing in early, middle, and late season. In particular, fruits were observed to be developing in greater numbers long after male flowers had dehisced and disappeared from the flowering population. This begs the obvious question, where is the pollen necessary for later season fruit setting flowers coming from? Dioecious plants (i.e. *T. testudinum*) do not allocate resources to both male and female function in a single plant. Resource allocation to sexual reproduction, respective of both sexes, effectively determines the number of flowers produced by each sex in a dioecious plant (Sutherland 1986a). Because male and female reproductive success is ultimately limited by the

number of flowers produced by the opposite sex, natural selection should favor a strategy that would optimize the relative number of male and female flowers produced, thus maximizing the probability of successful pollination while also maximizing plant vigor. This leads to many dioecious plant species producing more male flowers than female flowers. For certain terrestrial plants, fruiting success (i.e. fruit maturation and seed set) is largely limited by the number of male flowers (i.e. pollen limitation) present in the breeding population (Stephenson 1981), and this condition has been postulated for the *T. testudinum* breeding system (Les 1988). Generally speaking, FKNMS sites that were observed with high numbers of male flowers did not have high numbers of female flowers, and likewise, sites that maintained the highest number of female flowers and fruits were observed with little to no male flowers. This observed phenomenon may be a function of single large genets, contiguous and/or fragmented, that dominate the sites sampled with respect to short shoot numbers. It is unlikely that scarce male flowers dotted among larger populations of female flowers are solely responsible for the successful pollination of many or all female flowers in a given area, especially in middle to late season when male flowers have long since disappeared. Additionally, it makes little sense energetically for predominantly male populations to expend the energy to produce flowers and pollen that is likely to be wasted due to a scarcity of female flowers to pollinate. Rather, I would hypothesize that certain male populations may act as pollen sources to female flowers at greater geographic distances and to female flowers that develop later in the season relative to the timing of male anthesis. Since male floral anthesis and pollen dehiscence progresses quickly and early in the flowering season (about two weeks, pers. obs.), pollen would have to be viable for up to three months to

account for fruit development in the latest part of the season. Fruit maturation is typically eight weeks (pers. obs.), and fruit development is observed throughout the flowering season, however, the greatest number of fruits develop weeks after most male flowers have dehisced and disappeared. *T. testudinum* pollen is released in slimy "rafts" that makes its way through the water column moved by the direction of currents (Cox and Tomlinson 1988). While there is no literature on *T. testudinum* pollen viability, it is feasible that if pollen is viable for several weeks, it could effectively pollinate a female flower many miles and many weeks away. For example, site 239 in my investigation had the highest number of female flowers and fruits observed among all sites. Interestingly, not a single male flower was observed at this site in any of the months sampled, yet fruits continued to initially develop and mature very late in the season. My investigation was not designed to address pollination questions directly, however this idea would be an important research question to address in future studies relating to *T. testudinum* reproductive ecology. The vectors for the potential transfer of genetic information among these predominantly clonal, genetically uniform (and therefore sexually uniform) dioecious plants are potentially more important and complex than are presently understood.

T. testudinum flowering, as evidenced through age-reconstruction techniques, was spatially variable. Flowering frequencies, as measured by the number of flowering short shoots, were higher relative to observed flowering frequencies. These values are consistent with previously reported demographic core *T. testudinum* flowering frequency data. A report from the Mexican Carribean (1991) yielded 17% of shoots examined had flowered (Gallegos et al. 1992), three sites from Tampa Bay (1992) yielded flowering

frequencies of 3, 27.5, and 23.1% (Witz and Dawes 1995), and demographic core flowering frequency data obtained from Lower Laguna Madre Bay, TX (1996) was reported at 13.7 to 33% (Kaldy and Dunton 2000). The shoot flowering frequency data obtained in my investigation falls within the ranges reported in the literature. It is unclear, however, whether the flowering frequencies reported in the literature are calculated from shoot flowering frequencies or from floral scar frequencies. The important difference between the two is that shoot flowering frequencies are calculated only from the number of shoots that have flowered regardless of whether or not there are multiple floral scars on a particular shoot (i.e. reflowering), whereas floral scar frequencies are calculated from the total number of floral scars observed among the total number of shoots examined. *T. testudinum* short shoots have not been previously observed to flower multiple times in a single flowering season, therefore, short shoots bearing multiple floral scars cannot be counted as such in a discreet estimate of flowering from age reconstruction techniques. For my investigation, the functional measure of flowering used was the shoot flowering frequency, because I felt it was a more conservative measure. However, this type of measurement may only be used and interpreted as a type of "flowering index" encompassing many years of flowering history because a core of short shoots contains shoots of different ages that may or may not have flowered at a particular time. Alternatively, counting floral scars is useful for estimating and reconstructing historical flowering frequencies. In my study, based on the short shoots from the cores I obtained, I reconstructed flowering frequencies from 1990-2002. I took advantage of the fact that I could estimate the ages of short shoots as well as their ages at flowering, and I then converted those ages (in days) to discreet dates in time.

Therefore, if there is confidence in the age reconstruction of short shoots and dates of flowering, then all short shoots have the potential to record yearly flowering trends. Based on the age frequency histogram that I generated, inclusive of all short shoots examined, I made the assumption of an equal probability of shoot mortality among age classes after a short shoot reached an age of two years, and I made the implicit assumption that there was no flowering-related shoot mortality. I made the additional assumption that short shoots in an age cohort were distributed normally about all pooled short shoots examined in the cores, and therefore, I was able to reconstruct past flowering events with a limited number of short shoots and flowering short shoots in a particular age class with conservative confidence. Historical flowering estimates represent mean flowering for the FKNMS, inclusively, because there was not a normal distribution of short shoots among all age cohorts from specific site cores to reconstruct flowering trends at those respective sites with any measure of confidence. What is exciting to note is that the calculated historical flowering frequencies from 1990-2002 all fell around the mean observed FKNMS flowering frequency that I calculated from direct observation of flowers in the field, and I therefore maintain a reasonable measure of confidence that the techniques I employed are valid for this type of analysis. I would assert, based on the historical flowering frequencies calculated herein, that *T. testudinum* flowering frequencies are not significantly temporally variable.

In my investigation, I observed a high degree of variability and patchiness in *T. testudinum* floral sex ratios. The spatial and temporal pattern of variability was similar to what I observed with respect to flowering frequencies. A male biased ratio is expressed early in the season, and female biased ratios are expressed later in the season, consistent

with the timing of flowering. While I observed an overall female biased floral sex ratio in the FKNMS, it is important to note that several sites were observed with only males or females. Therefore, sampling on smaller spatial scales may lead to effectively skewing the sex ratio toward being more male or more female, depending on sampling location and timing. This fact further demonstrates the notion that the timing of sampling is important in estimating *T. testudinum* flowering, and improper attention to timing may lead to inaccurate sex ratio reports. Reports of sex ratios from the literature generally demonstrate a male bias and are sampled on small spatial scales. An investigation conducted in Biscayne Bay (1969) yielded a male ratio of 4 (Tomlinson 1969). Researchers reported a male ratio of 1.4 in Tampa Bay and 1.27 for three sites in the lower Florida Keys during the same year (1979) (Durako and Moffler 1985a). An overall male biased ratio from three sites in the Mexican Caribbean (1991) was 1.12 (van Tussenbrock 1994). Indeed, the literature consistently points to a male *T. testudinum* sex ratio, however, considering the data from my investigation, 16 of 44 site-specific ratios calculated were female biased, yet the total number of females observed in the FKNMS drove the overall ratio . Therefore, it is unlikely that a single sampling event could accurately estimate a sex ratio for a given area when flowering is patchy and sex-specific floral anthesis has a difference in timing. Whether the objective is to sample on a small spatial scale or on a regional scale, the differential timing of floral sex expression mandates that multiple samplings be performed in order to more accurately estimate *T. testudinum* sex ratios. *T. testudinum* sex ratios among separate populations may vary according to certain sex-specific phenological phenomena. Yearly differences in *T. testudinum* sex ratios have previously been attributed to the annual variation in the density

of males, as the density of females remained fairly constant temporally (Durako and Moffler 1985a; Durako and Moffler 1985b). Further temporal studies demonstrated that male biased sex ratios correlated with the year of highest seed output (Durako and Moffler 1985a). It is unclear, however, how floral and fruit abortion affects *T. testudinum* seed production and seedling success. Flower and fruit abortion as well as pollen:ovule ratios in flowering plants have been a subject of investigation. For example, in plants that practice selective fruit abortion, more flowers are produced and more immature fruits are initiated than can be finally matured as fruits (Cruden 1977; Stephenson 1981; Sutherland 1986a; Sutherland 1986b). The plant selectively retains only those fruits that are of a high genetic quality (Stephenson 1981). This may allow for the plant to conserve resources that may otherwise be wasted on failed fruits or fruiting structures, and may allow for better overall vigor and fitness of the individual plant. Additionally, reproductive resource allocation may be directed toward the development of solitary fruits, which may help to promote a higher percentage of fruit maturation. My study did not specifically address *T. testudinum* flower and fruit abortion nor did I quantify seed output. Floral abortion may play a significant role in spatial and temporal sex ratio trends, which may in turn, affect trends in seed production. Additionally, selective flower consumption by parrotfishes or other grazers (Fourqurean pers. comm.) may affect sex ratio trends and seed production. Future studies should include multi-year floral monitoring and seed quantification of specific *T. testudinum* populations in order to better describe trends in sex ratios and the relationship of sex ratios to fruit and seed production.

The demographic core male:female flowering shoot sex ratio, not surprisingly, was female biased. The mean calculated demographic core ratio was lower than that calculated from direct observation. Additionally, the male:female sex ratio of floral scars

was lower than the flowering shoot sex ratio, reflecting the gain in total female floral scars over male scars due to a higher female reflowering frequency. I expected that the demographic core sex ratios would overestimate the female bias in the same way that flowering frequencies were overestimated. Therefore, these values need not necessarily reflect the ratio obtained from direct observation. Rather, they should act as a record through time, preserving overall multi-year trends in sex ratios on a local as well as on a regional scale. I feel that the resolution from data obtained from the cores cannot be used to accurately describe trends in sex ratios in discreet time when compared to the resolution garnered from direct observation. However, with a large enough sample of short shoots in cohort classes, this would be theoretically possible. Given the relative size of cores likely needed to be collected in order to perform this type of analysis, it may only be possible to describe sex ratios in general terms as either male or female biased. For this study, I can only conclude that the demographic core data demonstrates a trend that supports what I directly observed in the field.

Many more female shoots were present in the cores relative to males. The mean female shoot flower frequency was over 3 times higher than the mean male shoot flower frequency. I had considered why significantly more female short shoots were represented in the cores. The relatively high cost of being a female that produces large fruits (relative to the size of a short shoot) with viviparous seedlings would appear to be a large risk for female short shoots to contend with in the management of the resources needed for photosynthesis and respiration in an oligotrophic environment. Consequently, one may expect that mortality rates are greater among female flowering short shoots compared to male flowering short shoots. However, fruits may actually be a benefit to female short

shoots because, while maturing, fruits contain chlorophyll, are photosynthesizing, and thus may contribute to overall plant vigor. Fruit production has been shown to be a positive benefit in certain terrestrial plants by acting as an additional photosynthetic source, and this benefit has been shown to offset and even exceed the energetic cost to the parent plant of producing those fruits (Bazzaz and Carlson 1979). It is possible that once a *T. testudinum* fruit obtains a critical mass, the energy needed to produce seedlings may originate entirely from the fruit itself. Nutrients may be translocated along the entire genet to meet the needs of the developing seedlings. Certain Philippine seagrasses maintain high rates of N and P translocation among flowering shoots on a common genet (Duarte et al. 1997). *T. testudinum* female genets may have one or more ramets producing fruits at the same time (pers. obs.), and resource translocation from non-fruit producing short shoots may be an important source of nutrients to fruit producing short shoots. Therefore, energetically speaking, female flowering short shoots need not necessarily be at a higher risk for mortality when compared to male flowering shoots. An alternative explanation for why more female flowering shoots are present in the cores may lie in the genetic programming of the plants themselves. Female flowering short shoots had a lower overall minimum age at first flowering compared to males. Additionally, female flowering short shoots had a lower overall average age at first flowering compared to males. This implies that females are flowering at earlier ages than males. Due to the nature of short shoot mortality, I would expect to find fewer older short shoots in a core, and therefore, I would expect to find fewer male flowering short shoots represented in the cores relative to female flowering short shoots. Plant resource allocation theory, as it pertains to sexual reproduction, contends that a parent plant needs

to obtain a "critical mass" before it can allocate precious resources to reproduction (Bazzaz and Grace 1997). Female flowering short shoots may be genetically predisposed to allocate resources toward sexual reproduction at a younger age and smaller size relative to male flowering short shoots. Indeed, if females are younger than males when they first start to produce flowers, it stands to reason that they may contain less biomass (above- and below-ground) when compared to males. Future studies in energetics should focus on the fate of resources in male and female plants as it pertains to sexual reproduction.

Another aspect of *T. testudinum* phenology that may be evidence of increased female shoot vigor lies in the higher observed female shoot reflowering frequency compared to male reflowering shoots. Of the 52 shoots that had reflowered, 45 were female shoots. Female shoots reflower at a higher frequency compared to males and, consequently, female reflowering shoots are more abundant compared to males. Additionally, further qualitative evidence suggests that female flowering shoots benefit significantly from the photosynthetic contributions made by fruits, and that producing fruits increases the overall health and vitality of female flowering shoots. Future studies on *T. testudinum* reproductive ecology and shoot vigor in flowering female short shoots should be aimed at quantifying the destiny of resources toward fruit production and the potential photosynthetic benefits of and consequences to plant resource management they may provide.

One of the objectives of this study was to compare two different ways of assessing *T. testudinum* flowering trends and evaluate the effectiveness and agreement of both methodologies. Direct observation offers significantly better resolution of spatial

and temporal flowering trends over that obtained by age reconstruction techniques. Additionally, direct observation allows for a description of sex-specific anthesis timing that is not possible with age reconstruction techniques. Fruit production may only be assessed through direct observation, and direct observation allows for a more accurate assessment of floral sex ratios. Through age reconstruction, I expressed the core data flowering frequencies by the relative number of flowering shoots. This is useful for describing historical flowering trends in a given area, provided that the sample size can be normalized, thus requiring a large sample. As age reconstruction depends on the ability to age particular shoot shoots using leaf scars and Plastochrone intervals, higher relative mortality in older cohort classes makes it impractical to assume that a single core holds enough short shoots from various age cohorts to accurately estimate flowering frequency. At nearly every site sampled in my investigation, demographic core shoot flowering frequencies were higher than observed flowering frequencies. The uneven distribution of shoot ages that I observed in my investigation further illustrates the notion that small sample sizes may lead to biased results on local spatial scales. Sites with an under-represented number of shoots of flowering age (i.e. high mortality and/or shoot turnover rate) tended to have low flowering frequencies calculated from the cores. Conversely, sites that had a higher relative proportion of older shoots of flowering age (i.e. low mortality and/or low recruitment) tended to have higher calculated flowering frequencies. Pooling short shoots from a larger geographic area, such as the FKNMS, with diverse local habitat differences and variance around a mean recruitment and mortality rate is the only manner to obtain a normal sample for this type of analysis. Another source of error in assessing flowering frequencies with demographic cores lies in

the nature of how male shoots flower. Individual male short shoots produce one to three flowers per shoot on average (pers. obs.), however demographic core analysis can only provide a single flower estimate per male short shoot per floral scar. Therefore, demographic male flower frequencies along with total flower frequencies may be inherently underestimated. I did not account for this in my presentation of the demographic data because I wanted to evaluate the methodology solely on the merit with which it is able to support itself through untransformed or extrapolated data. Given that demographic core flowering frequencies were higher than observed flowering frequencies, multiplying male floral scars by a value of two or three will further inflate flowering frequencies and create a greater discrepancy between the two data sets. It is noteworthy, that observational and demographic core mean flowering frequencies were correlated (data not shown). While this fact does little to illuminate observed patterns of site-specific flowering variability and patchiness, it may suggest that a synthesis of these two different methods of assessing flowering may be realized under certain conditions and assumptions - the most important of these being a normal sample of short shoots that reflects an accurate age structure. Additionally, this evidence may be used to further support the notion that mean *T. testudinum* flowering frequencies are temporally stable. Further investigation is necessary to better determine how observational and demographic reconstruction flowering data may be best synthesized to provide the most accurate and precise details related to *T. testudinum* phenology and sexual reproduction.

A different problem arises when attempting to compare floral sex ratios from both methodologies. Observed sex ratios were calculated on a male flower:female flower basis, and I feel that this estimate is proper. The lower male:female sex ratios calculated

from the demographic cores may be accounted for in one of two scenarios: 1) historically, the density of male flowers fluctuates, and any variation of the ratio calculated from flowering shoots or floral scars is a value that will simply reflect the temporal stability of female flowering shoots, or 2) due to the restricted resolution that floral scars impose on determining the number of male flowers (only one scar for up to 3 flowers), realized sex ratios will always be lower (i.e. more female biased) than the true historically represented ratio may actually be. For these reasons, I am uncertain that demographic core data can be accurately used to describe floral sex ratios aside from describing general trends.

Where demographic core data may be used and applied with confidence is in the historical reconstruction of flowering events. By having a large enough sample size and by normalizing flowering shoots into cohort classes, historical flowering reconstruction is permissible. In my investigation, it was not possible to examine historical flowering trends on a site by site basis because the samples taken from the sites were too small to include a normal distribution of shoots in a given cohort needed to make historical flowering estimates. Future investigations of historical flowering reconstruction at a smaller geographic scale should include a power analysis of the number of shoots necessary for having normally distributed shoots in a given cohort class, given the age frequency distribution of the short shoots in the area of focus, in order to insure that the proper number of random short shoots are collected.

Evidence for relationships between *T. testudinum* flowering and water quality, seagrass vitality, and nutrient availability. Flowering response variables were most significantly correlated to nitrogen variables as a group. Specifically, a negative

relationship to water quality nitrogen variables and leaf tissue N content existed suggesting that flowering is reduced with high N availability. This relationship was demonstrated in both observational and demographic flowering sets, yet each set was correlated to two separate groups of N variables. Demographic shoot flowering frequency and male shoot flowering frequency was related to the water quality variables while the observed total flowering frequency, observed total female flowering frequency, and observed fruit frequency was related to *T. testudinum* leaf tissue %N content and C:N. While phosphorus has generally been cited as the primary limiting nutrient in carbonate environments (Morse and Others. 1985; Short et al. 1985; Short et al. 1990), new evidence has been reported which suggests that nitrogen may play a larger role in nutrient limitation of coastal carbonate systems (Ferdie and Fourqurean 2004). The correlative evidence from my study suggests that N availability may be a determining factor in driving general trends in *T. testudinum* sexual reproduction, and as a limiting nutrient, N may significantly affect yearly *T. testudinum* flowering frequencies on smaller spatial scales. Limiting nutrients have been shown to play an important role in determining yearly flowering frequencies of Philippine seagrasses (Duarte et al. 1997). In the FKNMS, N availability, as evidenced from seagrass leaf tissue content and stoichiometric elemental ratios, has been shown to vary spatially and temporally (Fourqurean and Zieman 2002). Based on this apparent relationship between flowering and N availability, it may be possible to model yearly trends in *T. testudinum* sexual reproduction by using nutrient availability as the proxy. Furthermore, with increasing eutrophication threatening the nearshore coastal ecosystems of the Florida Keys, I would infer that increased N loading may affect future trends in *T. testudinum* sexual reproduction.

Interestingly, apex frequency was positively correlated with surface water NO_3^- and negatively correlated with the demographic female shoot flowering frequency. This is significant because it demonstrates a tradeoff where asexual reproduction may be increased at the expense of sexual reproduction in high N conditions. Asexual reproduction may be further inferred to be limited by N availability in a reciprocal manner to how sexual reproduction may be limited. Based on this evidence, there is now a basis to suggest that a direct relationship between asexual and sexual reproduction exists in *T. testudinum*. I would propose a hypothetical model for *T. testudinum* reproduction, based on N as the limiting resource, where eutrophic conditions (i.e. high N) illicit a response for short shoots to allocate resources toward producing more shoots and branching rhizome apices, thus increasing reproductive fitness without the energetic expense of sexual reproduction. High N availability has been implicated in driving higher short shoot turnover rates and younger short shoot age structure in *T. testudinum* monospecific and mixed seagrass beds in the FKNMS (Fourqurean et al. 1998). Conversely, when N is limiting, plants may become stressed and dedicate reproductive resources toward producing flowers and fruits in order to relocate their progeny to areas more conducive to asexual reproduction, thus improving reproductive fitness. Net fitness may be increased with high N because the production of new short shoots by asexual clonal propagation far exceeds the production of new short shoots from seedlings. Additionally, net reproductive effort is increased with high N conditions. Future studies regarding this interesting relationship between N availability and reproduction should focus on quantifying asexual fitness (clonal production) and sexual fitness (seedling production, dispersal, and survival) as a function of N availability.

Seagrass vitality data contained two significant negative relationships with observed fruit frequency and demographic male shoot flowering frequency, however, this group of predictor variables was correlated most strongly with response variables related to *T. testudinum* phenology. Specifically, short shoot density, various measures of primary productivity, and the Plastochrone interval were positively correlated with the average male shoot age at first flowering. The data suggests that males are older at first flowering at sites where productivity is higher. These results may be used to explain, in part, why males are more scarce among flowering shoots than females. Males may be environmentally selected to occur in areas where conditions are peak for growth (i.e. increased nutrients, light, and space), and as a result, sexual reproduction becomes reduced. Males may therefore be allocating more resources toward growth and asexual reproduction, and less effort toward producing flowers. In dioecious plants, males often exceed females in general vigor and size, reflecting the generally lower costs of being male (Bazzaz and Grace 1997), and *T. testudinum* appears to follow this pattern. To illustrate this, comparisons of leaf width between the sexes indicated that leaf width constituted a secondary sex character for the species (Moffler et al. 1981). Female short shoots tend to have narrower leaves than male short shoots, and based on leaf width sex characters, males may have a higher growth rate than females during floral production and maintenance (Williams and Lyon 1997). However, narrow leaf widths have also been attributed to low salinity, reduced plant vigor, and low-light conditions due to depth or turbidity (Zieman 1975). Anecdotal, yet significant evidence from my investigation suggests that TOC is negatively related to male flowering frequency. High TOC may mean high phytoplankton in the water and/or high epiphyte loading. This could

potentially increase light attenuation in the water column, thus decreasing incident light to the seagrass canopy. Light stress could potentially adversely affect male flowering. Other anecdotal evidence from my study suggests that the average female shoot age at first flowering is negatively correlated with Kd, and may be a further indication that poor light conditions affect *T. testudinum* flowering trends. Further evidence to suggest that first flowering episodes are being influenced by environmental conditions may be seen in that the average shoot age at first flowering and the average male shoot age at first flowering are negatively correlated with leaf tissue %N and positively correlated with leaf tissue C:N, where the average age for first flowering of shoots in general, and for male shoots in particular, is higher with lower relative N availability, based on leaf tissue %N and lower leaf tissue C:N. This trend stands as a bit of a contrast to the trend of lower flowering frequency with higher N availability. One may expect that lower flowering frequency with high N availability may be explained, in part, by the phenomenon of short shoots simply being older at first flowering, and since older shoots are generally less abundant, there is a lower likelihood of flowering. However, my data suggests short shoots that are first flowering in high N environments are, in fact, younger relative to short shoots that flower for the first time in low N environments. Rather, N as a limiting resource, may be a stressor to short shoots, and short shoots may need more time to grow to a critical mass before allocating resources for sexual reproduction in low N environments. Flowering short shoots in high N environments, while fewer in frequency when compared to flowering short shoots in low N environments, are reaching that critical mass at any earlier age and are therefore able to dedicate resources toward sexual reproduction at any earlier age. This appears to hold especially true for male

flowering shoots as a group. An alternative explanation may be argued based on higher *T. testudinum* short shoot mortality that is observed in areas of high N availability (Fourqurean and Zieman 2002). Short shoots may be flowering at an earlier age in high N conditions because there is a higher turnover of short shoots. There may be a greater genetic turnover of genets where the environment selects for short shoots that can flower at an earlier age in order to better insure the transfer of genetic information. Thus, while high N environments may provide favorable conditions for growth and asexual reproduction, sexual reproduction may be reduced as a function of high short shoot turnover with fewer older short shoots in the population to flower. Additionally, short shoots that do flower under these conditions flower at a younger age relative to short shoots in low N environments. Future studies relating to N availability and reproduction should consider how trends in genetics, demographics, and short shoot turnover rates (mortality and recruitment) affect this relationship.

N + P nutrient addition experiment. The results of this 15 month experiment demonstrated that flowering was significantly reduced at all sites where N + P were added. N + P plots also had significant decreases in short shoot density over the course of floral monitoring (see Appendix), however, flowering frequencies were calculated as the % of observed shoots, and therefore, the data is still valid and comparable to control plot flowering frequencies. While the N + P experimental data supports trends observed in the observed and demographic core data with respect to lower flowering frequencies with higher relative N availability, caution must be exercised in drawing conclusions on this data for comparison. The experimental design of this study did not test for the effects of N and P individually, and therefore, these results are confounded. Due to the

limited resolution that this experimental design allows for, I can only conclude that increasing nutrients, additively, had a negative effect on *T. testudinum* flowering across a natural nutrient gradient of nitrogen and phosphorus limitation. Future attempts to experimentally explore the role of limiting nutrients (N and P) across this natural nutrient gradient may include a repeated measures modified split plot design with random fixed whole plots of nearshore and offshore treatments spanning the natural nutrient limitation gradient. The number of subplots necessary for the proper investigation of flowering responses to N + P addition may be determined by a power analysis, with response variables explored by ANOVA and *t*-tests for significant interactions.

Chapter VI. CONCLUSION

T. testudinum flowering in the FKNMS is generally low and spatially variable. Flowering frequencies vary widely over the FKNMS with certain sites supporting no evidence of flowering. Within a flowering season, there is temporal variability in sex-specific floral anthesis, and the flowering season is staggered. Male flowers appear first in greater numbers compared to female flowers early in the season. These early season females set fruit, however peak female anthesis occurs after the peak male anthesis, and fruit development continues throughout the course of the flowering season, particularly after male flowers have disappeared. I have hypothesized that pollen may be viable for a longer period of time than previously considered in order to account for the higher number of fruits developing and maturing late in the flowering season. Floral abortion, fruit abortion, and selective grazing of floral structures may have a significant effect on overall seed production. Through historical reconstruction of flowering, I have demonstrated that mean flowering frequencies in the FKNMS are not temporally variable. It is not acceptable to determine the historical degree of spatial variability in local *T. testudinum* populations without a large sample size of short shoots normalized in shoot age cohorts. Male short shoots are generally older than female short shoots at the age of first flowering, however, females reflower at a higher frequency compared to males over the course of their lifetime. Female short shoots may benefit energetically from fruit production, and this may lead to increased female short shoot vigor compared to male short shoots.

The observed overall floral sex ratio in the FKNMS is female biased, however, sex ratios are spatially variable and temporally variable over the course of a flowering

season as dictated by sex-specific floral anthesis. It is unclear whether or not sex ratios are otherwise variable through time. Furthermore, it is unclear what specific factors or phenomena are responsible for affecting trends in floral sex ratios.

Age reconstruction techniques cannot provide accurate discreet estimates of flowering frequencies or sex ratios on small spatial scales because flowering frequencies are inherently overestimated and sex ratios are inherently underestimated due to the likely violation of assumptions ultimately related to an inadequate normal sample of short shoots. Additionally, male floral scars cannot offer the resolution of true floral numbers necessary to calculate sex ratios. Age reconstruction can be used, cautiously, to describe mean annual trends in flowering and broad trends in sex ratios on a given spatial scale provided the sample is normal.

N availability may affect *T. testudinum* flowering trends, based on statistically significant correlative evidence from long term water quality, seagrass leaf tissue content and stoichiometric elemental ratio data. In particular, flowering frequencies are lower with high N availability and higher with low N availability. Asexual reproduction is positively correlated to N availability. I would infer that *T. testudinum* asexual reproductive fitness is increased with a tradeoff being reduced sexual reproductive fitness in high N availability, and correspondingly, sexual reproductive fitness is increased with a tradeoff being reduced asexual reproductive fitness in low N availability. Total reproductive fitness is higher with high N availability relative to low N availability due to the prevalence of asexual clonal propagation. Short shoots, and particularly male short shoots, flower for the first time at an older age with low N availability and at a younger age with high N availability. An *in situ* N +P nutrient addition experiment conducted in

the upper Florida Keys where a natural gradient of N and P limitation exists resulted in decreased flowering in N + P treatments relative to control treatments. These results, while confounded by experimental design, support trends resultant from observed and demographic data that demonstrate low *T. testudinum* flowering frequency with high N availability and high flowering frequency with low N availability.

Table 1. Historical observations of *T. testudinum* flowering frequencies.

Location	year	# shoots observed	# flowers	frequency (%)
Miami, FL [a]	1964	—	—	> 1
Tampa Bay, FL [b]	1976	—	—	3.8, 2.3, 6.8
Cockroach Bay, FL [c]	1979	170	*na*	38
Egmont Key, FL	1979	923	*na*	26
Big Coppit, FL	1979	279	*na*	25
Lassing Park, FL	1979	4471	*na*	22
No Name Key, FL	1979	339	*na*	3
Egmont Key, FL [d]	1981	—	—	11.4
Egmont Key, FL	1982	—	—	20.7
Egmont Key, FL	1983	—	—	10
Egmont Key, FL	1984	—	—	24.4
Egmont Key, FL	1985	—	—	21.9
Quintana Roo, Mexico [e]	1991	307*	*na*	17
Puerto Morelos, Mexico [f]	1991	—	—	1.8 to 3.5
Cockroach Bay, FL [g]	1992	200*	6	3
Sunset Beach, FL	1992	429*	118	27.5
Sunshine Skyway, FL	1992	303*	70	23.1
Lower Laguna Madre Bay, TX [h]	1995	—	—	13.7 to 33.0
Lower Laguna Madre Bay, TX	1996	—	—	13.7 to 30.4

[a](Orpurt and Boral 1964); [b](Grey and Moffler 1978); [c](Durako and Moffler 1985a); [d](Durako and Moffler 1987); [e](Gallegos et al. 1992); [f](van Tussenbrock 1994); [g](Witz and Dawes 1995); [h](Kaldy and Dunton 2000); (*) Data based on floral scars from cores.

Table 2. Historical reports of *T. testudinum* floral sex ratios.

Site	year	male shoots	female shoots	total shoots	male:female	total male:female
Biscayne Bay, FL [a]	1969	—	—	—	—	4
Tampa Bay, FL [b]	1976	0	21	—	0:21*	
Tampa Bay, FL	1976	1	4	—	0.25	
Tampa Bay, FL	1976	6	10	—	0.6	
Tampa Bay, FL	1976	9	17	—	0.53	
Tampa Bay, FL	1976	9	7	—	1.29	
Tampa Bay, FL	1976	1	2	—	0.5	
Tampa Bay, FL	1976	0	3	—	0:3*	
Tampa Bay, FL	1976	0	3	—	0:3*	
Tampa Bay, FL	1976	2	19	—	0.11	
Tampa Bay, FL	1976	0	9	—	0:9*	
Totals		28	95	—	—	0.33
Lassing Park, Tampa Bay, FL [c]	1979	414	295	4471	1.4	
Cockroach Bay, Tamp Bay, FL	1979	—	—	—	1	
Egmont Key, FL	1979	164	96	923	1.71	
Big Coppit Key, FL	1979	15	47	279	0.32	
No Name Key, FL	1979	8	4	339	2	
Totals (excluding Cockroach Bay)		601	442	5706	—	1.40
Tampa Bay, FL [d]	1986	—	—	—	—	1.33
Tampa Bay, FL	1986	—	—	—	—	2.00
Puerto Morelos, Mexico [e]	1991	24	20	2080	1.2	
Puerto Morelos, Mexico	1991	3	3	1930	1	
Puerto Morelos, Mexico	1991	1	2	1520	0.5	
Totals		28	25	5530	—	1.12

[a] Tomlinson (1969); [b] Grey and Moffler (1978); [c] Durako and Moffler (1985a); [d] Moffler and Durako (1987); [e] van Tussenbrock (1994); * no ratio can be calculated due to no observed male flowers.

Plate 1. *T. testudinum* male flower.

Plate 2. *T. testudinum* female flower.

Plate 3. *T. testudinum* fruit and seedlings.

Fig. 1. Site location map of 30 permanent monitoring stations in the FKNMS.

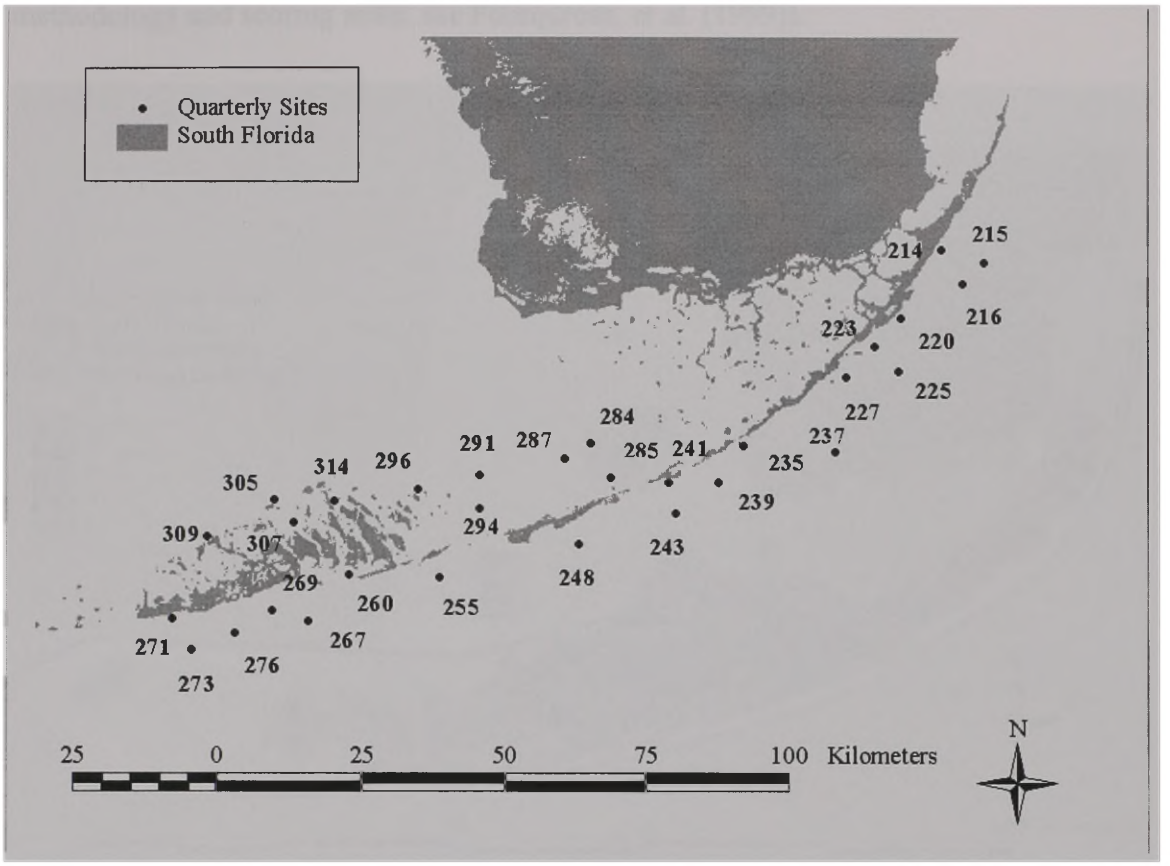


Fig. 2. Compiled *T. testudinum* density by Braun-Blanquet scores in the FKNMS. Scores based on % coverage by *T. testudinum* in a 0.25 m^2 quadrat. (For a description of methodology and scoring scale, see Fourqurean, et al. (1999)).

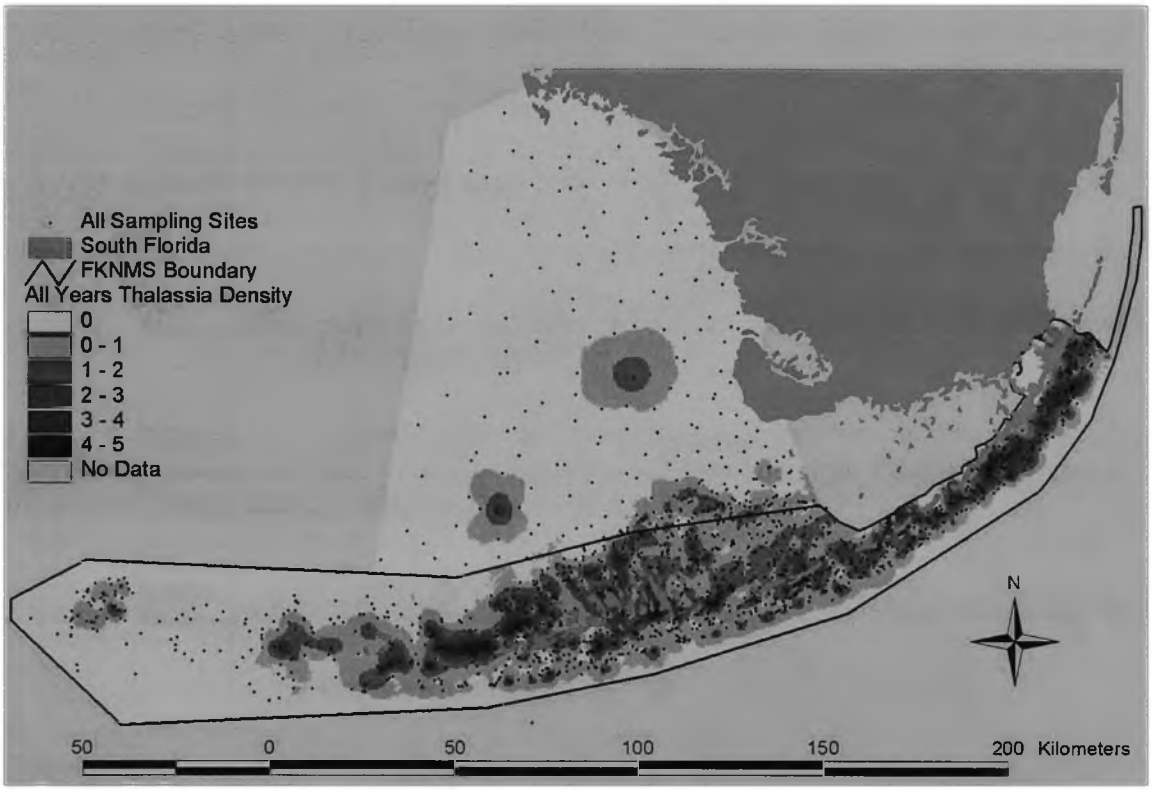


Fig. 3. Diagram of *T. testudinum* short shoots with detail of sex-specific male and female floral scars (modified from Cox and Tomlinson (1988)).

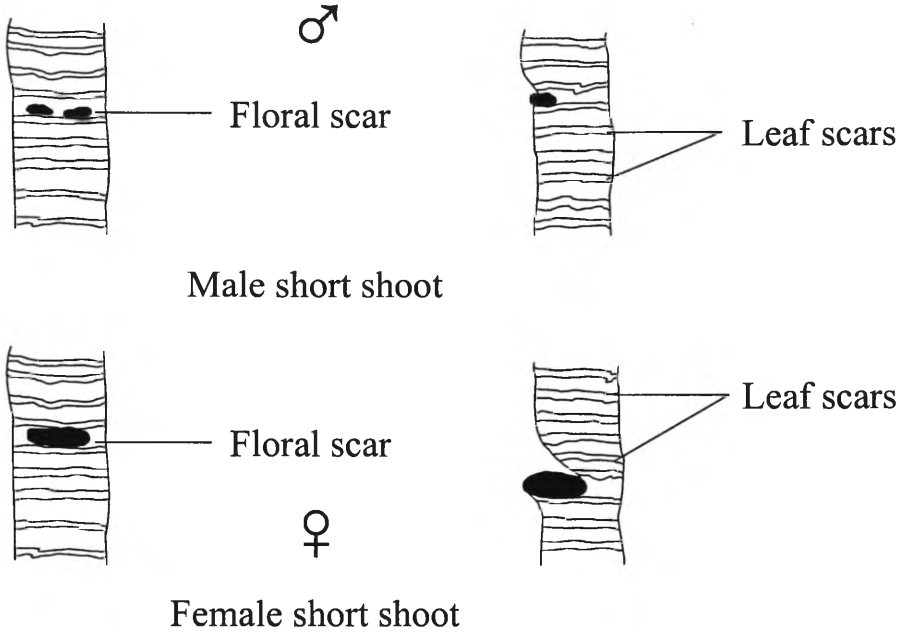


Fig. 4. Site location map of 10 *in situ* nutrient addition experiment sites in the upper Florida Keys (Key Largo).

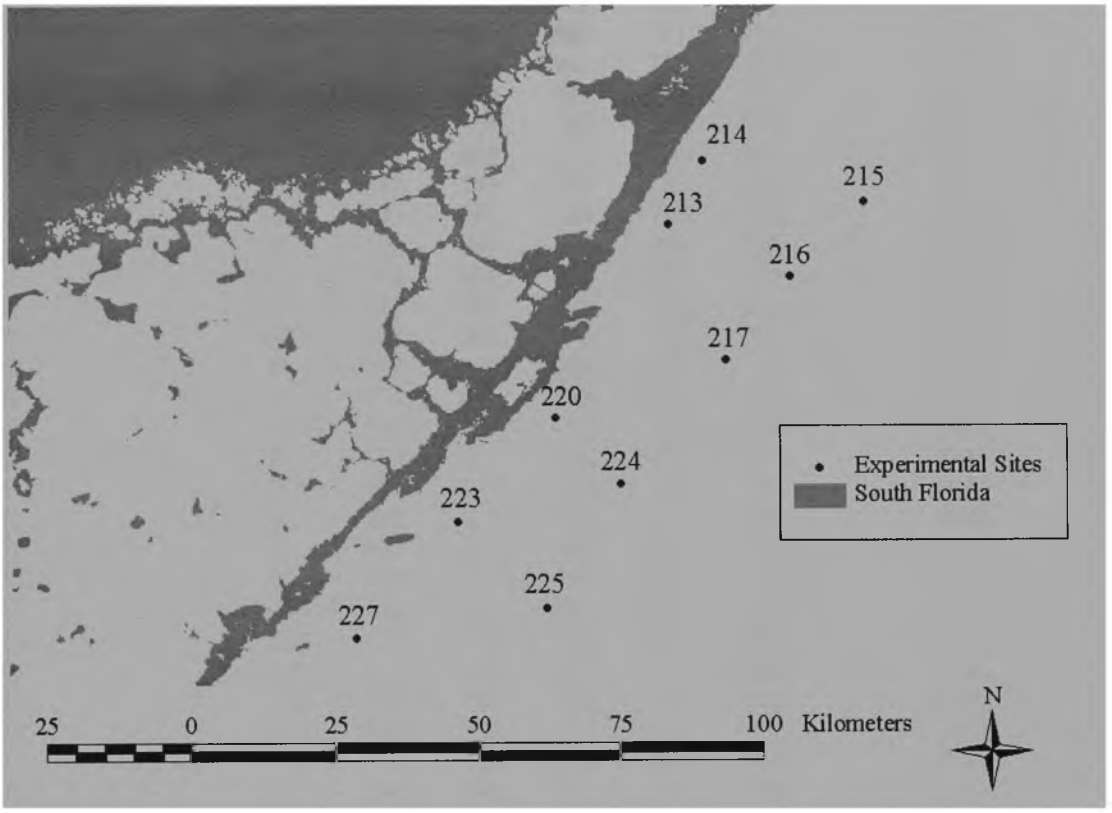


Table 3. *T. testudinum* short shoot observation totals by site and sampling period.

Site	Sample month	Feb '02	Mar '02	May '02	Jun '02	Sep '02	Jan '03	Total
	Decimal year	2002.12	2002.24	2002.35	2002.43	2002.68	2003.01	
214		753	761	838	766	765	700	4583
215		854	802	907	801	751	700	4815
216		759	641	810	801	720	520	4251
220		756	804	770	842	799	700	4671
223		755	728	754	792	710	700	4439
225		752	830	805	675	774	700	4536
227		775	815	753	787	751	700	4581
235		0	777	791	768	780	700	3816
237		0	752	864	806	682	700	3804
239		0	775	803	789	752	700	3819
241		0	724	757	732	738	700	3651
243		0	741	822	761	738	700	3762
248		0	746	887	764	804	700	3901
255		0	674	0	728	697	700	2799
260		0	806	0	762	752	700	3020
267		0	775	0	896	772	700	3143
269		0	775	0	896	772	700	3143
271		0	829	0	792	717	700	3038
273		0	805	0	748	804	700	3057
276		0	820	0	758	760	700	3038
284		725	775	734	780	700	600	4314
285		547	734	788	801	750	700	4320
287		779	803	732	730	700	500	4244
291		798	735	706	780	720	700	4439
294		712	740	756	800	725	520	4253
296		614	745	700	800	700	700	4259
305		0	795	549	839	750	700	3633
307		0	559	715	776	750	700	3500
309		0	576	720	943	700	0	2939
314		0	729	753	750	700	700	3632
Total		9579	22571	17714	23663	22233	19640	115400
n sites		13	30	23	30	30	29	

Table 4. Observed *T. testudinum* flower and fruit totals by sampling period.

Sex	Sample month Decimal year	Feb '02 2002.12	Mar '02 2002.24	May '02 2002.35	Jun '02 2002.43	Sep '02 2002.68	Jan '03 2003.01	Total
Males		0	1	272	114	0	0	387
Females		0	0	142	53	0	0	195
Fruits		0	0	30	196	142	0	368
Total female floral bodies		0	0	172	249	142	0	563
Total floral bodies		0	1	444	363	142	0	950
n sites		13	30	23	30	30	29	

Table 5. *T. testudinum* flowering frequencies calculated by site and sample period.

Site	Sample month	Feb '02	Mar '02	May '02	Jun '02	Sep '02	Jan '03
	Decimal year	2002.12	2002.24	2002.35	2002.43	2002.68	2003.01
214		0	0	1.43	0.78	0	0
215		0	0	1.32	0.50	0	0
216		0	0	0.37	6.62	0	0
220		0	0	4.03	1.31	0	0
223		0	0	4.64	2.53	0	0
225		0	0	3.48	3.26	4.13	0
227		0	0	0.27	1.27	0	0
235			0	0.88	0	0	0
237			0	0.46	0.12	0	0
239			0	6.10	24.21	4.92	0
241			0	0.79	0	0	0
243			0	1.34	0.66	1.36	0
248			0	0.56	2.62	3.98	0
255			0		0.41	4.45	0
260			0		0	0	0
267			0		0.33	0	0
269			0		0.52	0	0
271			0		0	0	0
273			0		0	0	0
276			0		0.13	0	0
284		0	0	0.82	0	0	0
285		0	0	2.16	0.12	0	0
287		0	0	3.83	0	0	0
291		0	0	1.56	0.26	0	0
294		0	0	6.08	0.75	0	0
296		0	0	0	0	0	0
305			$1.26e^{-3}$	21.86	0	0	0
307			0	0.28	0	0	0
309			0	0	0	0	
314			0	0.40	0	0	0
Mean		0	$4.43e^{-5}$	2.51	1.53	0.64	0

Mean pooled short shoot flowering frequency (May, June, and September) 1.49

Fig. 5. Observed *T. testudinum* flowering frequency for May 2002.

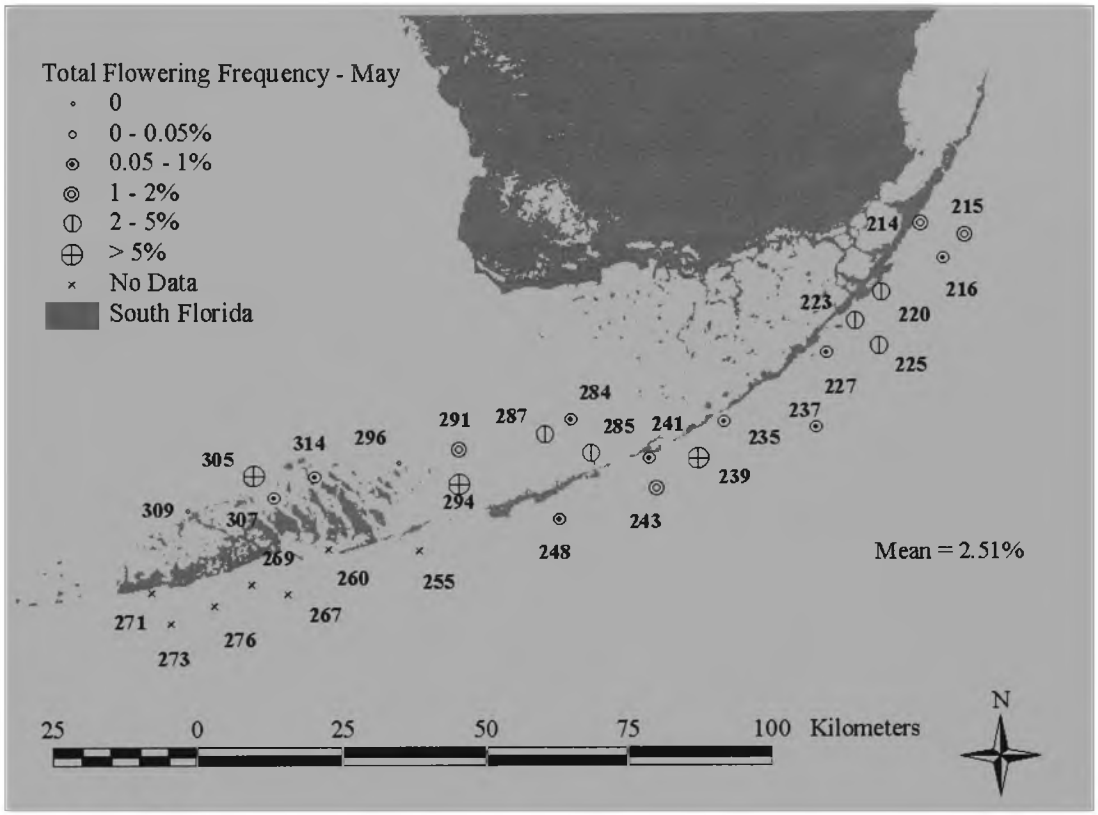


Fig. 6. Observed *T. testudinum* flowering frequency for June 2002.

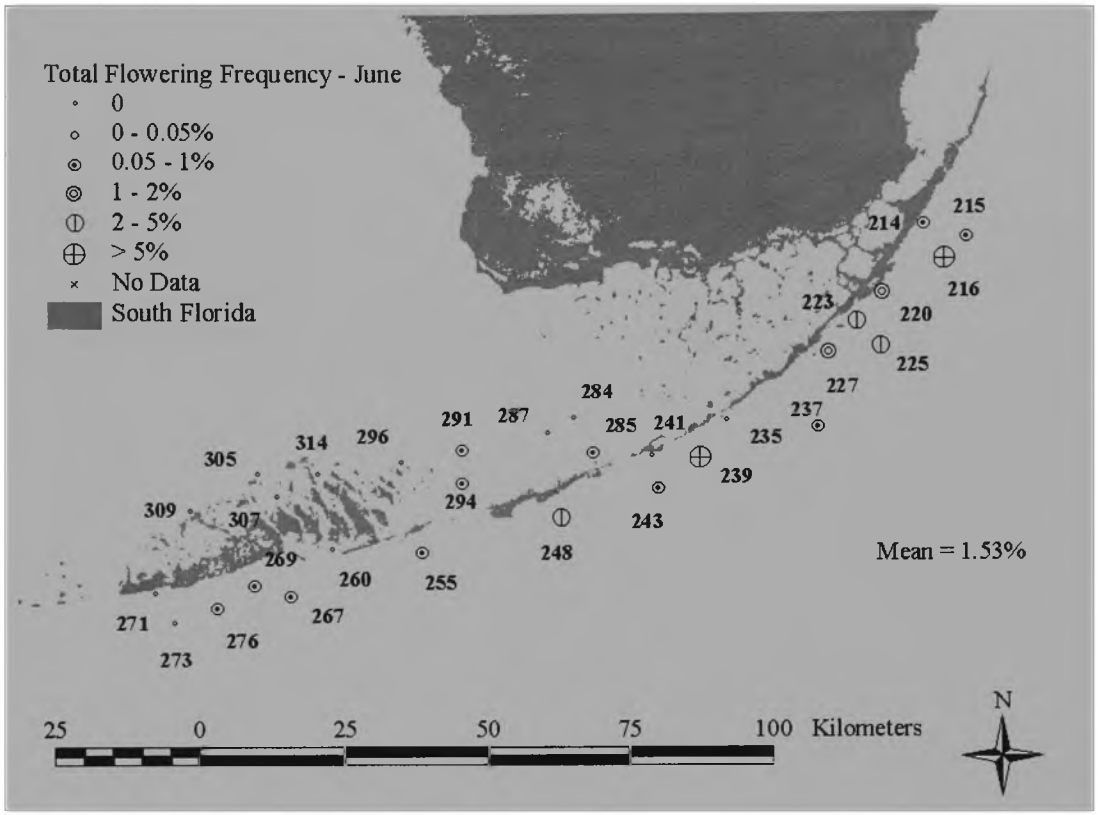


Fig. 7. Observed *T. testudinum* flowering frequency for September 2002.

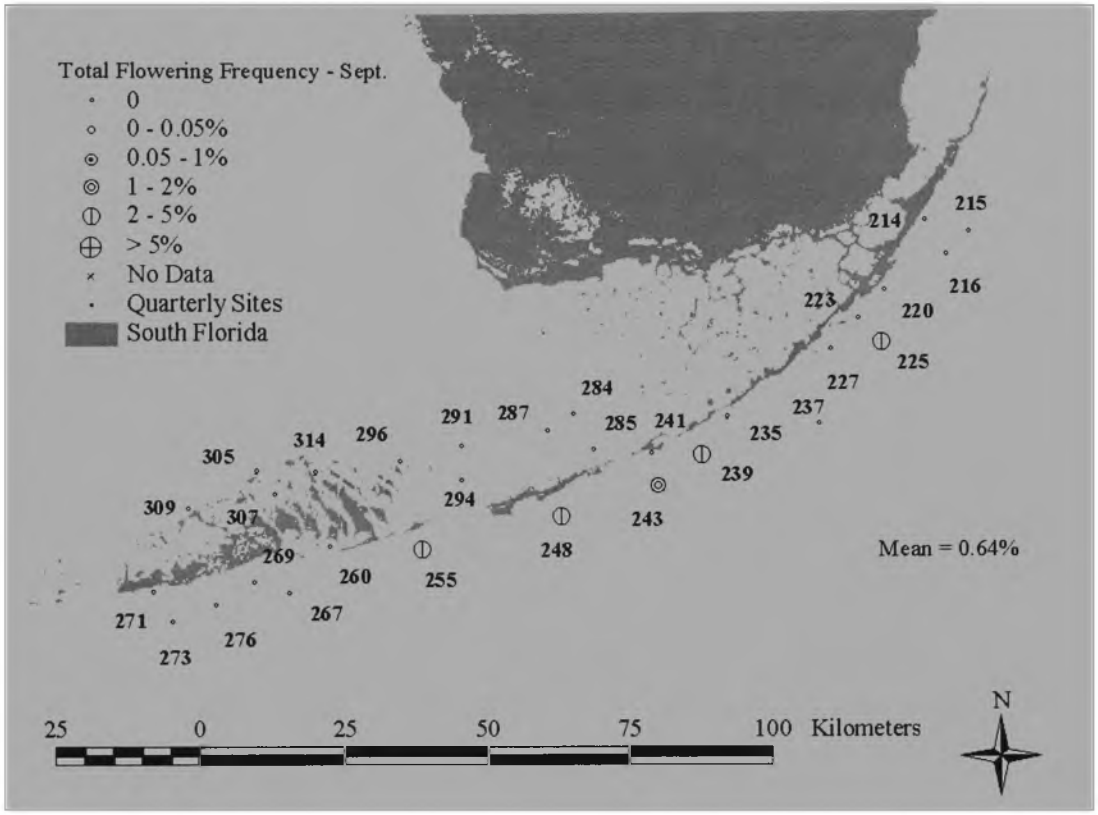


Fig. 8. Sex-specific floral anthesis of *T. testudinum*.

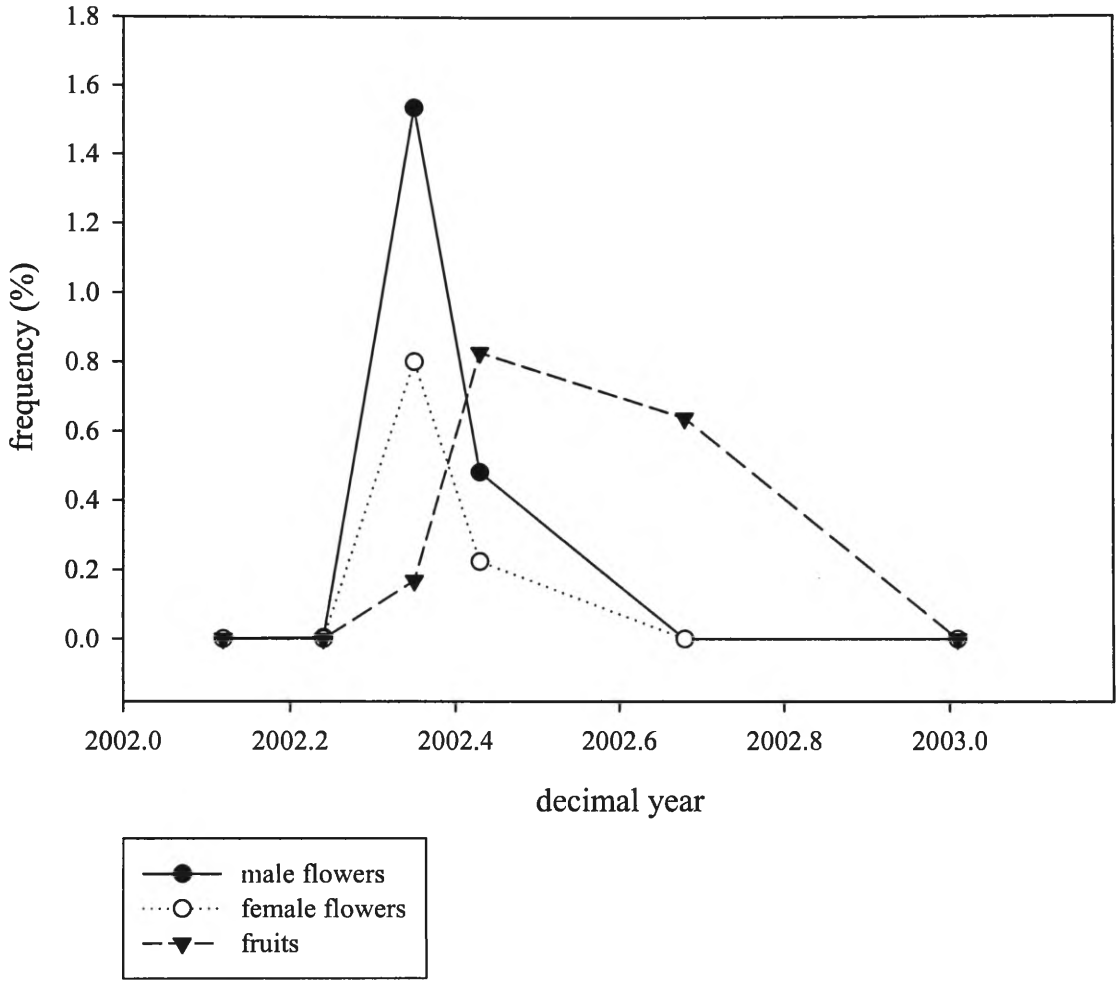


Table 6. Observed *T. testudinum* male:female sex ratios by site and sampling period. Site observations that yielded only one sex are reported as total floral numbers of that sex, respectively.

Site	Sample month Decimal year	Feb '02 2002.12	Mar '02 2002.24	May '02 2002.35	Jun '02 2002.43	Sep '02 2002.68	Jan '03 2003.01
214		0	0	11	6 to 0	0	0
215		0	0	12 to 0	3	0	0
216		0	0	0 to 3	53 to 0	0	0
220		0	0	6.75	0.83	0	0
223		0	0	7.75	2.33	0	0
225		0	0	1.55	1.75	0 to 32	0
227		0	0	2 to 0	0 to 10	0	0
235			0	7 to 0	0	0	0
237			0	1	0 to 1	0	0
239			0	0 to 49	0 to 191	0 to 37	0
241			0	2	0	0	0
243			0	10	5 to 0	0 to 10	0
248			0	1.5	0.54	0 to 32	0
255			0		2	0 to 31	0
260			0		0	0	0
267			0		0 to 3	0	0
269			0		1	0	0
271			0		0	0	0
273			0		0	0	0
276			0		0 to 1	0	0
284		0	0	1	0	0	0
285		0	0	0.55	1 to 0	0	0
287		0	0	27	0	0	0
291		0	0	4.5	2 to 0	0	0
294		0	0	0.18	0 to 6	0	0
296		0	0	0	0	0	0
305			1 to 0	2.64	0	0	0
307			0	2 to 0	0	0	0
309			0	0	0	0	
314			0	2	0	0	0
M:F		n/a	1 to 0	1.58	0.46	0 to 142	n/a
Pooled M:F (May, June, and September)				0.69			

Table 7. Site-specific *T. testudinum* number of leaves, number of leaf scars, total leaves, Plastochrone interval[a], and average short shoot age (days). All values are reported as site means.

Site	n shoots	# leaves (SS^{-1})	# leaf scars (SS^{-1})	Total leaves (SS^{-1})	PI	Average short shoot age (days)
214	115	4.10	35.11	39.13	44.92	1366
215	133	3.71	64.27	67.98	37.24	2474
216	130	2.42	40.85	43.27	31.94	1351
220	113	3.98	35.60	39.58	39.07	1365
223	142	3.33	46.00	49.33	36.67	1830
225	133	2.98	35.35	38.34	49.91	2109
227	99	3.39	35.56	38.95	41.43	1662
235	128	2.85	26.41	29.27	41.11	1100
237	119	2.61	28.25	30.87	52.89	1707
239	119	2.87	45.15	48.02	42.92	1999
241	125	3.62	21.03	24.66	33.97	834
243	126	3.15	39.17	42.33	46.68	2003
248	134	3.19	28.53	31.72	51.54	1402
255	108	2.71	30.69	33.40	47.97	1676
260	130	3.37	27.39	30.76	39.75	1107
267	112	2.83	40.80	43.63	45.32	1889
269	128	3.29	29.38	32.66	43.26	1493
271	116	2.78	31.08	33.86	39.99	1261
273	133	3.46	37.67	41.13	48.97	2045
276	121	2.75	29.51	32.26	39.25	1297
284	114	4.03	19.31	23.33	32.79	697
285	132	3.37	29.67	33.05	37.54	1123
287	126	3.18	41.06	44.25	38.43	1558
291	159	3.52	38.45	41.97	42.30	1531
294	124	3.13	38.48	41.60	39.16	1360
296	111	3.70	20.96	24.67	37.18	984
305	114	3.36	24.34	27.70	41.69	1136
307	158	4.07	31.13	35.20	37.39	1157
309	124	3.19	16.97	20.15	54.86	1128
314	132	3.73	36.23	39.97	63.73	1485
Mean	125	3.29	33.48	36.77	42.66	1471

[a]Plastochrone interval values are reported as means calculated from quarterly estimates of *T. testudinum* primary productivity measurements (1995-2003) from the Seagrass Status and Trends Monitoring Project.

Table 8. Site-specific *T. testudinum* demographic core total and sex-specific flowering short shoot totals and flowering frequencies. Frequency data are reported as % of shoots measured.

Site	n shoots	Total shoots	# Male shoots	# Female shoots		Total shoot flower frequency (%)	Male shoot flower frequency (%)	Female shoot flower frequency (%)
214	115	12	4	8		10.43	3.48	6.96
215	133	8	2	6		6.02	1.50	4.51
216	130	5	0	5		3.85	0	3.85
220	113	23	10	13		20.35	8.85	11.50
223	142	22	4	18		15.49	2.82	12.68
225	133	9	2	7		6.77	1.50	5.26
227	99	14	1	13		14.14	1.01	13.13
235	128	1	0	1		0.78	0	0.78
237	119	0	0	0		0	0	0
239	119	42	8	34		35.29	6.72	28.57
241	125	9	3	6		7.20	2.40	4.80
243	126	3	2	1		2.38	1.59	0.79
248	134	11	6	5		8.21	4.48	3.73
255	108	5	3	2		4.63	2.78	1.85
260	130	5	0	5		3.85	0	3.85
267	112	9	3	6		8.04	2.68	5.36
269	128	6	1	5		4.69	0.78	3.91
271	116	3	1	2		2.59	0.86	1.72
273	133	9	3	6		6.77	2.26	4.51
276	121	2	0	2		1.65	0	1.65
284	114	5	0	5		4.39	0	4.39
285	132	3	1	2		2.27	0.76	1.52
287	126	3	2	1		2.38	1.59	0.79
291	159	23	2	21		14.47	1.26	13.21
294	124	0	0	0		0	0	0
296	111	3	1	2		2.70	0.90	1.80
305	114	6	3	3		5.26	2.63	2.63
307	158	11	1	10		6.96	0.63	6.33
309	124	0	0	0		0	0	0
314	132	9	1	8		6.82	0.76	6.06
Total	3758	261	64	197	Mean	6.95	1.70	5.24

Fig. 9. Demographic core total *T. testudinum* shoot flowering frequencies.

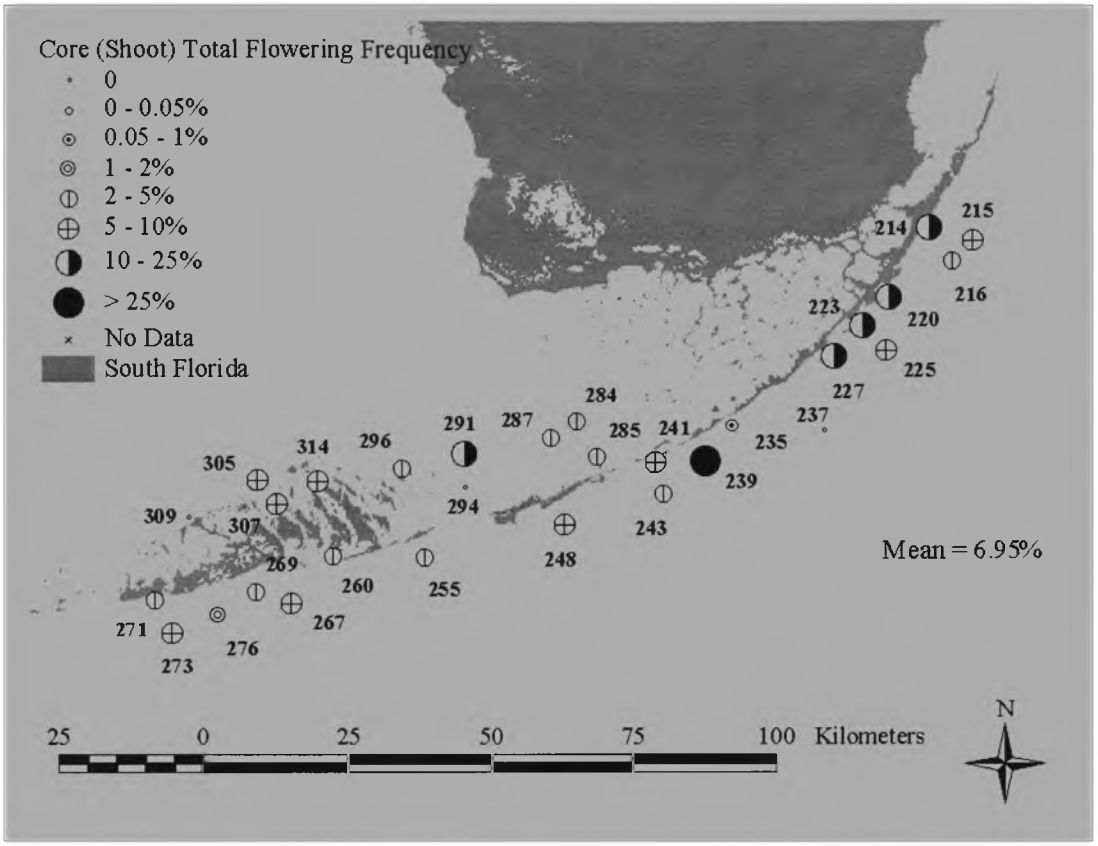


Table 9. Demographic core total and sex-specific *T. testudinum* minimum short shoot age at first flowering. All short shoot ages are reported in days and represent the single lowest flowering shoot age observed among all flowering shoots, respective of site and sex.

Site	Minimum short shoot age at 1st flowering	Minimum male short shoot age at 1st flowering	Minimum female short shoot age at 1st flowering
214	838	1012	838
215	1310	1310	1383
216	344	n/a	344
220	483	483	759
223	853	1669	853
225	770	1815	770
227	683	3286	683
235	2668	n/a	2668
237	n/a	n/a	n/a
239	416	1415	416
241	845	845	1015
243	852	1704	852
248	751	751	1060
255	853	1154	853
260	252	n/a	252
267	995	1169	995
269	457	914	457
271	1527	1527	1973
273	1094	1939	1094
276	2251	n/a	2251
284	269	n/a	269
285	952	1496	952
287	1444	1444	1549
291	620	1131	620
294	n/a	n/a	n/a
296	638	837	638
305	902	1763	902
307	723	789	723
309	n/a	n/a	n/a
314	817	2898	817
Mean	911	1425	962

Table 10. Demographic core total and sex-specific *T. testudinum* short shoot average age at first flowering. All short shoot ages are reported in days.

Site	Average short shoot age at 1st flowering	Average male short shoot age at 1st flowering	Average female short shoot age at 1st flowering
214	1289	1213	1327
215	2184	1565	2390
216	1181	n/a	1181
220	1431	1373	1475
223	1880	2096	1832
225	1602	2585	1627
227	1769	3286	1730
235	2668	n/a	2668
237	n/a	n/a	n/a
239	1726	2045	1650
241	1165	1229	1133
243	2193	2863	852
248	1651	1414	1935
255	1686	1890	1380
260	684	n/a	684
267	1900	1298	2200
269	754	914	722
271	1862	1527	2029
273	2248	2486	2129
276	3175	n/a	3175
284	1243	n/a	1243
285	1563	1496	1597
287	1713	1796	1549
291	1254	1167	1263
294	n/a	n/a	n/a
296	824	837	818
305	1735	2029	1588
307	1449	789	1515
309	n/a	n/a	n/a
314	1932	2898	1811
Mean	1658	1763	1611

Table 11. Site-specific *T. testudinum* demographic core raw floral scar totals, sex-specific short shoot reflowering totals, and reflowering frequencies. Frequency data are reported as % of pooled flowering shoots measured, respective of site and sex (n_{pooled} = 261). Values of "0" denote that no shoots of a particular sex had re-flowered, respective of site. Values of "n/a" denote that no flowers were observed, respective of site and sex.

Site	n flow. shoots	Total reflow. shoots	# Male reflow. shoots	# Female reflow. shoots		Total shoot re-flower frequency (%, n = 261)	Male shoot re-flower frequency (%, n = 64)	Female shoot re-flower frequency (%, n = 197)
214	12	4	1	3		33.33	25.00	37.50
215	8	2	0	2		25.00	0	33.33
216	5	0	0	0		0	n/a	0
220	23	3	2	1		13.04	20.00	7.69
223	22	7	1	6		31.82	25.00	33.33
225	9	3	0	3		33.33	0	42.86
227	14	6	0	6		42.86	0	46.15
235	1	0	0	0		0	n/a	0
237	0	0	0	0		n/a	n/a	n/a
239	42	11	1	10		26.19	12.50	29.41
241	9	0	0	0		0	0	0
243	3	0	0	0		0	0	0
248	11	3	1	2		27.27	16.67	40.00
255	5	0	0	0		0	0	0
260	5	0	0	0		0	n/a	0
267	9	0	0	0		0	0	0
269	6	1	0	1		16.67	0	20.00
271	3	0	0	0		0	0	0
273	9	1	0	1		11.11	0	16.67
276	2	0	0	0		0	n/a	0
284	5	0	0	0		0	n/a	0
285	3	0	0	0		0	0	0
287	3	0	0	0		0	0	0
291	23	9	0	9		39.13	0	42.86
294	0	0	0	0		n/a	n/a	n/a
296	3	0	0	0		0	0	0
305	6	1	1	0		16.67	33.33	0
307	11	1	0	1		9.09	0	10.00
309	0	0	0	0		n/a	n/a	n/a
314	9	0	0	0		0	0	0
Total	261	52	7	45	Mean	19.19	10.94	22.84

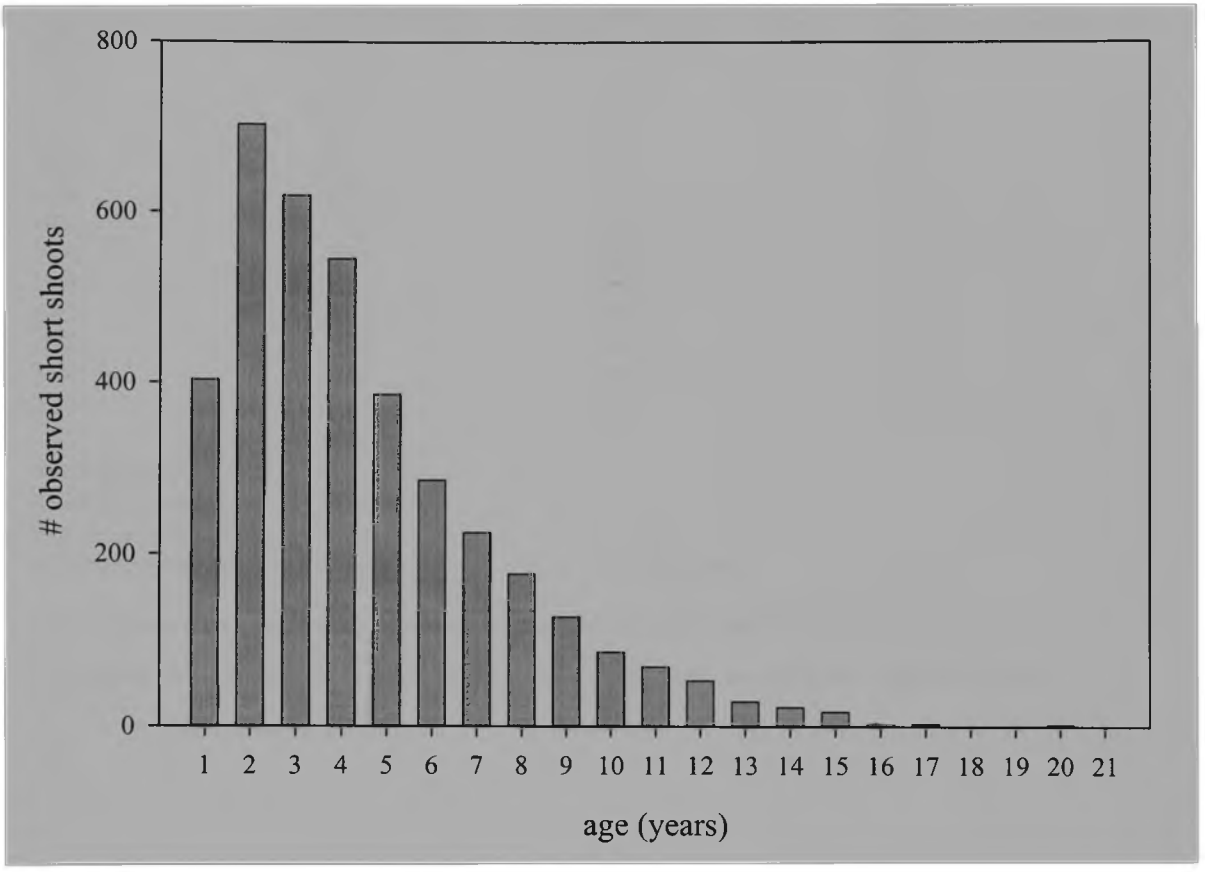
Table 12. Site-specific *T. testudinum* demographic core male:female flowering shoot sex ratios. Site observations that yielded only one sex are reported as total numbers of that sex, respectively.

Site	n shoots	Total flowering shoots	# Male flowering shoots	# Female flowering shoots	M:F flowering shoots
214	115	12	4	8	0.50
215	133	8	2	6	0.33
216	130	5	0	5	0 to 5
220	113	23	10	13	0.77
223	142	22	4	18	0.22
225	133	9	2	7	0.29
227	99	14	1	13	0.08
235	128	1	0	1	0 to 1
237	119	0	0	0	n/a
239	119	42	8	34	0.24
241	125	9	3	6	0.50
243	126	3	2	1	2
248	134	11	6	5	1.20
255	108	5	3	2	1.50
260	130	5	0	5	0 to 5
267	112	9	3	6	0.50
269	128	6	1	5	0.20
271	116	3	1	2	0.50
273	133	9	3	6	0.50
276	121	2	0	2	0 to 2
284	114	5	0	5	0 to 5
285	132	3	1	2	0.50
287	126	3	2	1	2
291	159	23	2	21	0.10
294	124	0	0	0	n/a
296	111	3	1	2	0.50
305	114	6	3	3	1.00
307	158	11	1	10	0.10
309	124	0	0	0	n/a
314	132	9	1	8	0.12
Total	3758	261	64	197	
Mean M:F flowering shoots					0.32

Table 13. Demographic core *T. testudinum* apical frequency. Data are reported as the % of short shoots.

Site	n shoots	Number of apices	Apical frequency (%)
214	115	39	33.91
215	133	22	16.54
216	130	17	13.08
220	113	11	9.73
223	142	6	4.23
225	133	22	16.54
227	99	13	13.13
235	128	27	21.09
237	119	13	10.92
239	119	10	8.40
241	125	12	9.60
243	126	43	34.13
248	134	20	14.93
255	108	24	22.22
260	130	0	0
267	112	13	11.61
269	128	26	20.31
271	116	27	23.28
273	133	35	26.32
276	121	22	18.18
284	114	12	10.53
285	132	18	13.64
287	126	0	0
291	159	13	8.18
294	124	12	9.68
296	111	28	25.23
305	114	28	24.56
307	158	32	20.25
309	124	27	21.77
314	132	13	9.85
Total	3758	585	
Mean apical frequency			15.57

Fig. 10. Pooled *T. testudinum* short shoot age frequency distribution in the FKNMS* (n = 3758, mean short shoot age = 4.03 years).



* 1 short shoot was observed in each of cohort classes 18, 19, and 21 years.

Table 14. *T. testudinum* demographic core historical reconstruction of flowering frequencies in the FKNMS. All flowering frequencies are reported as % flowering short shoots.

Year	Number of shoots eligible for flowering consideration	Number of floral scars observed in flowering year	Flowering frequency (%)
1990	143	1	0.70
1991	217	5	2.30
1992	308	5	1.62
1993	435	9	2.07
1994	628	9	1.43
1995	855	22	2.57
1996	1162	20	1.72
1997	1581	36	2.28
1998	2147	54	2.52
1999	2804	47	1.68
2000	3484	64	1.84
2001	3748	55	1.47
2002[a]	361	13	3.60
Mean observed FKNMS flowering frequency, (May, June, and September) 2002[b]	63610	949 (flowers)	1.49

[a] Short shoots were considered only from cores at sites 214, 220, and 273 collected in September 2002.

[b] Flowering data obtained from direct observation of short shoots during active flowering season.

Table 15. Comparison of observational and demographic *T. testudinum* flowering data in the FKNMS. All flowering frequencies are reported as % of short shoots. Historical flowering data is calculated from age-reconstruction techniques of demographic cores.

Site	Pooled observational flowering frequency (May, June, Sept.)	Core shoot flowering frequency	Year	Historical flowering frequency
214	0.76	10.43	1990	0.70
215	0.65	6.02	1991	2.30
216	2.40	3.85	1992	1.62
220	1.74	20.35	1993	2.07
223	2.44	15.49	1994	1.43
225	3.64	6.77	1995	2.57
227	0.52	14.14	1996	1.72
235	0.30	0.78	1997	2.28
237	0.21	0	1998	2.52
239	11.82	35.29	1999	1.68
241	0.27	7.20	2000	1.84
243	1.12	2.38	2001	1.47
248	2.32	8.21	2002	3.60
255	2.39	4.63		
260	0	3.85		
267	0.18	8.04		
269	0.24	4.69		
271	0	2.59		
273	0	6.77		
276	0.07	1.65		
284	0.54	4.39		
285	0.77	2.27		
287	1.30	2.38		
291	0.59	14.47		
294	2.28	0		
296	0	2.70		
305	5.61	5.26		
307	0.09	6.96		
309	0	0		
314	0.14	6.82		
Mean	1.49	6.95		

Table 16. Comparison of observational and demographic *T. testudinum* sex ratio data in the FKNMS. All ratios are reported as male:female. Sites where only one sex is represented are reported as the raw number to zero. Sites where no flowers or floral scars were observed are reported as n/a.

Site	Observational M:F (May, June, Sept.)	Core shoot M:F
214	17	0.50
215	15	0.33
216	17.67	0 to 5
220	3.20	0.77
223	4.50	0.22
225	0.61	0.29
227	0.20	0.08
235	7 to 0	0 to 1
237	0.67	n/a
239	0 to 277	0.24
241	2	0.50
243	1.36	2
248	0.21	1.20
255	0.06	1.50
260	n/a	0 to 5
267	0 to 3	0.50
269	1	0.20
271	n/a	0.50
273	n/a	0.50
276	0 to 1	0 to 2
284	1	0 to 5
285	0.64	0.50
287	27	2
291	5.50	0.10
294	0.16	n/a
296	n/a	0.50
305	2.64	1
307	2 to 0	0.10
309	n/a	n/a
314	2	0.12
Mean	0.69	0.32

Table 17. Correlation matrix of *T. testudinum* flowering variables versus water quality variables. For brevity, only the significant correlations are presented.

Variables		DSFF	DMFF	AAF1	AFF1	AF
NO_3^-	Pearson Correlation					0.449**
	Sig. (2-tailed)					0.013
	N					30
NO_2^-	Pearson Correlation	-0.381*	-0.439*			
	Sig. (2-tailed)	0.038	0.015			
	N	30	30			
TOC	Pearson Correlation		-0.369*			
	Sig. (2-tailed)		0.045			
	N		30			
TN	Pearson Correlation		-0.431*			
	Sig. (2-tailed)		0.018			
	N		30			
TON	Pearson Correlation		-0.427*	-0.384*		
	Sig. (2-tailed)		0.019	0.048		
	N		30	27		
Kd	Pearson Correlation			-0.449*	-0.388*	
	Sig. (2-tailed)			0.019	0.046	
	N			27	27	

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 18. Correlation matrix of *T. testudinum* flowering variables versus seagrass vitality variables. For brevity, only the significant correlations are presented.

Variables		OFrF	DMFF	AAF1	AMF1
Leaf number	Pearson Correlation	-0.396*		-0.465*	
	Sig. (2-tailed)	0.03		0.015	
	N	30		27	
Density	Pearson Correlation				0.531**
	Sig. (2-tailed)				0.011
	N				22
Productivity	Pearson Correlation		-0.362*		
	Sig. (2-tailed)		0.049		
	N		30		
Areal productivity	Pearson Correlation				0.424*
	Sig. (2-tailed)				0.049
	N				22
Leaf area productivity	Pearson Correlation				0.486*
	Sig. (2-tailed)				0.022
	N				22
PI	Pearson Correlation				0.495*
	Sig. (2-tailed)				0.019
	N				22

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 19. Correlation matrix of *T. testudinum* flowering variables versus nutrient availability variables. For brevity, only significant correlations are presented.

Variables		OTFF	OFFF	OFrF	AAF1	AMF1
%N	Pearson Correlation	-0.444**	-0.42*	-0.473**	-0.427*	-0.466*
	Sig. (2-tailed)	0.014	0.021	0.008	0.026	0.029
	N	30	30	30	27	22
C:N	Pearson Correlation	0.488**	0.464**	0.504**	0.393*	0.466*
	Sig. (2-tailed)	0.006	0.01	0.005	0.043	0.029
	N	30	30	30	27	22

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Fig. 11 Apex frequency versus demographic female flowering frequency.

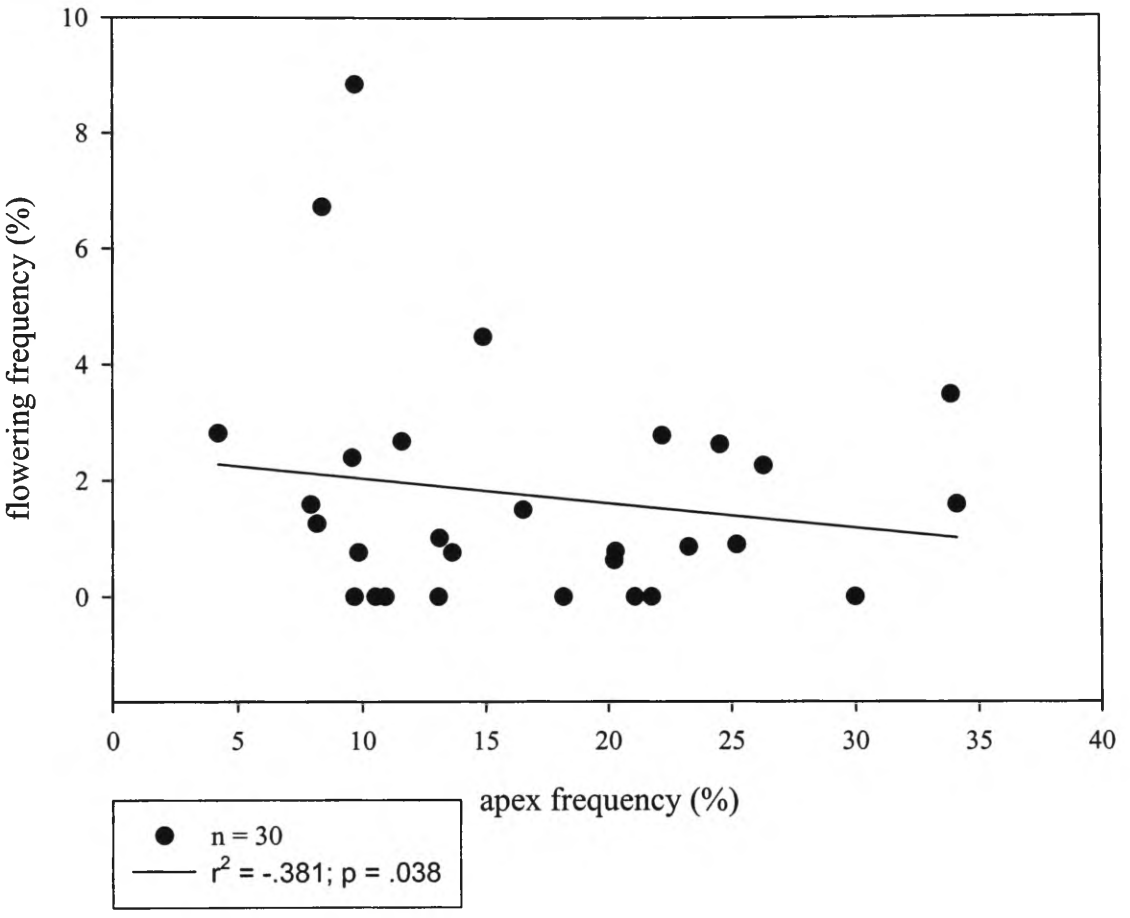


Table 20. Mean flowering frequencies by treatment. Sampling period totals represent the pooled mean of all ten sites, per treatment, respectively. Frequencies are reported as % of short shoots.

Treatment	Sampling period*				Mean
	April	May	June	July	
Inshore Control	0	6.18	1.19	0.62	2.11
Inshore N + P	0.24	1.13	0.38	0	0.46
Offshore Control	0.23	7.30	2.57	0.24	2.63
Offshore N + P	0	1.11	0.30	0	0.37

* January 2003 data not shown because there was no flowering observed.

Fig. 12. N + P addition site 213 flowering frequencies; $\mu_{control} = 3.36$, $\mu_{N+P} = 0$.

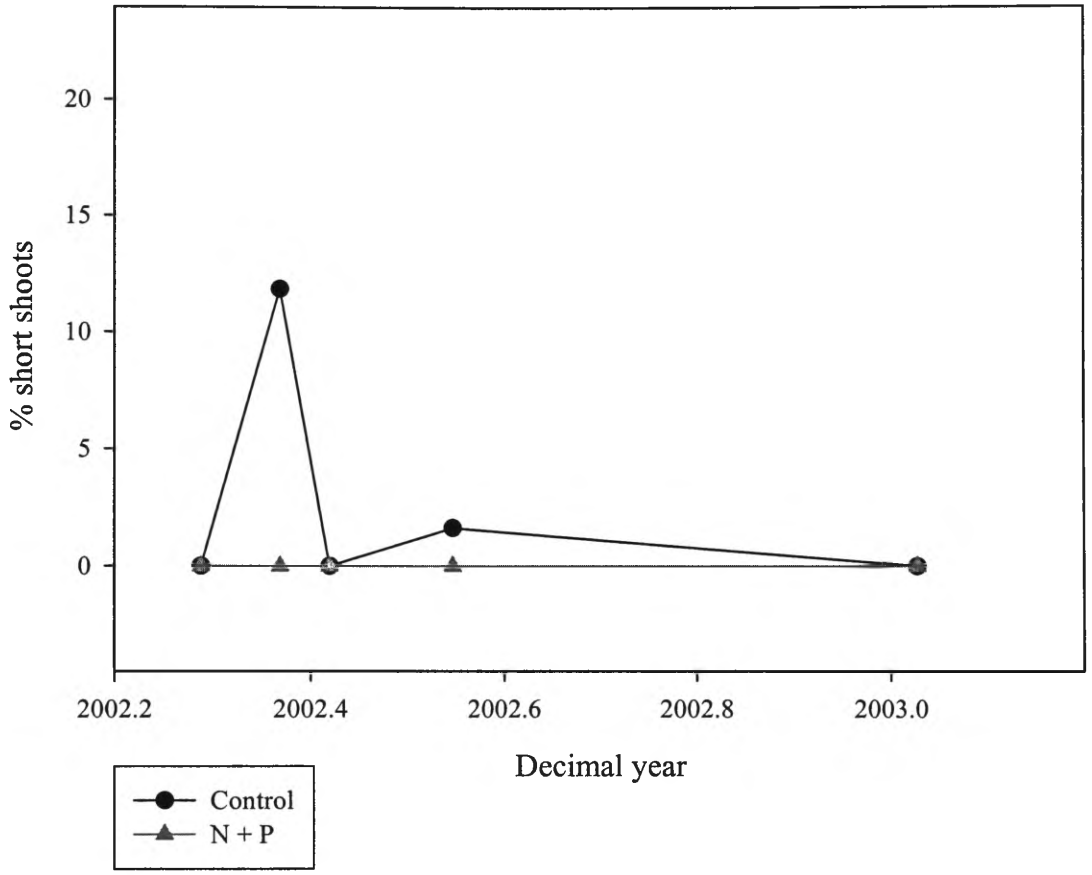


Fig. 13. N + P addition site 214 flowering frequencies; $\mu_{control} = 3.10$, $\mu_{N+P} = 0.71$.

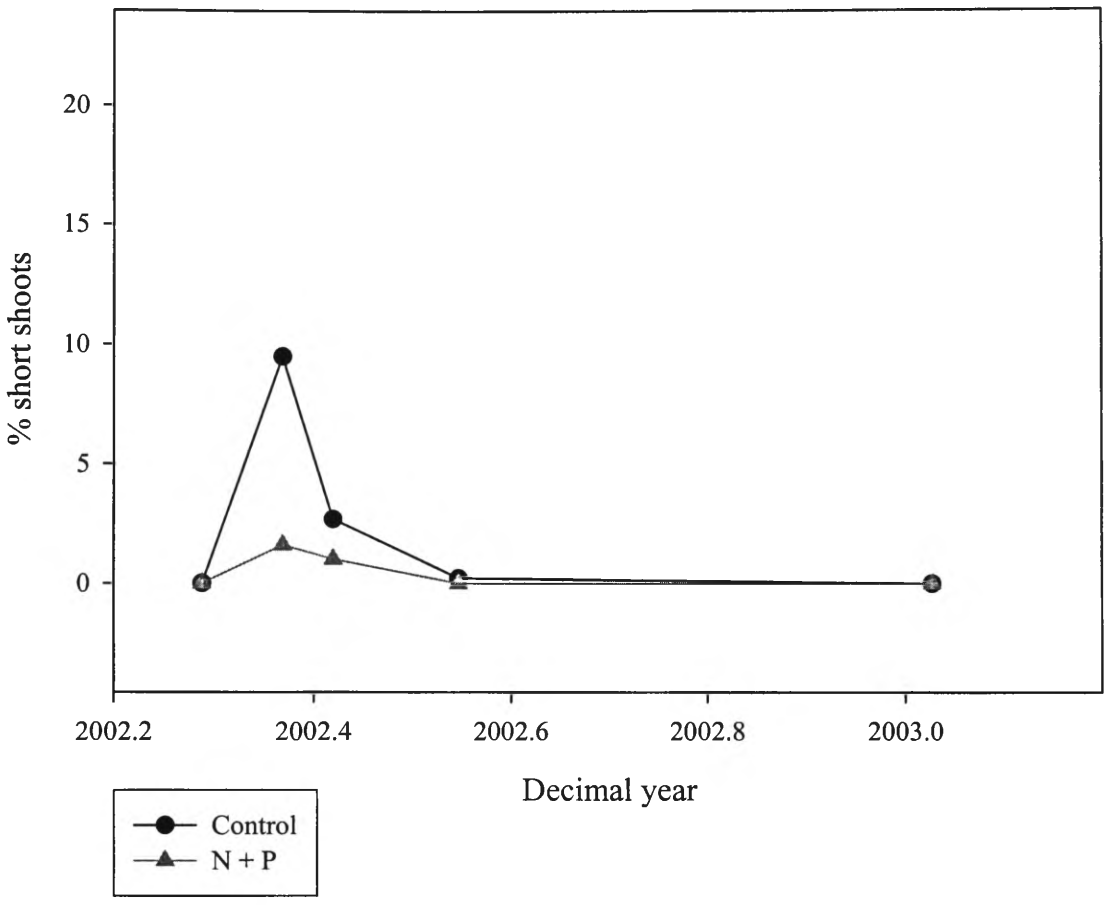


Fig. 14. N + P addition site 220 flowering frequencies; $\mu_{control} = 1.45$, $\mu_{N+P} = 0.18$.

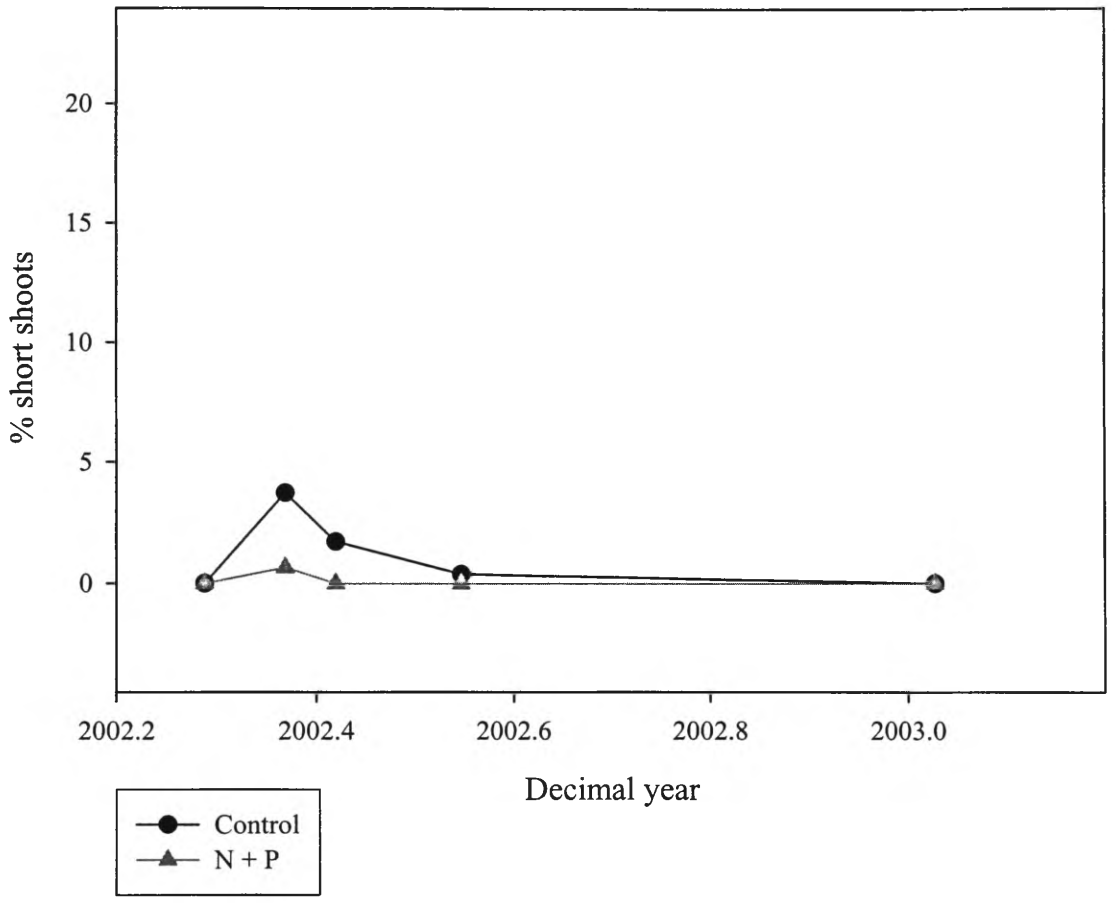


Fig. 15. N + P addition site 223 flowering frequencies; $\mu_{control} = 2.02$, $\mu_{N+P} = 1.12$.

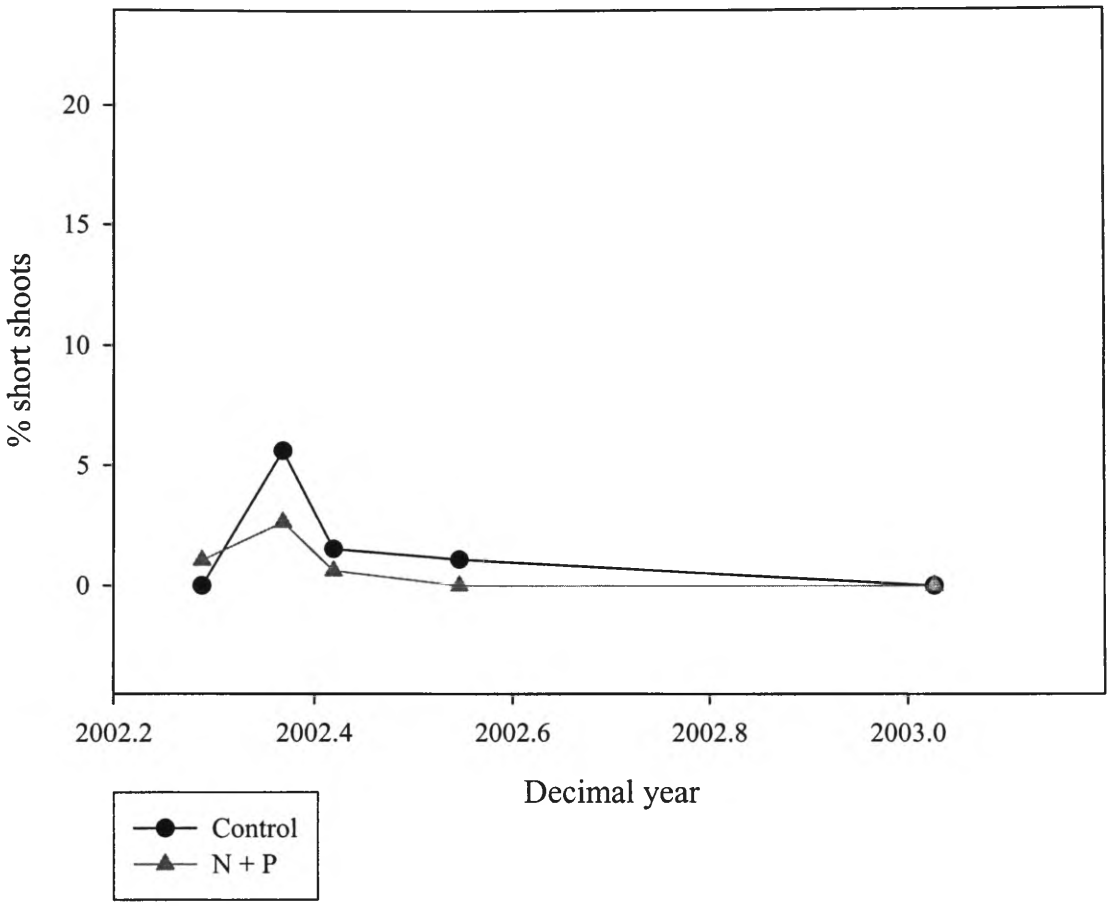


Fig. 16. N + P addition site 227 flowering frequencies; $\mu_{control} = 0.25$, $\mu_{N+P} = 0$.

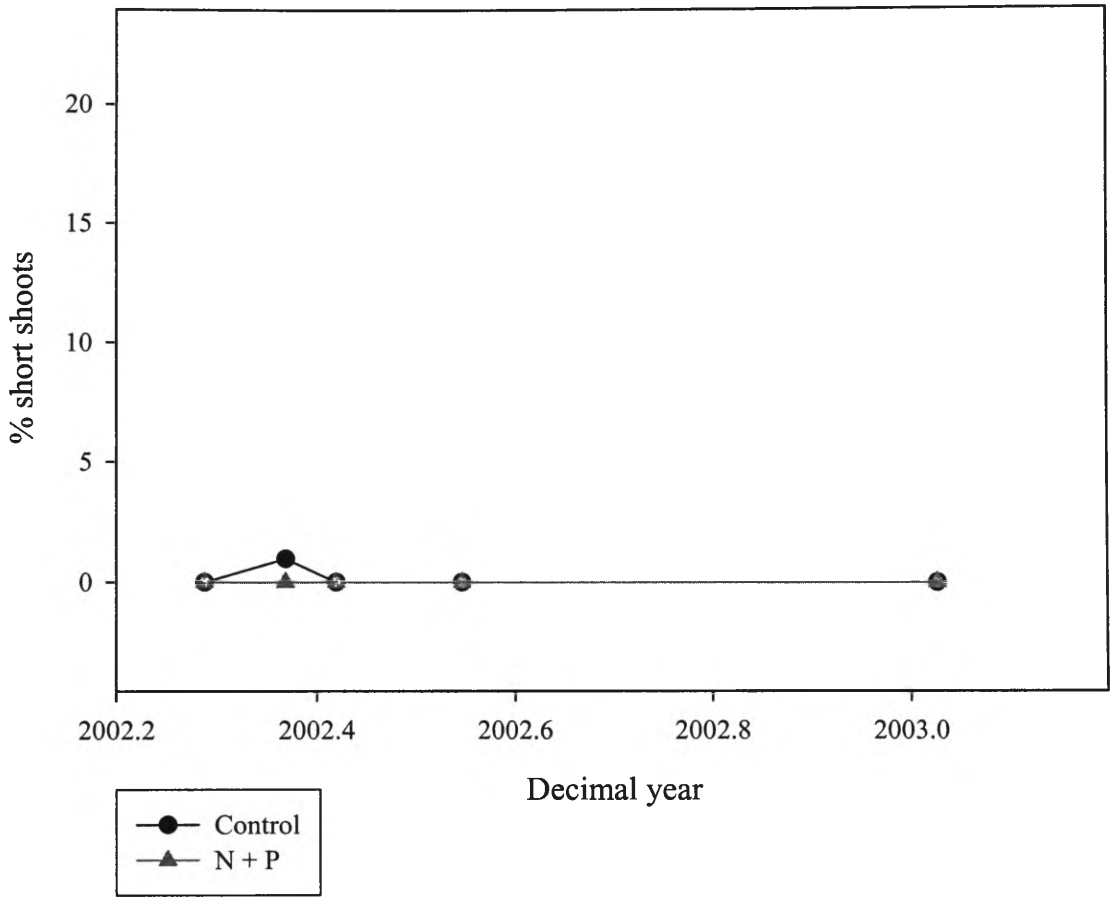


Fig. 17. N + P addition site 215 flowering frequencies; $\mu_{control} = 1.31$, $\mu_{N+P} = 0.06$.

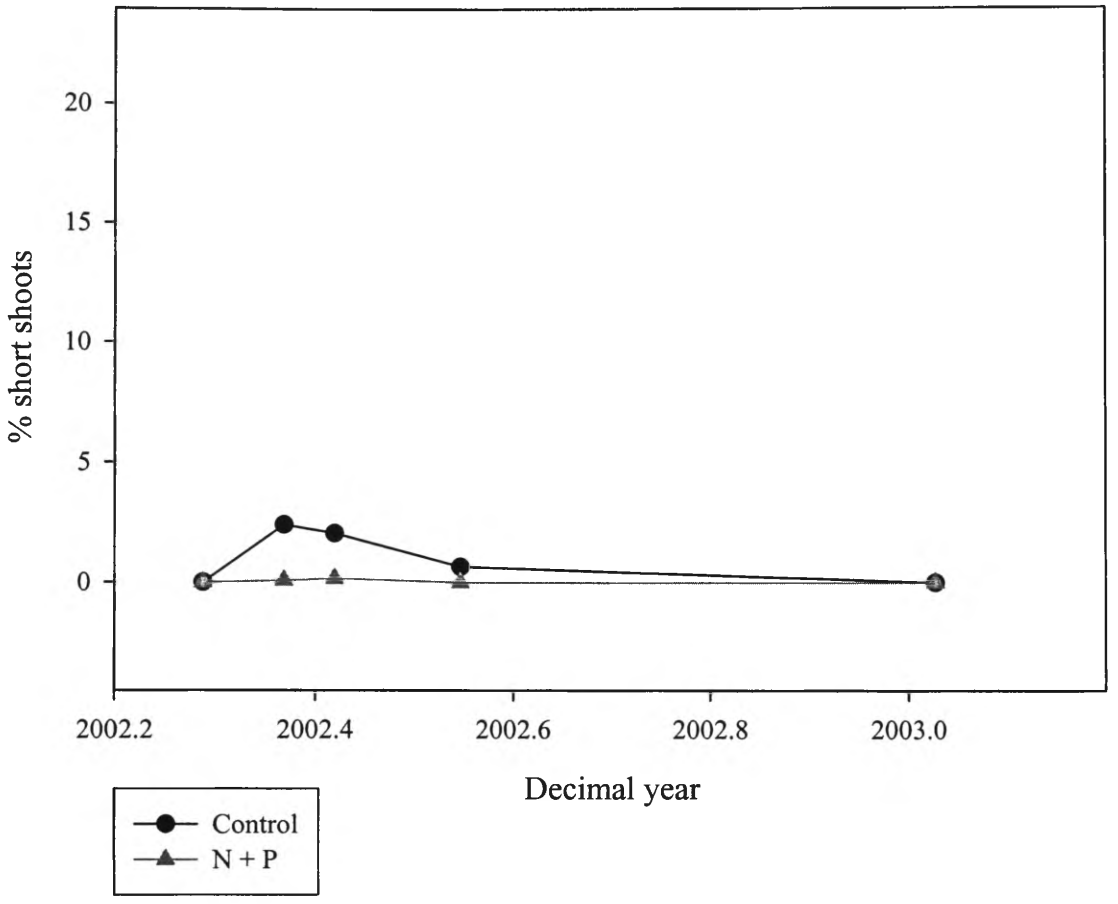


Fig. 18. N + P addition site 216 flowering frequencies; $\mu_{control} = 0.77$, $\mu_{N+P} = 0.61$.

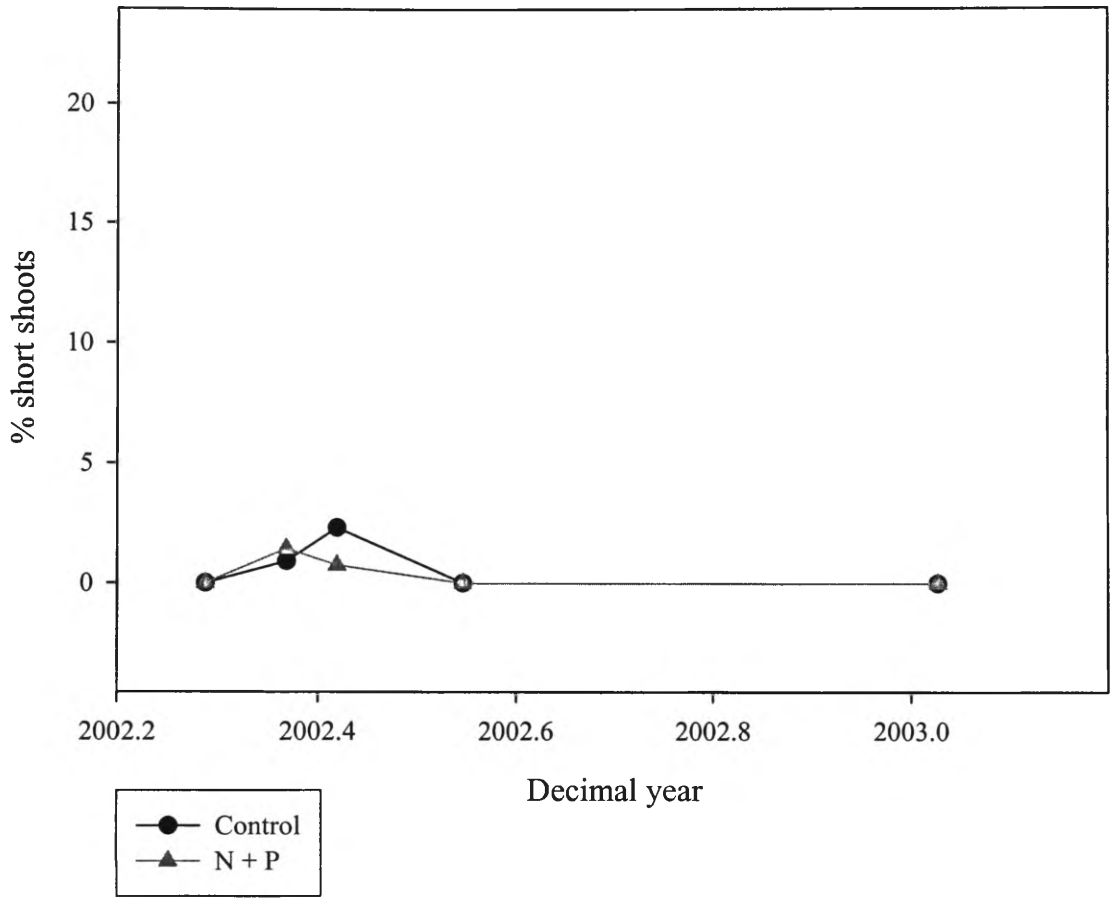


Fig. 19. N + P addition site 217 flowering frequencies; $\mu_{control} = 2.26$, $\mu_{N+P} = 0.15$.

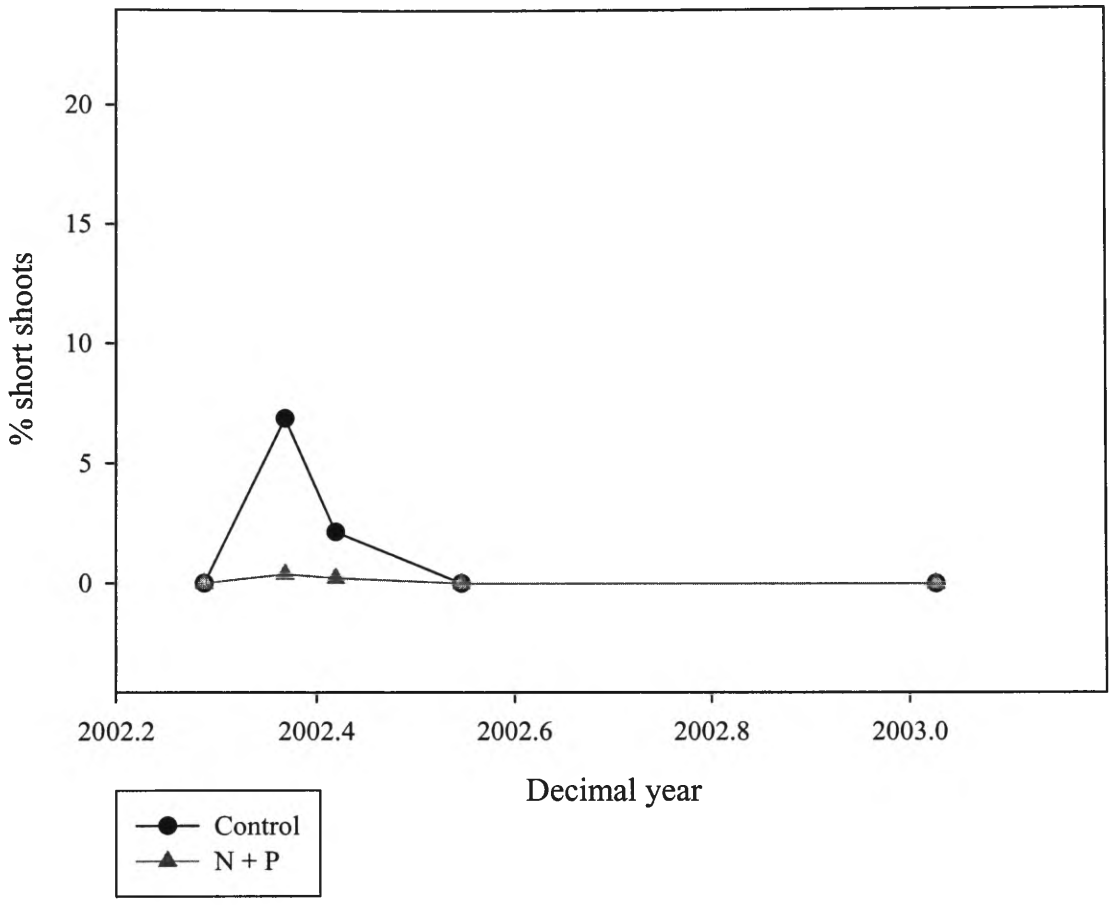


Fig. 20. N + P addition site 224 flowering frequencies; $\mu_{control} = 7.23$, $\mu_{N+P} = 0.59$.

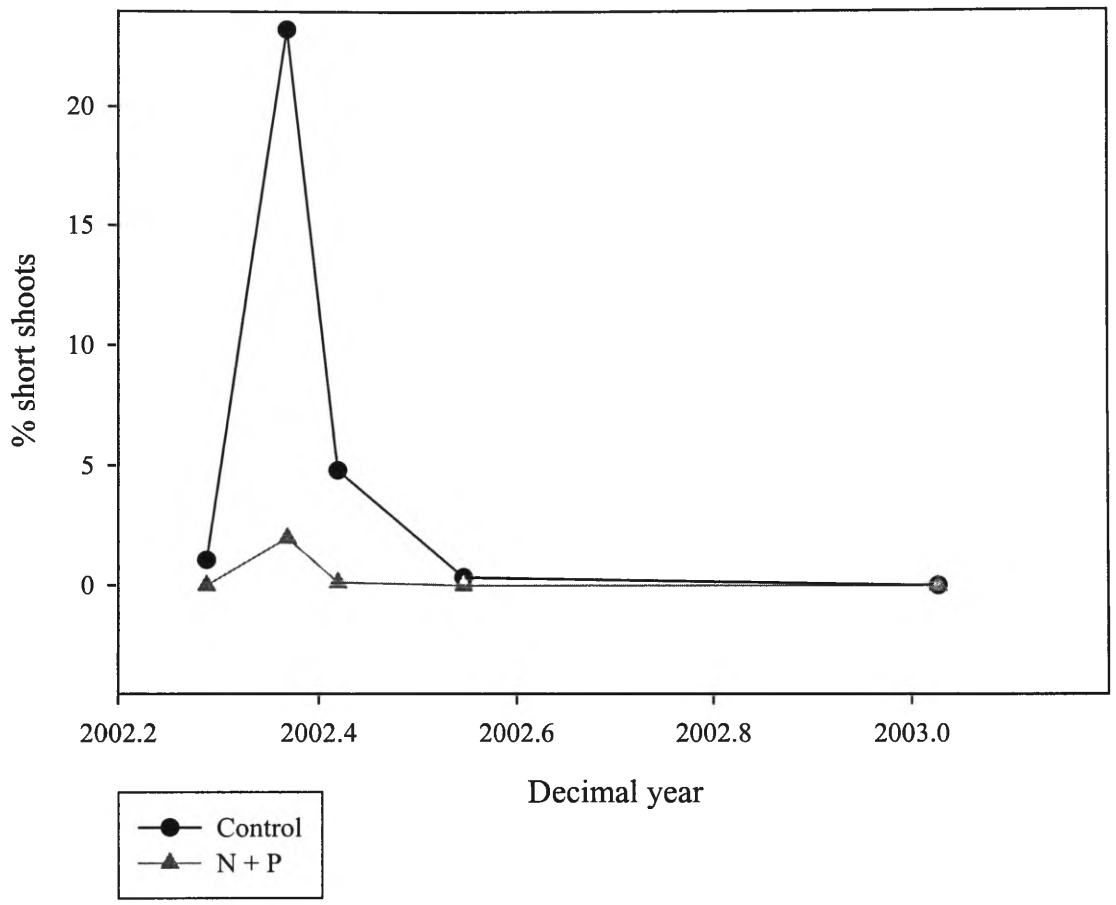


Fig. 21. N + P addition site 225 flowering frequencies; $\mu_{control} = 1.39$, $\mu_{N+P} = 0.73$.

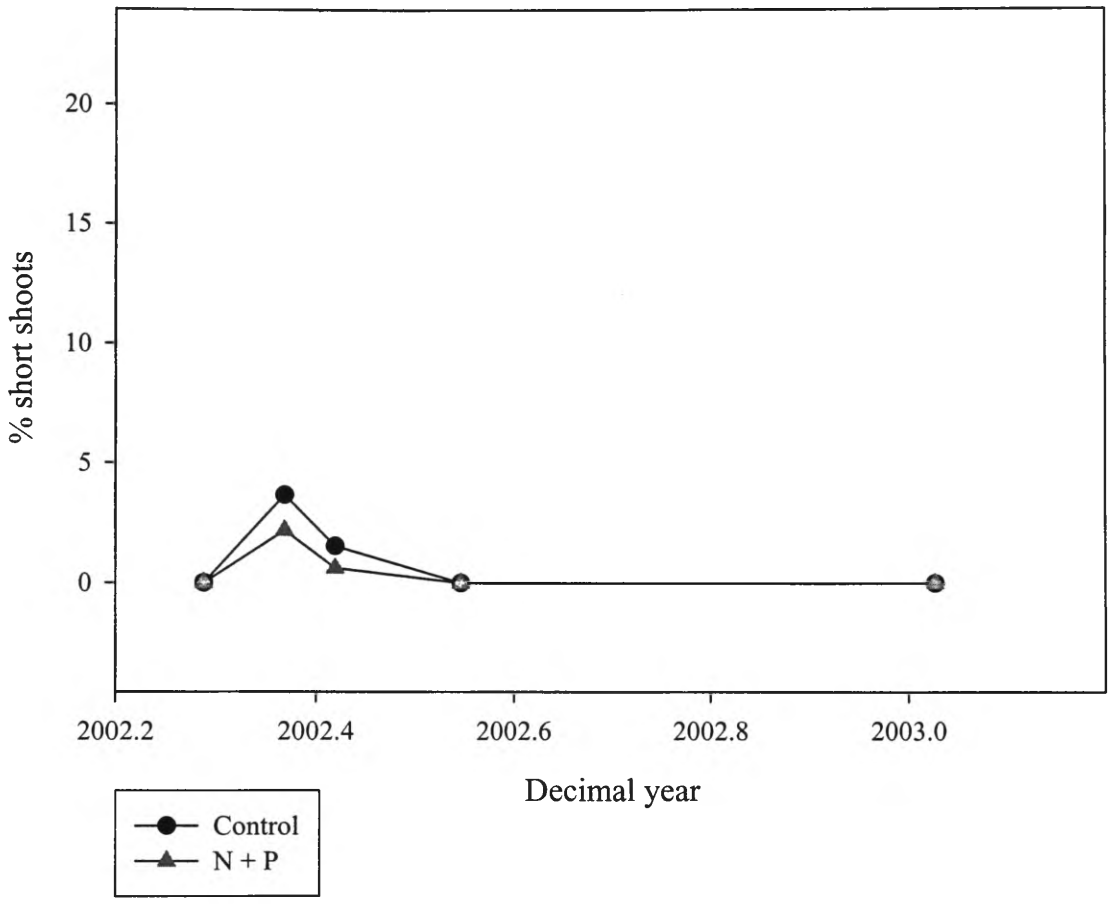


Table 21. Compiled floral numbers and sex ratios for inshore and offshore control and N + P sites.

Site/treatment	Male	Female	Fruit	Total female	M:F
Inshore control					
213	18	78	24	102	0.18
214	100	14	8	22	4.55
220	46	9	3	12	3.83
223	36	15	15	30	1.2
227	8	2	0	2	4
Totals	208	118	50	168	1.24
Inshore N + P					
213	0	0	0	0	n/a
214	14	7	4	11	1.27
220	5	0	0	0	5:0*
223	33	9	4	13	2.54
227	0	0	0	0	n/a
Totals	52	16	8	24	2.17
Offshore control					
215	17	46	17	63	0.27
216	0	22	8	30	0:30*
217	95	0	0	0	95:0*
224	318	32	10	42	7.57
225	64	2	1	3	21.33
Totals	494	102	36	138	3.58
Offshore N + P					
215	3	0	0	0	3:0*
216	15	0	0	0	15:0*
217	6	0	0	0	6:0*
224	24	2	0	2	12
225	32	3	3	6	5.33
Totals	80	5	3	8	10
Inshore + Offshore					
control	702	220	86	306	2.29
N+ P	132	21	11	32	4.13

* Ratio is expressed as either male only or female only.

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Appendix 1. Observed *T. testudinum* male flowers by site and sampling period.

Site	Sample month	Feb '02	Mar '02	May '02	Jun '02	Sep '02	Jan '03	Total
	Decimal year	2002.12	2002.24	2002.35	2002.43	2002.68	2003.01	
214		0	0	11	6	0	0	17
215		0	0	12	3	0	0	15
216		0	0	0	53	0	0	53
220		0	0	27	5	0	0	32
223		0	0	31	14	0	0	45
225		0	0	17	14	0	0	31
227		0	0	2	0	0	0	2
235			0	7	0	0	0	7
237			0	2	0	0	0	2
239			0	0	0	0	0	0
241			0	4	0	0	0	4
243			0	10	5	0	0	15
248			0	3	7	0	0	10
255			0		2	0	0	2
260			0		0	0	0	0
267			0		0	0	0	0
269			0		2	0	0	2
271			0		0	0	0	0
273			0		0	0	0	0
276			0		0	0	0	0
284		0	0	6	0	0	0	6
285		0	0	6	1	0	0	7
287		0	0	27	0	0	0	27
291		0	0	9	2	0	0	11
294		0	0	7	0	0	0	7
296		0	0	0	0	0	0	0
305			1	87	0	0	0	88
307			0	2	0	0	0	2
309			0	0	0	0		0
314			0	2	0	0	0	2
Total		0	1	272	114	0	0	387
n sites		13	30	23	30	30	29	

Appendix 2. Observed *T. testudinum* total female floral bodies (flowers and fruits) by site and sampling period. The value in () represents the number of fruits relative to the floral body total.

Site	Sample month	Feb '02	Mar '02	May '02	Jun '02	Sep '02	Jan '03	Total
	Decimal year	2002.12	2002.24	2002.35	2002.43	2002.68	2003.01	
214		0	0	1(1)	0	0	0	1(1)
215		0	0	0	1(1)	0	0	1(1)
216		0	0	3(0)	0	0	0	3(0)
220		0	0	4(1)	6(3)	0	0	10(4)
223		0	0	4(0)	6(3)	0	0	10(3)
225		0	0	11(5)	8(4)	32(32)	0	51(41)
227		0	0	0	10(6)	0	0	10(6)
235			0	0	0	0	0	0
237			0	2(0)	1(1)	0	0	3(1)
239			0	49(3)	191(164)	37(37)	0	277(204)
241			0	2(0)	0	0	0	2(0)
243			0	1(0)	0	10(10)	0	11(10)
248			0	2(0)	13(9)	32(32)	0	47(41)
255			0		1(1)	31(31)	0	32(32)
260			0		0	0	0	0
267			0		3(0)	0	0	3(0)
269			0		2(1)	0	0	2(1)
271			0		0	0	0	0
273			0		0	0	0	0
276			0		1(0)	0	0	1(0)
284		0	0	6(6)	0	0	0	6(6)
285		0	0	11(0)	0	0	0	11(0)
287		0	0	1(0)	0	0	0	1(0)
291		0	0	2(0)	0	0	0	2(0)
294		0	0	39(2)	6(3)	0	0	45(5)
296		0	0	0	0	0	0	0
305			0	33(12)	0	0	0	33(12)
307			0	0	0	0	0	0
309			0	0	0	0		0
314			0	1	0	0	0	1
Total		0	0	172(30)	249(196)	142(142)	0	563(368)
n sites		13	30	23	30	30	29	

Appendix 3. Observed *T. testudinum* male flowering frequencies by site and sample period.

Site	Sample month	Feb '02	Mar '02	May '02	Jun '02	Sep '02	Jan '03
	Decimal year	2002.12	2002.24	2002.35	2002.43	2002.68	2003.01
214		0	0	1.31	0.01	0	0
215		0	0	1.32	$3.75e^{-3}$	0	0
216		0	0	0	0.07	0	0
220		0	0	3.51	0.01	0	0
223		0	0	4.11	0.02	0	0
225		0	0	2.11	0.02	0	0
227		0	0	0.27	0	0	0
235			0	0.88	0	0	0
237			0	0.23	0	0	0
239			0	0	0	0	0
241			0	0.53	0	0	0
243			0	1.22	0.66	0	0
248			0	0.34	0.92	0	0
255			0		0.27	0	0
260			0		0	0	0
267			0		0	0	0
269			0		0.26	0	0
271			0		0	0	0
273			0		0	0	0
276			0		0	0	0
284		0	0	0.82	0	0	0
285		0	0	0.76	0.12	0	0
287		0	0	3.69	0	0	0
291		0	0	1.27	0.26	0	0
294		0	0	0.93	0	0	0
296		0	0	0	0	0	0
305			$1.26e^{-3}$	15.85	0	0	0
307			0	0.28	0	0	0
309			0	0	0	0	
314			0	0.27	0	0	0
Mean		0	$4.43e^{-5}$	1.54	0.48	0	0

Mean pooled male short shoot flowering frequency (May, June, and September) 0.60%

Appendix 4. Observed *T. testudinum* total female flowering frequencies (flowers and fruits) by site and sampling period. The value in () represents the frequency of fruits relative to the floral body total.

Site	Sample month Decimal year	Feb '02 2002.12	Mar '02 2002.24	May '02 2002.35	Jun '02 2002.43	Sep '02 2002.68	Jan '03 2003.01
214		0	0	0.12(0.12)	0	0	0
215		0	0	0	0.12(0.12)	0	0
216		0	0	0.37(0)	0	0	0
220		0	0	0.52(0.13)	0.71(0.36)	0	0
223		0	0	0.53(0)	0.76(0.38)	0	0
225		0	0	1.37(0.62)	1.19(0.59)	4.13(4.13)	0
227		0	0	0	1.27(0.76)	0	0
235			0	0	0	0	0
237			0	0.23(0)	0.12(0.12)	0	0
239			0	6.10(0.37)	24.21(20.79)	4.92(4.92)	0
241			0	0.26(0)	0	0	0
243			0	0.12(0)	0	1.36(1.36)	0
248			0	0.23(0)	1.70(1.18)	3.98(3.98)	0
255			0		0.14(0.14)	4.45(4.45)	0
260			0		0	0	0
267			0		0.33(0.13)	0	0
269			0		0.26(0)	0	0
271			0		0	0	0
273			0		0	0	0
276			0		0.13(0)	0	0
284		0	0	0	0	0	0
285		0	0	1.40(0)	0	0	0
287		0	0	0.14(0)	0	0	0
291		0	0	0.28(0)	0	0	0
294		0	0	5.16(0.26)	0.75(0.38)	0	0
296		0	0	0	0	0	0
305			0	6.01(2.19)	0	0	0
307			0	0	0	0	0
309			0	0	0	0	
314			0	1.33e-03(0)	0	0	0
Mean		0	0	0.97(0.17)	1.05(0.83)	0.64(0.64)	0

Mean pooled female short shoot flowering frequency (May, June, and September) 0.87%(0.57%)

Appendix 5. *T. testudinum* compiled short shoot morphometric measurements (n=3758). Values are reported as site means.

Site	n shoots	Rhizome diameter	SS length length (mm)	Avg. leaf length (mm)	Avg. leaf width (mm)
214	115	4.21	27.93	149.15	5.57
215	133	3.95	52.65	123.72	6.65
216	130	5.25	47.60	118.42	7.60
220	113	4.88	25.89	169.40	5.99
223	142	4.52	56.77	177.03	6.68
225	133	5.96	49.70	106.93	6.77
227	99	4.46	39.17	171.95	7.02
235	128	4.33	19.77	61.49	5.87
237	119	4.26	21.84	102.40	6.25
239	119	4.75	39.10	101.79	6.33
241	125	5.05	22.12	163.22	5.68
243	126	4.16	30.75	113.90	5.51
248	134	4.10	25.22	134.84	5.98
255	108	3.84	18.03	96.84	4.30
260	130	5.12	28.37	157.49	6.31
267	112	3.76	28.20	121.83	5.89
269	128	3.73	21.68	113.95	5.44
271	116	3.95	19.37	78.89	5.22
273	133	3.99	29.10	115.68	5.83
276	121	4.55	21.38	146.48	5.70
284	114	5.56	17.49	288.92	6.12
285	132	5.34	24.12	134.49	6.85
287	126	4.93	35.56	160.58	5.85
291	159	4.16	41.04	166.99	6.05
294	124	3.81	36.77	191.13	7.16
296	111	5.54	23.91	257.58	6.50
305	114	5.22	19.16	121.46	5.77
307	158	5.07	32.85	168.76	6.63
309	124	4.19	15.21	119.37	5.79
314	132	5.52	32.64	160.18	6.98
Pooled means	125	4.61	30.11	143.16	6.14

Appendix 6. *T. testudinum* compiled site-specific average leaf area per short shoot and pooled total leaf area for all leaves measured for each core, respectively.

Site	n shoots	Avg. leaf area per short shoot ($cm^{-2}SS^{-1}$)	Pooled total leaf area (cm^2)	Total leaves
214	115	39.99	18833.24	471
215	133	33.90	16714.35	493
216	130	24.65	7765.10	315
220	113	47.18	21229.56	450
223	142	44.11	20865.97	473
225	133	23.98	9521.21	397
227	99	44.78	15045.05	336
235	128	11.31	4126.95	365
237	119	18.90	5877.25	311
239	119	20.94	7141.27	341
241	125	38.35	17374.23	453
243	126	21.14	8393.78	397
248	134	28.69	12281.17	428
255	108	12.89	3776.66	293
260	130	38.58	16899.52	438
267	112	22.73	7206.49	317
269	128	22.47	9457.86	421
271	116	12.22	3945.48	323
273	133	26.48	12181.86	460
276	121	26.55	8842.32	333
284	114	82.36	37804.13	459
285	132	35.73	15899.23	445
287	126	33.39	13389.65	401
291	159	43.90	24537.48	559
294	124	48.45	18798.72	388
296	111	68.57	28183.48	411
305	114	27.09	10376.96	383
307	158	54.52	35053.88	643
309	124	26.94	10642.50	395
314	132	47.24	23289.02	493
Pooled means	125	34.27	14848.48	413

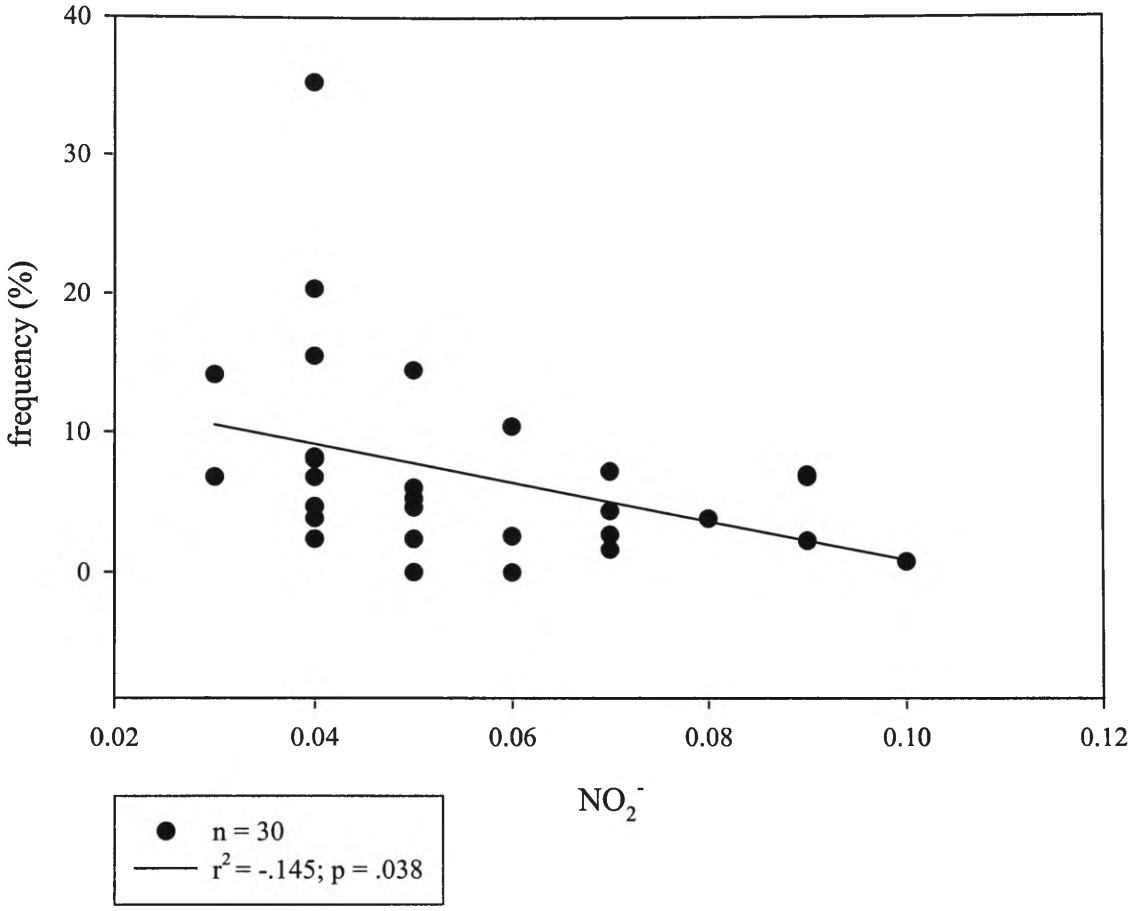
Appendix 7. Compiled demographic core total and sex-specific floral scar raw numbers.

Site	n shoots	Total floral scars	Male floral scars	Female floral scars
214	115	17	5	12
215	133	10	2	8
216	130	5	0	5
220	113	26	12	14
223	142	32	5	27
225	133	13	2	11
227	99	26	1	25
235	128	1	0	1
237	119	0	0	0
239	119	62	9	53
241	125	9	3	6
243	126	3	2	1
248	134	15	7	8
255	108	5	3	2
260	130	5	0	5
267	112	9	3	6
269	128	7	1	6
271	116	3	1	2
273	133	10	3	7
276	121	2	0	2
284	114	5	0	5
285	132	3	1	2
287	126	3	2	1
291	159	38	2	36
294	124	0	0	0
296	111	3	1	2
305	114	7	4	3
307	158	12	1	11
309	124	0	0	0
314	132	9	1	8
Total	3758	340	71	269

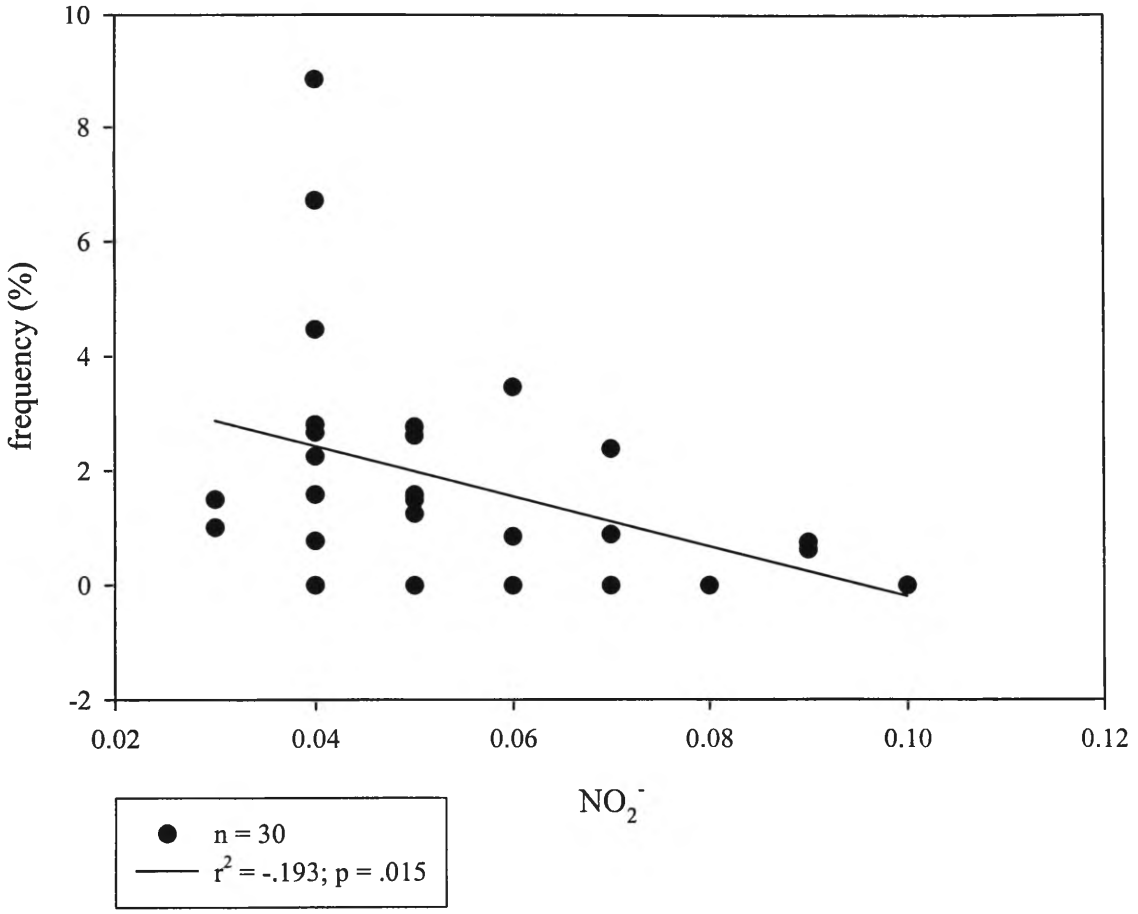
Appendix 8. *T. testudinum* compiled site-specific demographic core male:female floral scar ratios. Site observations that yielded only one sex are reported as total numbers of that sex, respectively.

Site	n shoots	Total floral scars	# Male floral scars	# Female floral scars	M:F floral scars
214	115	17	5	12	0.42
215	133	10	2	8	0.25
216	130	5	0	5	0 to 5
220	113	26	12	14	0.86
223	142	32	5	27	0.19
225	133	13	2	11	0.18
227	99	26	1	25	0.04
235	128	1	0	1	0 to 1
237	119	0	0	0	n/a
239	119	62	9	53	0.17
241	125	9	3	6	0.50
243	126	3	2	1	2
248	134	15	7	8	0.88
255	108	5	3	2	1.50
260	130	5	0	5	0 to 5
267	112	9	3	6	0.50
269	128	7	1	6	0.17
271	116	3	1	2	0.50
273	133	10	3	7	0.43
276	121	2	0	2	0 to 2
284	114	5	0	5	0 to 5
285	132	3	1	2	0.50
287	126	3	2	1	2
291	159	38	2	36	0.06
294	124	0	0	0	n/a
296	111	3	1	2	0.50
305	114	7	4	3	1.33
307	158	12	1	11	0.09
309	124	0	0	0	n/a
314	132	9	1	8	0.12
Total	3758	340	71	269	
Mean M:F floral scars					0.26

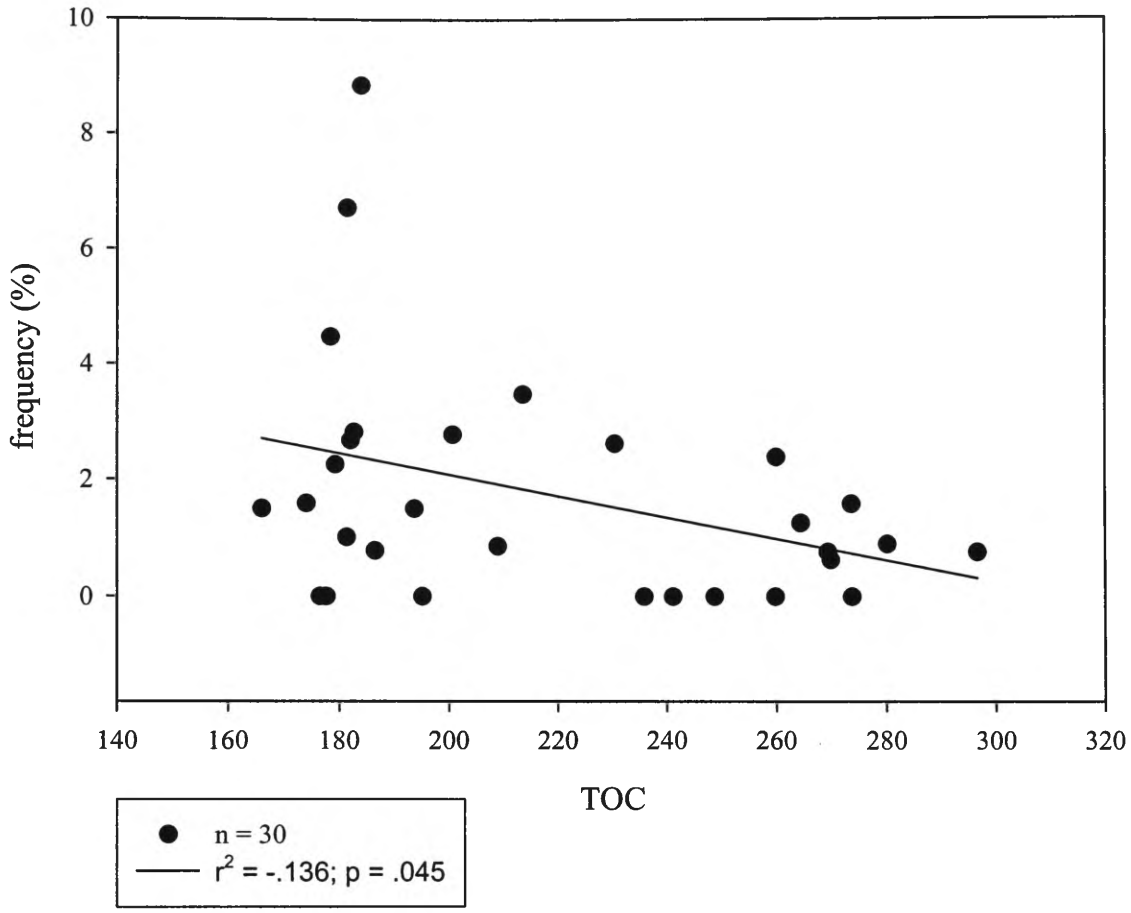
Appendix 9. Surface water NO_2^- versus demographic shoot flowering frequency.



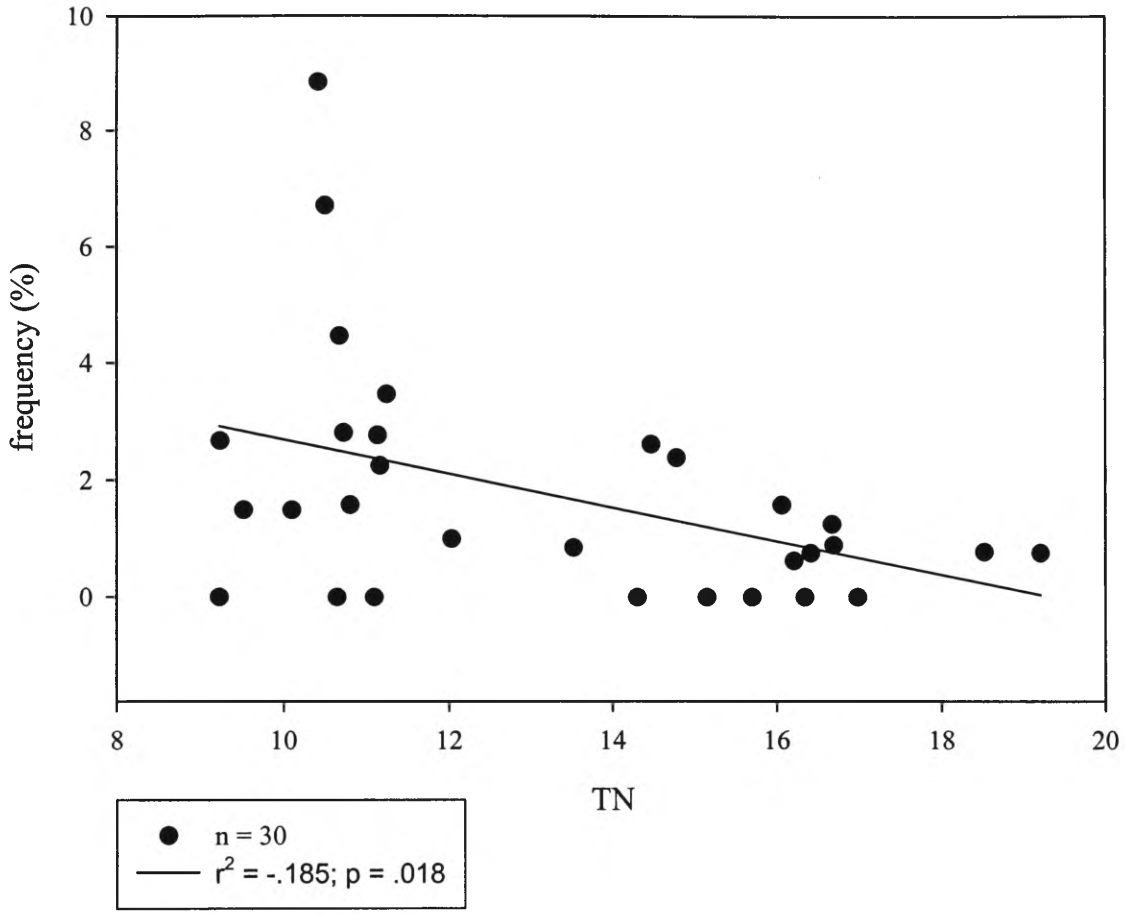
Appendix 10. Surface water NO_2^- versus demographic male shoot flowering frequency.



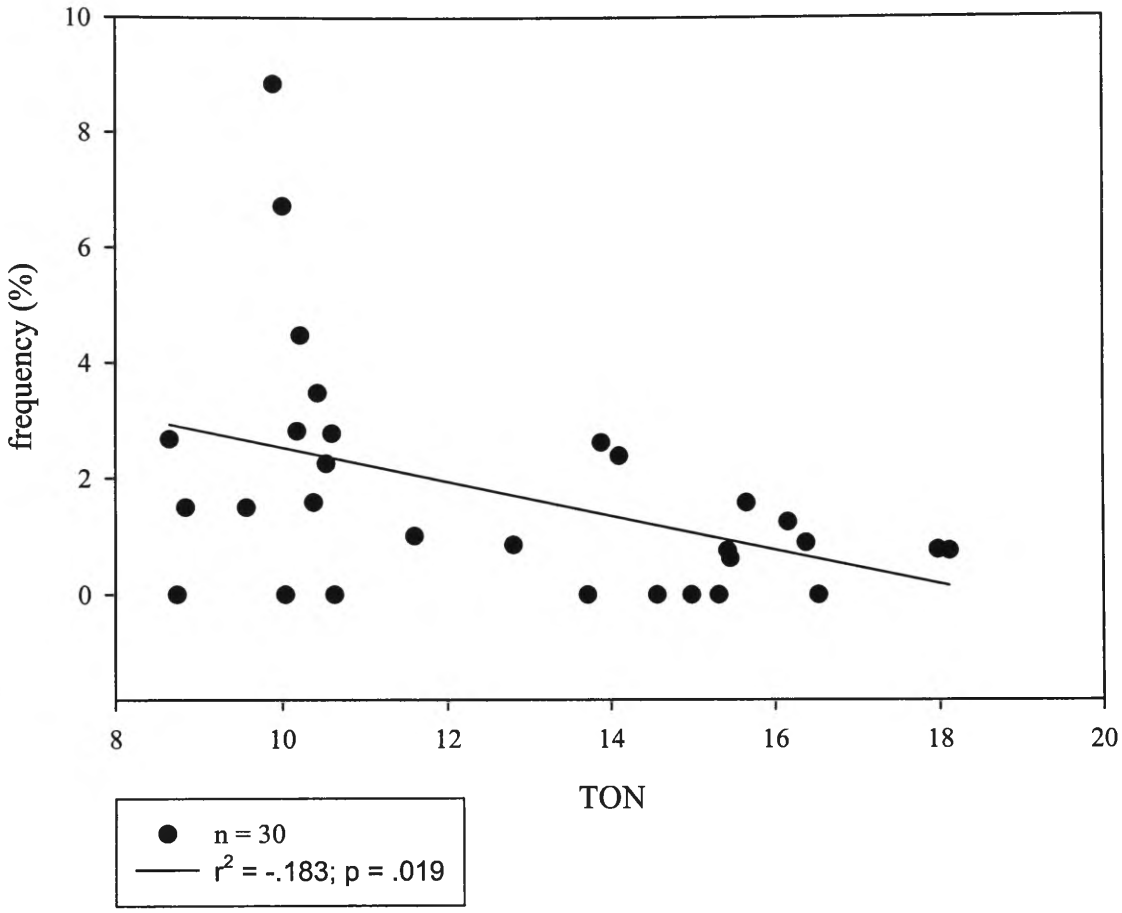
Appendix 11. Surface water TOC versus demographic male shoot flowering frequency.



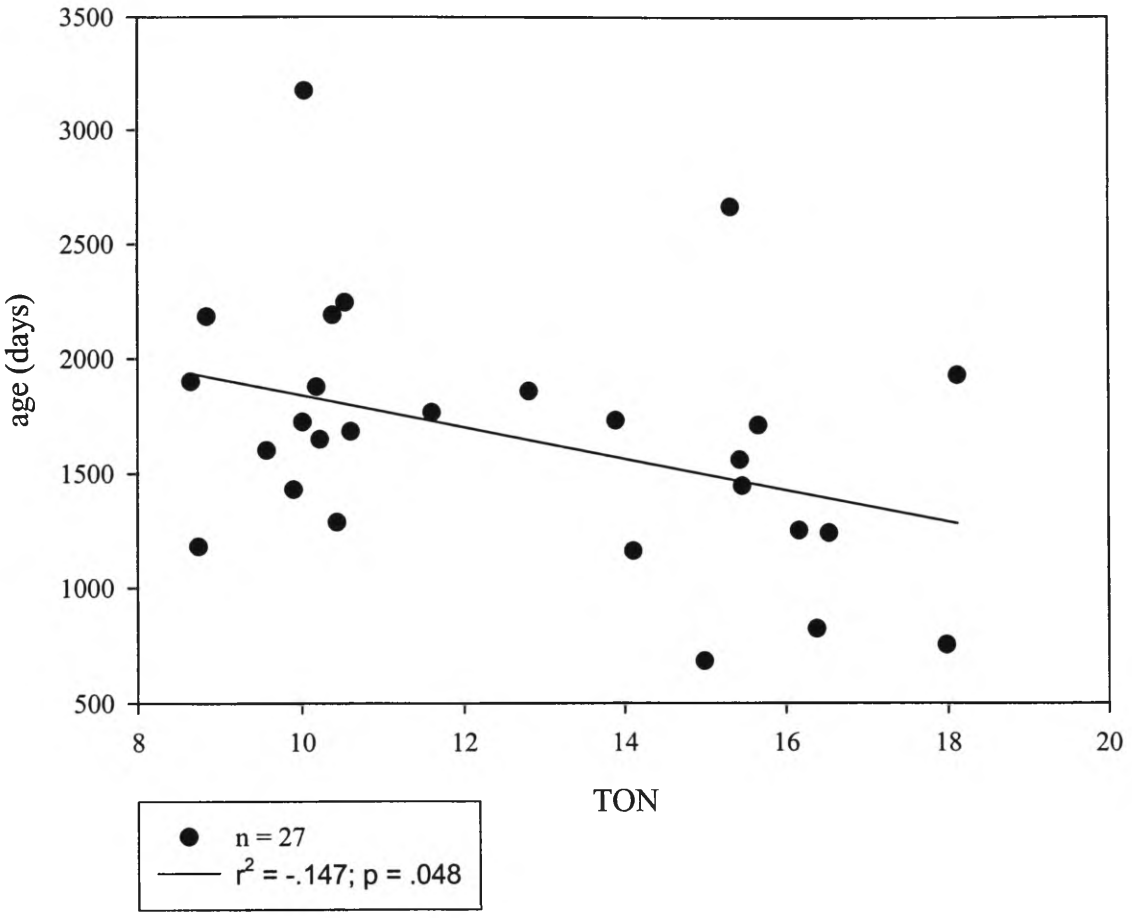
Appendix 12. Surface water TN versus demographic male shoot flowering frequency.



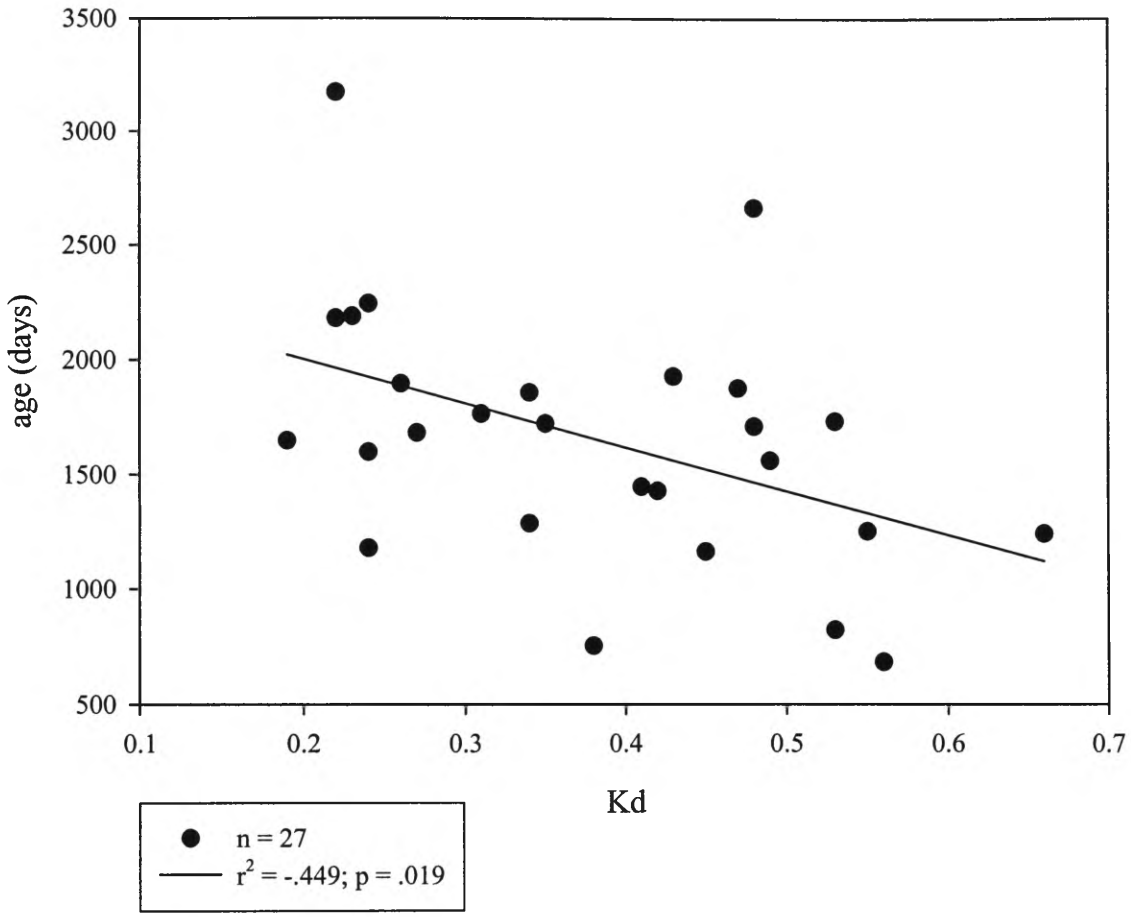
Appendix 13. Surface water TON versus demographic male shoot flowering frequency.



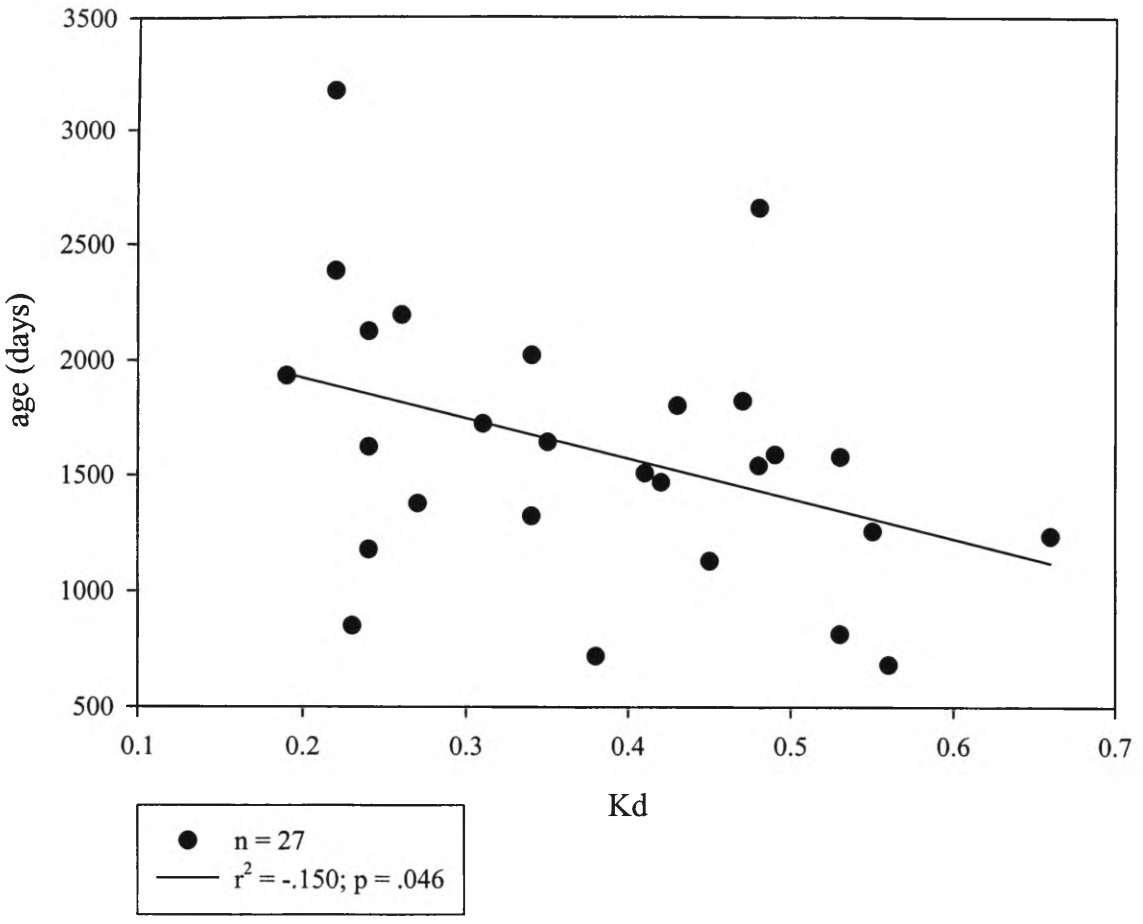
Appendix 14. Surface water TON versus average shoot age at first flowering.



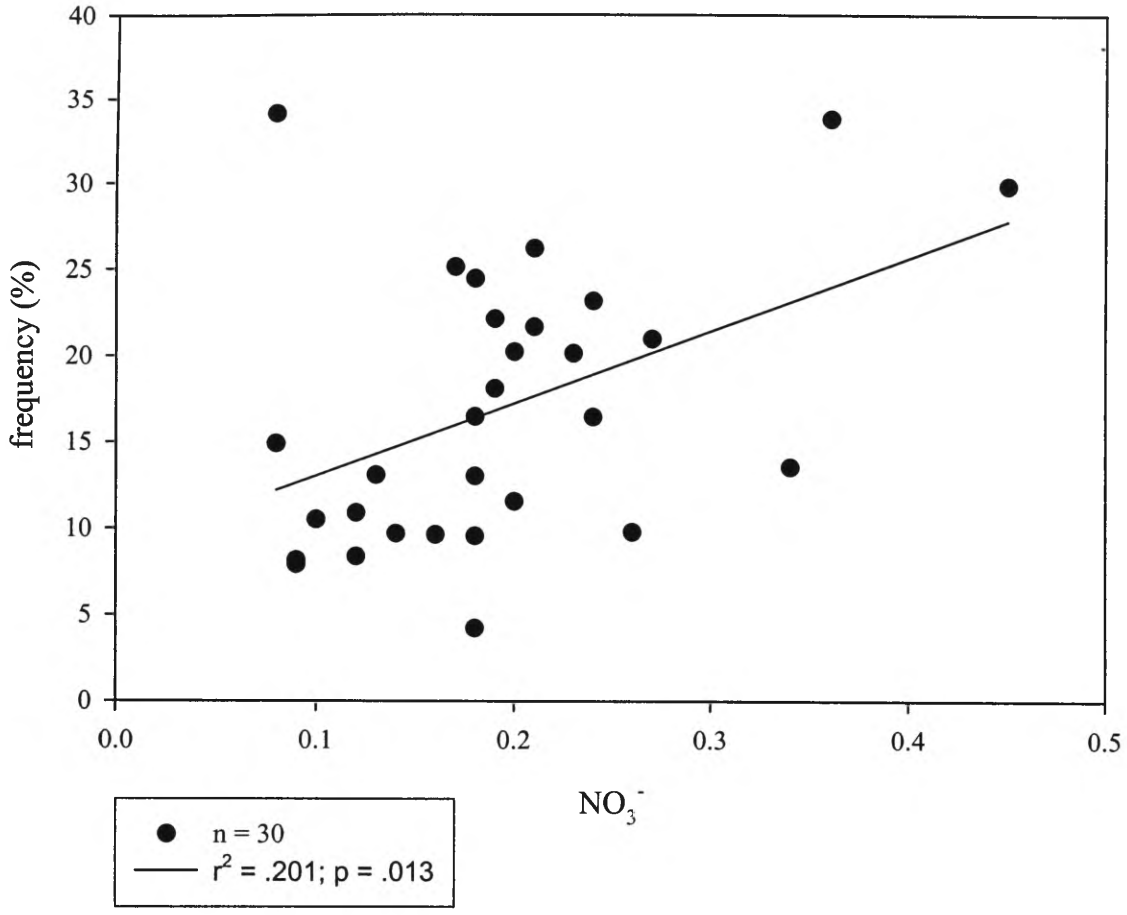
Appendix 15. Kd versus average female shoot age at first flowering.



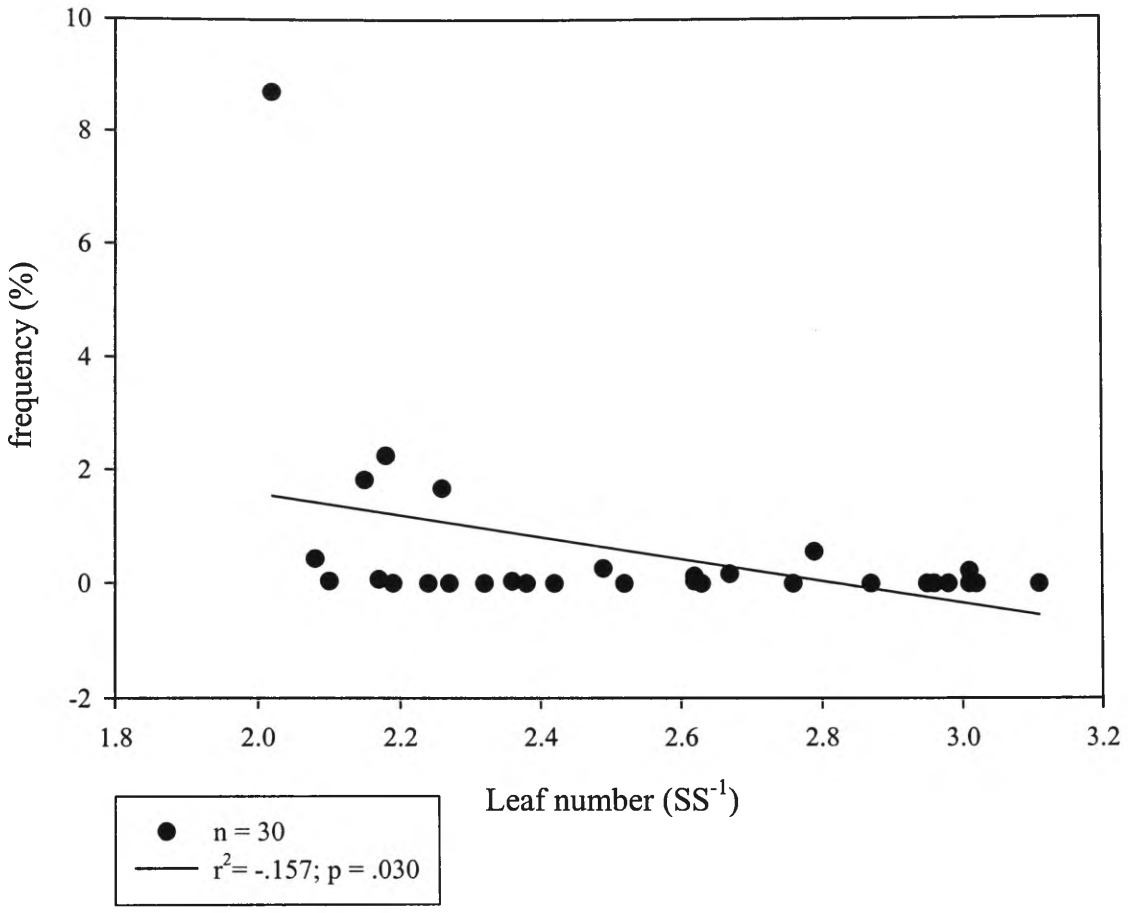
Appendix 16. Kd versus average female shoot age at first flowering.



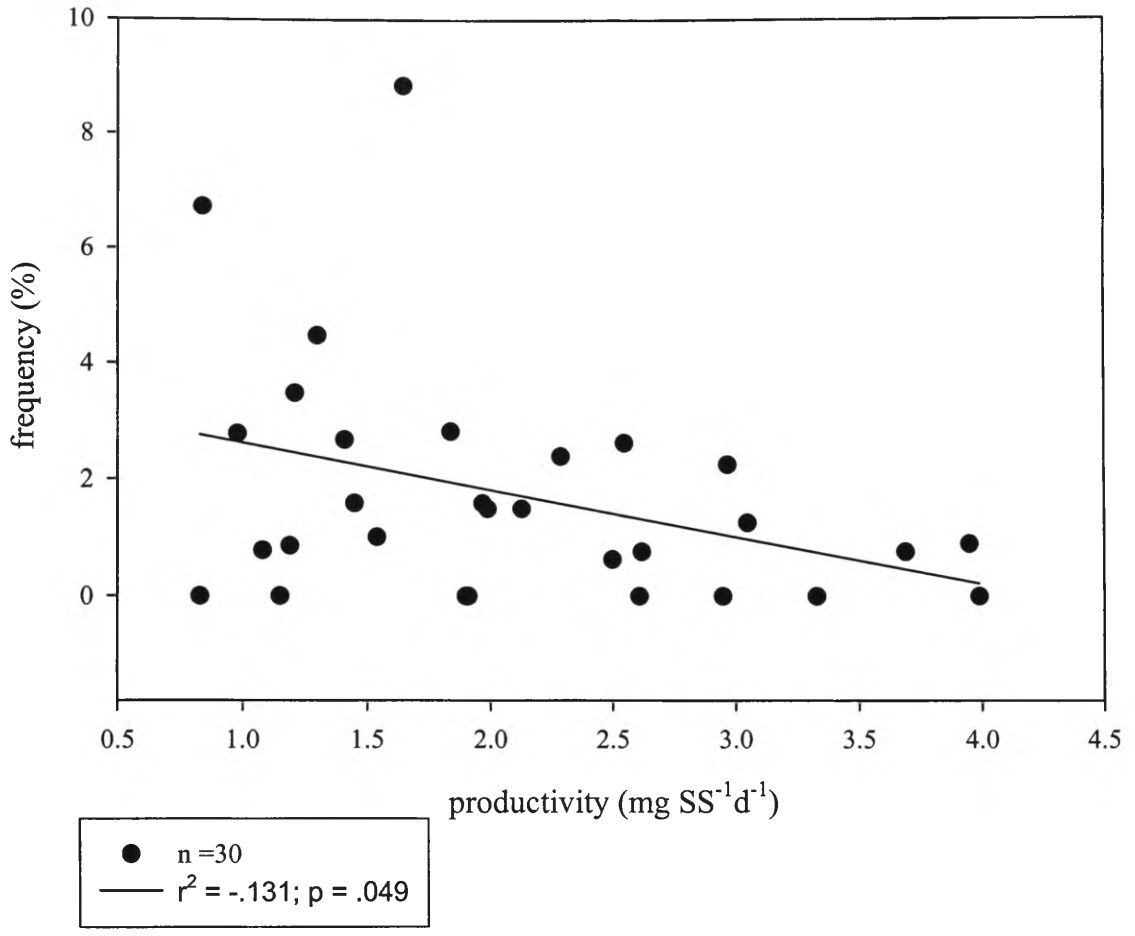
Appendix 17. Surface water NO_3^- versus apex frequency.



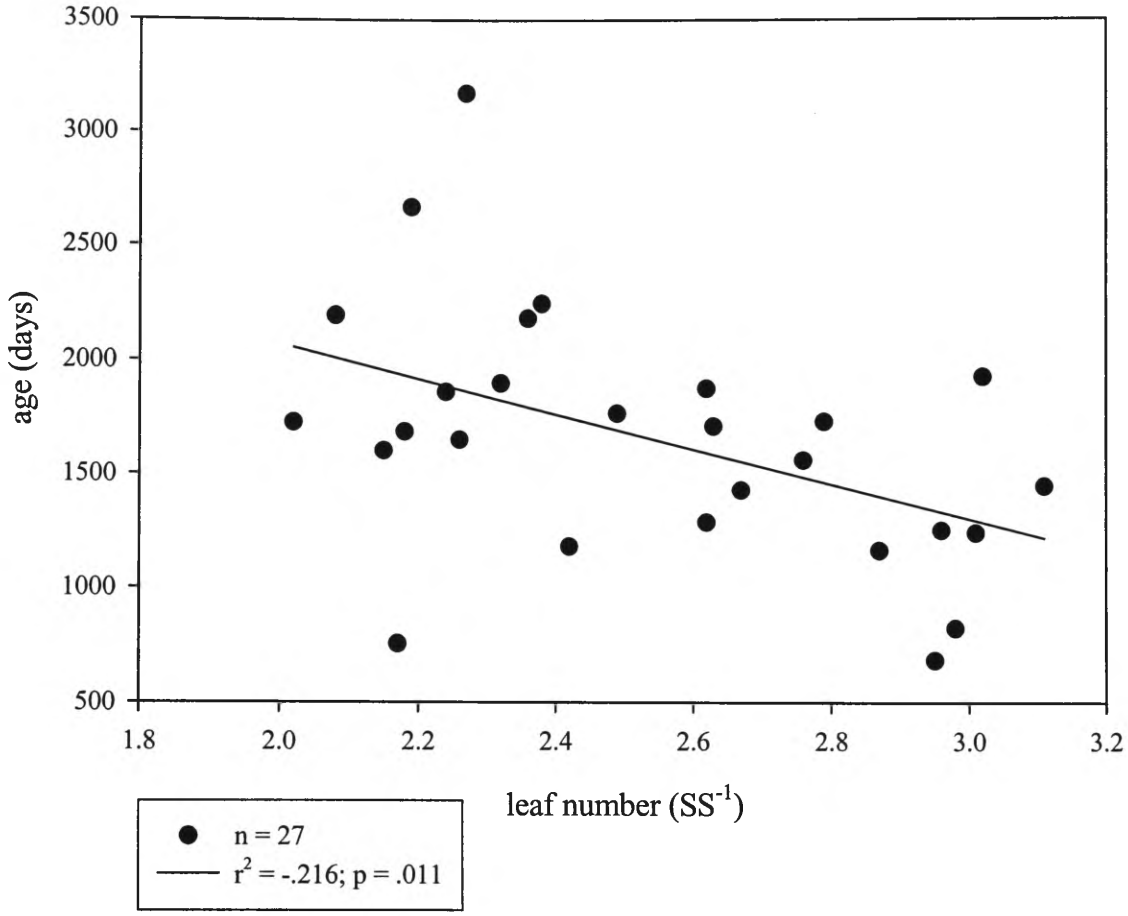
Appendix 18. Leaf number versus observed fruit frequency.



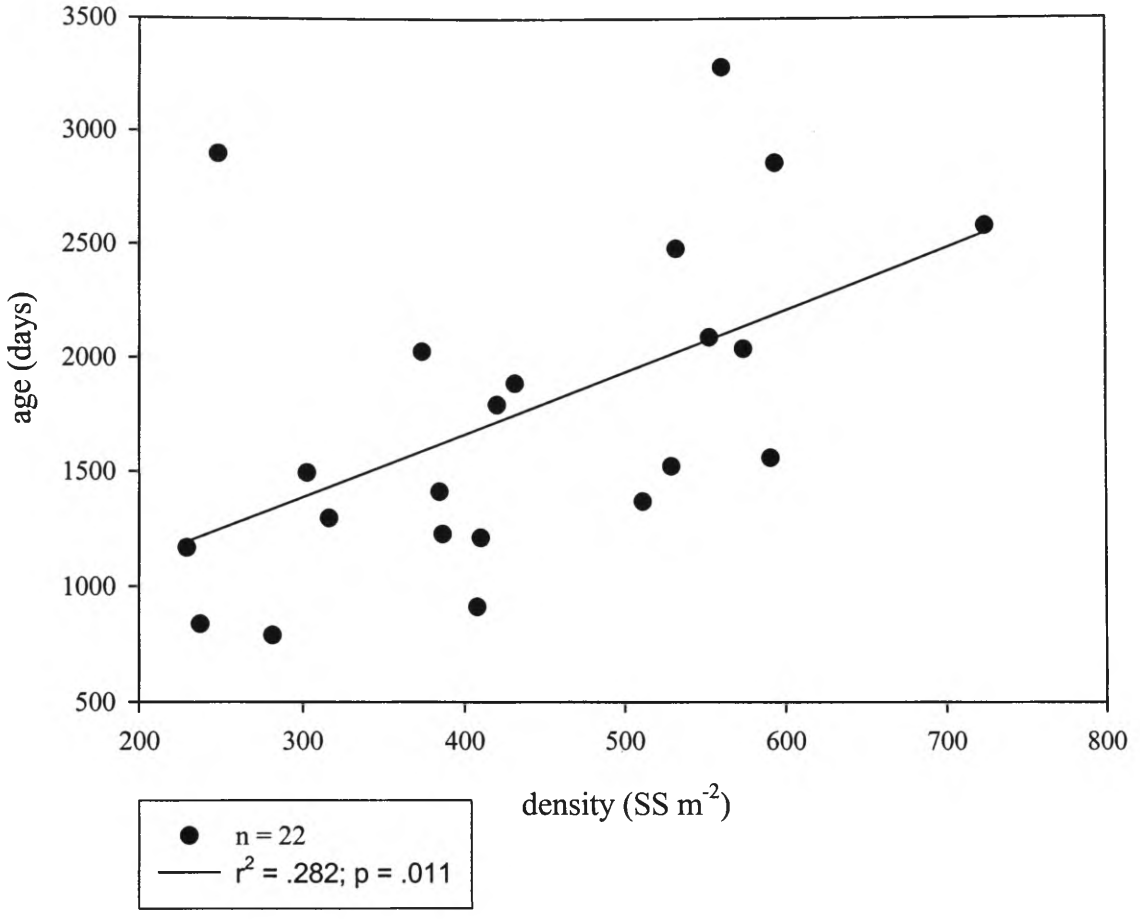
Appendix 19. Productivity versus demographic male shoot flowering frequency.



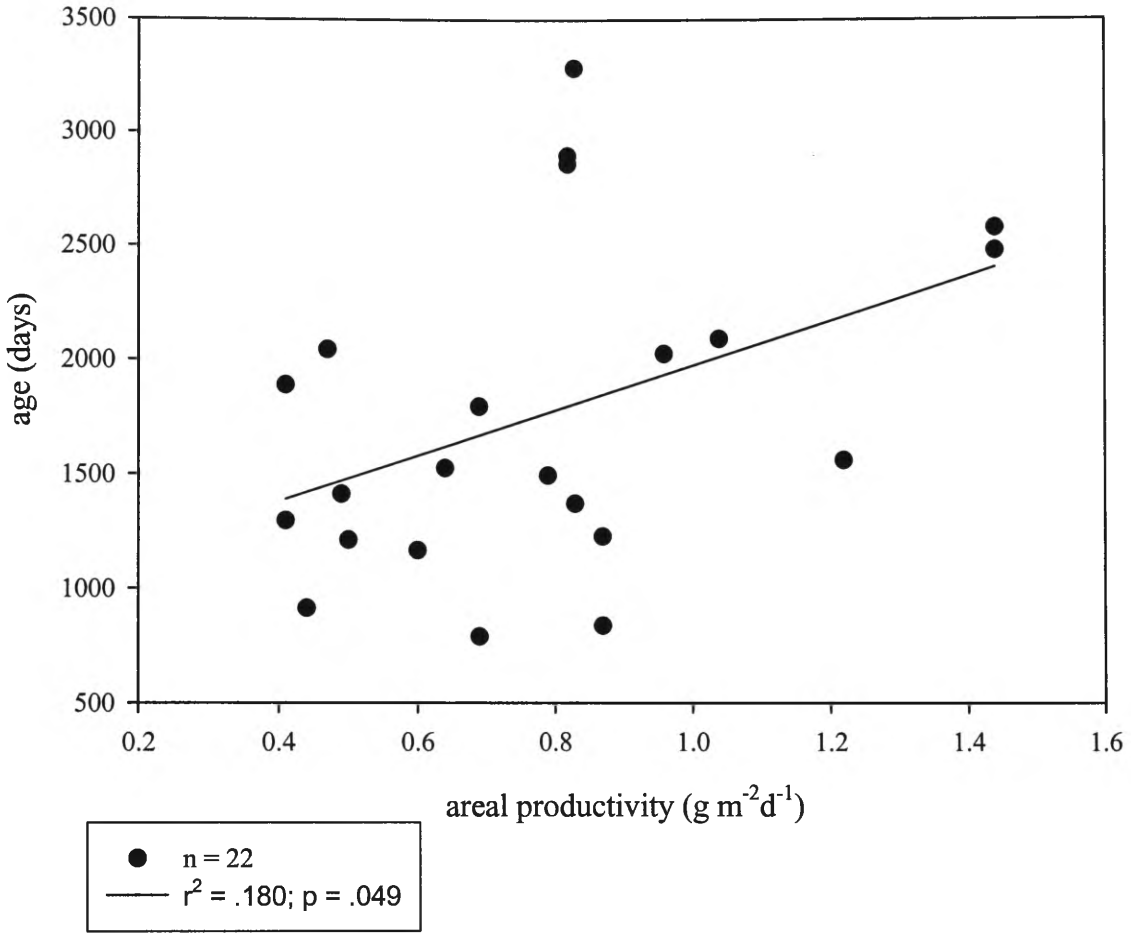
Appendix 20. Leaf number versus average shoot age at first flowering.



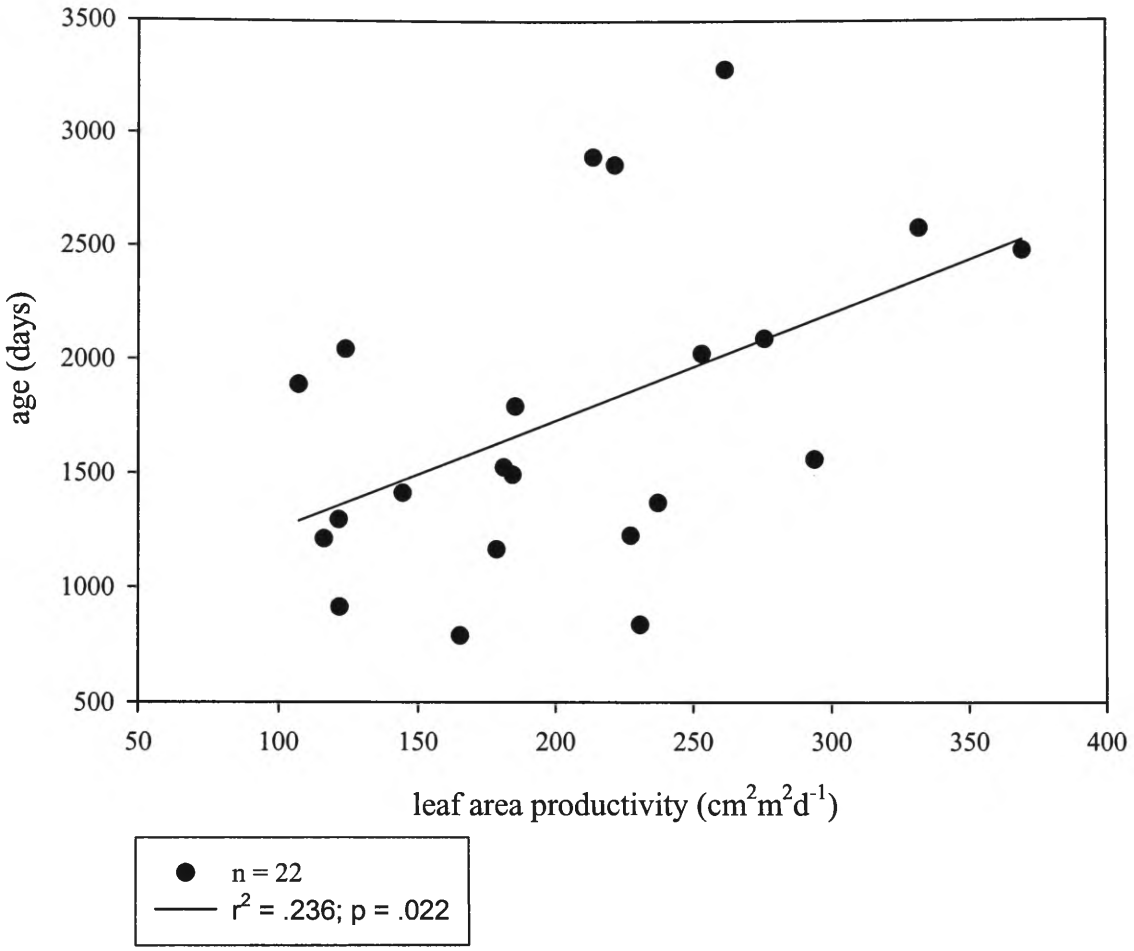
Appendix 21. Density versus average male shoot age at first flowering.



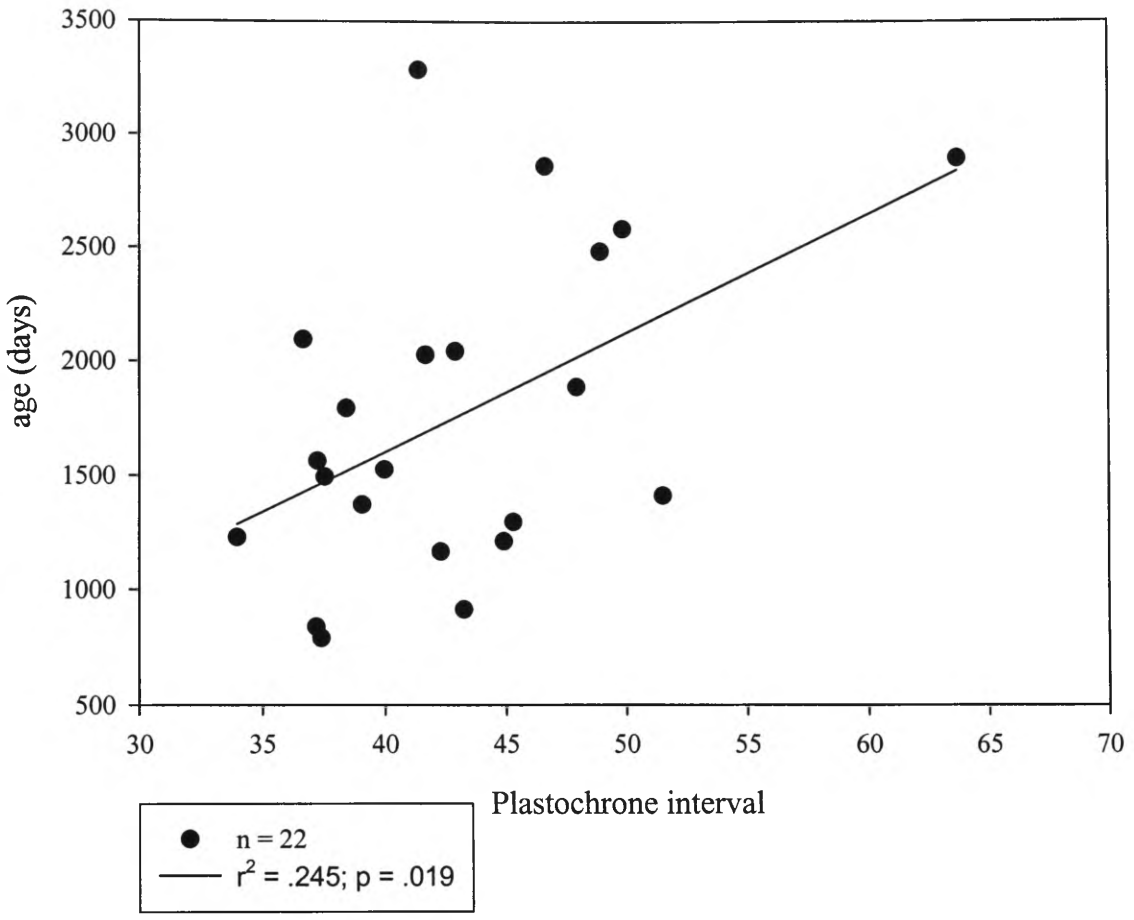
Appendix 22. Areal productivity versus average male shoot age at first flowering.



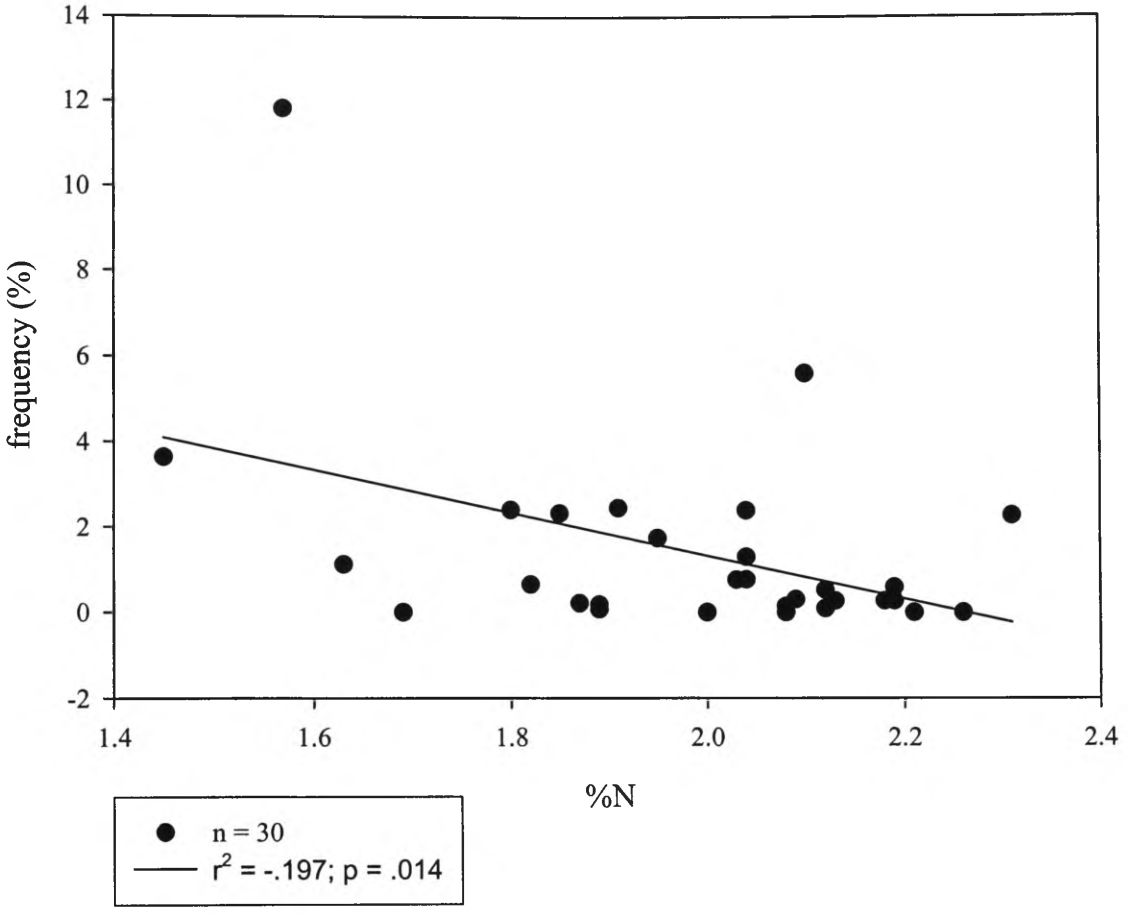
Appendix 23. Leaf area productivity versus average male shoot age at first flowering.



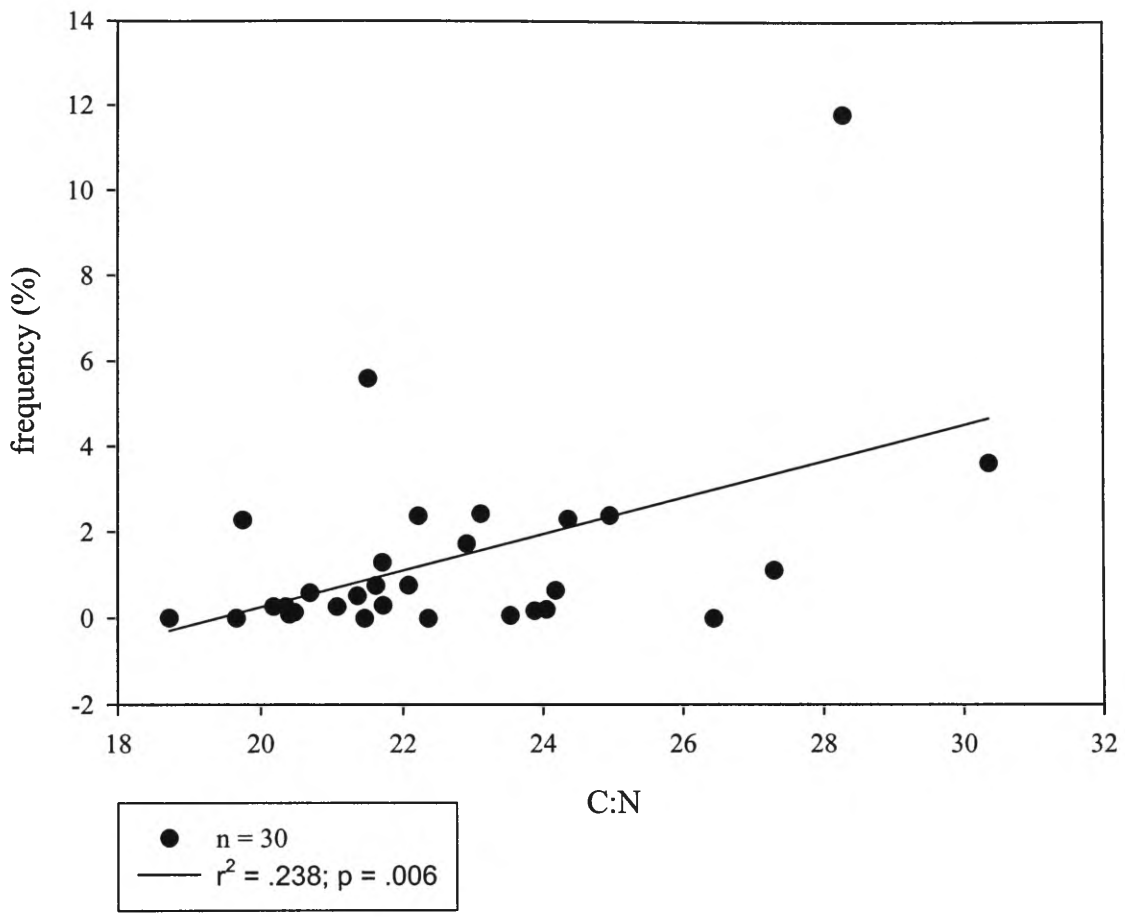
Appendix 24. Plastochrone interval versus average male shoot age at first flowering.



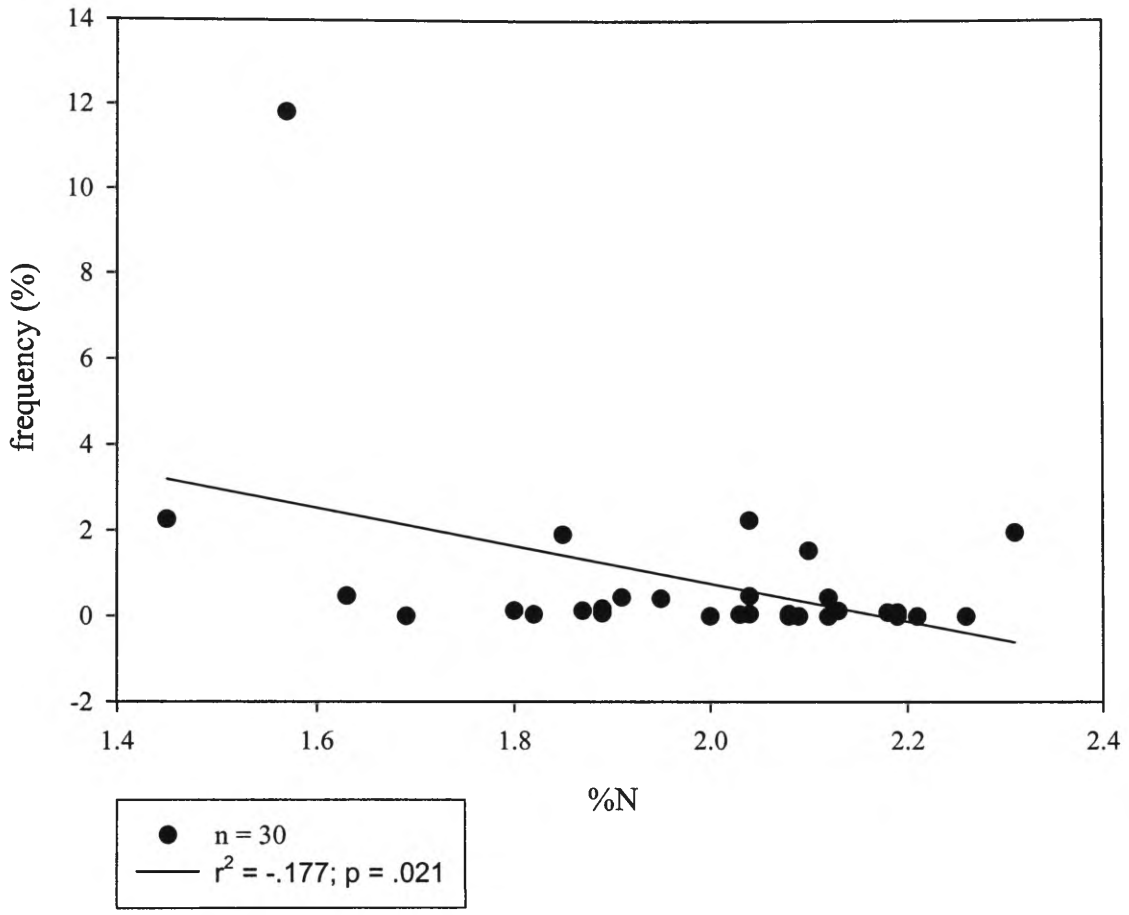
Appendix 25. *T. testudinum* leaf tissue %N versus observed total flowering frequency.



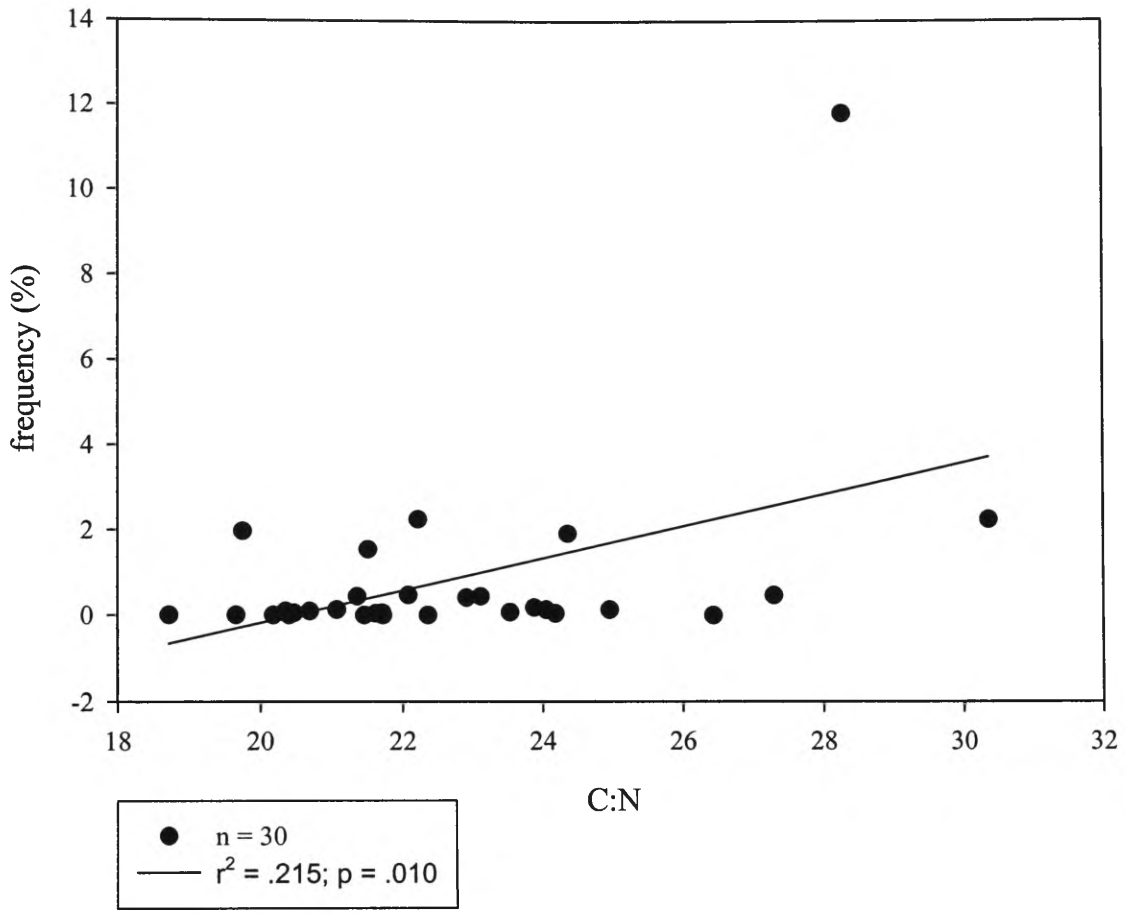
Appendix 26. *T. testudinum* leaf tissue C:N versus observed total flowering frequency.



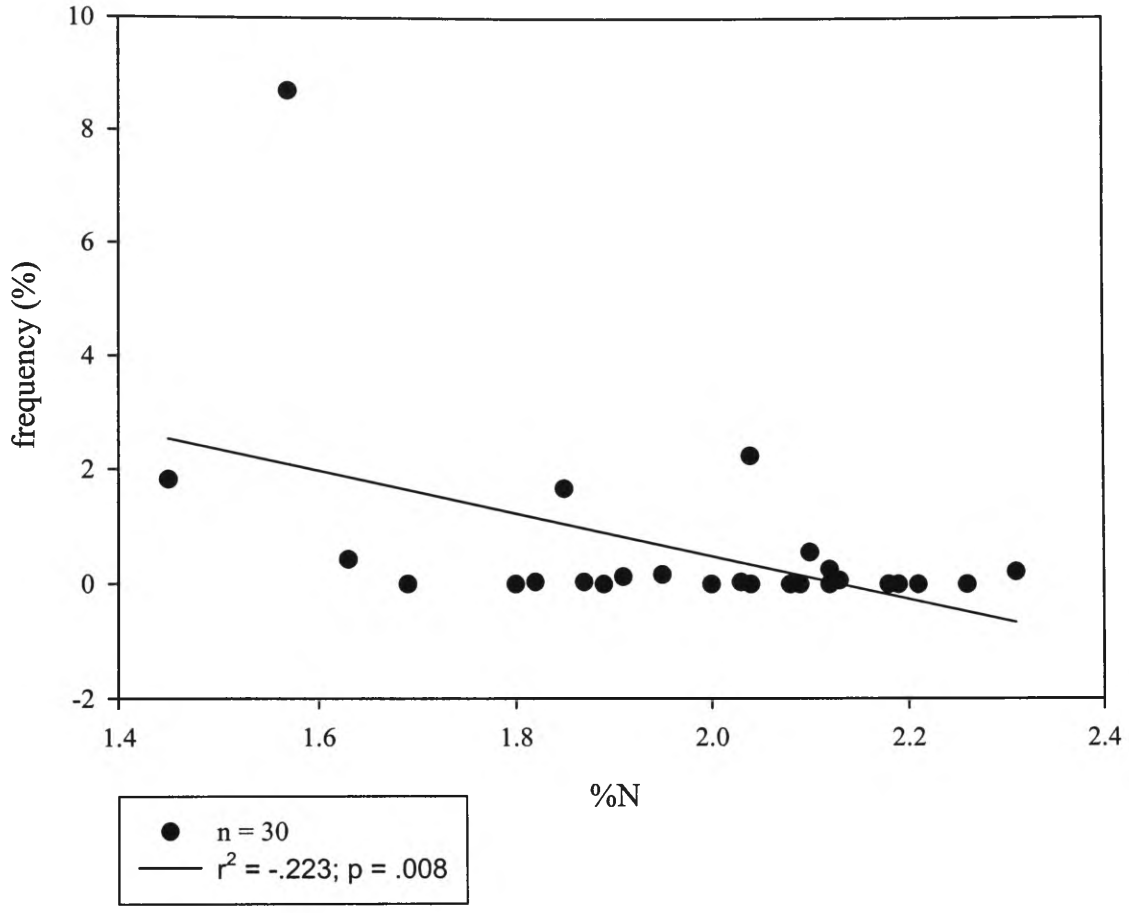
Appendix 27. *T. testudinum* leaf tissue %N versus observed total female flowering frequency.



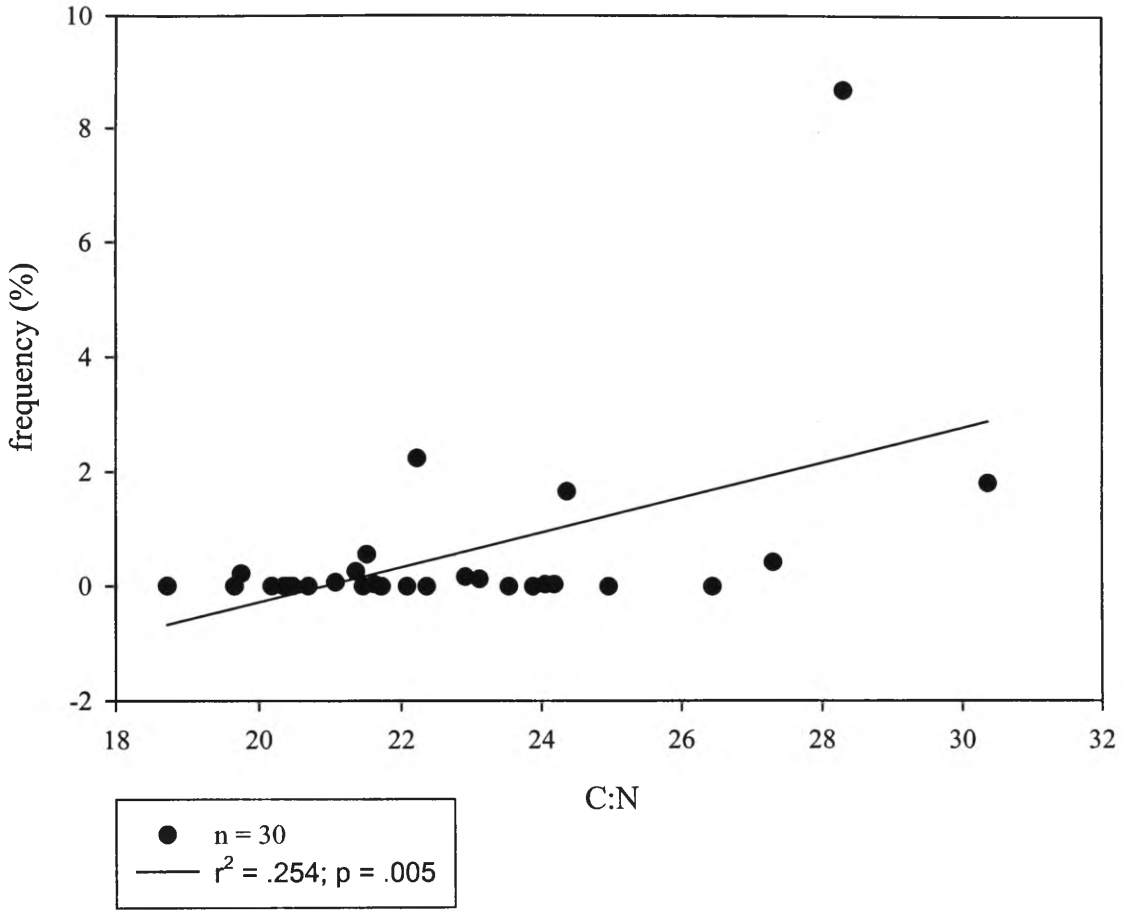
Appendix 28. *T. testudinum* leaf tissue C:N versus observed total female flowering frequency.



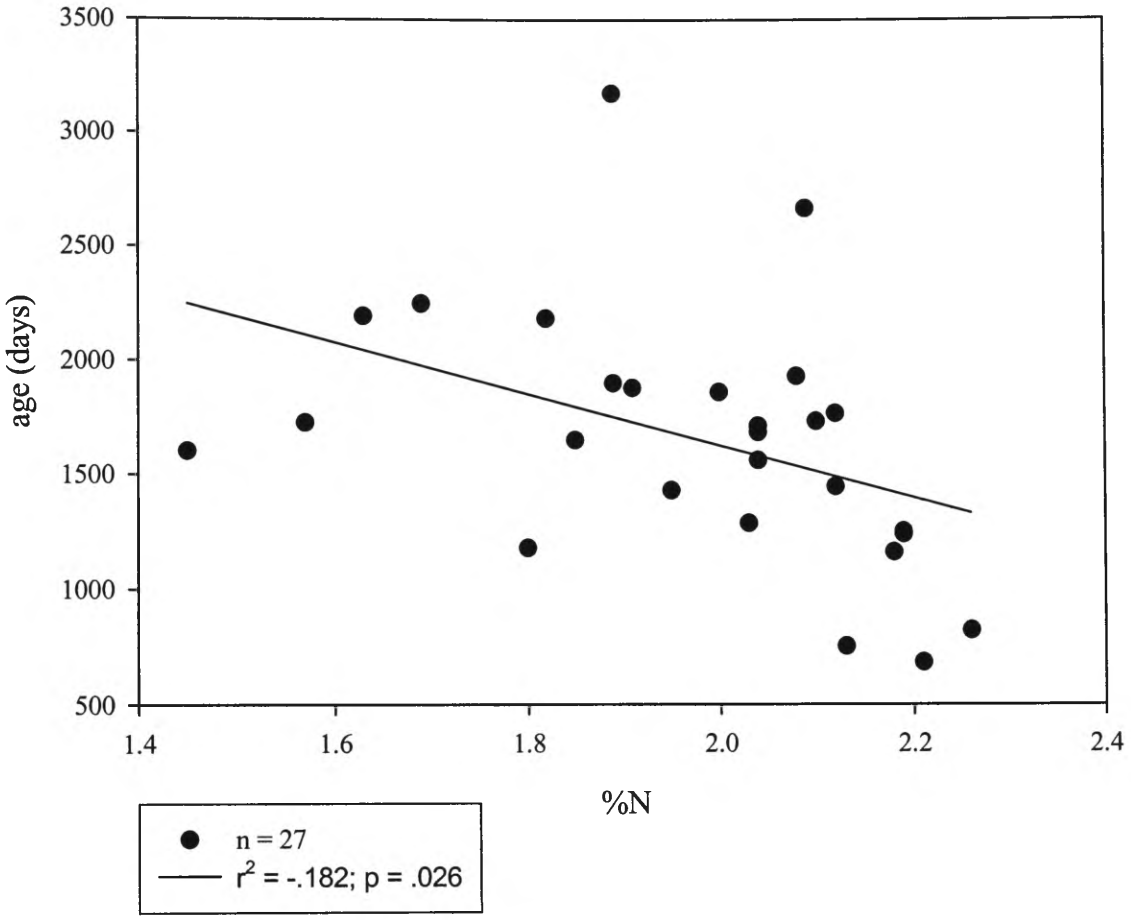
Appendix 29. *T. testudinum* leaf tissue %N versus observed total fruit frequency.



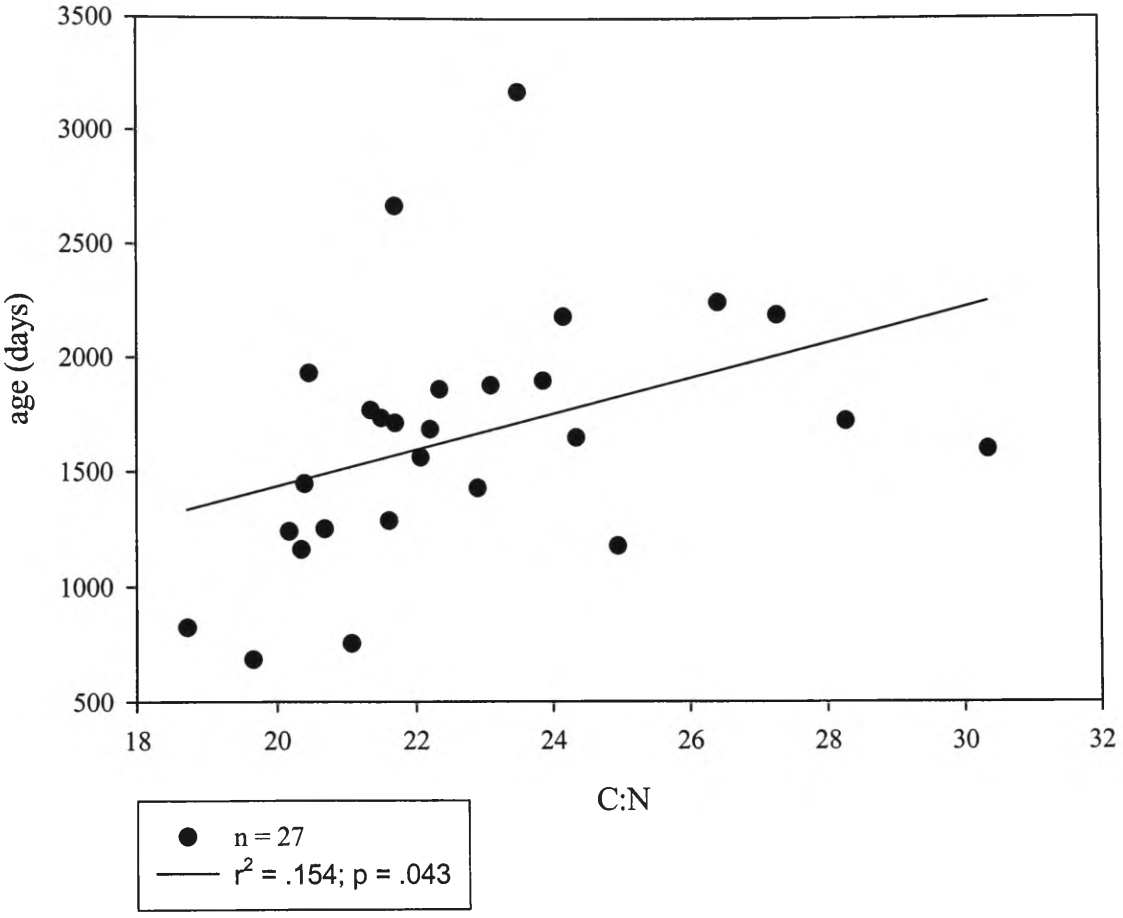
Appendix 30. *T. testudinum* leaf tissue C:N versus observed fruit frequency.



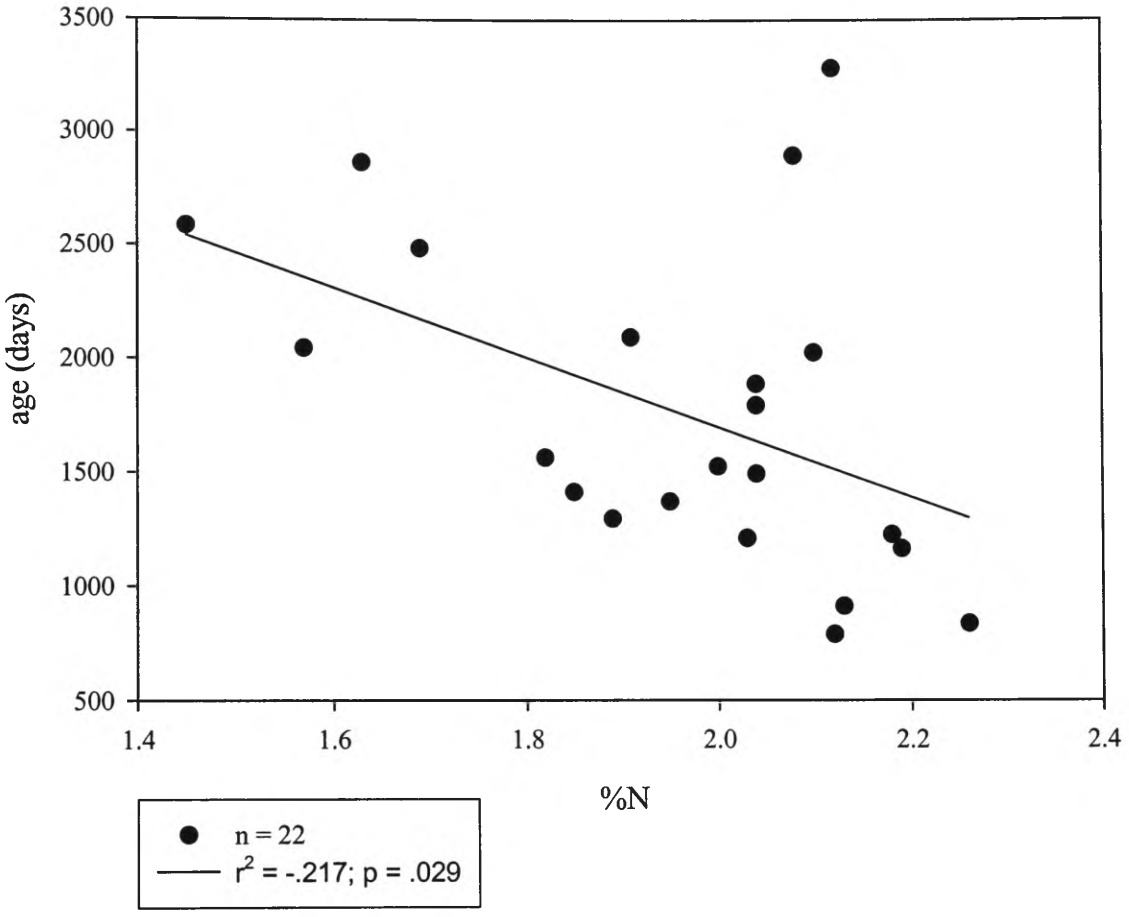
Appendix 31. *T. testudinum* leaf tissue %N versus average shoot age at first flowering.



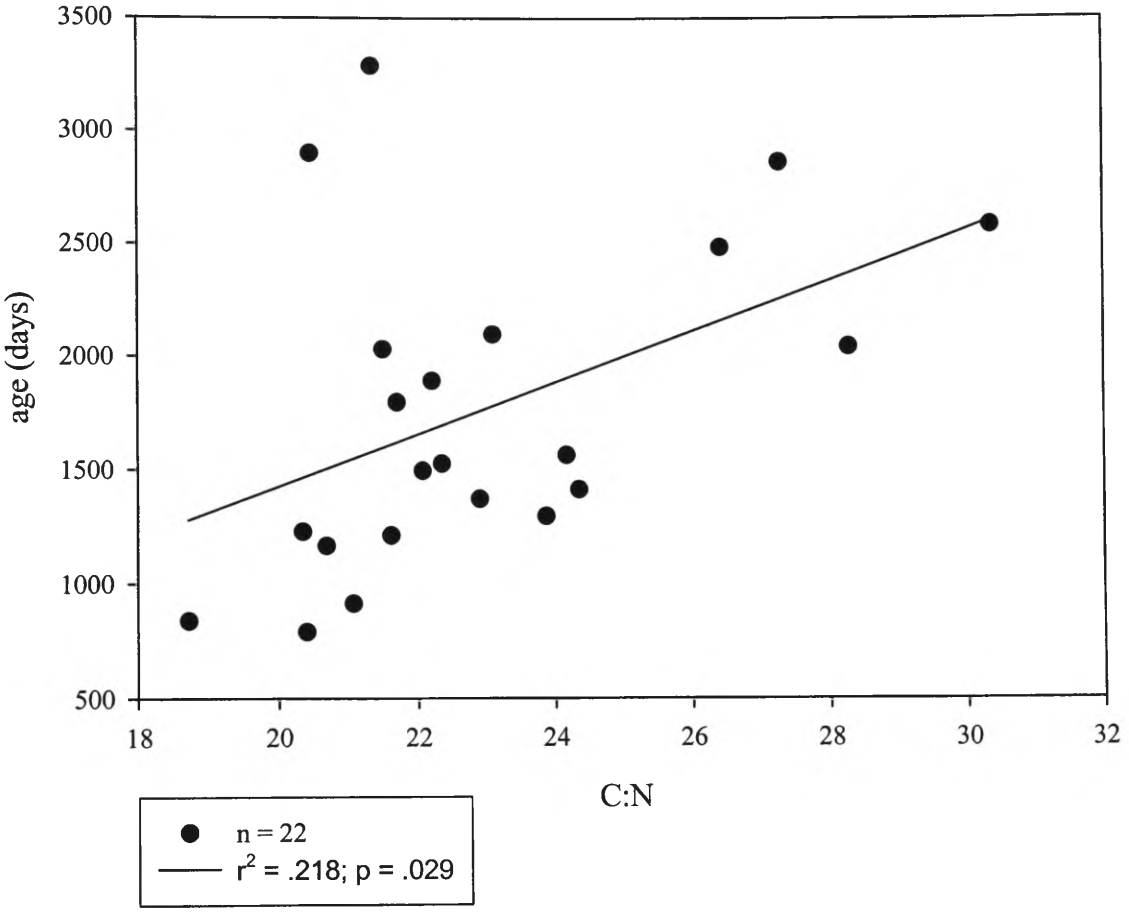
Appendix 32. *T. testudinum* leaf tissue C:N versus average shoot age at first flowering.



Appendix 33. *T. testudinum* leaf tissue %N versus average male shoot age at first flowering.



Appendix 34. *T. testudinum* leaf tissue C:N versus average male shoot age at first flowering.



Appendix 35. *T. testudinum* flowering frequencies for control and N + P treatments.

Site/treatment	Sampling period*				Mean
	April	May	June	July	
Inshore sites					
213 Control	0	11.84	0	1.62	3.36
213 N + P	0	0	0	0	0
214 Control	0	9.49	2.69	0.21	3.10
214 N + P	0	1.60	1.01	0	0.71
220 Control	0	3.73	1.72	0.38	1.45
220 N + P	0	0.65	0	0	0.18
223 Control	0	5.62	1.52	1.08	2.02
223 N + P	1.05	2.64	0.62	0	1.12
227 Control	0	0.97	0	0	0.25
227 N + P	0	0	0	0	0
Offshore sites					
215 Control	0	2.41	2.05	0.65	1.31
215 N + P	0	0.07	0.16	0	0.06
216 Control	0	0.90	2.30	0	0.77
216 N + P	0	1.43	0.74	0	0.61
217 Control	0	6.89	2.13	0	2.26
217 N + P	0	0.37	0.21	0	0.15
224 Control	1.05	23.25	4.80	0.33	7.23
224 N + P	0	1.98	0.12	0	0.59
225 Control	0	3.66	1.53	0	1.39
225 N + P	0	2.18	0.62	0	0.73

* January 2003 data not shown because there was no flowering observed.

Appendix 36. *T. testudinum* floral numbers and sex ratios for inshore control and N + P treatments.

Site/treatment	Sample period					Total	M:F
	April	May	June	July	January 2003		
213 Control							
Male	0	18	0	0	0	18	
Female	0	74	0	4	0	78	
Fruit	0	14	0	10	0	24	
Total female	0	88	0	14	0	102	0.18
213 N + P							
Male	0	0	0	0	0	0	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	n/a
214 Control							
Male	0	85	15	0	0	100	
Female	0	8	6	0	0	14	
Fruit	0	0	6	2	0	8	
Total female	0	8	12	2	0	22	4.55
214 N + P							
Male	0	14	0	0	0	14	
Female	0	3	4	0	0	7	
Fruit	0	0	4	0	0	4	
Total female	0	3	8	0	0	11	1.27
220 Control							
Male	0	38	8	0	0	46	
Female	0	0	6	3	0	9	
Fruit	0	0	2	1	0	3	
Total female	0	0	8	4	0	12	3.83
220 N + P							
Male	0	5	0	0	0	5	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	5:0
223 Control							
Male	0	31	5	0	0	36	
Female	0	11	4	0	0	15	
Fruit	0	3	3	9	0	15	
Total female	0	14	7	9	0	30	1.2
223 N + P							
Male	2	26	5	0	0	33	
Female	7	1	1	0	0	9	
Fruit	2	1	1	0	0	4	
Total female	9	2	2	0	0	13	2.54

Appendix 36 (cont.). *T. testudinum* floral numbers and sex ratios for inshore control and N + P treatments.

Site/treatment	Sample period					Total	M:F
	April	May	June	July	January 2003		
227 Control							
Male	0	8	0	0	0	8	
Female	0	2	0	0	0	2	
Fruit	0	0	0	0	0	0	
Total female	0	2	0	0	0	2	4
227 N + P							
Male	0	0	0	0	0	0	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	n/a

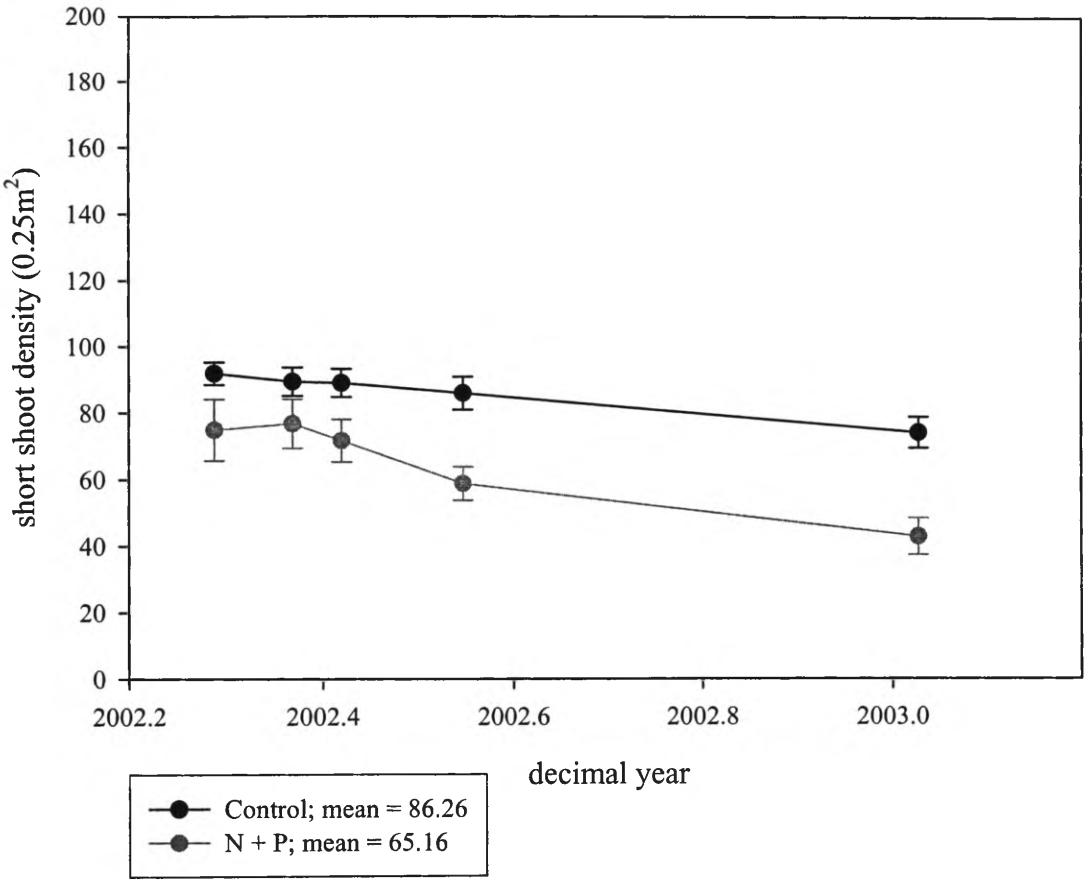
Appendix 37. *T. testudinum* floral numbers and sex ratios for offshore control and N + P treatments.

Site/treatment	Sample period					Total	M:F
	April	May	June	July	January 2003		
215 Control							
Male	0	16	1	0	0	17	
Female	0	24	18	4	0	46	
Fruit	0	1	10	6	0	17	
Total female	0	25	28	10	0	63	0.27
215 N + P							
Male	0	1	2	0	0	3	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	3:0
216 Control							
Male	0	0	0	0	0	0	
Female	0	9	13	0	0	22	
Fruit	0	0	8	0	0	8	
Total female	0	9	21	0	0	30	0:30
216 N + P							
Male	0	11	4	0	0	15	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	15:0
217 Control							
Male	0	75	20	0	0	95	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	95:0
217 N + P							
Male	0	4	2	0	0	6	
Female	0	0	0	0	0	0	
Fruit	0	0	0	0	0	0	
Total female	0	0	0	0	0	0	6:0
224 Control							
Male	7	280	38	0	0	318	
Female	7	10	15	0	0	32	
Fruit	0	2	4	4	0	10	
Total female	7	12	19	4	0	42	7.57
224 N + P							
Male	0	24	0	0	0	24	
Female	0	1	1	0	0	2	
Fruit	0	0	0	0	0	0	
Total female	0	1	1	0	0	2	12

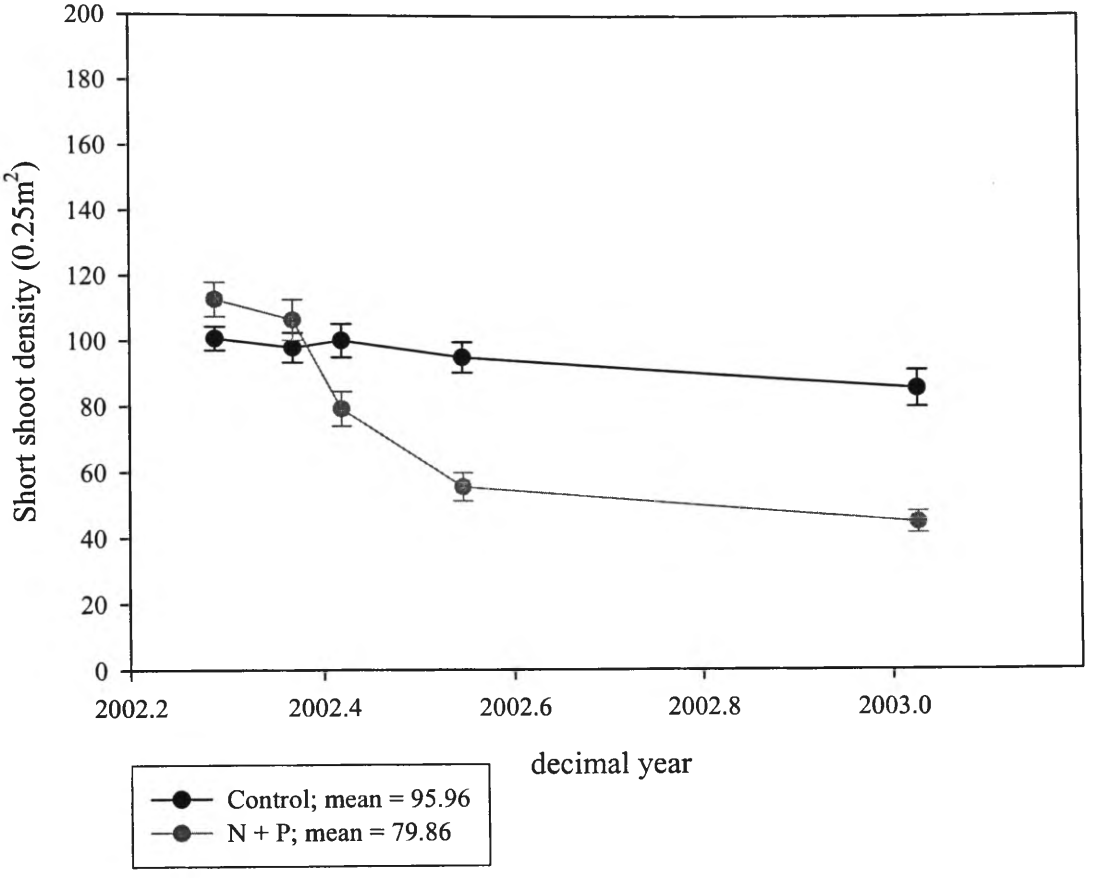
Appendix 37 (cont.). *T. testudinum* floral numbers and sex ratios for offshore control and N + P treatments.

Site/treatment	Sample period					Total	M:F
	April	May	June	July	January 2003		
225 Control							
Male	0	48	16	0	0	64	
Female	0	1	1	0	0	2	
Fruit	0	0	1	0	0	1	
Total female	0	1	2	0	0	3	21.33
225 N + P							
Male	0	28	4	0	0	32	
Female	0	1	2	0	0	3	
Fruit	0	1	2	0	0	3	
Total female	0	2	4	0	0	6	5.33

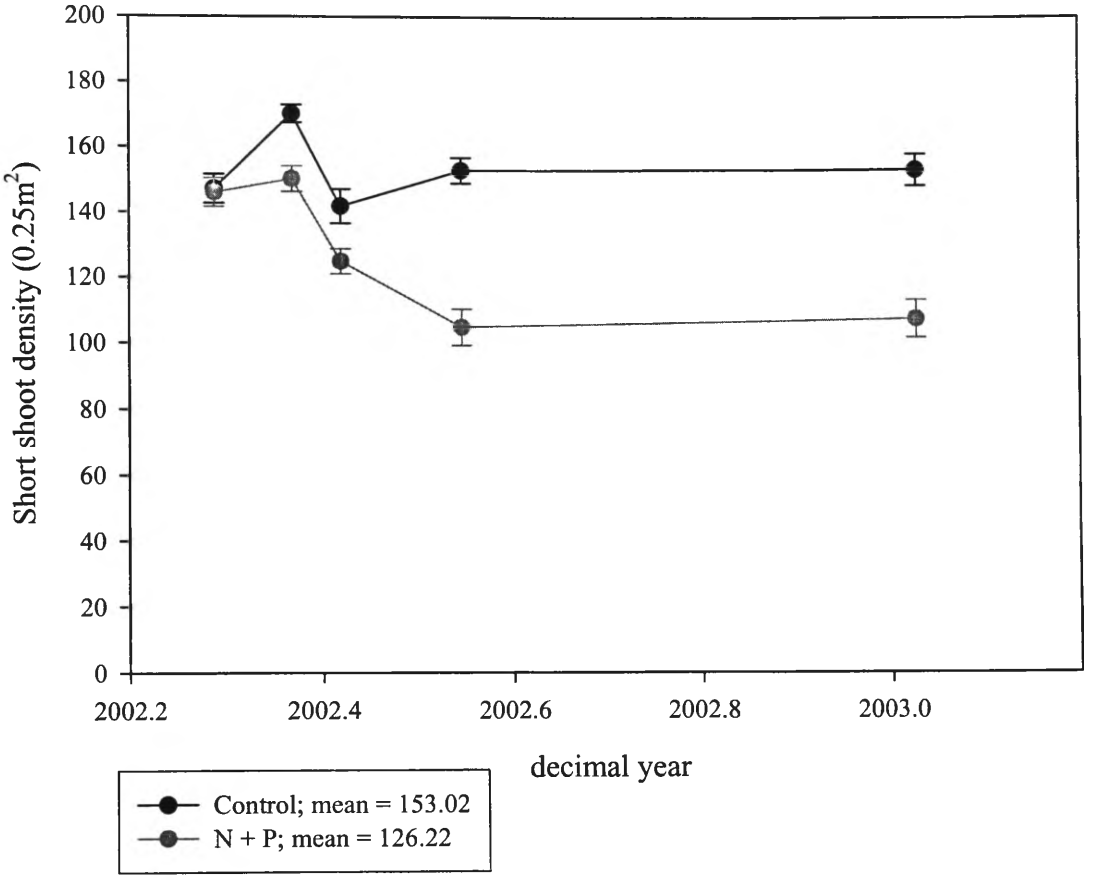
Appendix 38. *T. testudinum* control and N + P short shoot densities at site 213. Error bars represent ± 1 SE.



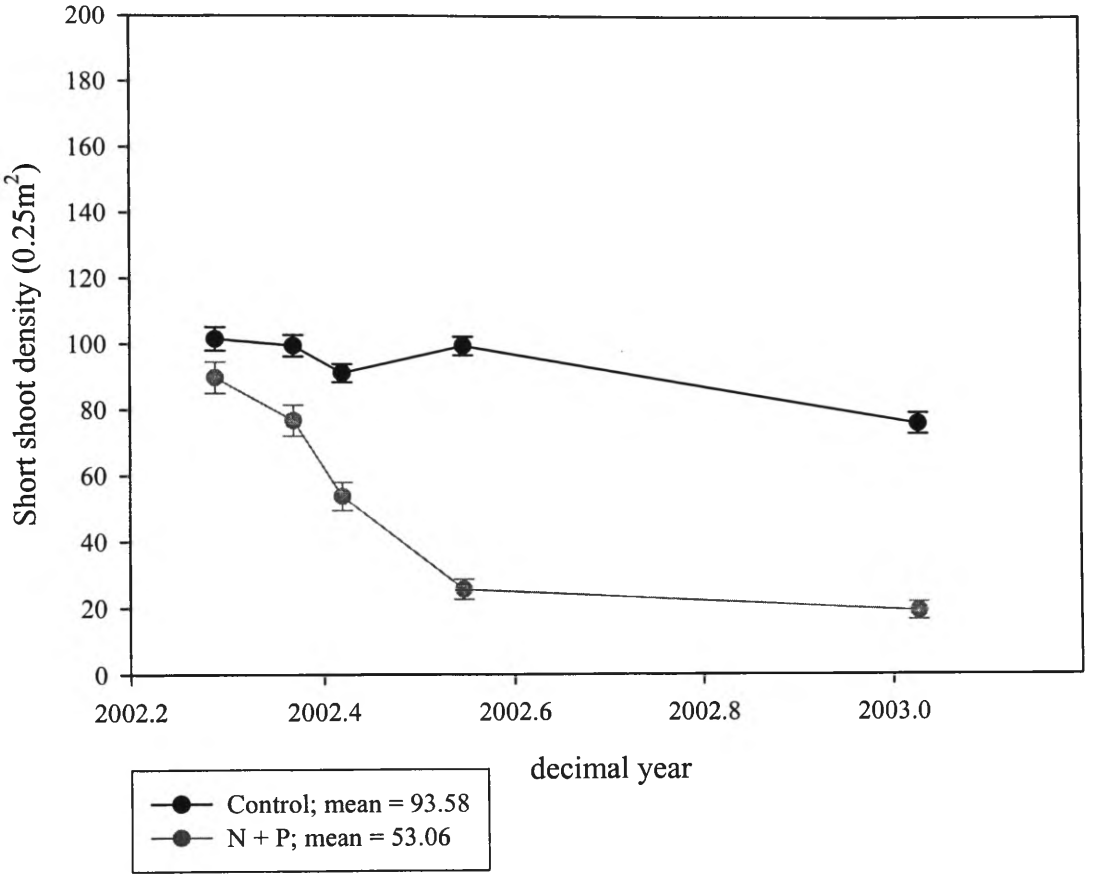
Appendix 39. *T. testudinum* control and N + P short shoot densities at site 214. Error bars represent ± 1 SE.



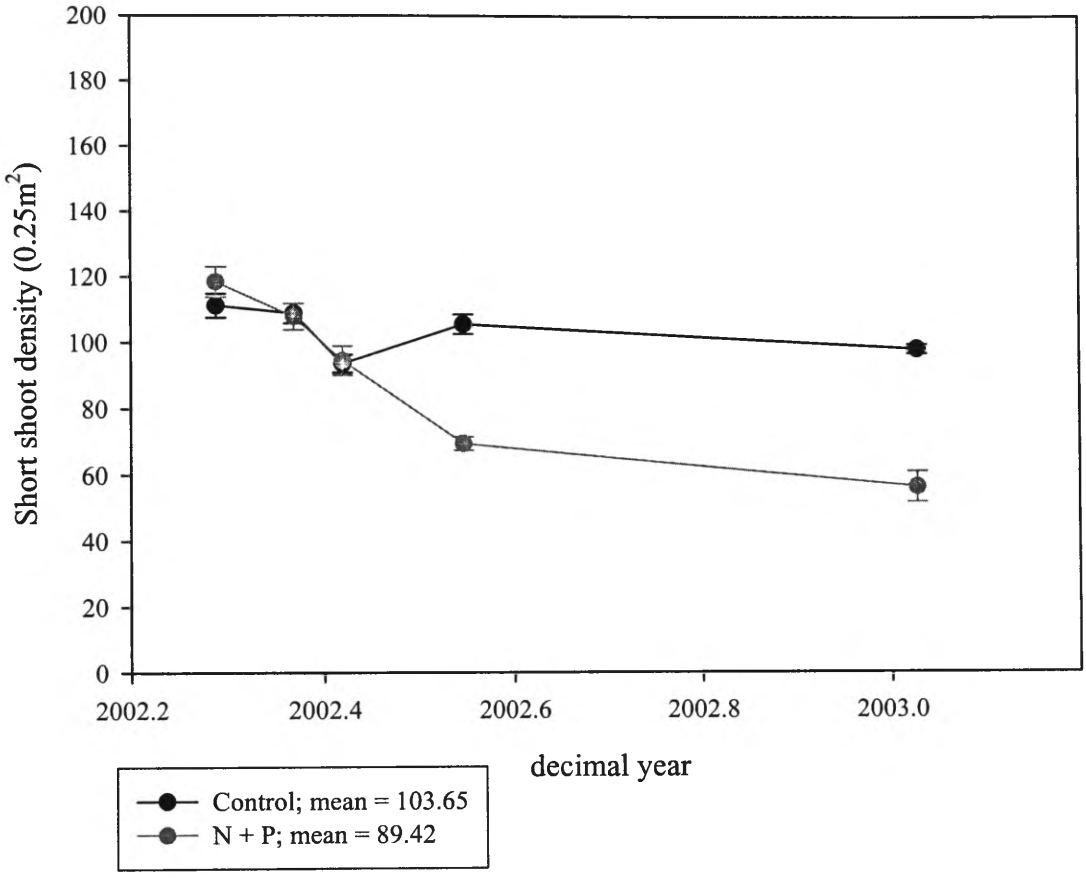
Appendix 40. *T. testudinum* control and N + P short shoot densities at site 215. Error bars represent ± 1 SE.



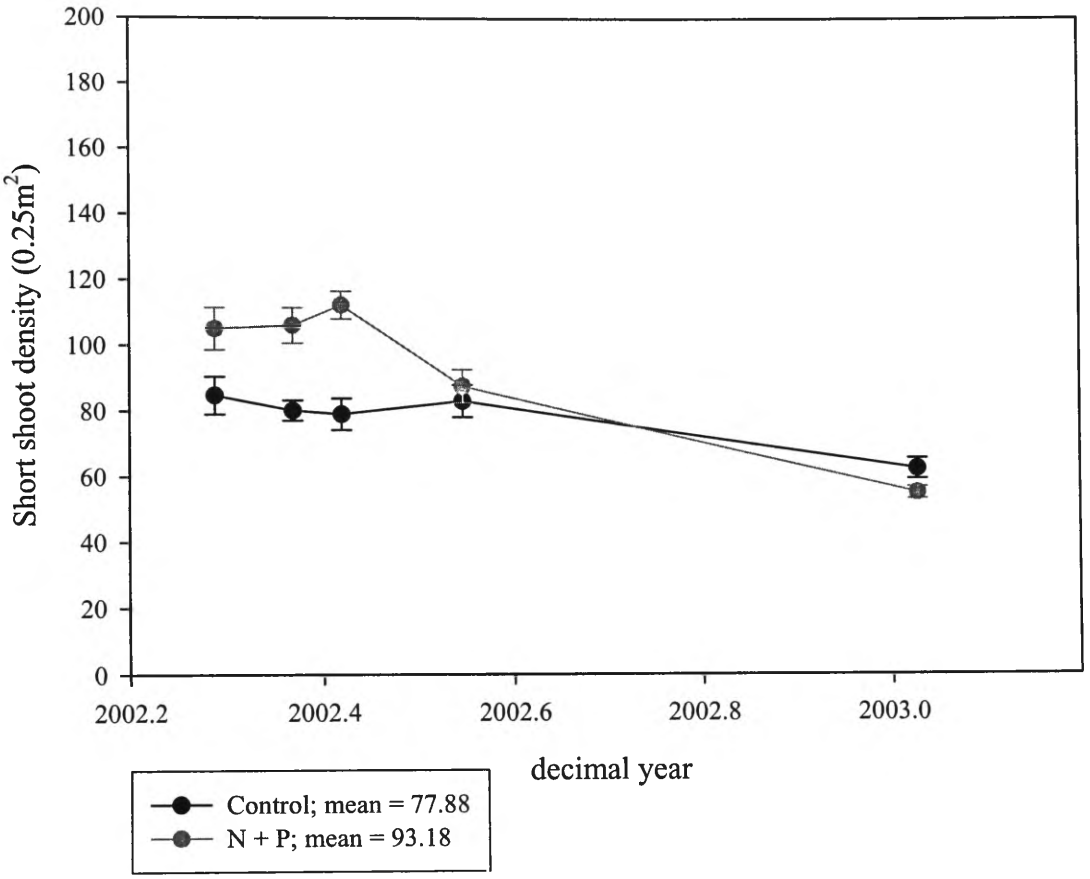
Appendix 41. *T. testudinum* control and N + P short shoot densities at site 216. Error bars represent ± 1 SE.



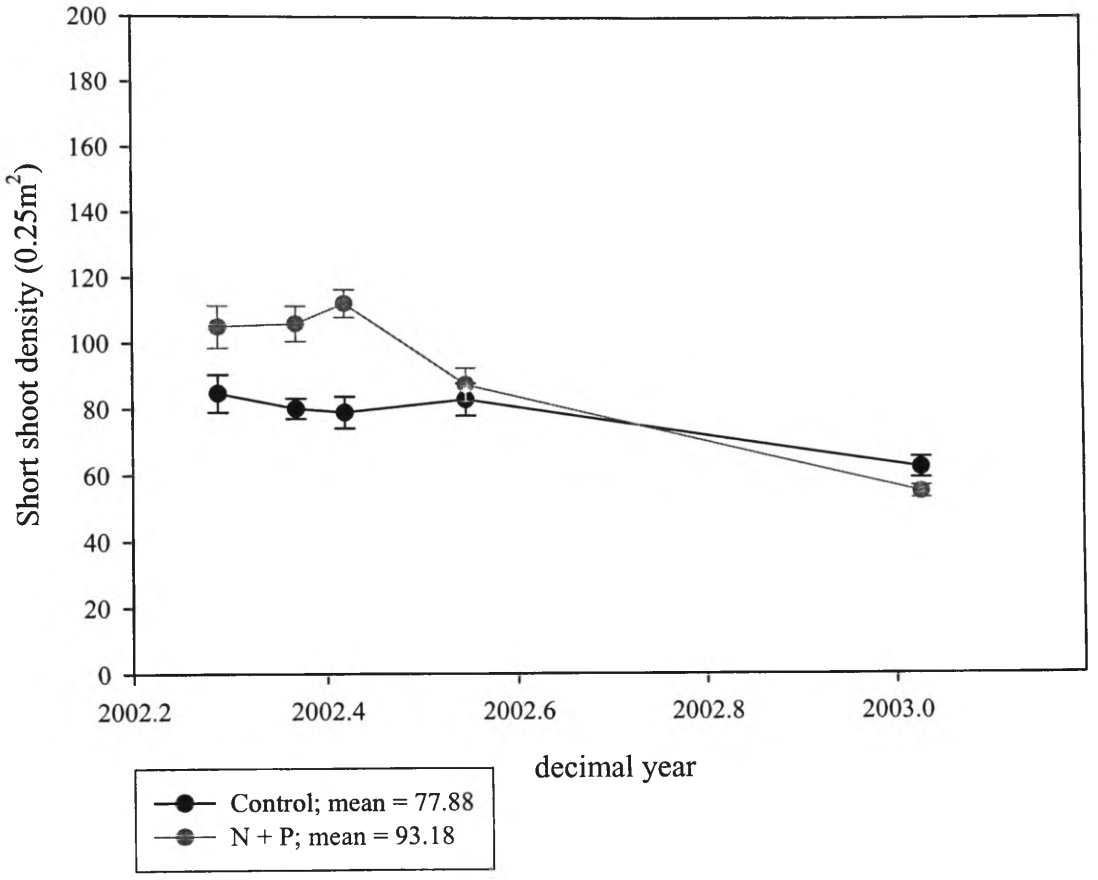
Appendix 42. *T. testudinum* control and N + P short shoot densities at site 217. Error bars represent ± 1 SE.



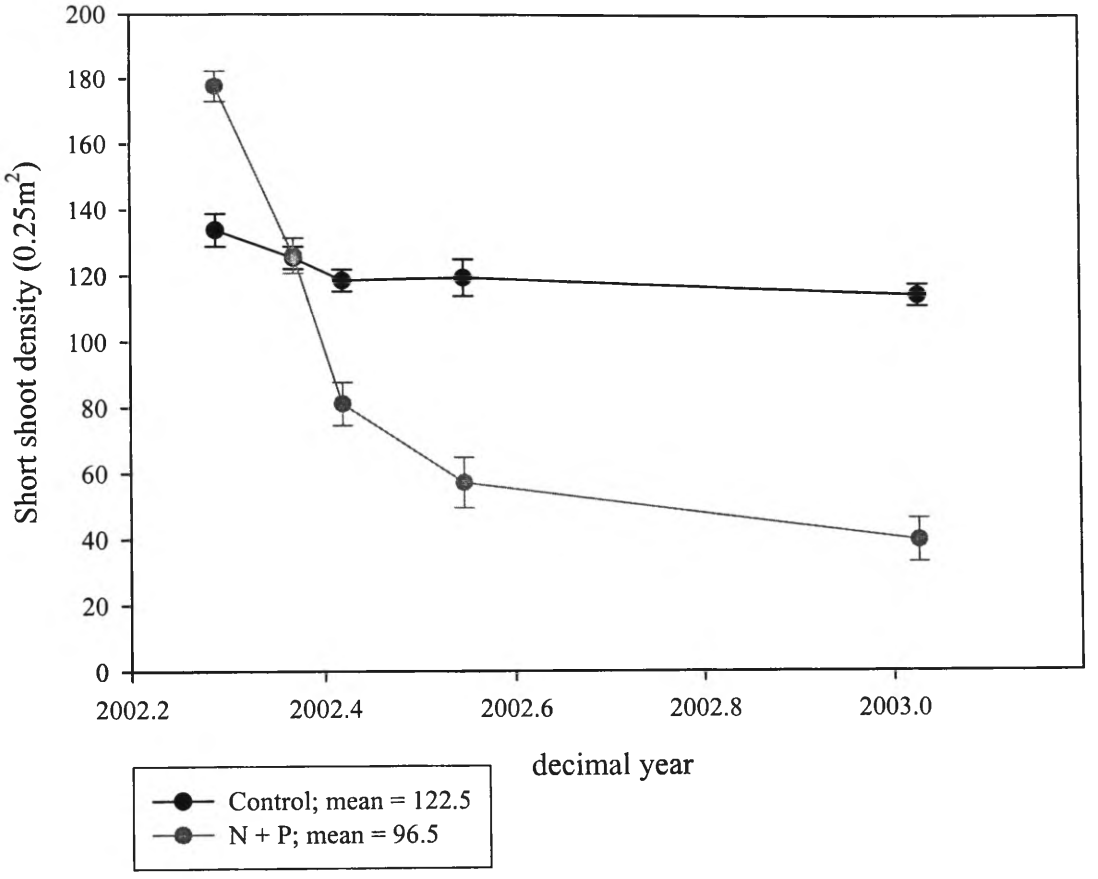
Appendix 43. *T. testudinum* control and N + P short shoot densities at site 223. Error bars represent ± 1 SE.



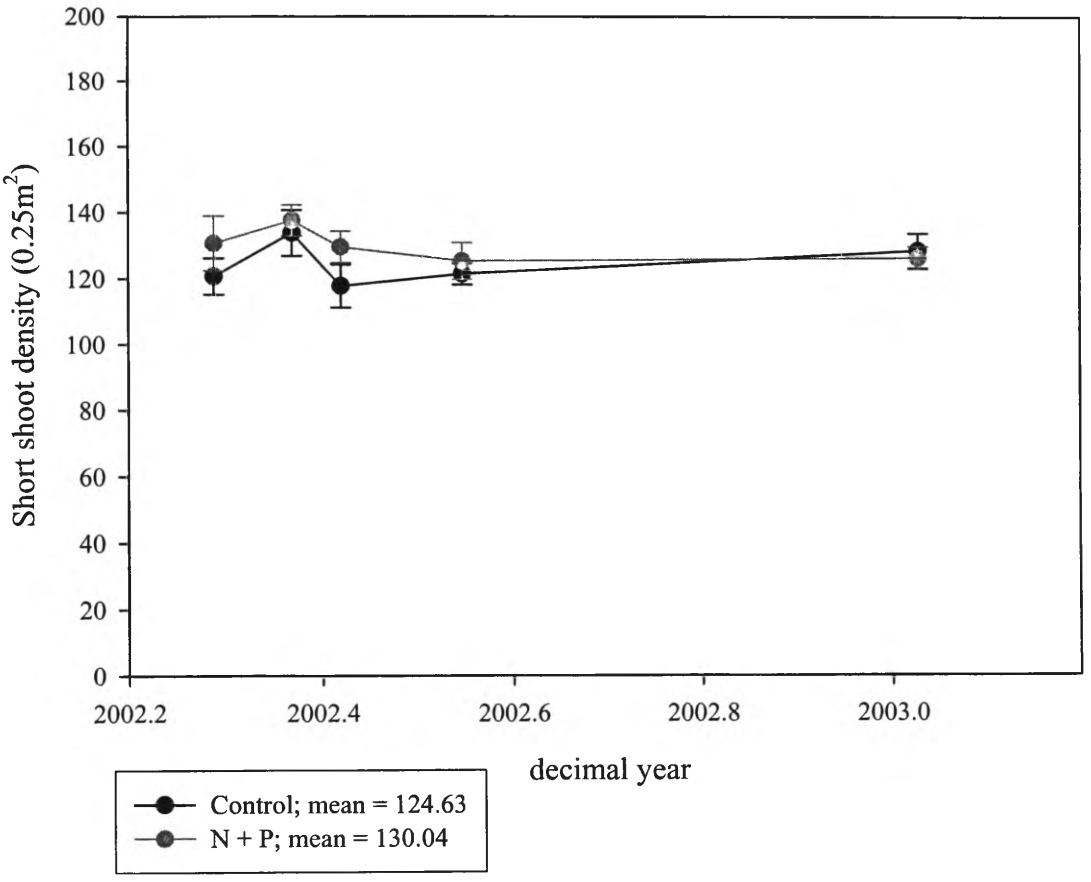
Appendix 44. *T. testudinum* control and N + P short shoot densities at site 223. Error bars represent ± 1 SE.



Appendix 45. *T. testudinum* control and N + P short shoot densities at site 224. Error bars represent ± 1 SE.



Appendix 46. *T. testudinum* control and N + P short shoot densities at site 225. Error bars represent ± 1 SE.



Appendix 47. *T. testudinum* control and N + P short shoot densities at site 227. Error bars represent ± 1 SE.