

BOLUS LIBRARY
C24 0007 8968



Pragmatic strategy of amphicarpic Sedge

Trianoptiles solitaria



Ryan Thomas de Roo
Botany Honours
University of Cape Town
2002

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

Abstract

Trianoptiles solitaria is an amphicarpic sedge which displays some interesting deviations from the typical characteristics of amphicarpy as suggested by Cheplick (1987). Consequences of amphicarpy in most plants can be seen as a dichotomy between a pessimistic and an optimistic life history strategy. The pessimistic strategy would involve early production of basal seeds and fewer numbers of basal seeds under non-stressful conditions and basal spikelets that are cleistogamous. *T. solitaria* is shown to utilise a more pragmatic strategy of reproduction: the equal production of both basal and aerial seeds phenologically, the equal production of both basal and aerial seed numbers by the seasons end, and potentially cross-pollinating basal spikelets.



UCT LIBRARIES DATE DUE

~~0 MAY 2003~~
~~11 NOV 2003~~
M 0 JUN 2005
28/07/07
RETURNED

Introduction

Amphicarpy

Amphicarpy is the production of two kinds of fruit (Usher 1966). Bruhl (1994) considers amphicarpy to include the production of aerial and basal flowers where the basal fruit/seeds are larger than the aerial. Cheplick (1987) uses a stricter definition of amphicarpy. Amphicarpy being a specialised form of dimorphism found in certain plant species where, in addition to aerial fruits on the same individual, some of the fruits and seed are produced below the soil surface on specialised reproductive structures (van der Pijl 1982). Cheplick (1987) found that additional attributes shared by most amphicarpic plants are self-fertile basal flowers that produce few large seeds with limited dispersal capability. In contrast aerial flowers that usually cross-pollinate producing many small seeds with greater dispersal capability (Cheplick 1987).

Cheplick (1987) proposed four hypotheses for the selection of seed produced below the soil surface. (i) The placement of basal seeds in a suitable site as defined by the occurrence of a parent plant. (ii) The protection of the basal seeds from the extremes of microclimate results in a higher maintenance of viability over time of the basal seeds relative to the aerial seeds. (iii) The protection of the basal seeds from predation and (iv) the protection of the basal seeds from perturbations, such as fire. Since amphicarpic plants also have aerial seed production, there are various hypotheses for the selection of seed produced above the soil surface. (i) A reduced energy costs of aerial seeds (since aerial seeds are lighter than basal seeds). (ii) An increased chance of out-crossing for the formation of aerial seeds (allowing higher

levels of gene exchange). (iii) A higher dispersal capability found for aerial seeds, which reduces the occurrence of phenomena like sibling competition and also allows for greater chance of long-distance dispersal and the founding of new populations. Finally, (iv) there are potential edaphic (soil) influences (like compacted soils) which would result in selection for aerial seeds (Cheplick 1987).

Cheplick (1987) argues that subterranean seeds are produced early in the plant ontogeny, prior to the production of aerial seeds, because of their importance to individual fitness. As a life history strategy, Zeide (1978) termed the early production of subterranean propagules a 'pessimistic' strategy. In unpredictable or highly disturbed habitats, survival to the end of the growing season (or length of the growing season) is uncertain. Seed production is completed as soon as possible, regardless of the total quantity of seed. In contrast the aerial seed production can be seen as an 'optimistic' strategy, whereby reproduction is delayed until the end of the growing season if the plant has had time in suitable growth conditions to accumulate sufficient energy resources (Cheplick 1987). Often aerial seed production is more strongly related than basal fruit production to plant biomass (Cheplick 1987). This combined strategy is seen as a pessimistic-opportunistic dichotomy because of the differing eco-genetic consequences of amphicarpic plants (Cheplick 1987).

Table 1. Cheplick's view of the eco-genetic consequences of amphicarpy and associated life cycle attributes (a pessimistic-opportunistic strategy). (Modified from Cheplick 1987)

	Aerial	Basal	Comments
I Seed and seedling biology			
Seed size	Small	Large	

Seed weight	Low	High	Basal seeds range from 2 to 20 times as heavy
Seed number per plant	High	Low	Not true under stressful conditions where aerial seeds may not develop
Seed number per fruit	High	Low	
Seed bank	Transient	Persistent	
Seed germinability	Low	High	Refers to newly harvested seeds; variable in some species
Seedling size	Small	Large	
II Population biology			
Survivorship	Low	High	
Competitive ability	Low	High	
Stress tolerance	Low	High	
Fitness	Low	High	
III Phenology			
Seed germination	-	-	Usually no difference in timing of seed germination
Flower and seed production	Late	Early	
IV Reproductive biology			
Flowers	Chasmogamous or cleistogamous	Cleistogamous	
Breeding system	Potentially cross pollinating	Selfing	
% Seed set	Low	High	
Fruit weight	Low	High	
Seed dispersal potential	High	Low	
V Life history variation			
Flower and seed production	High phenotypic plasticity	Low phenotypic plasticity	Aerial reproduction is more strongly affected by environmental conditions
Flower and seed characteristics	High genetic variation	Low genetic variation	In one species, heritabilities of aerial reproductive traits were higher

***Trianoptiles* Fenzl. an amphicarpic sedge**

***Trianoptiles solitaria* Levyns**

The plants of the genus are annuals, tufted in habit (See figure a of plant habit). *T. solitaria* grows up to 20 centimetres in height and many of the spikelets are short and hidden among the leaves. In 1943 Levyns provided a short revision of the genus *Trianoptiles* with the discovery of basal female flowers. (See figure b of detail of plant) There are two kinds of spikelet; the aerial spikelets are bisexual and basal spikelets female (partially hidden among basal leaves). Aerial spikelets are greenish in colour and of a monopodial structure (Haines and Lye 1977). They consist of

three to five bracts more or less arranged in two vertical rows. In most cases two perfect spikelets are associated with the two uppermost bracts. There are three stamens and the style has three long branches. Seed formed are triangular in cross-section with perianth scales which are linear with dense long weak hairs possibly assisting in dispersion. The Basal spikelet consists of two to four tightly sheathed bracts and a terminal flower. The basal portion of the spikelet is subterranean, a narrow tubular tip of the uppermost bract projects above the ground. No growth pushes the fruit away from the plant and seed ripen in the axils of the leaves close to the culms. The style branches protrude through the tubular uppermost bract. There are no perianth scales and fruit are more or less spherical. The species is found, among other areas of the cape flats, on Rondebosch Common where the habitat is mesophytic (specifically wet/dry seasonally damp areas).

This study documents the flowering and seed-set of the amphicarpic sedge *Trianoptiles solitaria* Levyns. The aim is to assess whether *T. solitaria* employs a dichotomous pessimistic-opportunistic strategy. The following questions are addressed. Flowering. Are aerial spikelets protogynous or protandrous and do aerial spikelets self-pollinate? Is there basal self-fertilisation? Which spikelets flower first? Is aerial spikelet height growth for improved pollen dispersal, pollen interception or seed dispersal? Seed-set. Are aerial seeds smaller than basal seeds? Are basal seed less dormant (seed coat thickness) than aerial seeds? Are different numbers of basal and aerial seeds produced? Are basal seeds produced earlier than aerial seeds? Is there lower seed-set of aerial seeds in isolated plants than in

clumped plantings? Are seedlings of basal seeds larger than seedlings aerial seed origin?

Methods

Study site

The *Trianoptiles solitaria* population found on Rondebosch Common was used for harvests and collections of individuals. The initial harvest and collections took place on the 31st of July (2002). For the purposes of this study that is day one and all following harvests both from the field and in the glass-house follow from day one. All plants grown in the glass-house were grown in polystyrene seed trays. Soil medium consisted of four parts soil from habitat, one part sand, one part peat, and one part potting soil. Growing in the glass-house plants were kept in standing water.

Data collection and analysis

Flowering sequence and spikelet development

To document the sequence of events of flowering and spikelet development of *T. solitaria*, 14 isolated plants were observed each day in the glass house from the 20 August to 28 September (day 21 to 60 = 40 days). All plants initially lacked inflorescences. Events recorded daily were the number of basal spikelets with receptive stigmas, the number of aerial spikelets with dehiscent anthers and/or receptive stigmas, and the height of any aerial spikelets. Stigmas were assumed receptive from emergence until drying and/or turning a darker colour (observed using a hand lens).

Spikelet temporal development

1. Aerial spikelet flowering sequence

Dehiscent anthers were recorded as present at initial dehiscence. Receptive stigmas were recorded as present from initial emergence. The time *from* initial anther dehiscence *to* stigma receptivity was recorded to test for protandry, and *visa versa* for protogyny. Observed temporal separation is recorded as temporal separation differences greater than or equal to one day. Each observation is equivalent to an individual spikelet. The null is no temporal difference (or temporal co-occurrence) between the initial occurrence of dehiscent anthers and receptive stigmas and *visa versa*. The alternative is that the anthers begin dehiscence before or after the stigmas are receptive.

2. Aerial self-pollination potential

Differing only subtly from the sequence of anther dehiscence and stigma receptivity, the observed *overlap* of dehiscent anthers and receptive stigmas is used to test for the achieved avoidance of potential selfing; the observed temporal overlap is recorded for times greater than or equal to one day. The null is that the stigmas are receptive during anther dehiscence. The alternative is for avoidance of temporal co-occurrence of dehiscent anthers and receptive stigmas.

3. Potential for basal self-fertilisation

Similarly the observed temporal overlap of dehiscent anthers and receptive basal stigmas is used to test for the avoidance of potential selfing. The null is that the

basal stigma is receptive during anther dehiscence. The alternative is for minimal temporal co-occurrence of dehiscent anthers and receptive basal stigmas.

4. Timing of basal spikelets

To show the period or length of time from basal stigma receptivity to aerial stigma receptivity is examined, observed temporal separation is recorded as varying temporal separation differences greater or equal to one day before and after stigma receptivity. The null is for temporal co-occurrence of receptive basal and aerial stigmas. The alternative is that the basal stigmas are receptive before or after the aerial stigmas.

To test these hypotheses observed versus expected X^2 frequency tests were used. Expected frequencies for the null hypotheses are (1) based on all observations being 0 days time from anther dehiscence to stigma receptivity or visa versa, (2) all observations being greater or equal to one day's time from anther dehiscence and stigma co-occurrence, (3) all observations being greater or equal to one day's time from anther dehiscence and basal stigma co-occurrence, and (4) all observations being equal to 0 days time from basal stigma receptivity to aerial stigma receptivity co-occurrence.

Spikelet spatial development

5. Illustrating Spikelet height growth

Since aerial spikelets within and between plants appeared at different times, in order to compare the development of these spikelets across all plants, the

temporal scale was standardised to begin with the appearance of each first, second and third spikelets. Graphical representations of aerial spikelet growth and corresponding flowering are shown only for the first, second and third spikelets (few plants formed more). A combined representation is shown of all three spikelets based on the combining of standardised days for all three spikelet cases.

6. Height at anthesis, stigma receptivity and maximum

Difference in the aerial spikelet height was assessed for spikelets at anthesis and when stigmas emerge. Similarly, difference in spikelet height while flowering (the maximum either at stigma emergence or anthesis depending on the previous result) and maximum height attained was examined. To test for this a t-test for dependent samples is used.

Seed-set, seed morphology and seedlings

To document the sequences of events of seed-set, seed morphology and seedlings of *T. solitaria*, sampling consisted of six harvests following stopped watering in the glass-house (Days 9, 19, 29, 39, 49, and 59; a total 106 individuals) and four harvests from the field (Days 1, 18, 34 and 50; a total 88 individuals). To document effects on seed-set of plant isolation 14 individuals were grown in isolation and 10 individuals grown in a clumped planting (harvested on day 59). Harvested individuals from the field were oven-dried at 60 degrees C for 48 hours, all other individuals were allowed to dry in the soil. Conditions recorded include the dry mass of the entire plant, the number of basal spikelets, the number and

mass of basal seeds, the number of aerial spikelets, the number and mass of aerial seeds.

Dry masses amounts were determined using a balance precise to 0.1mg. From these conditions seed mass for basal and aerial seeds (average seed mass) and vegetative dry mass (excluding seeds) was calculated. For all individuals harvested, roots were excluded (cut off) since harvesting consistently resulted in incomplete roots harvested.

Eight of each basal and aerial seed (of roughly the same mass) were dissected and seed coat thickness was measured using an eyepiece ruler on a Zeis stemi SR microscope precise to the nearest 10 micrometers.

For the first two harvests from the field the presence or absence of basal seed coat remains was recorded.

Seed morphology

a. Difference in seed size

To assess difference in seed size between aerial and basal seeds, data from the final stop-watering and clumped planting harvests (Day 59) are used. This was tested using a T-test for independent samples.

b. Are basal seed coats thinner than aerial seed coats?

To assess the difference in seed coat thickness between aerial and basal seeds a T-test for independent samples was used.

Seed-set

c. Seed number differences

To assess the difference in amount of seed-set between basal and aerial seeds were tested using a T-test for independent samples. Again data from the final stop-watering and clumped planting harvests (Day 59) was used.

d. Seeds first produced

To assess which seeds are produced first both field and greenhouse treatments are used. Box and whisker plots are created for seed numbers, total seed mass, and seed mass for basal and aerial seed and a graph of the relationship between plant mass and seed numbers.

The difference between the mean conditions for each harvest are compared using breakdown and one way ANOVA. This is used to assess for each treatment, when basal and aerial seeds reach their respective optimum seed conditions (optimum taken to be the seed condition for the last harvest).

Harvest periods were identified in which plants yielded the first seeds, whether aerial or basal for both treatments. The difference in seed number, total seed mass and seed mass between aerial and basal seeds for the period identified was tested using t-tests for independent samples.

For stopped watering harvests any differences in seed mass found in section for the day 59 harvest (section 'a') is used as an expected seed mass (taken to be optimum seed mass). The difference between this and the seed mass for the treatment with first seeds produced is established for each seed type using a t-test for independent samples. The ratio of seed mass to expected (optimum) seed mass is used in addition to assess which seed is produced first.

e. Lower seed-set of aerial seeds in isolated plants versus clumped plantings?

To assess the difference in seed-set of aerial seed harvested from isolated plants and clumped plantings a t-test for independent samples was used.

Seedlings

f. Seedling size with and without seed coats attached

Difference in seedling size between seedlings with and without remnant basal seed coats were tested using a t-test for independent samples.

Results

Flowering sequence and spikelet development

Spikelet temporal development

1. Aerial spikelet flowering sequence

Data collected provided a sample size of 29 observations (14 plants - some spikelets with two sets of anthers emerging, most with a 1st and 2nd spikelet). For 22 aerial spikelets a day or more of time from initial anthesis to stigma receptivity was observed, for 7 aerial spikelets no temporal separation was observed, and for no aerial spikelets did stigma receptivity precede anthesis. The chi-square value shows that for the first and second spikelets the observed pattern is significantly different from that of the null hypothesis (Chi-square = 16.6897, df = 1, $p < 0.000044$). The null hypothesis of temporal co-occurrence of initial dehiscence and stigma emergence is rejected. The anther dehiscence of *T. solitaria* occurs before stigmas emerge and are receptive, it is protandrous.

2. Aerial self-pollination

Again data collected provided a sample size of 29 observations. For 16 aerial spikelets a day or more of co-occurring anthesis and stigma receptivity was observed and for 13 aerial spikelets no co-occurrence was observed. The chi-square value shows that for the first and second spikelets the observed pattern is significantly different from that of the null hypothesis (Chi-square = 5.8276, df = 1, $p < 0.0157$). The null hypothesis that the stigmas are mainly receptive during anther dehiscence is discarded. *T. solitaria* aerial spikelets have the potential to self pollinate based on the co-occurrence of dehiscent anthers and receptive stigmas.

Despite being protandrous, there is temporal overlap in gender expression for aerial florets.

3. Basal self-fertilisation

Testing for temporal overlap with dehiscent anthers and basal stigma receptivity data collected provides a sample size of 36 observations (from 14 plants). For 29 basal spikelets a day or more of co-occurring anthesis and stigma receptivity was observed and for 7 basal spikelets no co-occurrence was observed. The chi-square value shows that the observed pattern is not significantly different from that of the null hypothesis (Chi-square = 1.3611, $df = 1$, $p < 0.2434$). The null hypothesis that the basal stigma is receptive during anther dehiscence is accepted.

4. Timing of basal spikelets

Testing whether basal spikelets are receptive to pollen first, data collected provides a sample size of 14 (for 14 plants - since there is only one initial basal and aerial spikelet per plant). Twelve basal spikelet stigmas emerged a day or more before aerial spikelet stigmas, no basal spikelets emerged on the same day as aerial spikelet stigmas and two basal spikelet stigmas emerged after aerial spikelet stigmas. The chi-square value shows that the observed pattern is significantly different from that of the null hypothesis (Chi-square = 14, $df = 2$, $p < 0.000913$). The null hypothesis of co-occurrence or post occurrence of the basal stigma receptivity to aerial stigma receptivity is rejected. The basal stigma is receptive before the aerial spikelet stigma.

Spikelet spatial development

5. Aerial spikelets

A graphical representation of aerial spikelet growth is shown in figure one. The pattern emerging is an apparent initial linear culm height increase followed by a period when culms cease growing taller (as seen by a levelling off in the graph). The first to third spikelets together based on the standardised temporal scale. The first spikelet gains height most rapidly, the second and third spikelets gain less respectively.

6. Flowering followed by height growth

Sample size for each condition is 14. Significant difference is found between the spikelet height at anthesis and when the stigma emerges (t-value = -4.0909, df = 13, $p = 0.001275$). Spikelets are significantly further from the ground when stigmas emerge than during anthesis. See Appendix (Figure two). Since the emergence of receptive stigmas occurs when the spikelet is higher, this height is compared to the maximum spikelet height attained. Significant difference is found between the spikelet height at stigma emergence and maximum height (t-value = -15.6411, df = 13, $p = 0$). Spikelets are significantly higher post-flowering. See Appendix (Figure three).

Seed-set, seed morphology and seedlings

Seed morphology

a. Difference in seed size

Data collected provides a sample size of 36 seeds for both aerial and basal seeds. Deviation from normal distribution was found so a Kolmogorov-Smirnov two-sample test was used. There is significant difference between basal and aerial seed mass ($p < 0.001$). The null hypothesis of no difference between the two is rejected. See Appendix (Figure four).

b. Basal seed coats thinner than aerial seed coats

For basal and aerial seed coats data collected provides a sample size of 8 for each seed type. Normal distributions and equal variances were found so a standard t-test was used. There is significant difference in seed coat thickness for the basal and aerial seeds (t-value = -9.1, df = 14, $p = 0$). The null hypothesis of no difference is rejected. See appendix (Figure five)

Seed-set

c. Seed number produced

Data collected provides a sample size of 37 individual plants for both aerial and basal seed numbers. Normal distribution was found with equal variances so a standard t-test was used. There is no significant difference between basal and aerial seed numbers produced (t-value = -1.3039, df = 72, $p = 0.1964$). The null hypothesis of no difference between the two is accepted. See appendix (Figure six).

d. Timing of production of basal and aerial seeds

Box and whisker plots are shown for and seed mass, seed numbers, total seed mass for basal and aerial seed for both field and greenhouse treatments. See Appendix (Field harvests: Figures 7-12; Stopped watering harvests: Figures 13-18). For the stopped watering harvests a box and whisker plot of vegetative mass and a graph of the relationship between plant mass and seed numbers is shown. See Appendix (Figures 19, 20).

The results of the breakdown one way ANOVA. are shown with the corresponding box and whisker plots for both the seed conditions of the field and stopped watering harvests. See Appendix (Field: Tables 1-6; Stopped watering: Tables 7-13).

Field harvests

It is clear that the day 34 harvest contains the plants yielding the first seeds, both aerial and basal. From day 18 to day 34 basal and aerial seed mass and total seed mass does not increase significantly. For the same harvests days basal and aerial seed number does increase significantly. Between day 34 and day 50 harvests basal and aerial seed mass increases significantly. Likewise the same is found for total basal and aerial seed mass. For basal seed number between these harvests a significant increase is seen, while aerial seed number does not increase significantly.

Between seed types for the day 34 harvest, data collected provides a sample size of 12 seed yielding plants. For seed number, normal distribution and equal variance were found so a standard t-test was used. There is no significant difference found between aerial and basal seed number (t-value = -0.2231, df = 22, p = 0.8255). The null hypothesis of no difference between the two is accepted. See appendix (figure 21).

For total seed mass, normal distribution and unequal variances were found so a t-test with separate variance estimates was used. There is no significant difference found between aerial and basal total seed mass (t-value = -0.8485, df = 22, p = 0.4066). The null hypothesis of no difference between the two is accepted. See appendix (figure 22).

For average seed mass, normal distribution and unequal variances were found so a t-test with separate variance estimates was used. There is no significant difference found between aerial and basal average seed mass (t-value = -0.2883, df = 18.8, p = 0.7762). The null hypothesis of no difference between the two is accepted. See appendix (figure 23).

Stop watering harvests

From the box and whisker plots it is clear that the day 29 harvest contains the plants yielding the first seeds. From day 19 to day 29 basal and aerial seed mass and seed number increases significantly. For the same harvests basal and aerial and total seed mass does not increase significantly. Between day 39 and day 49 basal

seed mass increases significantly while aerial seed mass does not. Likewise the same is found for total basal and aerial seed mass. No significant increases or decreases were found for plant vegetative mass over any of the harvests. A linear relationship between seed number and plant mass for basal and aerial seeds has R^2 values of 0.4133 and 0.5077 respectively.

Between seed types for the day 29 harvest, data collected provides a sample size of 16 seed yielding plants. For seed number, normal distribution and equal variance were found so a standard t-test was used. There is no significant difference found between aerial and basal seed number (t-value = -0.4860, df = 30, p = 0.6304). The null hypothesis of no difference between the two is accepted. See appendix (figure 24).

For total seed mass, normal distribution and unequal variances were found so a t-test with separate variance estimates was used. There is significant difference found between aerial and basal total seed mass (t-value = 4.1855, df = 19.4, p = 0.000483). The null hypothesis of no difference between the two is rejected. See appendix (figure 25).

For average seed mass, normal distribution and unequal variances were found so a t-test with separate variance estimates was used. There is significant difference found between aerial and basal average seed mass (t-value = 5.4246, df = 17.4, p = 0.000042). The null hypothesis of no difference between the two is rejected. See appendix (figure 26).

Since there is a significant difference between average seed mass of aerial and basal seeds a Kolmogorov-Smirnov two-sample tests were carried out between the stop watering 'crucial' period and day 59 aerial and basal seeds. Data provided a sample size of 36 and 16 for the day 59 and stop watering 'crucial' harvests respectively (for both aerial and basal seeds). There is significant difference found between two. For both aerial and basal $p < 0.001$. The null hypothesis of no difference is rejected. See appendix (figures 27,28).

For the day 29 stop watering harvest, both aerial and basal seeds are found to be just over 0.4 times the size of the day 59 harvest seed sizes.

e. Equal seed-set of aerial seeds in isolated plants and clumped plantings

Data collected provides a sample size of 10 and 12 samples for clumped and isolated plant harvests. Normal distribution and significantly different variances were found so a t-test with separate variance estimates was used. There is no significant difference in aerial total seed mass for clumped and isolated plantings (t-value = -2, df = 10.6, $p = 0.07$). The null hypothesis is accepted. See appendix (figure 29).

f. Larger seedling size with seed coats attached

Data collected provides a sample size of 17 plants with a seed coat and 6 plants without. Normal distribution and significantly different variances so a t-test with separate variance estimates was used. There is significant difference in seedling mass with and without seed coats (t-value = -5.7, df = 19.7, $p = 0$). The null

hypothesis for no difference is rejected. Seedlings with seed coats are significantly larger. See appendix (figure 30).

Discussion

Flowering

Spikelet temporal development

Haines & Lye (1977) describe *T. solitaria* as being protogynous. Since results in this study show that the anther dehiscence occurs before stigmas emerge and are receptive it is clear that *T. solitaria* aerial spikelets are protandrous rather than protogynous in flowering sequence.

While the flowers of *T. solitaria* are protandrous, there appears to be some degree of temporal overlap in pollen availability and stigma receptivity. This allows for self-pollination (not forgetting self-fertilisation from other spikelets can also occur). This assumes pollen remains viable beyond a day following initial dehiscence and it also assumes self-compatibility. The results show that the tendency is not for maximum temporal co-occurrence of dehiscent anthers and receptive aerial stigmas. Thus protandry for *T. solitaria* avoids selfing and promotes out-crossing for aerial spikelets, while still leaving a window of opportunity for selfing to occur. If one assumes pollen viability decreases past a day after anther dehiscence then the effectiveness of protandry as a strategy to promote out-crossing increases.

Results show *T. solitaria* basal spikelets have co-occurrence of dehiscent anthers and receptive basal stigmas. Similarly the potential for basal spikelets to be self-fertilised by the aerial spikelets can be seen by the degree of temporal overlap in aerial pollen availability and basal stigma receptivity. Again this assumes pollen

remains viable beyond a day following initial dehiscence and self-compatibility. For basal stigmas the tendency is for high temporal co-occurrence of dehiscent anthers and receptive basal stigmas. This suggests high potential of basal spikelet self-fertilisation, while still allowing for cross-pollination.

Given the protandrous nature of the aerial flowering and the co-occurrence of stigmas of the basal spikelets and aerial anthers it is logical to presume that stigmas of the basal spikelets emerge before those on the aerial spikelets. The results confirm this and this suggests that basal spikelets are the first to be fertilised.

Spikelet spatial development

Aerial spikelets of *T. solitaria* flowers are near to the base of the plant. Culm extension occurs after flowering which suggests that aerial spikelet height growth is for improved dispersal capability of aerial seed. The combined graph of the three aerial spikelets over time shows that the maximum heights attained by the spikelets decreases from the first to the third formed aerial spikelet. This might be due to fewer resources available to allocate to spikelet height development at the later stage in the plant life (each successive spikelet arising later in the season).

While is evident from the graphic representations of the spikelet flowering and height growth that flowering takes place when the aerial spikelets first form (low in height) and increase significantly in height during and well after flowering. This

confirms the tendency for basal spikelet self-fertilisation based on spatial co-occurrence of basal stigmas and dehiscent anthers.

While it is clear that anther dehiscence takes place when the spikelet height is close to the ground, the protandrous nature of flowering of *T. solitaria* could also be in order to increase the height at which spikelets are borne when stigmas are receptive improving pollen interception. This assumes that increased stigma height improves the pollen interception from other individuals. Results show that the average spikelet heights with anthers and with stigmas are not significantly different. This indicates that protandry in the aerial spikelets does not result in significantly higher aerial spikelets. It follows that the height increase of spikelets when stigmas are receptive, brought about by protandry, does not result in increased pollen interception.

Seed morphology, seed-set and seedlings

Seed morphology

Seeds produced by basal spikelets of *T. solitaria* are about 2.3 times heavier on average than those produced by the aerial spikelets, a difference that is highly significant. Basal seeds with a mean mass of 2.8mg and aerial seeds with a mean mass of 1.2mg, this result shows a basal: aerial weight ratio of 2.2826:1. This could result in basal seeds being able to emerge from greater depths below the soil surface (Bond et. al. 1999). This is obviously important since basal seeds develop below the soil surface, which would presumably result in basal seed more likely developing at greater depths than aerial seeds. Also if larger seeds produce larger

and more vigorous seedlings (Cheplick, 1987) than *T. solitaria* basal seeds will have this advantage.

Aerial seeds have significantly thinner seed coats than basal seeds. It has been found that for sedges of the genus *Carex*, observed differences in dormancy were found to be related to the thickness and hardness of the seed coat (Schutz 2000). This difference in thickness could result in basal seeds having greater potential lengths of dormancy that potentially increases the survival of basal seeds.

Seed-set

Results show that the same numbers of aerial and basal seeds are produced by day 59 harvests.

Field harvests

The results show for the initial seed production there is no significant difference in seed-set for seed number, total seed mass, and average seed mass between the aerial and basal seeds. It is important to note that the field harvests were immediately oven dried on removing the plants from the soil.

Stopped watering harvests

The results for this treatment show no significant difference in the numbers of seeds produced by aerial and basal spikelets. However, the results do show significant difference in seed-set for total seed mass and average seed mass between the two seed types.

There is significant difference between these average seed sizes and the seed sizes found for basal and aerial seeds of day 59 harvests. This is probably because by the day 34 stop watering harvest any seeds produced failed to reach an optimum seed size (as reflected by seed size found by the day 59 stop watering harvest).

Both aerial and basal seeds are the same proportionate size of the end harvest seed size. Since they both make up the same fraction of 'optimality' there is no winner in the race to be the first seed.

This shows that both aerial and basal seeds are produced at the same time in the same numbers for both the field and stopped watering treatments. The lack of any difference for total seed mass and seed mass between the two types of seed for the field harvest may reflect the fact that plants were oven dried. In this sense the field harvests show the instantaneous seed masses during this 'crucial' seed development period. In contrast the stopped watering harvest while preventing any further plant growth, still allows for further development of seeds based on resources within the plant. Should this be the case and since 'instantaneously' during the initial seed production aerial and basal seeds are of the same number and mass; with the onset of conditions no longer suitable for further plant development the basal seeds receive an increased amount of resources relative to the aerial seeds in order to reach a presumed equal state of maturity (based on the optimum mass).

It was found that aerial seeds reached their optimum on day X harvest and basal seeds reached their optimum on day Y.

Seedlings

Results show a significant difference in seedling size between seedlings with seed coats attached and those without. Since all seed coats found attached are clearly of basal seed origin it is assumed that those seedlings without seed coats attached are of aerial seed origin. If this assumption is correct then the observed difference reflects that basal seeded seedlings are larger than aerial seeded seedlings. If the assumption is correct then it is also interesting to note that 6 aerial and 17 basal seeded individuals were sampled in the first field harvest. Since plants harvested were generally growing in clumps and isolated individuals were avoided; this shows that while there are more basal seeded plants there are also aerial seeded plants growing within the clumps.

A pessimistic-optimistic dichotomy?

To establish whether amphicarpic *T. solitaria* utilises the 'same' strategy as most other amphicarpic plants which can be seen as a pessimistic-opportunistic dichotomous strategy; the table of eco-generic consequences of amphicarpy and associated life-cycle attributes is re-drawn for *T. solitaria*. Aspects of the table not dealt with in this study are shown in grey, deviations from the table are shown in red.

Table 1. The eco-genetic consequences of amphicarpy and associated life-cycle attributes (*T. solitaria*)

	Aerial	Basal	Comments
I Seed and seedling biology			
Seed size	Small	Large	

Aerial

Basal

Seed weight	Low	High	Basal seeds 2.3 times as heavy
Seed number per plant	Equal	Equal	True even under stressful conditions
Seed number per fruit	Up to two	One	
Seed bank	Transient	Persistent	Based on seed coat thickness relating to dormancy
Seed germinability	High	High	Based on assumptions of finding both aerial and basal resultant seedlings
Seedling size	Small	Large	Based on assumptions of finding both aerial and basal resultant seedlings
II Population biology			
Survivorship	Low	High	
Competitive ability	Low	High	
Stress tolerance	Low	High	
Fitness	Low	High	
III Phenology			
Seed germination	-	-	Usually no difference in timing of seed germination
Flower and seed production	Equal	Equal	
IV Reproductive biology			
Flowers	Chasmogamous	Chasmogamous	
Breeding system	Potentially cross pollinating	Potentially cross pollinating	
% Seed set	Low	High	
Fruit weight	Low	High	
Seed dispersal potential	High	Low	
V Life history variation			
Flower and seed production	High phenotypic plasticity	Low phenotypic plasticity	Aerial reproduction is more strongly affected by environmental conditions
Flower and seed characteristics	High genetic variation	Low genetic variation	In one species, heritabilities of aerial reproductive traits were higher

Conclusion

Consequences of amphicarpy for *T. solitaria* are shown to differ in many ways from those of the amphicarpic plants from Cheplick's study (1987). Particular attributes which reduce the pessimism of *T. solitaria* basal reproduction: the equal production of both basal and aerial seeds phenologically, the equal production of both basal and aerial seed numbers by the last harvest, and potentially cross-pollinating basal spikelets. The pessimistic strategy would involve early production of basal seeds and fewer numbers of basal seeds under non-stressful conditions, and cleistogamous basal spikelets. The same attributes reduce the optimism of *T. solitaria* aerial spikelets.

References

- Van der Pijl, L. (1982). Principles of Dispersal in Higher Plants. Springer-Verlag.
- Cheplick, G.P., Quinn, J.A. (1982). *Oecologia*. 52:327-332
- Cheplick, G.P. (1987). The Ecology of Amphicarpic Plants. *Trends in Ecology and Evolution*. 2(4):97-101.
- Usher, G. (1966) *The Wordsworth Dictionary of Botany* (1996). Wordsworth Editions Ltd. Hertfordshire.
- Bond, W.J., Honig, M., Maze, K.E. (1999) Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia*. 121(1):132-136.
- Bruhl, J.J. (1994) Amphicarpy in the Cyperaceae, with Novel Variation in the Wetland Sedge *Eleocharis caespitosissima* Baker. *Australian Journal of Botany*. 42:441-448.
- Schutz, W (2000) Ecology of seed dormancy and germination in sedges (*Carex*). *Perspectives in Plant Ecology*. 3(1):67-89.

Appendix

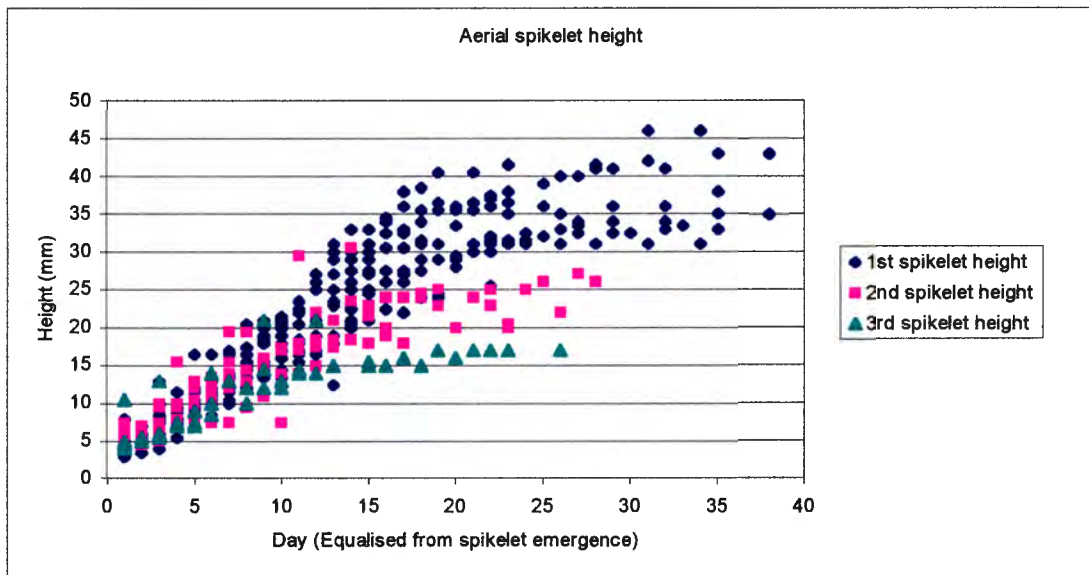


Figure 1. 1st, 2nd and 3rd spikelet development. There is a sample size of fourteen first spikelets, eleven second spikelets, and 4 third spikelets (since all plants observed produced first spikelets and only some produce more).

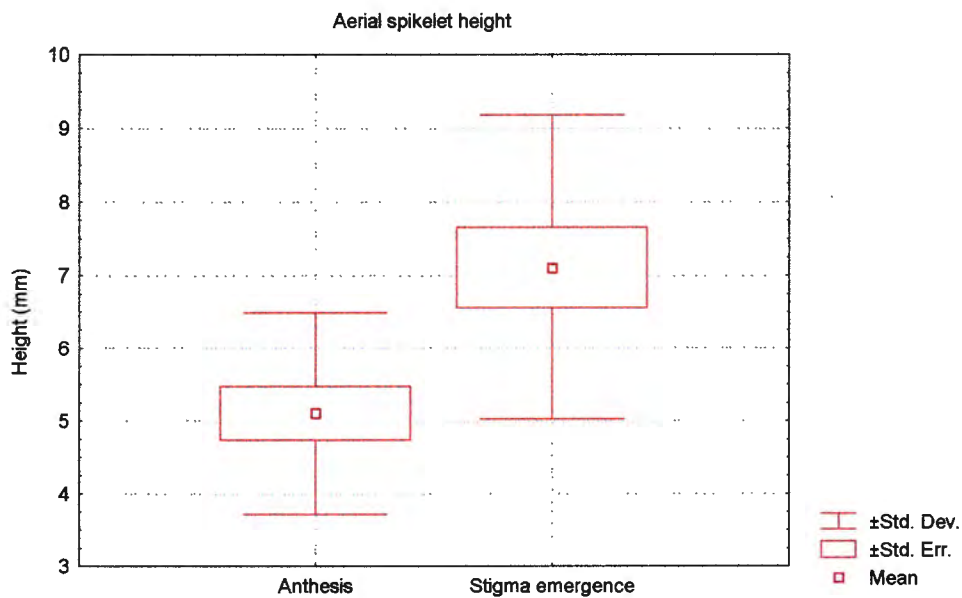


Figure 2. Box and whisker spikelet height with and post anthers

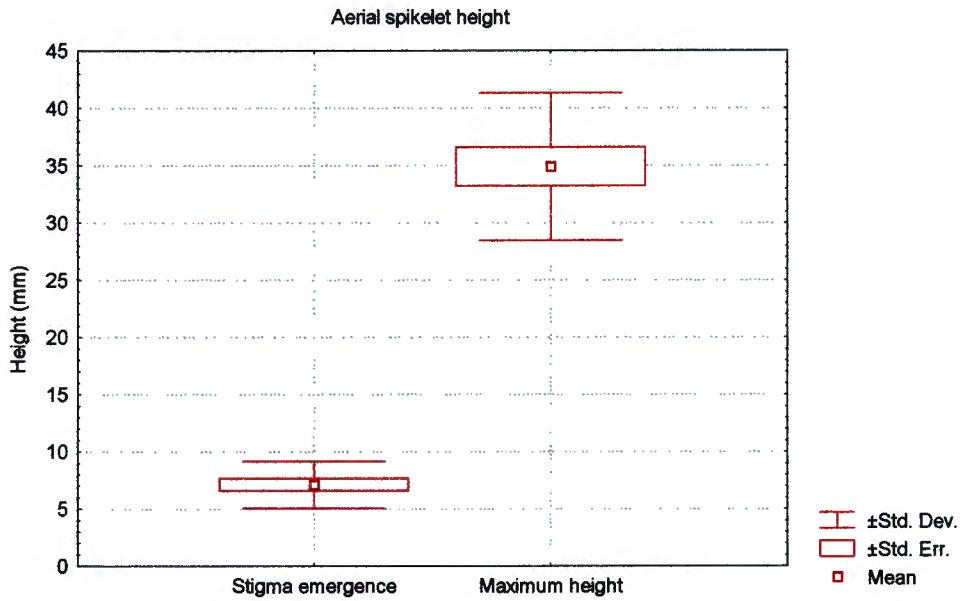


Figure 3. Box and whisker of spikelet height at spikelet height and maximum

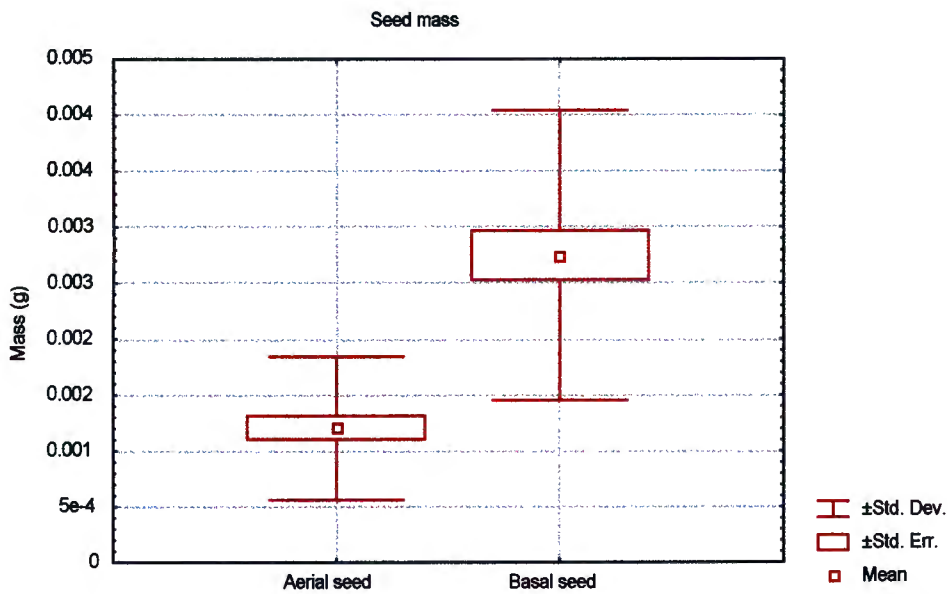


Figure 4. Box and whisker aerial and basal seed mass

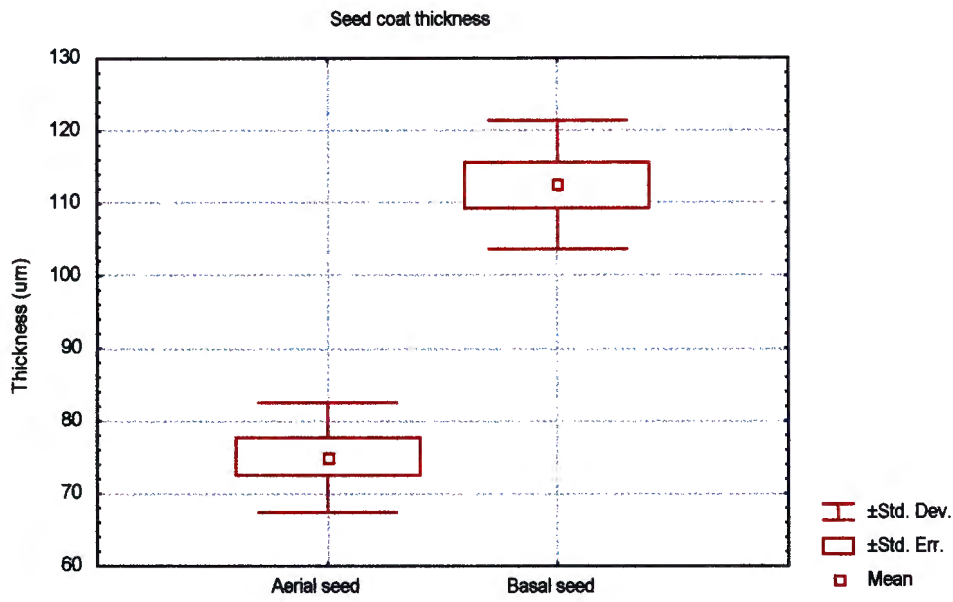


Figure 5. Aerial and basal seed coat thickness

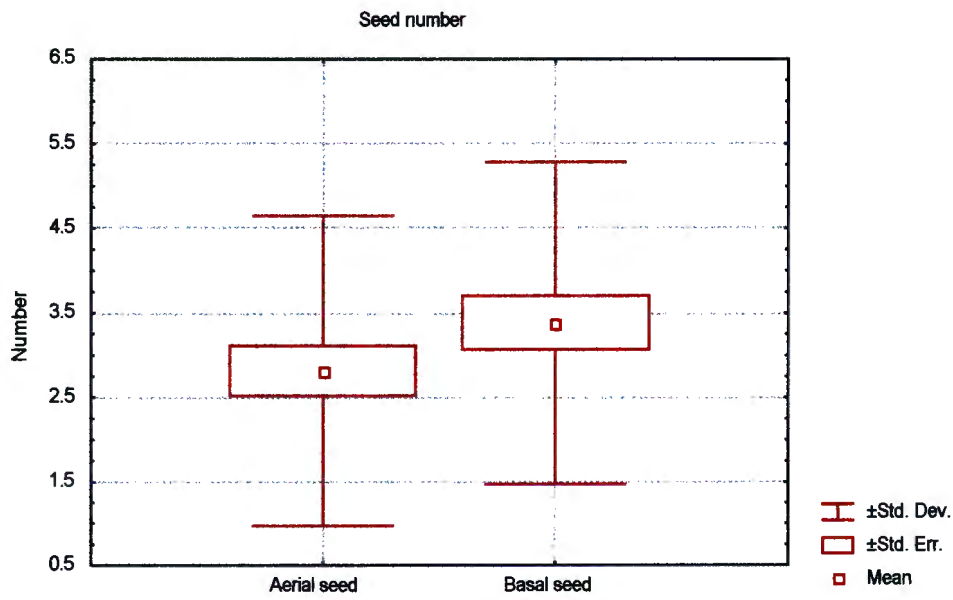


Figure 6. Box and whisker aerial and basal seed number

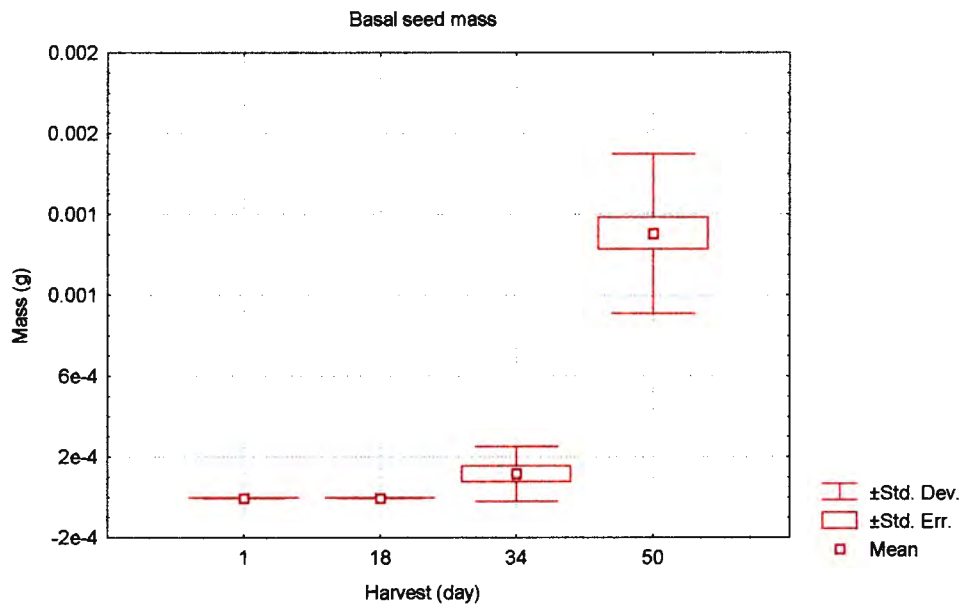


Figure 7. Basal seed mass (field harvests)

Table 1.
Scheffe Test; Variable: Basal seed mass (Field)

	{1}	{2}	{3}	{4}
	M=0.0000	M=0.0000	M=.00012	M=.00131
G_1:1 {1}		1.000000	.508570	0.000000
G_2:18 {2}	1.000000		.480766	0.000000
G_3:34 {3}	.508570	.480766		.000000
G_4:50 {4}	0.000000	0.000000	.000000	

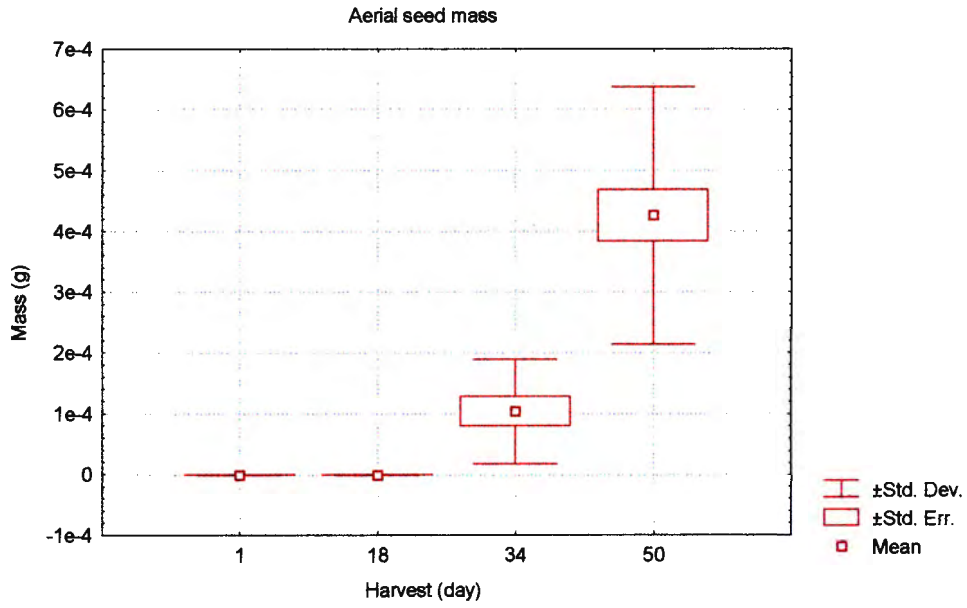


Figure 8. Aerial seed mass (Field harvests)

Table 2.

Scheffe Test; Variable: Aerial seed mass (Field)

	{1}	{2}	{3}	{4}
	M=0.0000	M=0.0000	M=.00010	M=.00043
G_1:1 {1}		1.000000	.109292	.000000
G_2:18 {2}	1.000000		.092549	.000000
G_3:34 {3}	.109292	.092549		.000000
G_4:50 {4}	.000000	.000000	.000000	

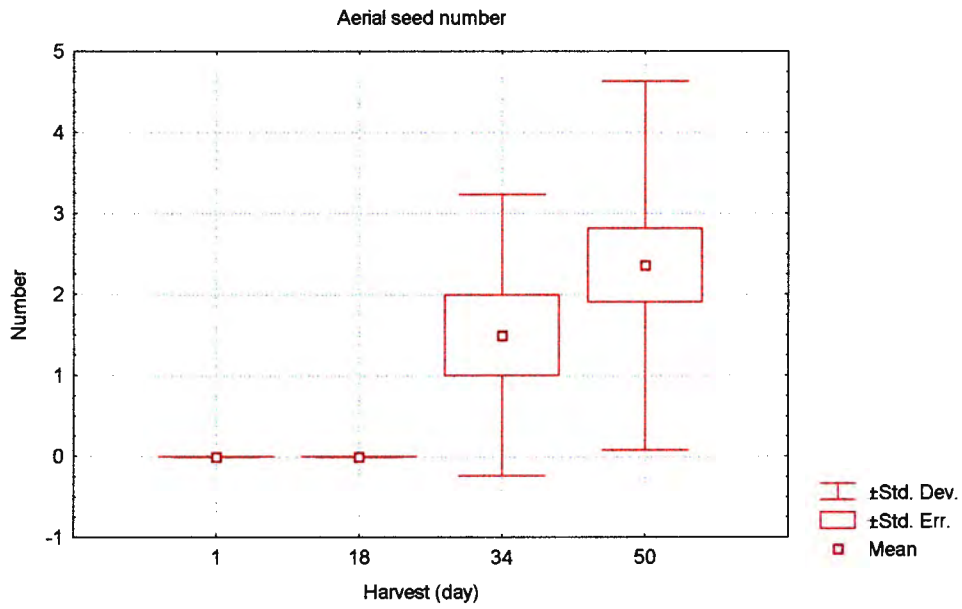


Figure 8. Aerial seed number (Field harvests)

Table 3.

Scheffe Test; Variable: Aerial seed number (Field)

	{1}	{2}	{3}	{4}
	M=0.0000	M=0.0000	M=1.5000	M=2.3600
G_1:1 {1}		1.000000	.028507	.000001
G_2:18 {2}	1.000000		.022107	.000000
G_3:34 {3}	.028507	.022107		.365852
G_4:50 {4}	.000001	.000000	.365852	

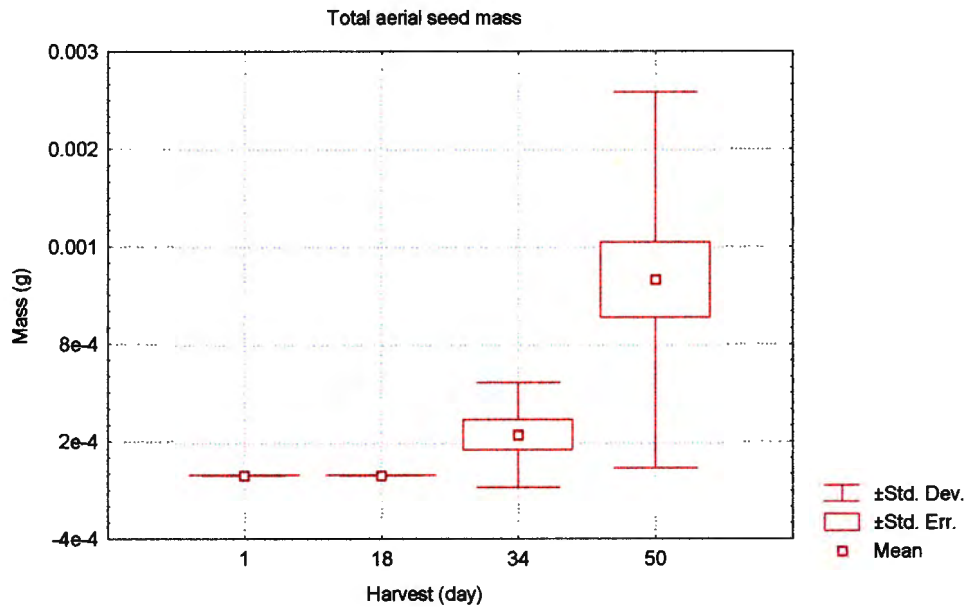


Figure 9. Total aerial seed mass (Field harvests)

Table 4.

Scheffe Test; Variable: Total aerial seed mass (Field)

	{1}	{2}	{3}	{4}
	M=0.0000	M=0.0000	M=.00025	M=.00120
G_1:1 {1}		1.000000	.740320	.000000
G_2:18 {2}	1.000000		.721055	.000000
G_3:34 {3}	.740320	.721055		.000732
G_4:50 {4}	.000000	.000000	.000732	

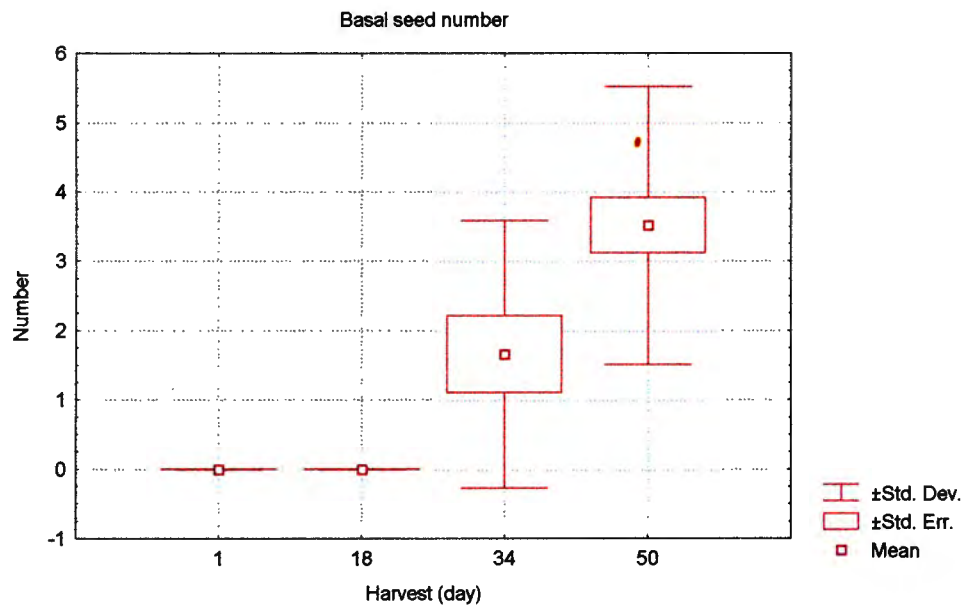


Figure 10. Basal seed number (Field harvests)

Table 5.

Scheffe Test; Variable: Basal seed number (Field)

	{1}	{2}	{3}	{4}
	M=0.0000	M=0.0000	M=1.6667	M=3.5200
G_1:1 {1}		1.000000	.005745	.000000
G_2:18 {2}	1.000000		.004037	.000000
G_3:34 {3}	.005745	.004037		.001341
G_4:50 {4}	.000000	.000000	.001341	

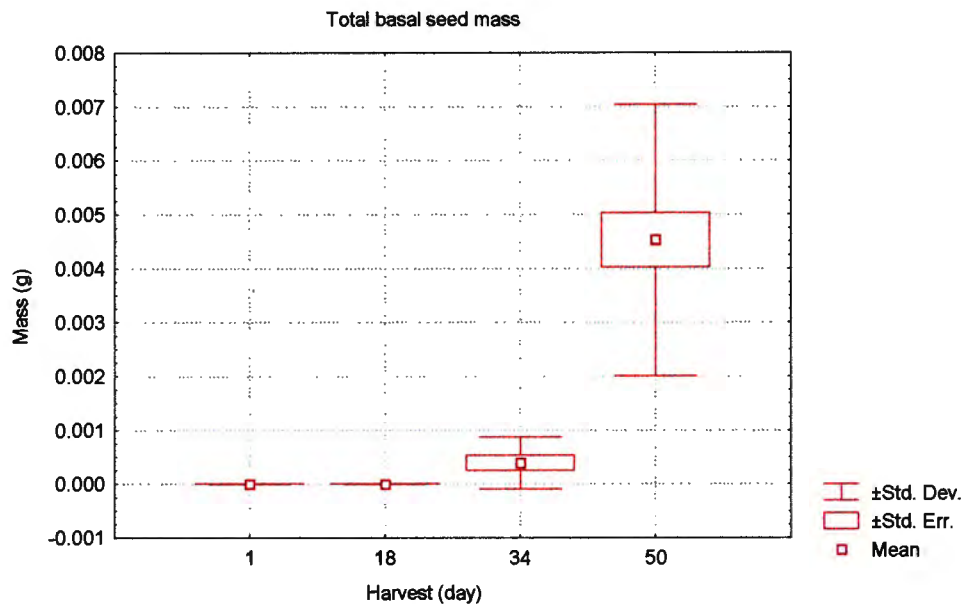


Figure 11. Total basal seed mass (Field harvests)

Table 6.

Scheffe Test; Variable: Total basal seed mass (Field)

	{1}	{2}	{3}	{4}
	M=0.0000	M=0.0000	M=.00039	M=.00453
G_1:1 {1}		1.000000	.881867	.000000
G_2:18 {2}	1.000000		.871774	.000000
G_3:34 {3}	.881867	.871774		.000000
G_4:50 {4}	.000000	.000000	.000000	

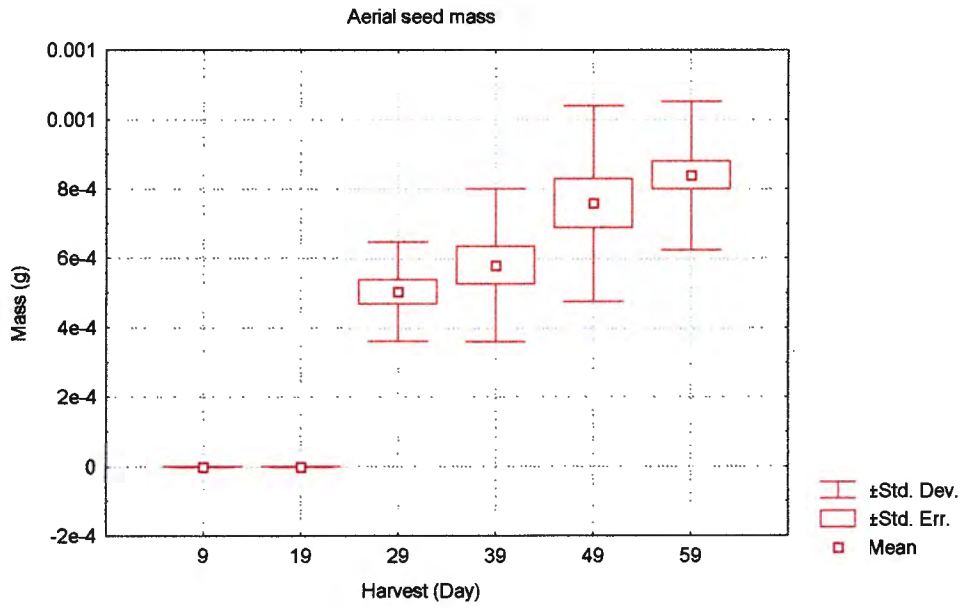


Figure 12. Aerial seed mass (Stop watering harvests)

Table 7.

Scheffe Test; Variable: Aerial seed mass (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=0.0000	M=0.0000	M=.00051	M=.00058	M=.00076	M=.00084
G_1:9 {1}		1.000000	.000000	.000000	.000000	.000000
G_2:19 {2}	1.000000		.000000	.000000	.000000	.000000
G_3:29 {3}	.000000	.000000		.929287	.013633	.000024
G_4:39 {4}	.000000	.000000	.929287		.197529	.002592
G_5:49 {5}	.000000	.000000	.013633	.197529		.860928
G_6:59 {6}	.000000	.000000	.000024	.002592	.860928	

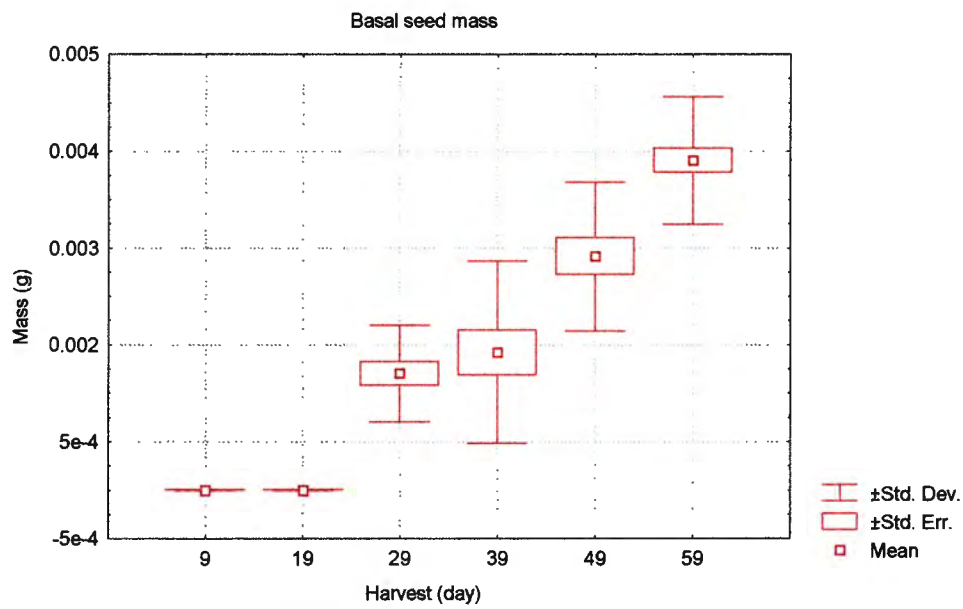


Figure 13. Basal seed mass (Stop watering harvests)

Table 8.

Scheffe Test; Variable: Basal seed mass (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=0.0000	M=0.0000	M=.00121	M=.00143	M=.00241	M=.00341
G_1:9 {1}		1.000000	.000056	.000001	.000000	.000000
G_2:19 {2}	1.000000		.000039	.000001	.000000	.000000
G_3:29 {3}	.000056	.000039		.961411	.000044	.000000
G_4:39 {4}	.000001	.000001	.961411		.001645	.000000
G_5:49 {5}	.000000	.000000	.000044	.001645		.000221
G_6:59 {6}	.000000	.000000	.000000	.000000	.000221	

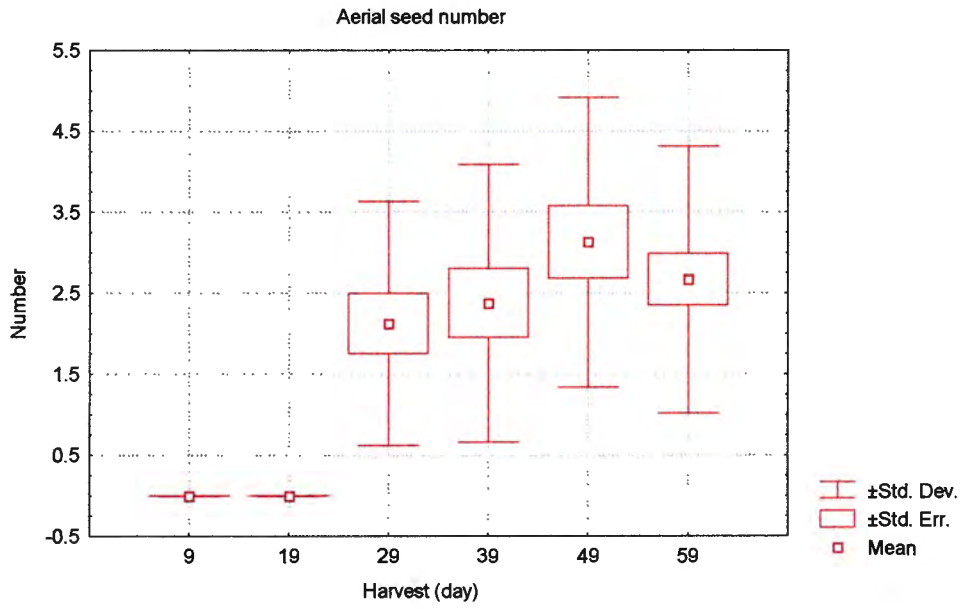


Figure 14. Aerial seed number (Stopped watering harvests)

Table 9.

Scheffe Test; Variable: Aerial seed number (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=0.0000	M=0.0000	M=2.1250	M=2.3750	M=3.1250	M=2.6667
G_1:9 {1}		1.000000	.005077	.001015	.000003	.000012
G_2:19 {2}	1.000000		.004091	.000777	.000002	.000007
G_3:29 {3}	.005077	.004091		.998335	.538629	.910665
G_4:39 {4}	.001015	.000777	.998335		.804430	.994053
G_5:49 {5}	.000003	.000002	.538629	.804430		.954886
G_6:59 {6}	.000012	.000007	.910665	.994053	.954886	

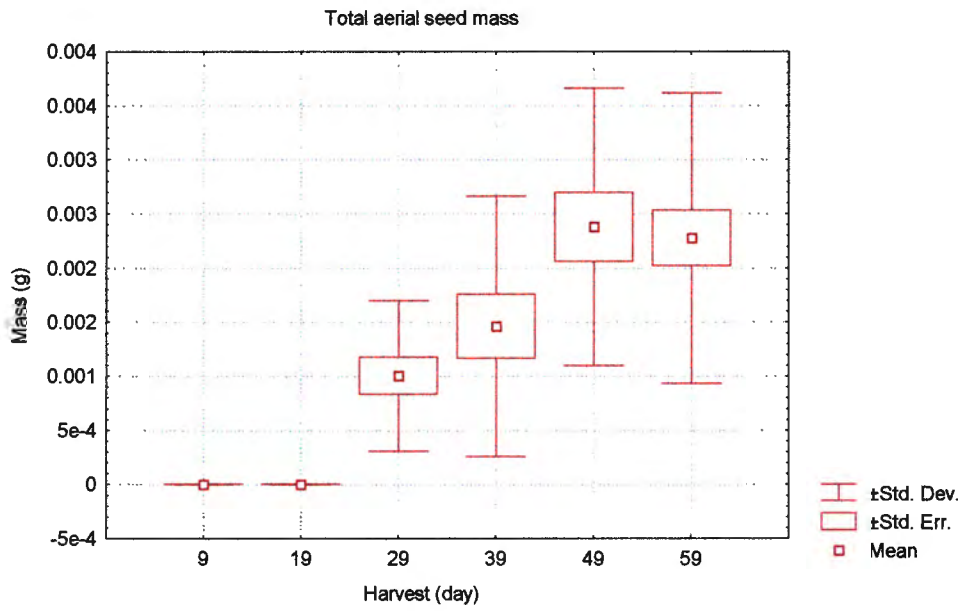


Figure 15. Total aerial seed mass (Stopped watering harvests)

Table 10.

Scheffe Test; Variable: Total aerial seed mass (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=0.0000	M=0.0000	M=.00101	M=.00146	M=.00238	M=.00228
G_1:9 {1}		1.000000	.177598	.008426	.000001	.000000
G_2:19 {2}	1.000000		.163047	.006907	.000000	.000000
G_3:29 {3}	.177598	.163047		.892583	.014111	.009442
G_4:39 {4}	.008426	.006907	.892583		.251307	.257271
G_5:49 {5}	.000001	.000000	.014111	.251307		.999800
G_6:59 {6}	.000000	.000000	.009442	.257271	.999800	

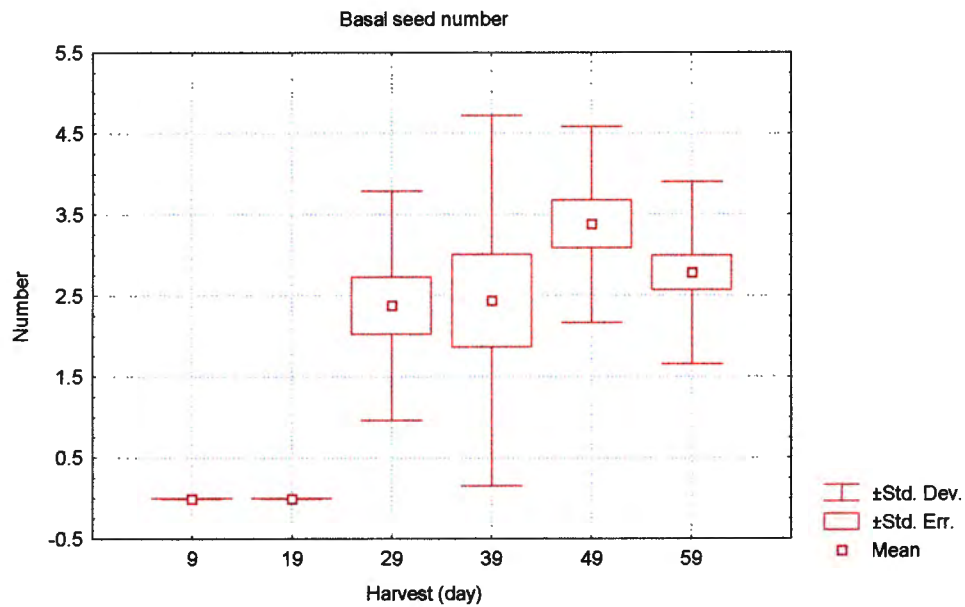


Figure 16. Basal seed number (Stopped watering harvests)

Table 11.

Scheffe Test; Variable: Basal seed number (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=0.0000	M=0.0000	M=2.3750	M=2.4375	M=3.3750	M=2.7778
G_1:9 {1}		1.000000	.000199	.000120	.000000	.000000
G_2:19 {2}	1.000000		.000145	.000086	.000000	.000000
G_3:29 {3}	.000199	.000145		.999997	.429609	.961321
G_4:39 {4}	.000120	.000086	.999997		.505816	.981528
G_5:49 {5}	.000000	.000000	.429609	.505816		.818089
G_6:59 {6}	.000000	.000000	.961321	.981528	.818089	

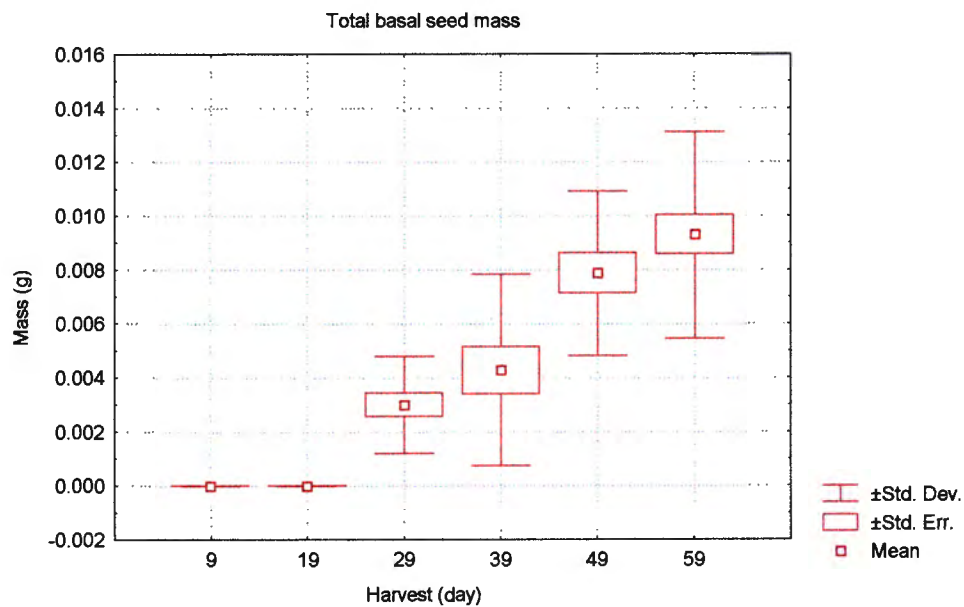


Figure 17. Total basal seed mass (Stopped watering harvests)

Table 12.

Scheffe Test; Variable: Total basal seed mass (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=0.0000	M=0.0000	M=.00302	M=.00429	M=.00788	M=.00930
G_1:9 {1}		1.000000	.106239	.003520	.000000	.000000
G_2:19 {2}	1.000000		.095520	.002802	.000000	.000000
G_3:29 {3}	.106239	.095520		.884526	.000385	.000000
G_4:39 {4}	.003520	.002802	.884526		.023554	.000021
G_5:49 {5}	.000000	.000000	.000385	.023554		.746388
G_6:59 {6}	.000000	.000000	.000000	.000021	.746388	

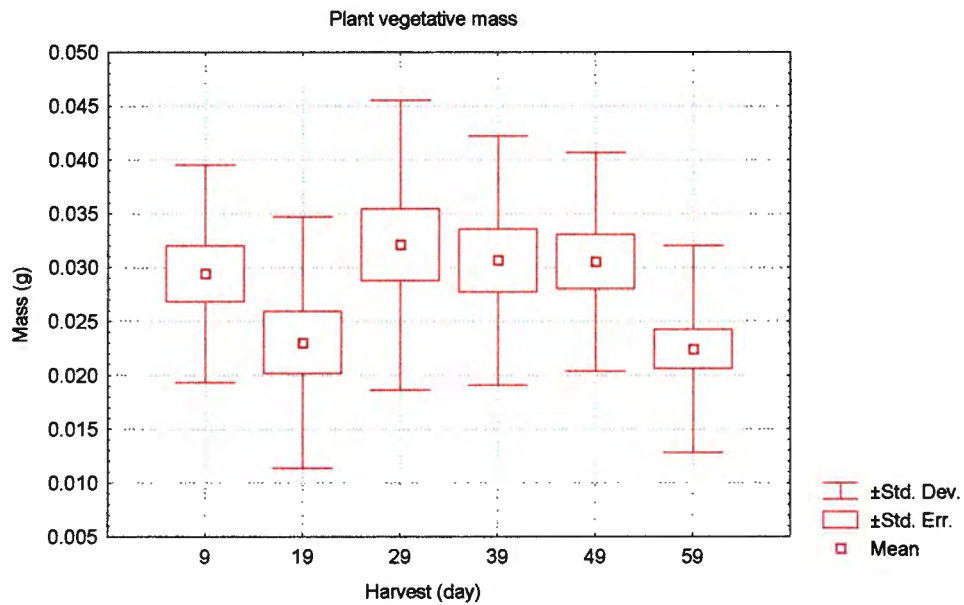


Figure 18. Plant vegetative mass (Stopped watering harvests)

Table 13.

Scheffe Test; Variable: Vegetative mass (Stopped watering)

	{1}	{2}	{3}	{4}	{5}	{6}
	M=.02945	M=.02305	M=.03212	M=.03066	M=.03053	M=.02242
G_1:9 {1}		.757108	.993398	.999853	.999916	.560702
G_2:19 {2}	.757108		.370839	.575133	.594121	.999989
G_3:29 {3}	.993398	.370839		.999613	.999409	.177037
G_4:39 {4}	.999853	.575133	.999613		1.000000	.349581
G_5:49 {5}	.999916	.594121	.999409	1.000000		.368438
G_6:59 {6}	.560702	.999989	.177037	.349581	.368438	

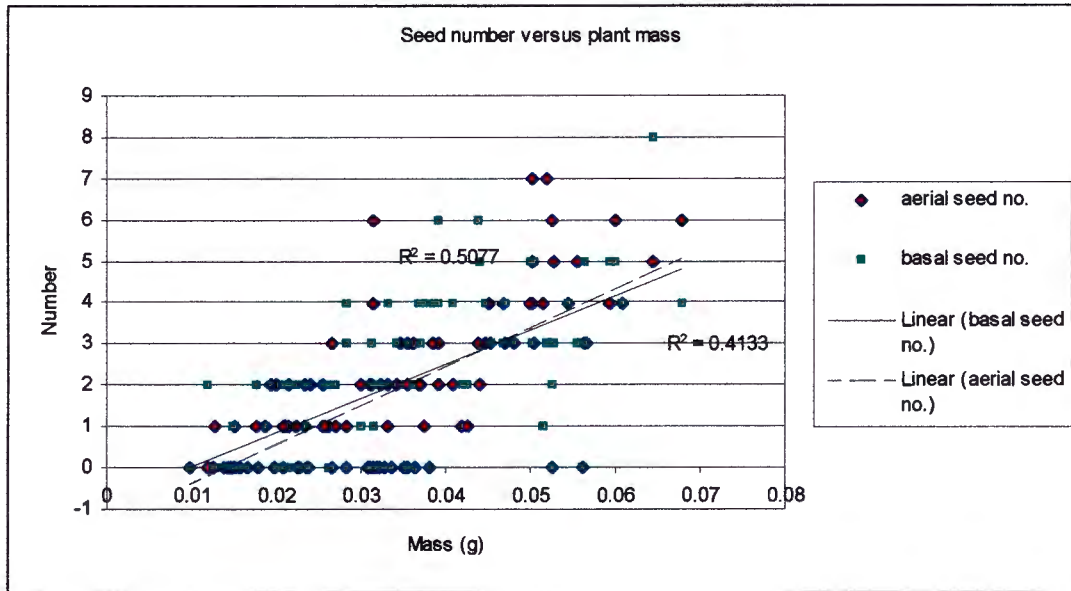


Figure 19. Aerial and basal seed numbers versus plant mass

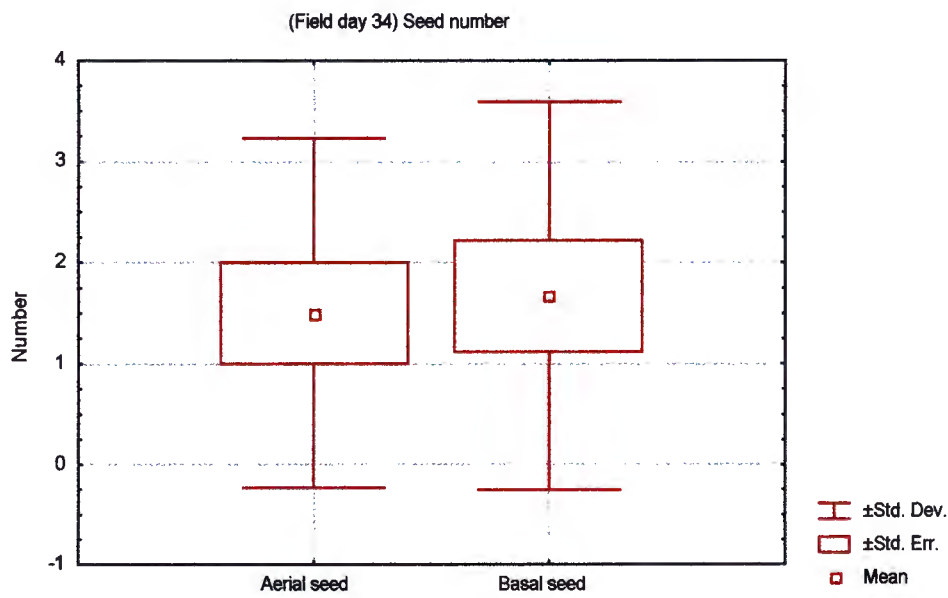


Figure 20. (Field day 34) Seed number of aerial and basal seeds

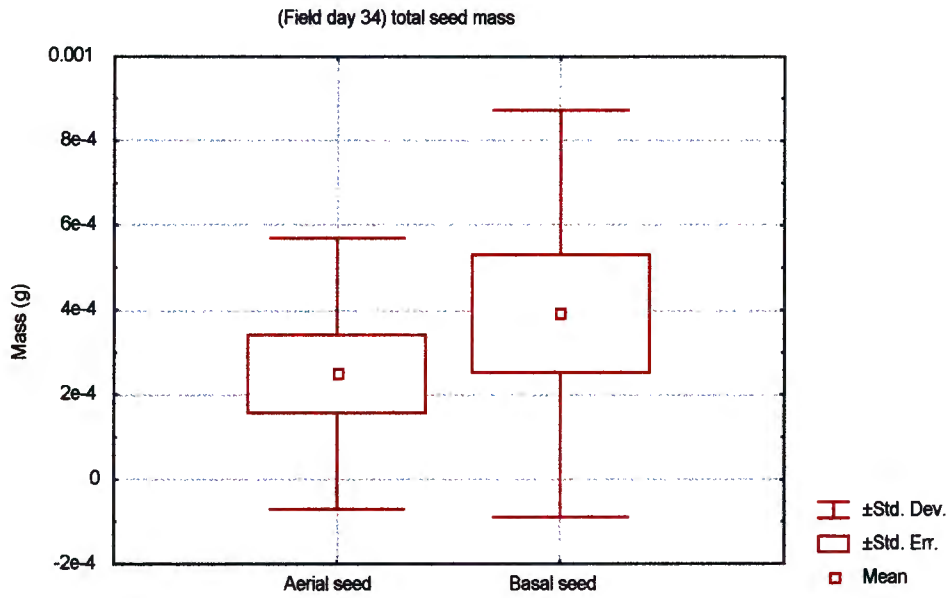


Figure 21. (Field day 34) Total seed mass for aerial and basal seeds

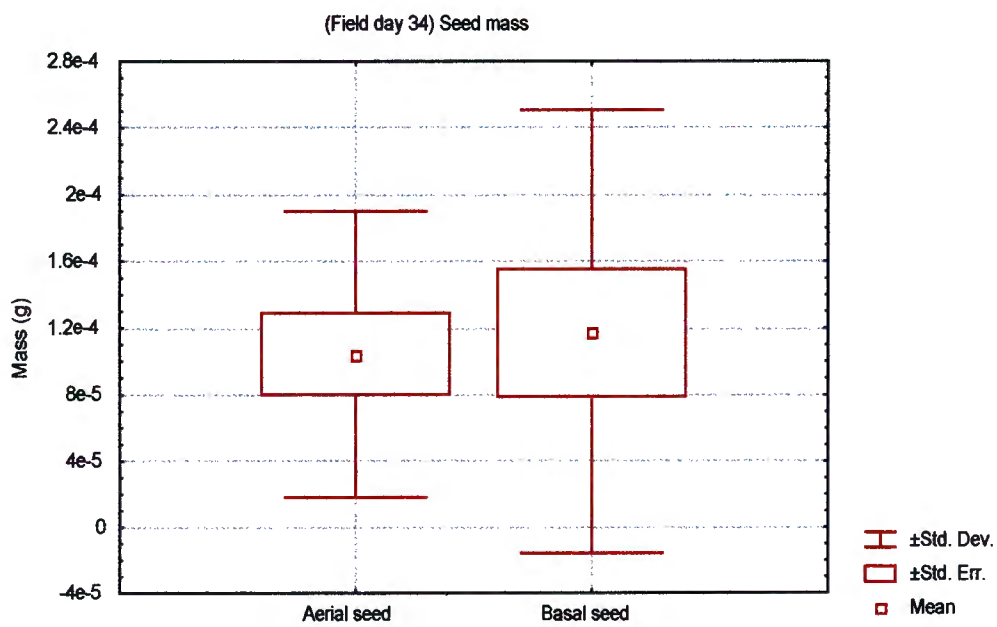


Figure 22. (Field day 34) Seed mass for aerial and basal seeds

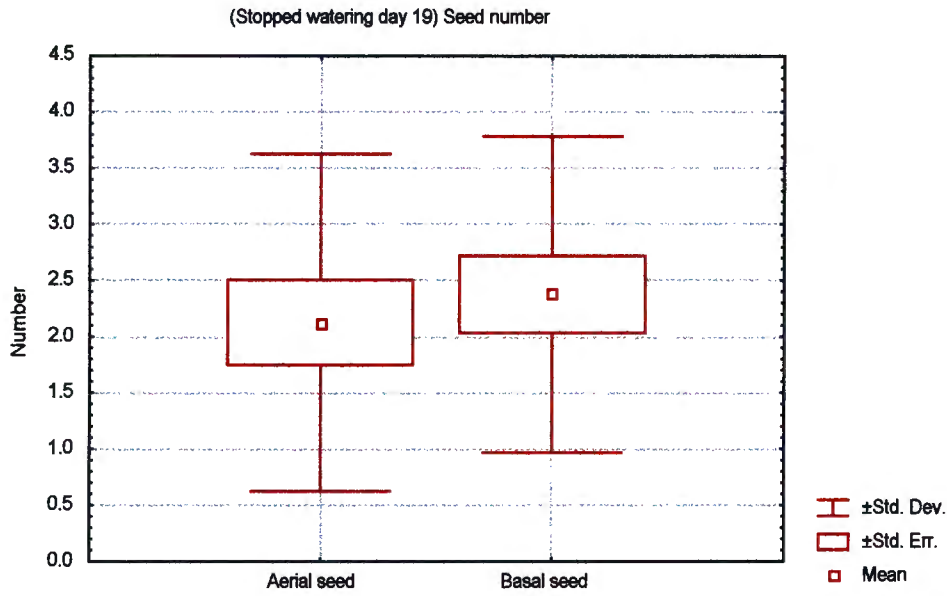


Figure 23. (Stopped watering day 19) Seed number for aerial and basal seeds

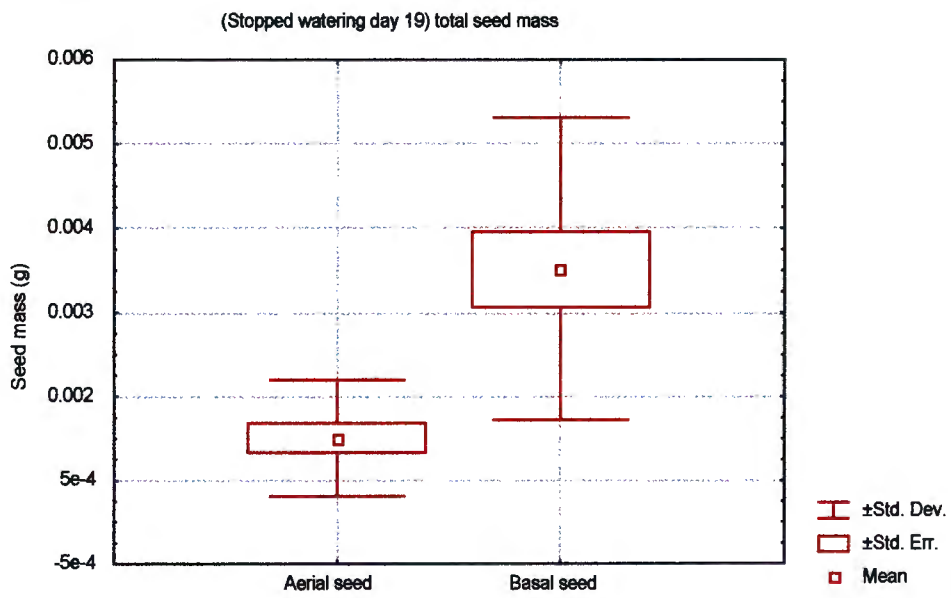


Figure 24. (Stopped watering day 19) Total seed mass for aerial and basal seeds

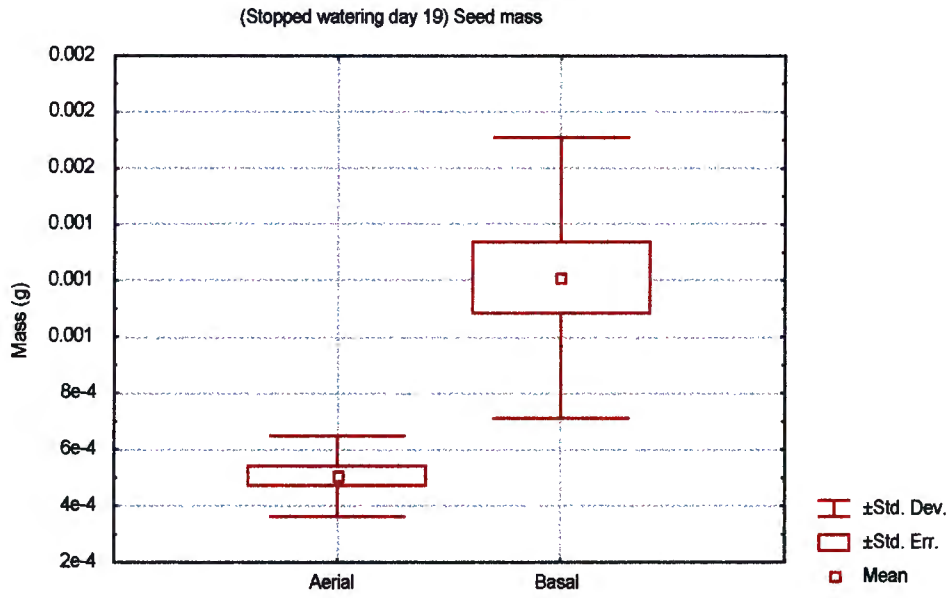


Figure 25. (Stopped watering day 19) Seed mass for aerial and basal seeds

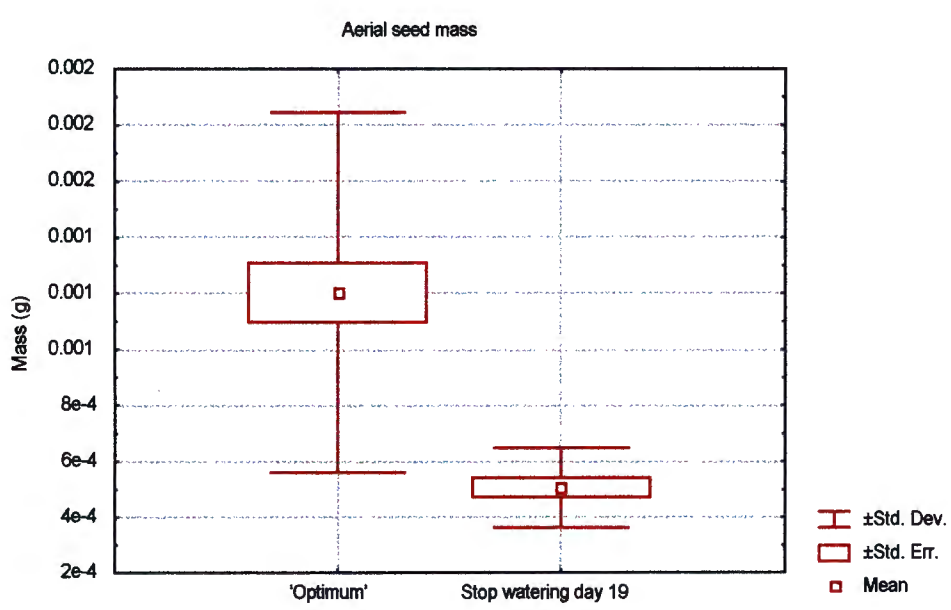


Figure 26. Aerial seed size of the optimum and stop watering day 19 harvests

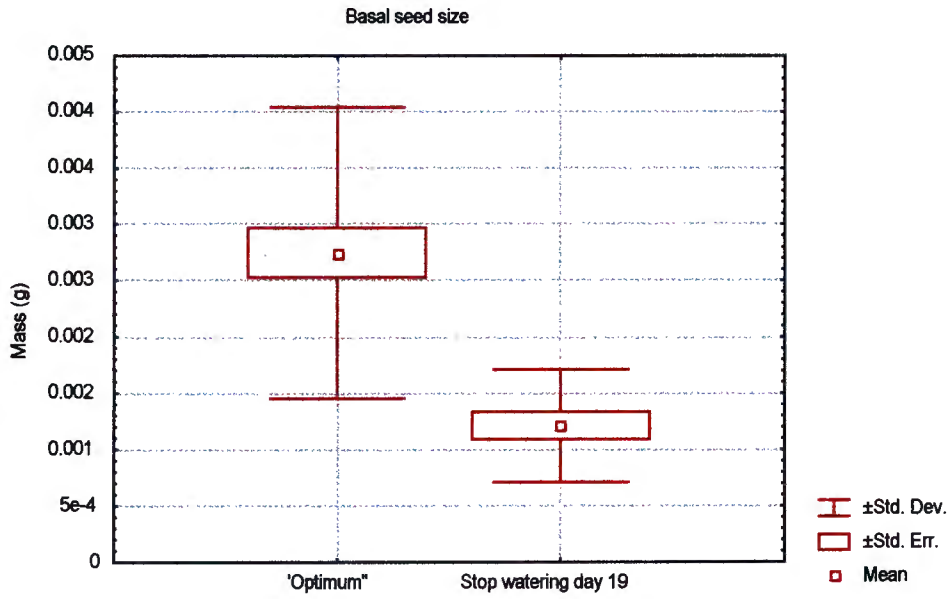


Figure 27. Basal seed size of the optimum and stop watering day 19 harvests

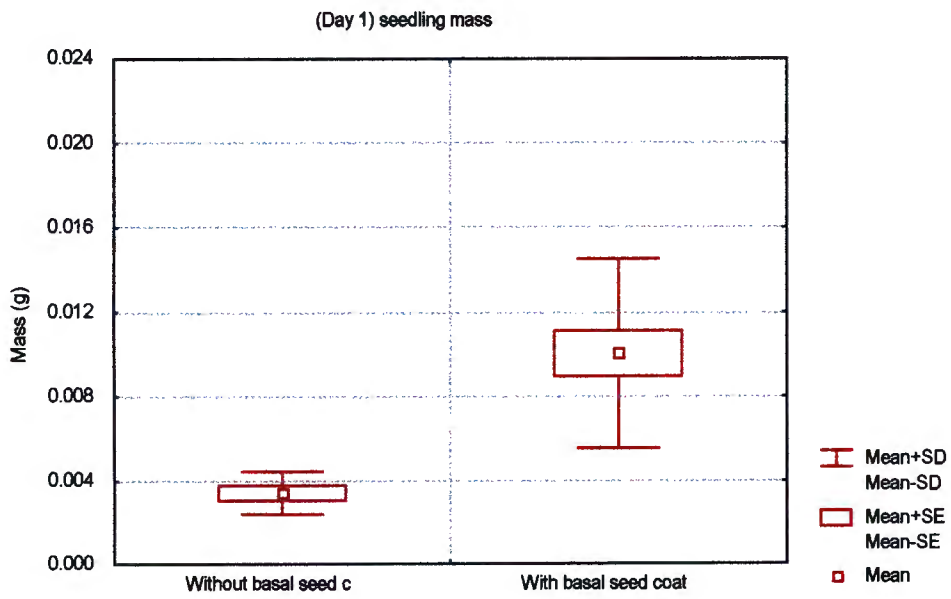


Figure 28. Seedling mass with and without old seed coat

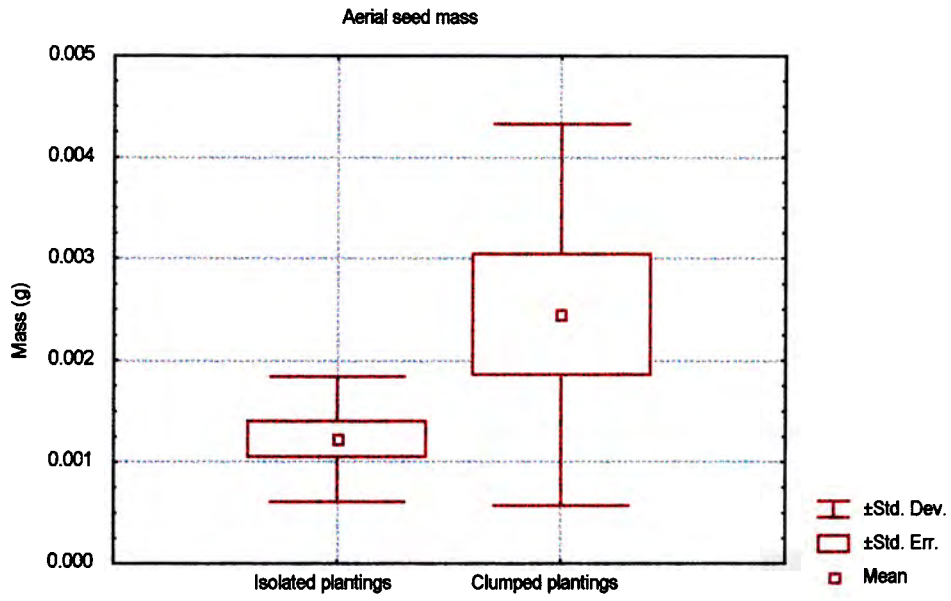


Figure 29. Aerial seed mass for isolated and clumped plantings



Figure a. *Trianoptiles solitaria* habit



Figure b. detail of *Trianoptiles solitaria*