# Final Report

**For the 2001 contract period through November 30th, 2001.**

## An evaluation of the life-histories of invading populations of *Ardisia crenata* in north Florida to improve our understanding of their invasive impacts and management.

To
The Department of Environmental Protection

From
Alison M. Fox
Agronomy Department, UF/IFAS
P.O. Box 110500, 304 Newell Hall,
Gainesville, FL 32611-0500
Tel: 352-392-1811 x.207; Fax: 352-392-1840;
e-mail: amfox@gnv.ifas.ufl.edu

and

Kaoru Kitajima
Botany Department, UF
P.O Box 118526, 220 Bartram Hall,
Gainesville, FL 32611
Tel: 352-392-4234; Fax: 352-392-3993
e-mail: kitajima@botany.ufl.edu

## Executive Summary

1. There is no evidence of internal seed dormancy mechanisms or soil seed banks in *A. crenata*.

2. Seeds are capable of surviving for at least two years in moist conditions that are too cold for germination (e.g., 3$^{o}$C) but at typical field temperatures in north Florida (around 26$^{o}$C), germination occurs within two months.

3. Seeds have poor tolerance for moisture loss with seed death occurring when moisture content falls below about 50%. Moisture loss may be faster in the absence of the fruit skin and pulp, although it is not evident that this significantly prolongs seed viability in dry conditions. It is unlikely that this species would invade drier upland habitats such as scrub or sand-hills.

4. Over 90% of *A. crenata* seeds that are deposited in sites with suitable conditions are capable of germinating. There may be some seasonal variation in this germination rate in the field (data still being collected) but preliminary analyses suggest that 25% to 65% of fruit lost from field plants will develop into seedlings.

5. Mature fruit of *A. crenata* can remain on the plant up to a year but most losses occur during the spring and early summer. Flowering occurs in the summer, but new fruit that are formed in the early fall do not fully mature to their red coloration until December. In typical field conditions, up to 150 fruit are produced per plant.

6. Preliminary studies of the fate of *A. crenata* fruit indicate that 70% - 80% land directly below the parent. It is possible that bird and animal activities (e.g., predation or dispersal) may account for some of the missing fruit.

7. The distribution of different sized *A. crenata* plants mapped in populations in a mesic hardwood, show a close association of small seedlings with parent plants and relatively few plants over 5 m from the main populations. Mechanisms of long-distance dispersal of *A. crenata* are unknown, but despite being infrequent such events may be very important in relation to the frequency that new infestations occur in more remote areas.

8. Populations of *A. crenata* have large numbers of seedlings up to 10 cm tall (100-200 plants $m^{-2}$) but there is high mortality of these seedlings, especially between February and June, so that densities of plants over 10 cm in height have densities of less than 5 plants $m^{-2}$. Stems over 80 cm tall also had high mortality rates but the tracking of plant longevity is complicated by the production of multiple stems from root crowns.

9. Plants grown in an irrigated common garden experiment showed poor growth under full sunlight where frost and photo-injury occurred. Optimal growth was observed at 18-45% of full sunlight.

10. Relative growth rates estimated from tagged plants growing in the field, were much lower than for the plants in the common garden experiment (1-1.5 mg/g/day and 6 – 13 mg/g/day, respectively) indicating that factors other than light availability may be limiting growth rates in field conditions.

11. The slow growth rates recorded in the field indicate that plants were much older than expected. Reproductive plants of 20cm in height may be 10 years old, and stems starting to die-back at over 80 cm in height may be over 20 years old. These estimates assume that growth rates are not faster in wetter years or when light gaps appear due to tree falls. Based on the common garden experiment, it is likely that *A. crenata* plants are able to rapidly grow and exploit such light gaps.

12. Although *A. crenata* roots have some if the highest starch concentrations recorded for shrubs (up to 60% of root dry mass), they are not capable of re-sprouting if severed from the root crown.

13. Root carbohydrate storage is maximal in March following high levels of photosynthesis when the tree canopy is thinnest, and least in September following stem elongation, and leaf and flower production. Stem regrowth from root crowns may be reduced in September due to this reduced storage capacity, but root crown removal is necessary to ensure the prevention of regrowth from plants that are cut or treated with contact herbicides.

14. Data from studies of the plant communities in and around infestations of *A. crenata* indicate that there is a negative correlation of the abundance of native understory plants with *A. crenata*.

## Table of Contents

*Citations for bi-annual reports.* *The following bi-annual reports to the DEP on* Ardisia crenata *are referred to in this text by superscript numbers 1-3:*

1. *Status Report for the 2000 contract period through June 1st, 2000.*
2. *Final Report for the 2000 contract period through November 30th, 2000.*
3. *Status Report for the 2001 contract period through June 1st, 2001.*

## I. Life History of *Ardisia crenata*

### 1. Seed dormancy

Seeds of *A. crenata* have been germinated in growth chambers on numerous occasions, and there has been no evidence of a dormancy mechanism inhibiting immediate germination of freshly collected, mature, red fruit. The proportion of such seeds that are viable is typically over 90%.

### 2. Influence of seed moisture on seed germination and viability

Preliminary observations that seeds allowed to air-dry for 30 days lost viability[2] prompted a series of studies to quantify the relationship between seed moisture content and germination.

Before the initiation of the experiment, moisture contents of fruits and seeds were determined immediately following the collection in the field in February, 2001. The moisture content on dry mass basis (water content / dry mass) was much higher for pulp (mean ± s.d. = 7.4 ± 2.9) than for seeds (1.02 ± 0.05). Seeds cleaned of pulp were buried in moist sand and stored for 1 month at 3°C for cold stratification and to saturate seed moisture contents (moisture content = 1.05 ± 0.05). Seeds would not germinate at this low temperature but maintain viability for over one year. Then, seeds were stored in paper envelopes (70 seeds per envelope) in the air conditioned room (22°C), or refrigerator (3°C) for 1-120 days. After drying under each condition for specified periods, 10 seeds were used for determination of fresh mass and dry mass (after drying to constant mass at 65°C). Remaining 60 seeds were divided to three replicates and tested for germination in petri dishes lined with moist filter paper. For each storage duration treatment, 3 replicates of 20 seeds stored in cold moist sand were also planted as control.

The rate of drying was much slower in the refrigerator with lower temperature and higher relative humidity (Fig. 1). Both rate of germination and final germination level became lower as desiccation progressed. For example, after 32 days of storage, cold-dried seeds germinated slowly but eventually reached 100% germination, while seeds dried in the room temperature (with lower moisture contents) had slower germination and lower final germination level (mean ± sd = 87 ± 10% - Fig. 2.). The difference was apparently due to difference in moisture contents between the two drying conditions.

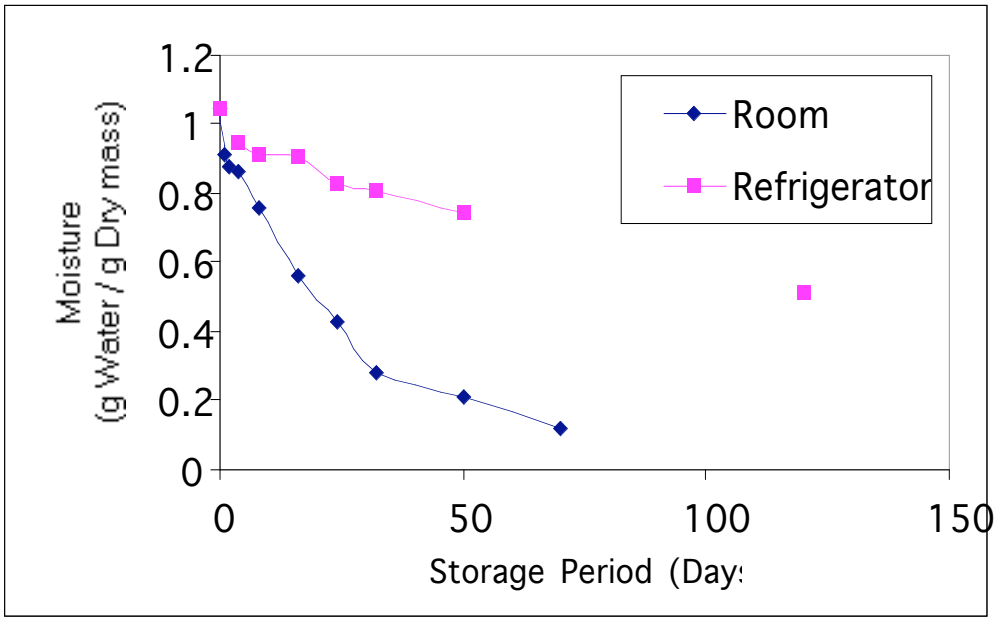


Fig. 1. Loss of seed moisture content during storage in paper envelopes in air-conditioned room vs. refrigerator.

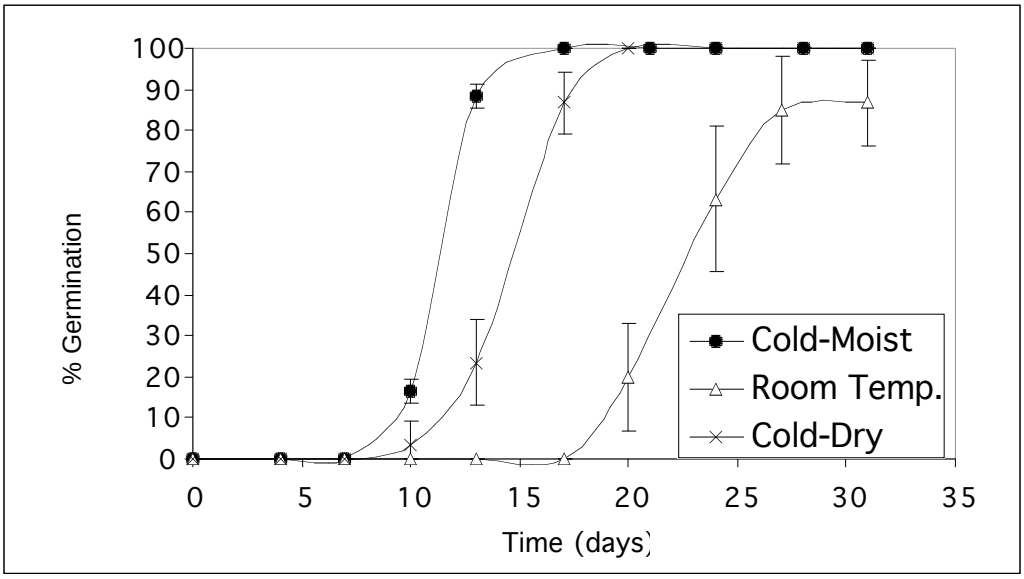


Fig. 2. Germination of seeds stored in three different conditions for 32 days. Cold-moist seeds were stored in moist sand in refrigerator (3°C), while seeds were stored in paper envelopes in an air-conditioned room (21°C) or refrigerator (3°C).

Thus, the rate of germination and final germination were analyzed as functions of seed moisture contents. The rate of germination was quantified as the average time for 50% of the seeds to germinate (LD50) after fitting a logistic curve to the percentage germination against time plotted together for three replicates.

Seed germination was slower for seeds with lower moisture contents in a linear relationship (Fig. 3). However, final seed germination level was 100% until seed moisture content dropped below 0.4 on dry mass basis. Below this level, final germination dropped rapidly and at the moisture content of 0.2 (51 day drying at room temperature), all seeds were dead.

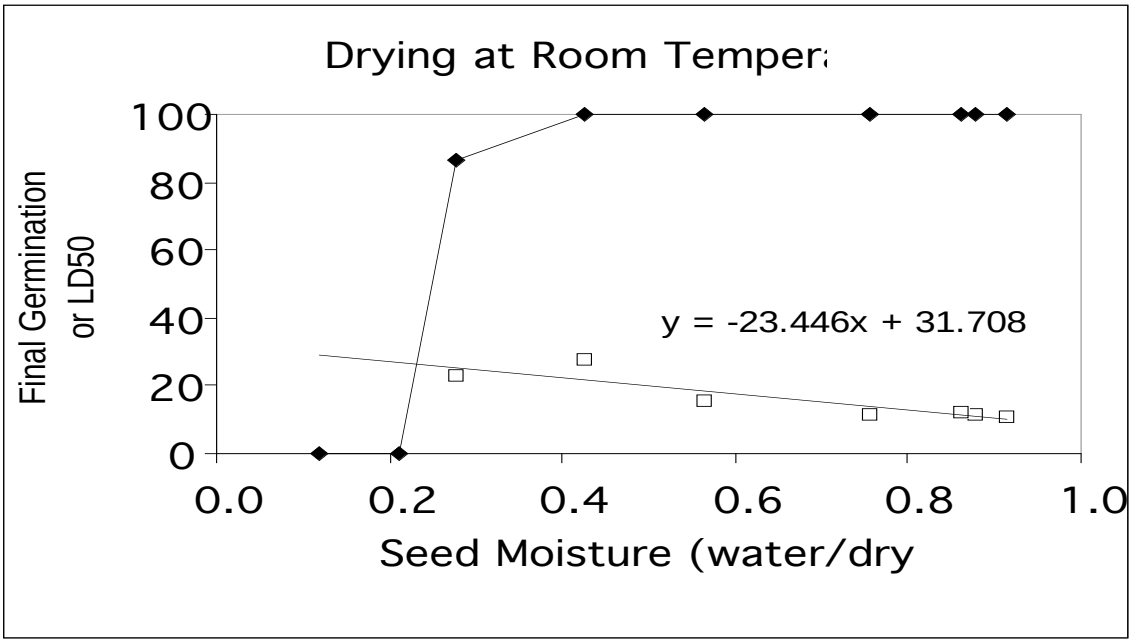


Fig. 3. Effects of seed moisture contents on final germination percentage (diamond) and time (days) for 50% germination (open square) for seeds dried in paper envelopes in an air-conditioned room.

Seeds dried in paper envelopes in the refrigerator showed similar a similar relationship between germination time (LD50) and seed moisture content (Fig. 4). Seed moisture contents did not reach 0.5 in this slower drying condition. However, final germination percentage dropped below 100% at a moisture content of 0.51 (for 120 day drying), while seeds dried at room temperature more rapidly had 100% germination at a comparable moisture level (0.56 for 16 day drying and 0.43 for 25 day drying). Thus, duration of time for seeds to remain in dry condition negatively impacts seed viability.

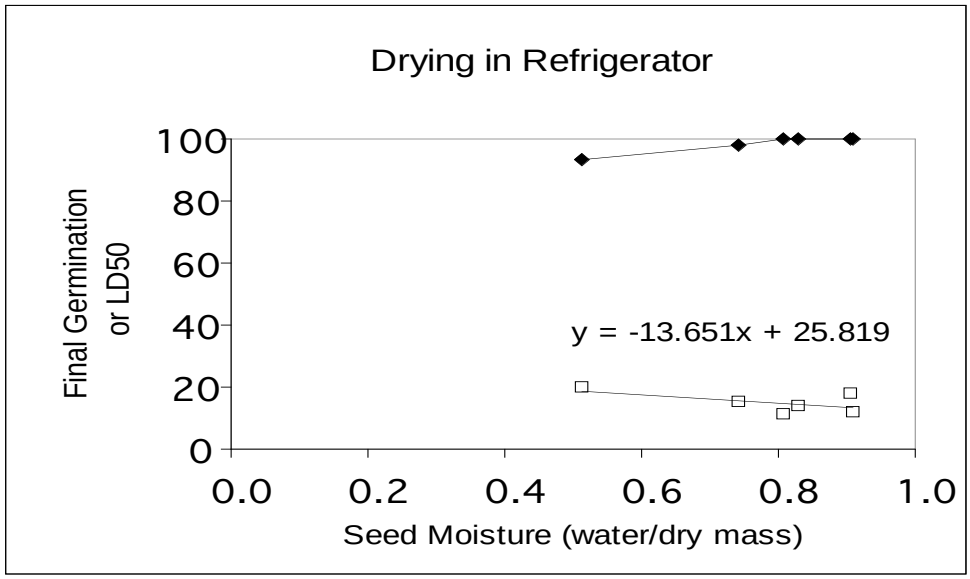


Fig. 4. Effects of seed moisture contents on final germination percentage (diamond) and time for 50% germination (open square) for seeds dried in paper envelopes in a refrigerator (3°C).

Control seeds stored at 3°C in moist sand maintained 100% viability (Fig. 5), but beyond 1 year, viability was declining even when seed moisture content was maintained high (water/dry mass ratio = 1.14, higher than the freshly collected seeds). Curiously, batches of seeds stored in sands of different moisture contents (as sands dried gradually in some bags that were partially open) exhibit a humped relationship between seed moisture contents and final germination level (Fig. 6). It was not surprising to find lower seed germination levels for seeds with lower moisture contents. However, above moisture contents of 1.0, seed viability also declines with higher moisture contents.

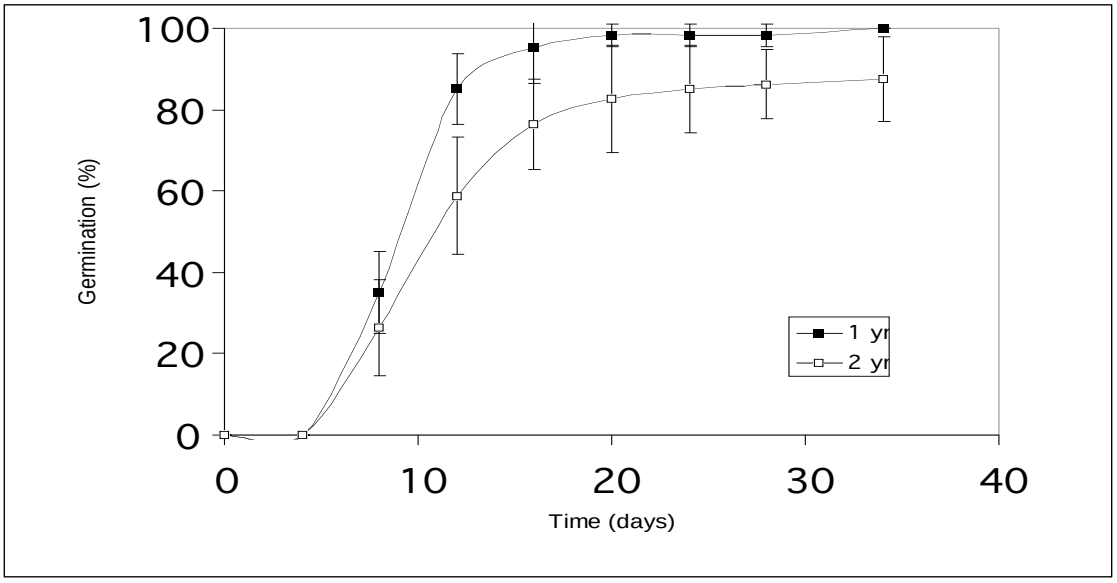


Fig. 5. Effects of long-term storage in moist chilled condition (3°C, buried in moist sand).

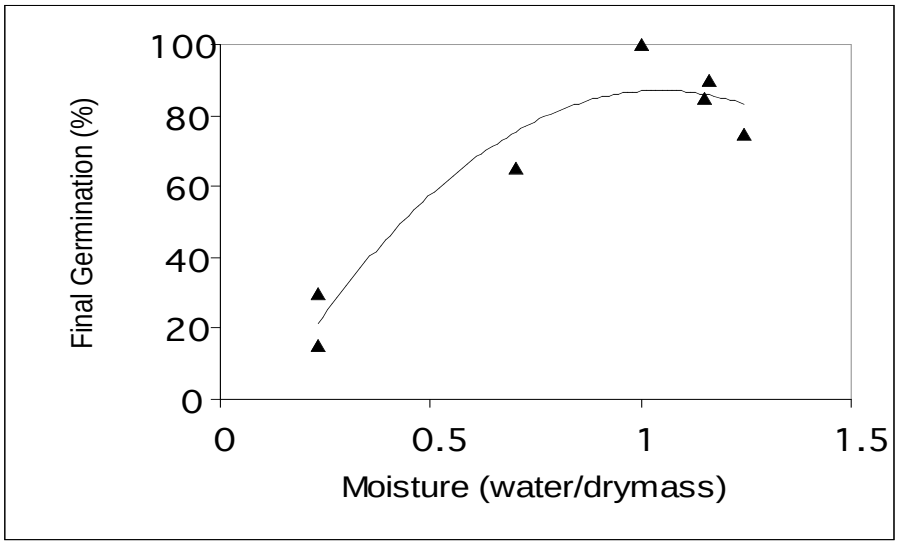


Fig. 6. Effects of seed moisture on final germination (%) for chilled storage for 2 yrs in sand of various moisture contents.

In conclusion, seeds of *A. crenata* seeds are recalcitrant. They cannot tolerate desiccation. For a seed to remain viable, it has to be in moist conditions. High moisture contents of pulp are critical in maintaining the viability of seeds inside intact fruits on plants. Although pulp contains higher moisture contents than seeds, seeds do not germinate in intact fruits on plants. The water uptake balance of seeds may be regulated by higher osmotic potential that maintains the water potential of seeds and pulp to be at an equilibrium. However, when seeds are exposed to soil medium with sufficient moisture contents, they germinate if the temperature is favorable (between 10 and 30°C). Beyond inhibition of water uptake of seeds by pulp, there is no dormancy mechanism to keep seeds from germination. If soil is too dry for seeds to maintain critical moisture content (above water / drymass = 0.5), then seed viability declines. Thus, the species is unlikely to colonize dry sites (i.e., scrub, sand-hills).

3. Influence of fruit pulp on the rates of seed moisture loss and germination

Since moisture content has such an important influence on the viability of *A. crenata* seeds and the fruit pulp has such a high moisture content, it is possible that the maintenance of an intact fruit is critical for seed survival. Under drought conditions in the field, it was observed that the pulp of fruit retained on *A. creanta* plants could wither to contain less than 50% of the moisture of un-withered fruit, while the seed moisture content was reduce by almost 20% in the withered fruit.[2]

A study was established in which seeds were stored in air with or without their fruit pulp, and in moist sand at room temperature without the pulp.[3] Data have not yet been collected from

the final sampling times, but initial results confirm the findings of the previous study showing that there was a significant decline in seed moisture content in air-dried seeds as compared to those stored in moist sand (Fig. 7). The rate of moisture loss for seeds that retained their pulp and fruit skin was shown by analysis of variance to be significantly slower than for exposed seeds but was still significantly faster than seeds maintained in the moist sand.

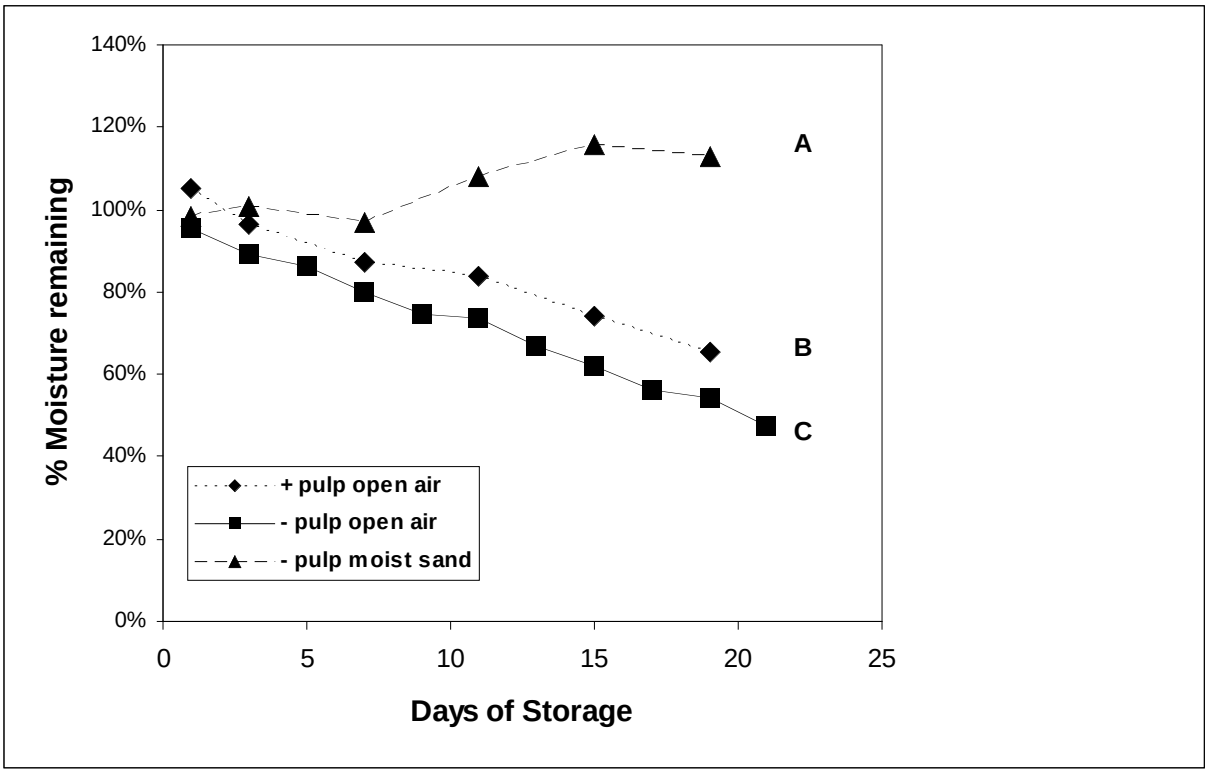


Fig. 7. Percentage of moisture remaining in seeds (with or without pulp) as compared to freshly collected fruit after varying lengths of time stored in air or moist sand. Treatments with different letters are significantly different. Data not available for sampling times 23 – 29 days.

The proportion of seeds that germinated after storage in sand remained very high including, for the later samples, a majority that germinated in the sand (Fig. 8). The proportion of the air-dried seeds that germinated remained high up to 11 days of storage but declined sharply after that time. There was much variation in the proportions of seeds germinating in these two treatments between days 11 and 21 so it will be important to see whether the reduction in germination declines to consistently low values at the later dates (not shown). An analysis of variance of these data so far, showed that the differences in germination were significant between the treatments stored in air and moist sand, but not between the two air-dried treatments.

Although seed viability was not measured directly (e.g., with a vital stain), seeds that were left for several weeks under ideal germination conditions in the growth chamber eventually began to decay, indicating a loss of viability rather than an induced dormancy.

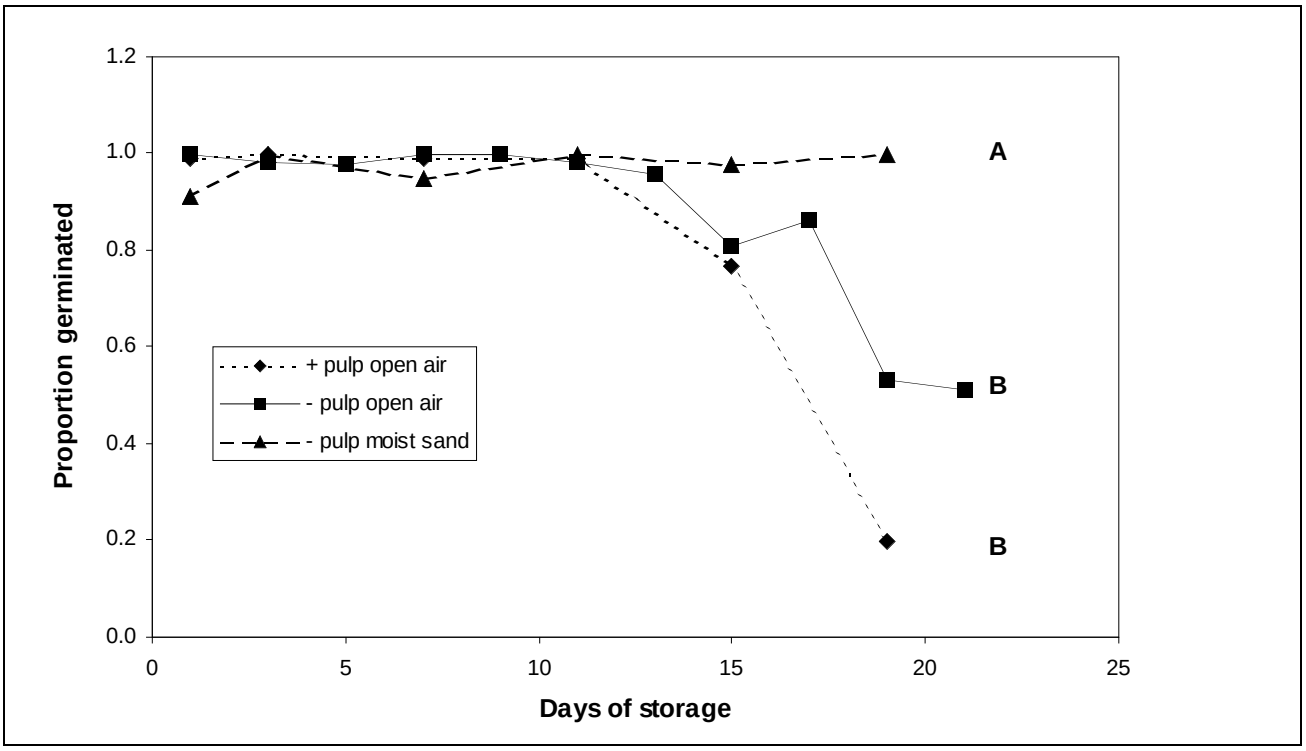


Fig. 8. Proportion of seeds (with or without pulp) germinating after varying lengths of time stored in air or moist sand. Treatments with different letters are significantly different. Data not available for sampling times 23 – 29 days.

The relationship between seed moisture content and viability will be examined as the final results (up to day 29 of storage) are compiled but there is every indication that, as seen in the previous study, there will be a threshold of seed moisture content (40-60%) below which seed viability is suddenly lost. Once the collection of data is complete, they will be analyzed with the percentage of moisture remaining correlated with proportion of seeds germinating to establish this relationship. The results from other air-dried treatments where intermediate amounts of pulp were removed (skin of the fruit was damaged by a single incision or half the pulp was removed) will also be analyzed.

While it appears that the rate of seed moisture loss is slowed by the presence of the fruit skin and pulp, it does not appear that this protects the seeds from mortality for a significantly longer period of exposure under these types of conditions.

## 4. Influence of temperature on seed germination

When germination rates were compared at different constant temperatures, over 90% of seeds had germinated after two weeks at 26$^{o}$C. A similar percentage of germination was only

achieved at 16 °C after seven weeks but at 33 °C, only 40% of the seeds had germinated by seven weeks.[2] These data indicate that germination studies are best conducted at about 26 °C, and they reflect the warm temperate distribution of *A. crenata* in its native range in Japan and China.

5. The structure of *A. crenata* populations based on stem height

A study to quantify the density of different sized *A. crenata* plants was initiated in June 2000 and was repeated at 6-month intervals.[1, 2] Within the first year, data were collected at two sites, but due to logging at the Law School site, subsequent data have only been collected at the Paynes Prairie site. In Fall 2000, one large and two small trees fell in the plots in where these studies were conducted and consequently a number of stems were lost or killed. The data have been analyzed regardless of this, since these were natural events, but the misfortune that two out of five plots were affected is likely to have negatively influenced the overall rate of survival and resulted in high variability in the data.

Results for three surveys at the Paynes Prairie site are presented in Fig. 9. These show a large decrease in the density of stems between the 5-10cm and 10-20cm size classes and a slight seasonal variation in the density of the 0-5cm size class. These data may indicate periods of high mortality for the smaller plants (e.g., in the summer), a periodic flush of germination and growth into the shortest size classes (e.g., in fall/winter), and/or selective growth, whereby only a portion of the shorter plants continue to grow into the taller size classes, leaving some seedling in a relatively dormant state. It will only be possible to evaluate which of these scenarios is most likely, once all the data on stem densities, seedling recruitment, survivorship, and growth rates have been fully analyzed.

The densities of stems in the size classes taller than 60cm appear to be decreasing over time. This indicates a rate of mortality that is not being compensated for by recruitment from shorter size classes. This could indicate the selective loss of taller stems over the year, possibly resulting from drought conditions, the hard freezes in January, or particular susceptibility to the tree falls in some plots. These data could also reflect the demise of some unusually numerous year-classes that had resulted from exceptionally high seedling recruitment and/or survival in previous years. More detailed analysis of these data in relation to the cohort study may help to narrow down the possible causes.

Many aspects of the population and growth dynamics of *A. crenata* are complicated by the fact that this plant is capable of producing multiple stems from a root crown. Most of these data are reported on a per stem basis, but this may overestimate the numbers of genetically distinct plants that have survived from seedlings. However, in terms of ecological impact and management, stem densities may be more important than distinguishing between individual plants.

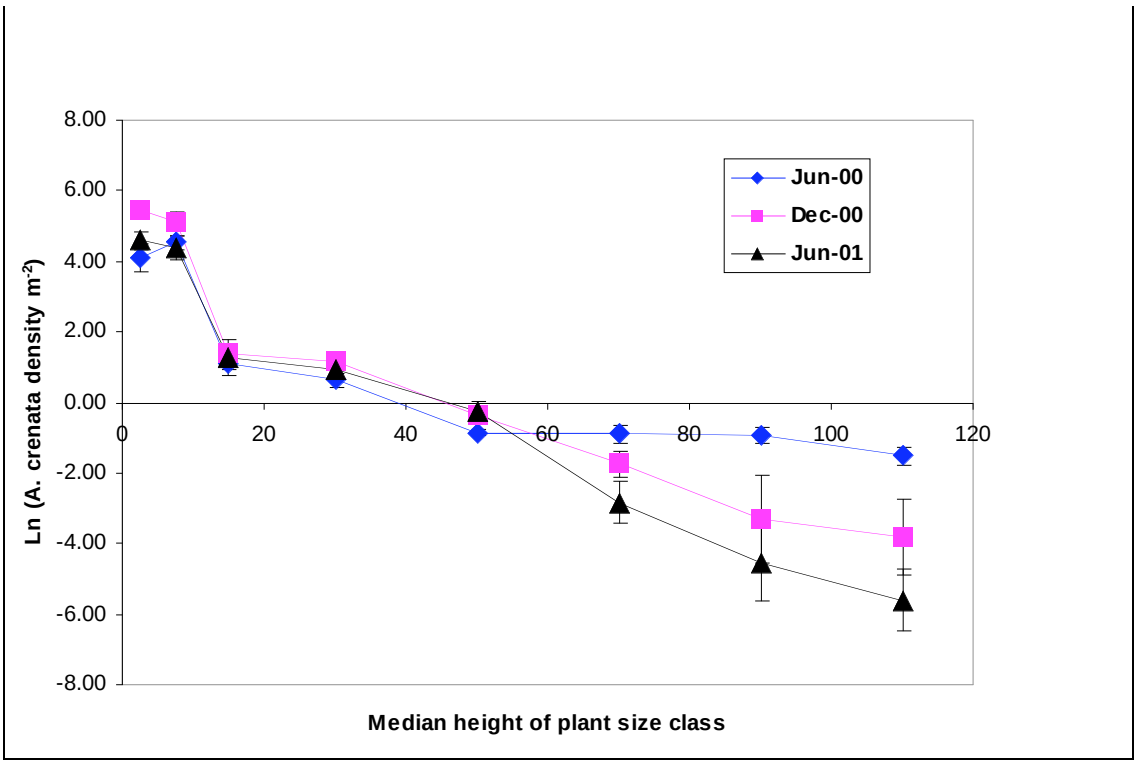


Fig 9. Densities of *A. crenata* stems per size class at the Paynes Prairie site. Error bars = SE. (Note Ln scale on Y axis).

6. Survivorship of plants with different stem heights

Concurrent with the density study, several *A. crenata* stems within each size class were tagged, and have been measured at 6-month intervals for parameters such as plant height, basal diameter, number of branches, fruit production, etc.[1, 2] After the logging at the Law School sites, data collection was only continued at the Paynes Prairie site. These plants would also have been affected by the tree falls in some of the plots in Fall 2000.

Compared to the number of stems initially tagged in June 2000, the percentage surviving in February and June 2001 are shown, per size class in Fig. 10. There was a larger decrease in survivorship of the shortest stems between February and June 2001 than for the other short size classes, possibly as a result of springtime drought, increased competition, etc. Stem mortality was high is both of the tallest size classes, particularly between June 2000 and February 2001. This may be in part due to their susceptibility to damage from the tree falls and freezes, but also these taller stems are likely to be reaching the end of their lifespan. Survivorship was similar between the mid-height size classes.

Further data are being collected in Dec 2001, so there should be enough information to compare the net and gross survivorship results from the respective density and cohort studies, and develop statistical life-history tables. Other parameters measured on the tagged stems (e.g.,

basal diameter, fruit and leaf counts, etc.) will be analyzed in addition to survivorship and growth based on stem height.

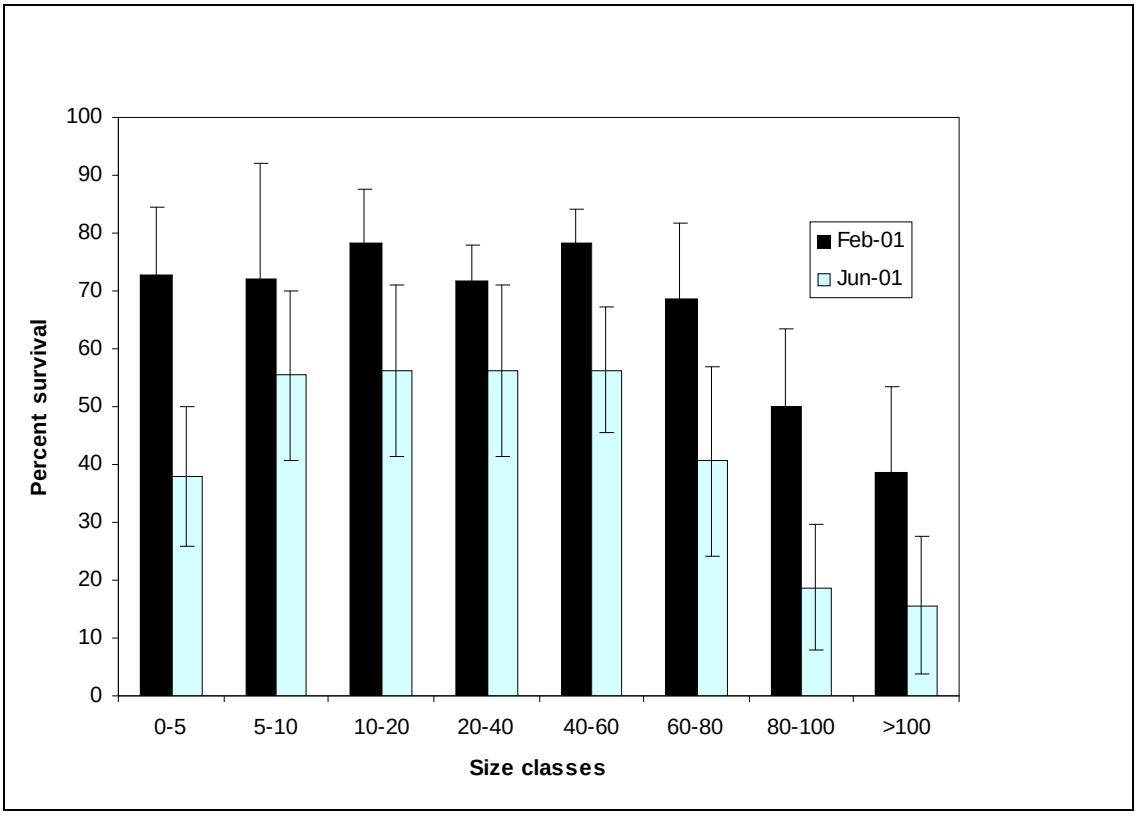


Fig. 10. The percentage survival of stems tagged in June 2000 by February and June 2001 at Paynes Prairie. Error bars = SE.

## 7. Growth rates of plants with different stem heights

Based on stem height data collected in the cohort study, a preliminary analysis is presented of the rates of stem growth as measured under field conditions. These are based only on stems surviving for at least one year and while this included between 13 and 33 stems in most size classes, in the 80-100cm and >100cm classes there were only 6 and 4 stems, respectively. These analyses have not been conducted by block and most calculations have been conducted on average rather than individual stem data, so between-stem variation is not presented. Another set of data will be collected in December 2001, and the full results will be subject to more rigorous analysis within the next 6 months, but some indication of the likely trends may be provided by these preliminary analyses.

The average initial height for all stems that survived for a year was estimated for each size class. The growth (increase in height) for stems surviving after a year was averaged and found to peak in the 40-60cm size class (Fig. 11). Growth in the tallest size class was not much greater than in the shortest size classes.

When estimated as a relative growth rate (RGR) based on initial stem height, the shortest plants had a much higher rate of growth than the other size classes (Fig. 11). Stems between 5 and 60cm in height showed a fairly constant RGR but in stems taller than 60cm it declined.

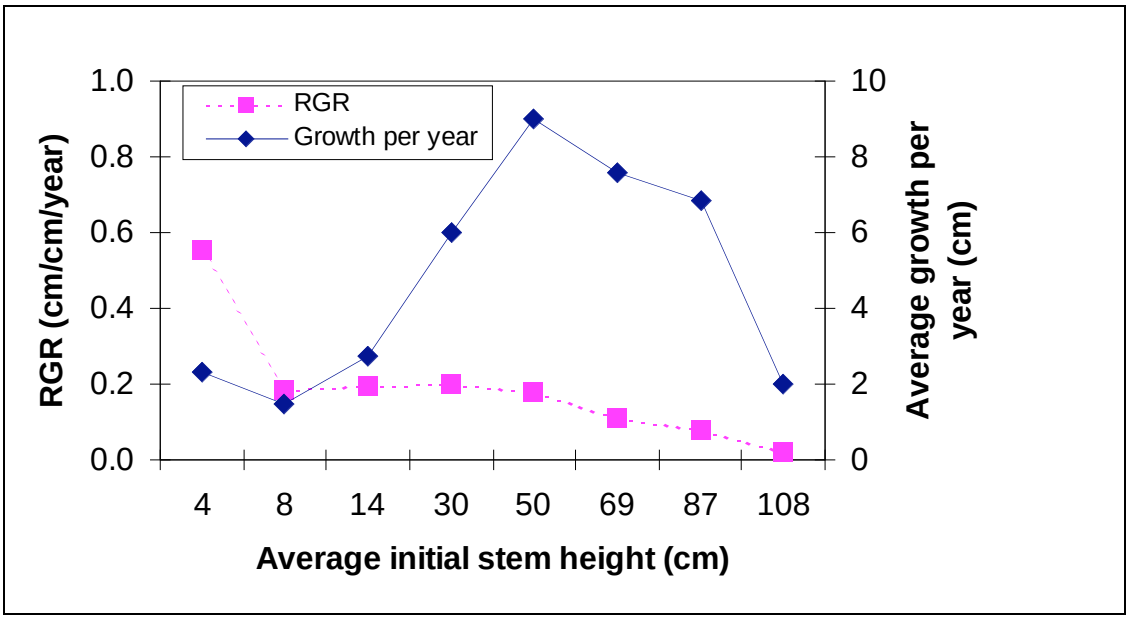


Fig. 11. Average annual growth and relative growth rates as related to average initial stem height within each size class. Data from the Paynes Prairie site. Number of stems (n) for each size class starting with the 0-5cm class are: 23, 33,18,18,18,13, 6 and 4.

## 8. Age estimates based on stem height

Over the two-year schedule of this project, it was not possible to track the growth of seedlings into mature plants. By following the growth of stems starting in different size classes and calculating their RGR, it is possible to estimate the growth of a hypothetical stem over several years. Based on the assumption that a 2.5 cm seedling was a year old (and this is reasonable based on the 0.55 cm/cm/yr RGR for the 0-5cm size class), the growth of a hypothetical stem is presented in Fig. 12.

Perhaps the most surprising aspect of these results is that the stems in the tallest size classes would be over 20 years old, and a stem at the size of first fruit production (20cm tall) may be 10 years old. The slow growth pattern for this hypothetical stem illustrates how low the RGR are for these plants growing in the field. Plants grown in greenhouses had reached 20cm in height and produced fruit within 2 to 3 years of germination. The slow growth of the tagged plants in these studies probably resulted from field factors such as, competition, shading by overstory plants, and drought conditions.

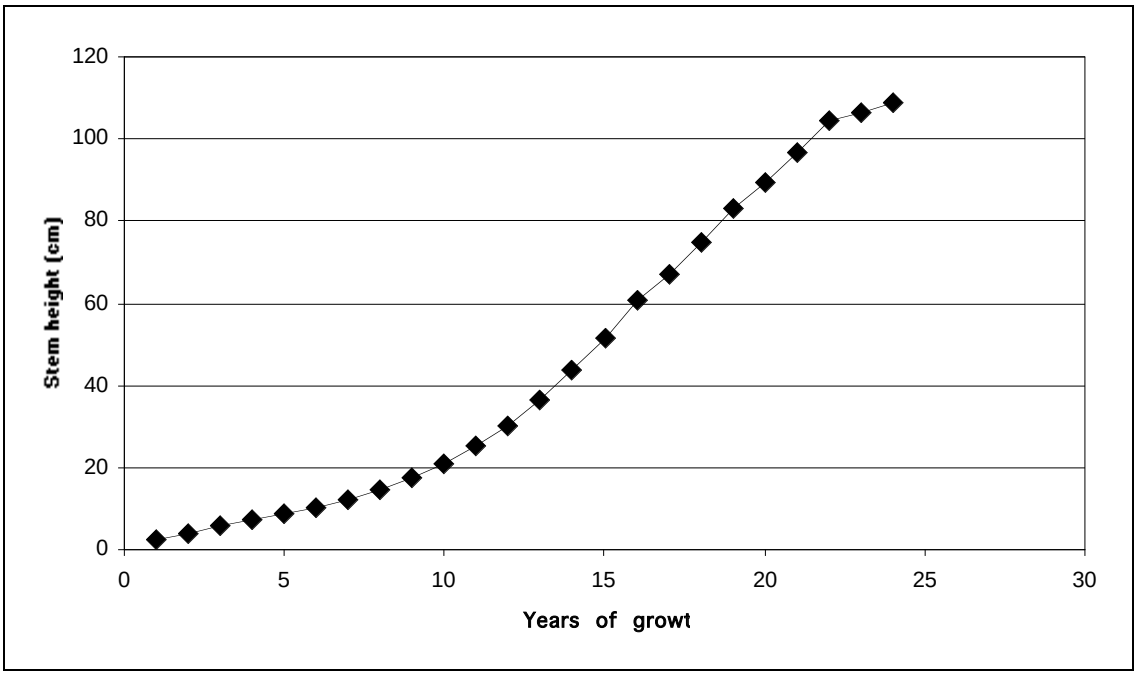


Fig. 12. The hypothetical growth curve for an *A. crenata* stem, based on the RGRs estimated for stems of various heights growing under field conditions at Paynes Prairie.

These data also do not distinguish between plants that have single stems and those that may be derived from large root crowns that are substantially older than any of their individual stems. Of all the stems tagged initially, it was found that on average, 52% of them were attached to root crowns and other stems. Thus, the stem growth rate may have reflected an average for both single and multiple stemmed plants. In considering the growth of whole, individual plants, particularly in relation to resource supply and storage, it would be necessary to track and sum the growth of multiple stems.

## 9. Relationship between plant height and biomass

Allometric relationships were established between the height of *A. crenata* plants and total or root dry biomass from material sampled in the mesic hardwood forest of the University of Florida Natural Areas Teaching Laboratory (NATL).[1] Plant height predicted total and root biomass very well ($r^2$ values of 0.95 and 0.91, respectively) as well as total leaf number ($r^2$ = 0.86). The proportion of roots in the total biomass was found to remain constant over the range of plant heights tested (5 – 90 cm; $r^2$ = 0.99) with a mean root to shoot ratio of 1.59 across all plant sizes.[3]

Thus, stem growth based on increases in stem height (Fig. 11) could be converted using the regression equation relating total biomass to height (Ln[total dry mass] = -4.375 + 2.139 Ln [height]) to growth based on biomass (Fig. 13). Given the use of a linear equation to make the

conversion from height extension to biomass increase, it is not surprising that these curves are similar (Fig. 11 and 13) but the size class in which biomass growth peaked was the 80-100cm stems.

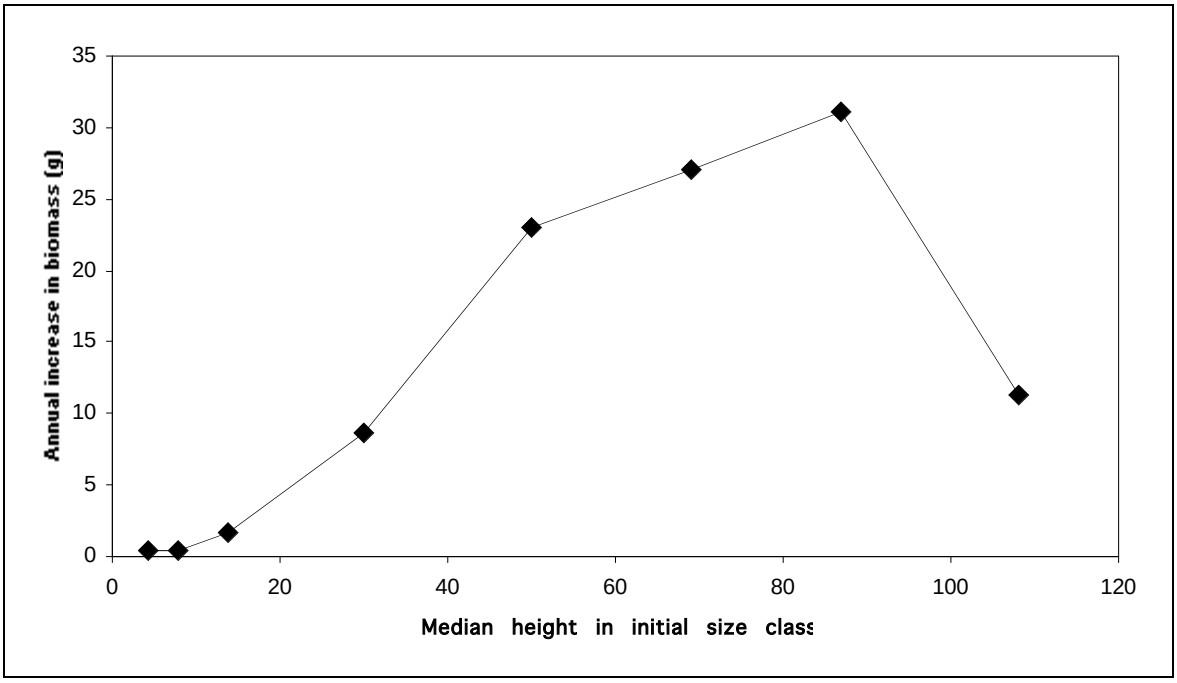


Fig. 13. Estimated annual increase in biomass for various sized *A. crenata* stems tracked at Paynes Prairie. Values calculated from changes in stem height and the linear relationship between plant height and biomass.

10. Growth and survival of *A. crenata* seedlings in a common garden study with varying light availability

Two experiments were conducted in an outdoor plant growing area with an irrigation system. Five light treatments (3, 6, 15, 40 and 100 % of full sun) were established in random order within each of the three blocks.[3] The first experiment was conducted with field-collected seedlings grown from October through March, in which we observed negative effects of freezing in plants in 100% open condition.[3]

The second experiment was started on 17 April, using 7-month old seedlings raised from seeds in a growth chamber in moderate light (200 μmol $m^{-2}$ $s^{-1}$ for 12 hours per day). Seedlings were harvested approximately 1 and 3 months later.

Leaf area of plants in the 100% open treatment was significantly lower than other treatments, as a result of initial leaf loss due to photo-injury during the first 1 month (Fig. 14). Although no plants died and they did produce new leaves, leaves were dwarfed and severely

bleached. However, there was no significant effect of light on leaf area in treatments ranging from 3 to 45 % full sun.

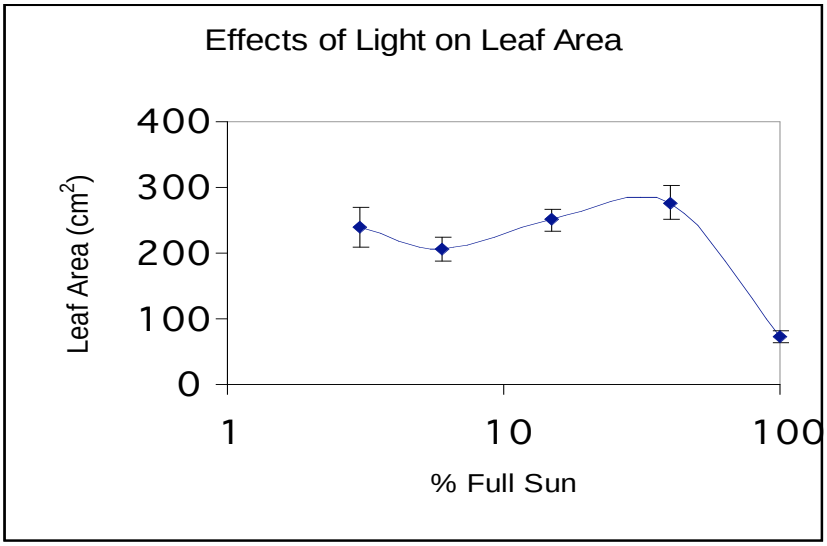


Fig. 14. Effect of light treatments (3, 6, 18, 45, 100%) on the total leaf area of seedlings 3 months after the initiation of the experiment. Mean ± s.e. Horizontal axis is in log scale.

In contrast to total leaf area, total seedling mass increased from 3% to 45% full sun, then declined sharply at 100% full sun (Fig. 15). Plants raised under 18 and 45% full sun are more robust and had greater root mass (Fig. 16).

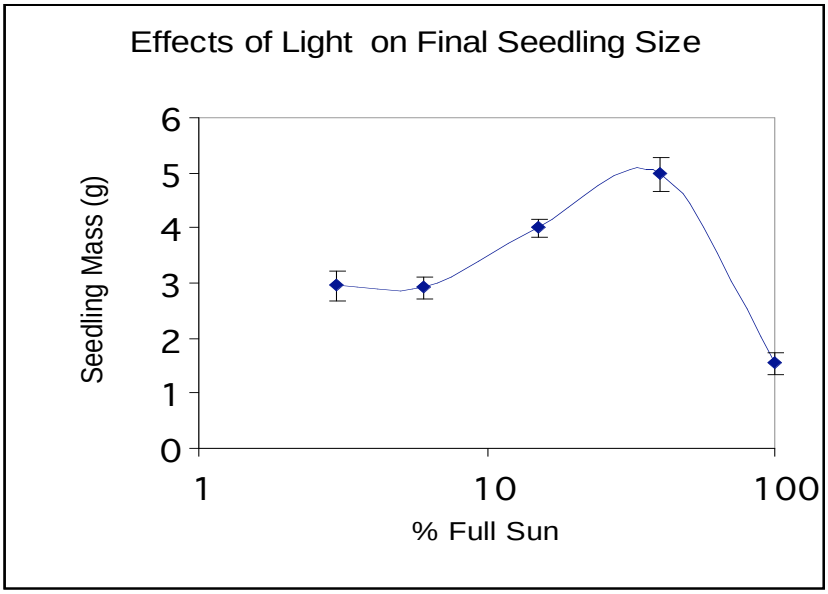


Fig. 15. Effect of light treatments (3, 6, 18, 45, 100%) on the total dry mass of seedlings 3 months after the initiation of the experiment. Mean ± s.e. Horizontal axis is in log scale.

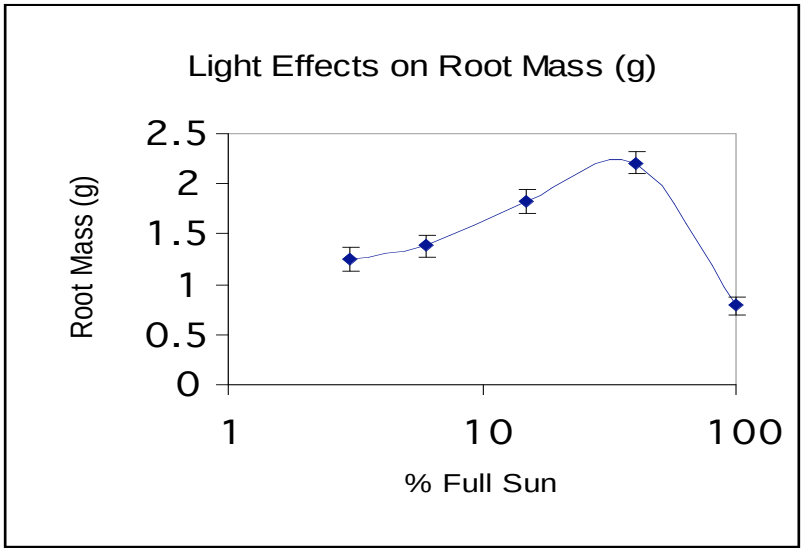


Fig. 16. Effect of light treatments (3, 6, 18, 45, 100%) on the root dry mass of seedlings 3 months after the initiation of the experiment. Mean ± s.e. Horizontal axis is in log scale.

The relative growth rates (RGR) trends in response to light treatment (Fig. 17) paralleled the final total biomass (Fig. 15). This is because biomass at 1 month was not significantly different among 3 - 45% full sun treatments. The negative RGR of plants in 100% full sun indicate that plants continuously suffered from photo-injury after the first month following transfer from moderate to high light.

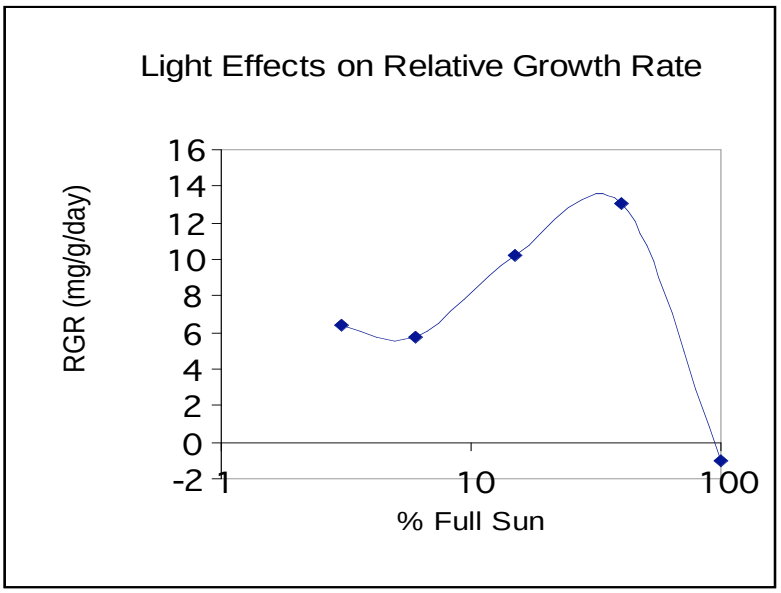


Fig. 17. Effect of light treatments (3, 6, 18, 45, 100%) on relative growth rate of seedling biomass between 1 and 3 months after initiation of the Experiment 2. Horizontal axis is in log scale.

In summary of the two experiments, one during fall-winter and another during spring-summer months, *A. crenata* is not tolerant of completely open condition because of photoinjury. However, in light condition similar to that found in forest edges and inside large treefall gaps (e.g., 30-40%), their biomass growth is enhanced compared to the deep shade. This result is consistent with observations by Japanese scientists that in its native habitat the frequency of this species increases in response to treefall gap disturbance. It appears that this species exhibits a conservative strategy of allocating extra carbon income under partly open canopy condition to carbohydrate storage in roots (Fig. 16), rather than enhancement of leaf area growth. Increased carbon storage, however, will contribute to the next year's growth and accelerate the time required for the plant to reach reproductive size.

11. Relationship between relative growth rates in common garden and field conditions

Based on the cohort data collected from the *A. crenata* stems tagged at Paynes Prairie, and the allometric relationship between plant height and biomass[3], relative growth rates based on stem height (cm/cm/year – Fig. 11) for the different size classes could be equated to relative biomass increases (mg/g/day - Fig. 18). For any size of stem other than the 0-5cm tall ones, these RGRs based on biomass were considerably lower than the RGR observed for plants grown in the common garden experiment (range where not photo-injured 6 – 13 mg/g/day in Fig. 17). These lower growth rates undoubtedly reflect the less optimal conditions for growth in the field, where competition, drought (the common garden plants were regularly irrigated), and perhaps limited nutrients would have prevented the *A. crenata* stems from growing at the full potential indicated in the common garden experiment. The RGR of 4.28 mg/g/day of the youngest seedlings measured in the field approached the RGR values obtained in the common garden experiment at low light levels. It would be useful to measure the light intensity at seedling level in the field sites to see how it compares with the lower intensities used in the common garden experiment (3, 6 and 18% of full sunlight).

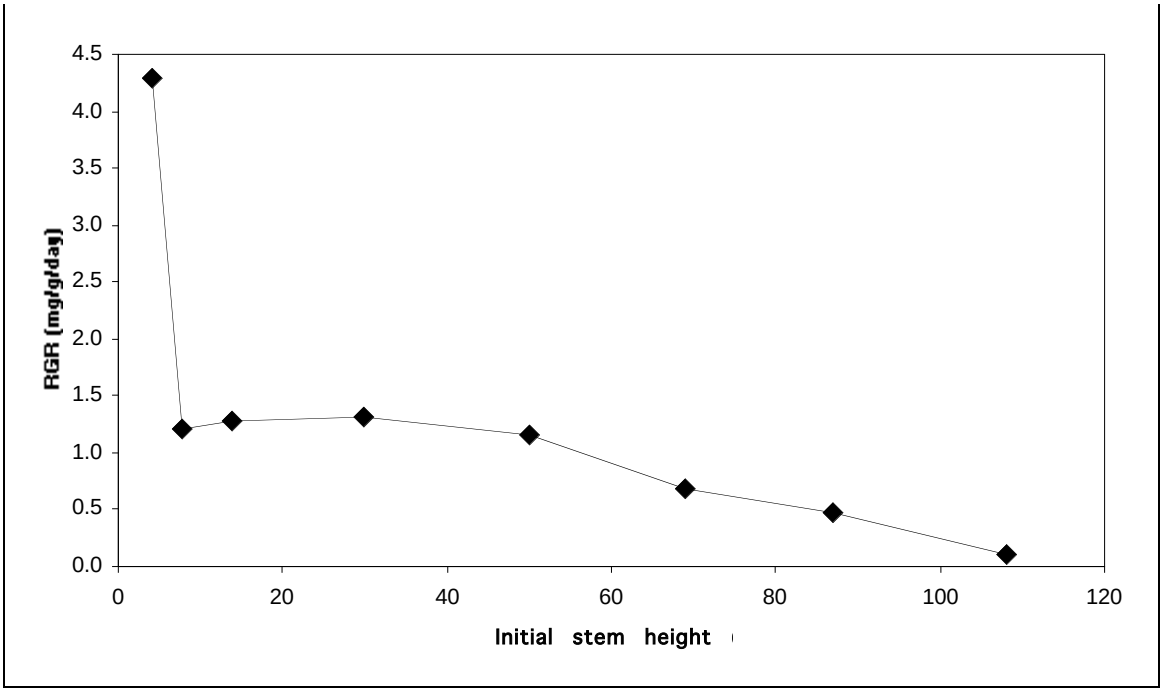


Fig. 18. Relative growth rates (biomass) for various sized *A. crenata* stems tracked at Paynes Prairie. Values calculated from changes in stem height and the linear relationship between plant height and biomass.

## II. Ecological influence of *Ardisia crenata* in native plant communities

The relationships between the coverage and density of *A. crenata* and the frequency and coverage of native plant species were investigated at five sites in the Gainesville area. The first survey was conducted in Spring 2001 and there was a significant negative correlation between the coverage of *A. crenata* and the number of native understory species ($r^2 = 0.19$, n = 157).[3]

These surveys were repeated in the Summer 2001 so that late emerging native species were not missed from the description of the native plant community. The summer survey included five more species than observed in the spring. (Site and species list in Appendix.) The negative correlation between *A. crenata* cover and number of native species in the understory was also significant in the late summer (Fig. 19, n=152). Negative correlations between the density of *A. crenata* plants (over 20 cm in height) and number of native species in the understory were also significant in spring and late summer ($r^2 = 0.185$ and 0.153, respectively). To check the validity of quantifying the presence of *A. crenata* by measuring the density of only plants over 20 cm in height, this parameter was regressed against *A. crenata* coverage. These resulted in the expected positive correlations with high coefficient values (Fig. 20; spring $r^2=0.824$, summer $r^2=0.708$)

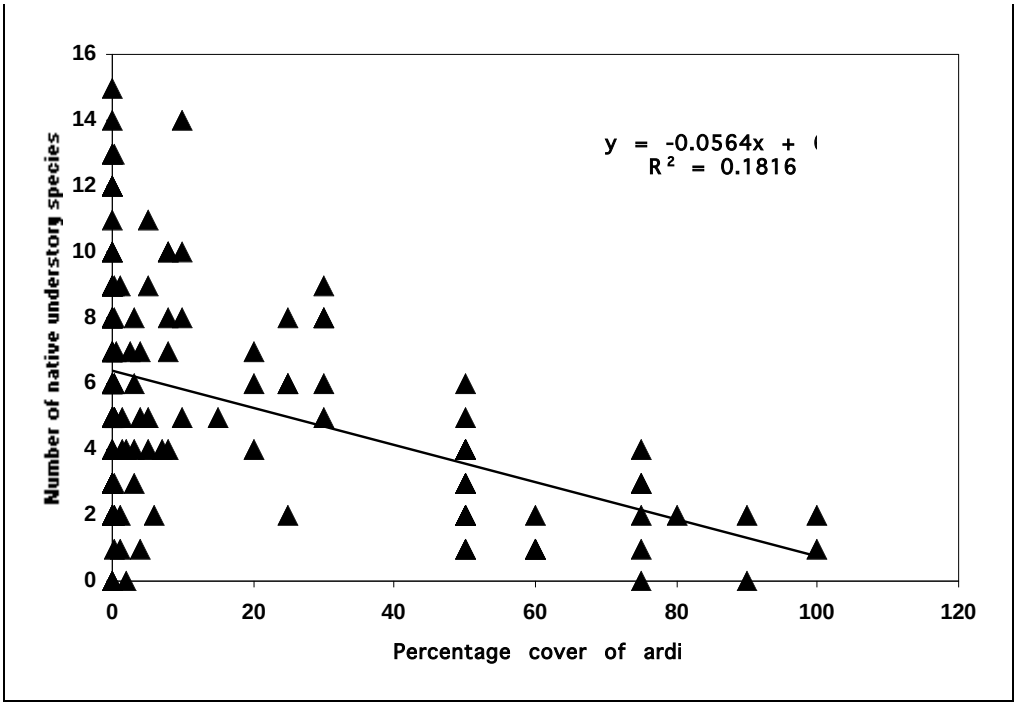


Fig. 19. Regression of the percentage cover of *A. crenata* with the number of native understory species in late summer 2001.

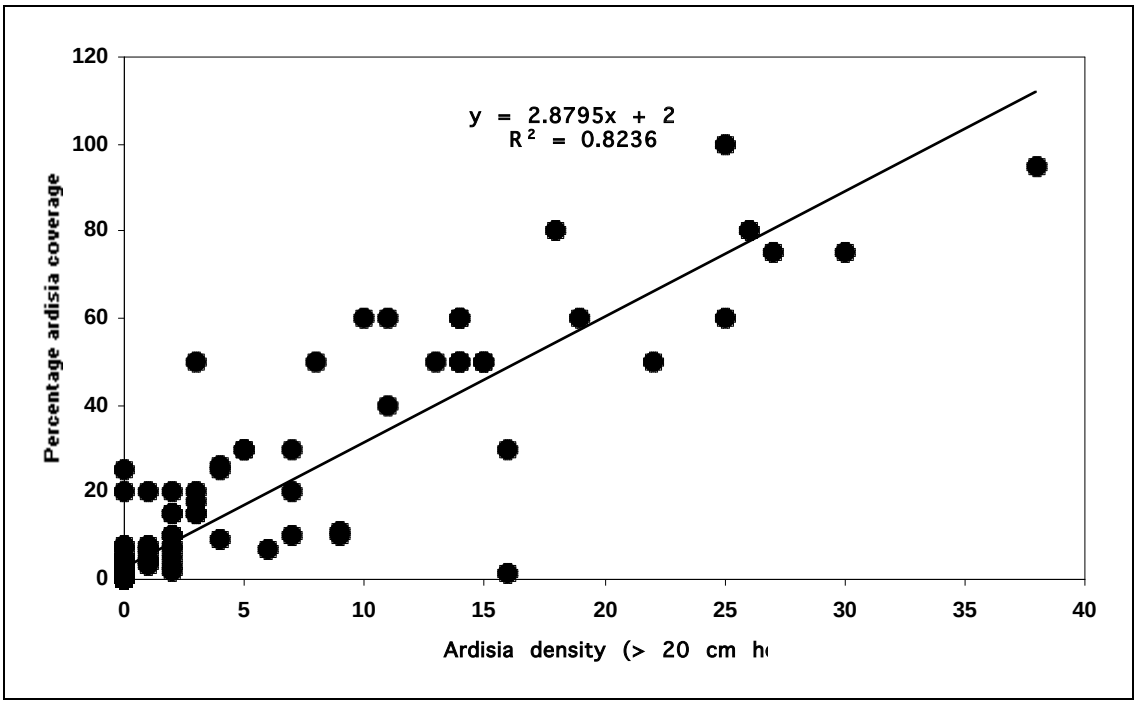


Fig. 20. Regression of the density and percentage cover of *A. crenata* in spring 2001.

When the cover of *A. crenata* was regressed against the total coverage of native understory species, there were significant negative correlations at both sampling times, but the correlation coefficient was much lower in the late summer (spring $r^2$=0.156, summer $r^2$=0.076 – Fig. 21) largely due to some high percentages of species coverage in samples that also had significant amounts of *A. crenata* (total cover can exceed 100% due to overlapping layers of vegetation) . When examined further it was found that at the Paynes Prairie site the cover of understory plants was higher than the other sites, especially in the summer, because of a dominating stand of *Petiveria alliacea*. Average plot cover with *A. crenata* at this site was 12.3% in the spring and 21.7% in the late summer. In the same plots, *P. alliacea* coverage was 17.7% in the spring and an impressive 53.3% in the late summer.

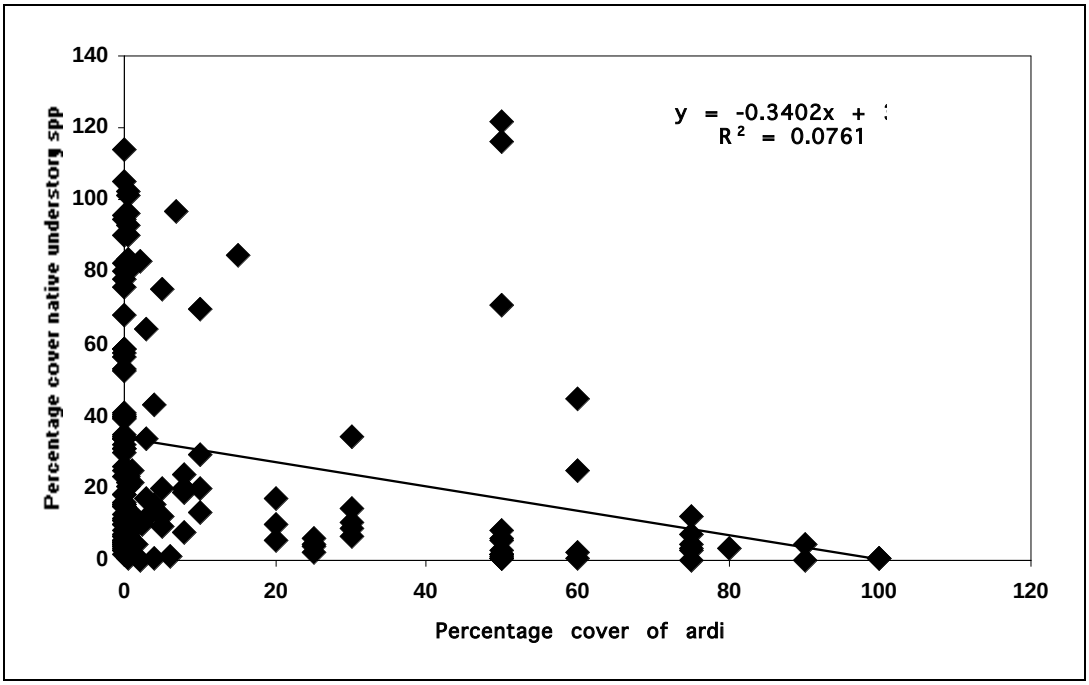


Fig. 21. Regression of percentage cover of of *A. crenata* with the total percentage cover of native understory species in late summer 2001.

Within the Paynes Prairie plots, there was a significant negative correlation between the coverage of the two species (Fig. 22) but when included in the whole dataset, *P. alliacea* may be acting as a covariate to the *A. crenata.* All regressions of *A. crenata* and native plant parameters were repeated without the Paynes Prairie data to ensure that these relationships were not being unduly influenced by the effects of *P. alliacea* acting as a covariate. Although most correlation coefficients were reduced in the smaller dataset, all regressions remained significant.

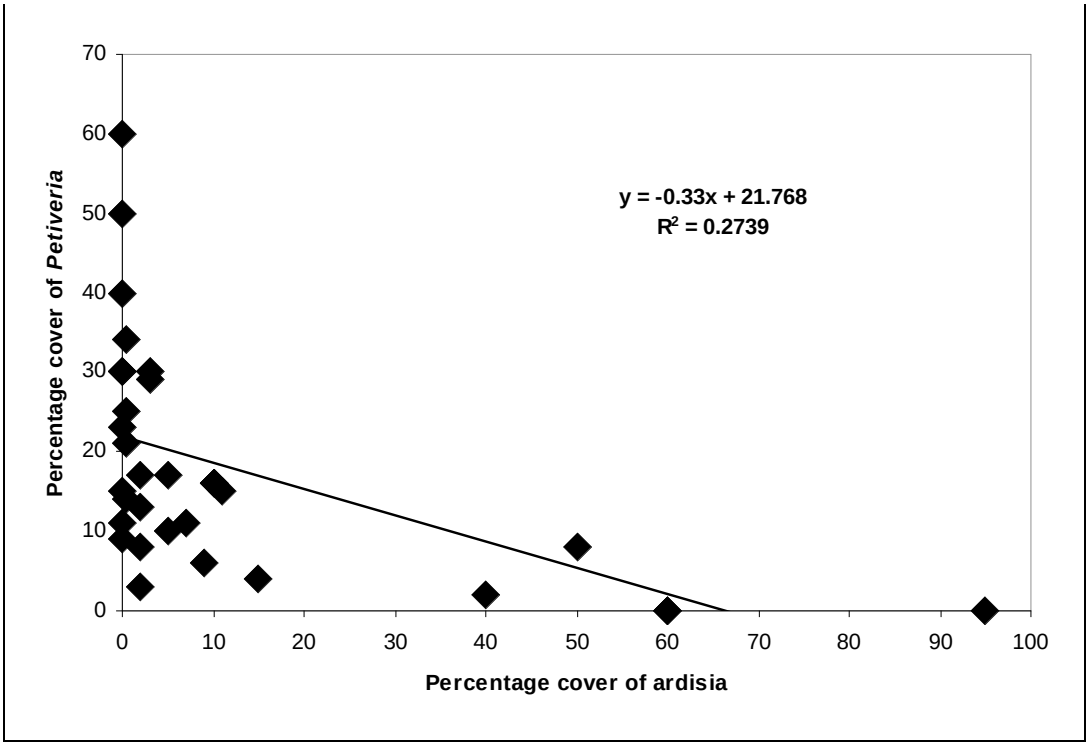


Fig. 22. Regression of the percentage cover of *A. crenata* and *P. alliacea* at the Paynes Prairie sites in spring 2001.

All analyses thus far have used linear regressions. These data will be further analyzed to investigate non-linear relationships and with the subdivision of sampling units according to factors such as light availability and soil moisture. The influence of *P. alliacea* and any other species that could also dominate the community, especially when *A. crenata* is not dominant, will be also be investigated.

Although there was great variation in these data, the consistent detection of a negative relationship between the amount of *A. crenata* and the diversity of the native understory vegetation, is important in quantifying *A. crenata*'s detrimental influence in natural areas of north Florida.

## III. Factors influencing the effective management of *Ardisia crenata*

### 1. Seed longevity

As described in earlier in this report (Sections I. 2 and I. 3), seeds of *A. crenata* will tend to germinate immediately if kept moist and warm, or desiccate and lose viability if kept in dry conditions. Seeds have been maintained with approximately 80% viability for two years in moist and cold ($3^{o}$C) conditions (Fig. 6), but with immediate germination once moved to warm conditions, this is conditional, rather than physiological or physical, dormancy. This indicates

that extended longevity of these seeds is possible, but is this likely to occur under the typical field conditions found in north Florida?

A series of studies was conducted to investigate the longevity of *A. crenata* seeds buried in field conditions at a site where *A. crenata* has invaded.[1, 2] Seeds were buried in bags in three soil types, dry, medium, and wet, that had respectively, 10.3%, 29.6% and 482.2% moisture compared to the soil dry weight. In all three studies, there was no long-term survival of seeds in the soil because they either died (0 - 20%) or had germinated within two months of burial.[1, 2] In the third study, the proportion of buried seeds that had germinated within one month (averaged over all soil types) was 55%, significantly lower than the 90% that germinated in the soil after two months. Most of the un-germinated seeds that were recovered after one month of burial, germinated after three weeks in the growth chambers, but none of the un-germinated seeds recovered from the soil after two months was viable.

In the third study, there was some variation in seed viability depending upon the conditions of burial. By two months after burial there was a significantly lower number of seeds surviving in the driest soil (percentage germination 80.8%, 97.3% and 91.3% in the dry, medium, and wet respectively). However, it cannot be assumed that soil moisture was the only parameter varying between burial conditions; for example, pH and soil composition would have been covariates.

Prior to the third burial study, a laboratory experiment was conducted to evaluate whether light was necessary for the germination of *A. crenata*. In germination studies, green light is considered a "safe" light to use to avoid affecting the seed because the phytochrome response of seeds to light is based on the relative exposure to red and far red light. In a dark room, a cardboard box with its bottom end removed was lined inside and out with green acetate and placed over the ceiling light. Shade cloth was also suspended below the light to reduce the light intensity as low as possible. To evaluate what wavelengths of light were passing through the acetate, a small piece was placed in a spectrophotometer and the wavelengths of light that passed through were measured. The transmittance of the wavelengths of red (660 nm) and far red (730 nm) were low as compared to the majority of light that passed through the acetate, and it was assumed that these values were too small to affect germination

After seeds were removed from the pulp of freshly collected fruit in this environment, 6 replicates with 20 seeds each were established in petri dishes that were placed in a growth chamber, either fully exposed to light, or tightly bound in foil to keep out any light. All replicates showed 100% germination except one of the dark replicates that had 95% germination. Thus, there was no significant difference in germination between seeds exposed to light and those that were not.

By establishing that light is not necessary for the induction of germination in *A. crenata* seeds, it is shown to be unlikely that the action of collecting and preparing seeds for the burial studies would have induced germination that might not have occurred in fruit that was buried naturally. In summary, the accumulated data relating to lack of seed dormancy or requirement

for light for germination, indicate that it is very unlikely that *A. crenata* will produce a long-term soil seed bank under the field conditions found in north Florida.

2. Fruit production and loss of *A. crenata* under field conditions

Flowers are produced on *A. crenata* plants in the field in May – July and green fruit are observed on over 90% of plants by early September.[2] These fruit do not typically ripen to their mature red coloration until late December. Red fruit have been observed to remain on the plants for several months after the new crop of green fruit develop, so that there may only be a one or two month period in November to December when there are no mature fruit on the plant.

In a study conducted to determine the rate and seasonal timing of fruit loss from the plant, the number of fruit was tracked on three branches of each of 100 plants randomly tagged along permanent transects at the Newnan's lake site. The number of fruit remaining on the plants as a percentage of the initial count (average 12 fruit per branch on January 15th, 2001) decreased at rate showing a sigmoidal curve (Fig. 23) with greatest losses in spring (March to June). A few red fruit remained on 4% of the plants until November.

Similar rates of decline (data not shown) were noted in a second study where the total number of fruit per plant were tracked over time for 20 plants in each of two sites. These plants were isolated by removing all other *A. crenata* plants within a 1m radius. This second study did not commence until February 2001, when there were 102 and 72 red fruit per plant at the Payne's Prairie and Newnan's Lake sites respectively. By November, the new crop of green fruit averaged 100 and 153 per plant at these respective sites.

In both studies, there may have been some loss of red fruit prior to the initial counts particularly because there were hard freezes on January 1st and 10th, 2001. Once the final counts that include red fruit are finished and incorporated into the data-set, the rate of fruit loss from the isolated plants will be compared with that from the permanent transects. This may indicate if the exposure of fruit on the isolated plants made them more susceptible to predation or losses due to a less stable micro-climate than those plants in the crowded and relatively undisturbed transects.

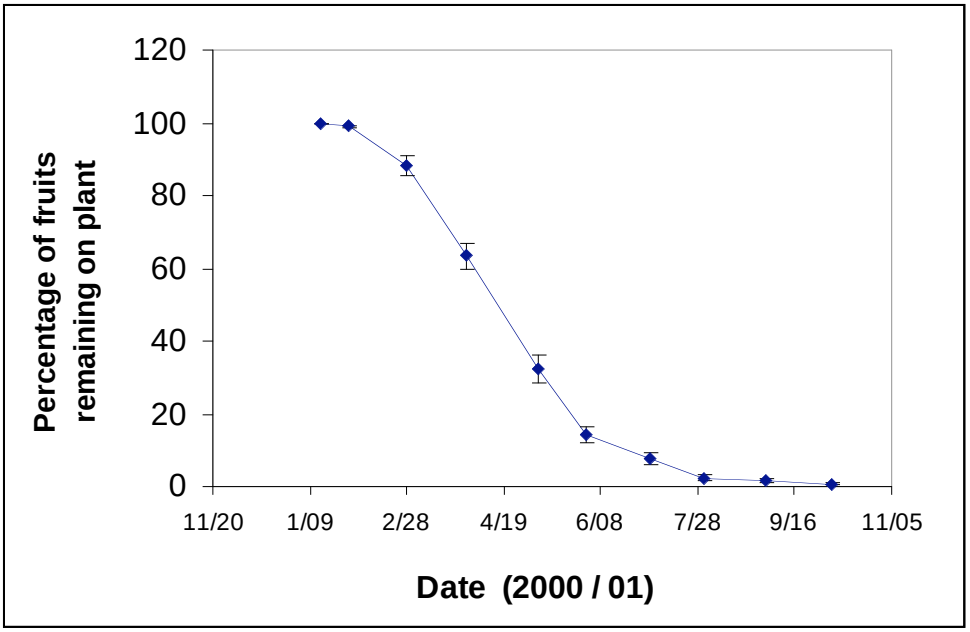


Fig. 23. Percentage of fruit remaining on three branches each of 100 plants. Error bars = standard error.

3. Estimation of fruit removal from *A. crenata* plants in the field

This study was conducted in the summer of 2001 (May - September) at the Paynes Prairie site. Prior to this study, fruit fall had been observed but nothing was known as to what percentage of fruit may remain near or be dispersed from the plant. Damaged fruit were also observed, but nothing was known as to what species may account for the damage and possible removal of fruit.

The methods for this study were simple in design, yet very effective. Ten plants were chosen at random, marked with flagging tape and numbered with an aluminum tag. Initial fruit counts were made and depending on fruit number and placement, 1 or 2 black plastic nursery trays (12" x 24") were placed around the base of each plant. Trays were secured with aluminum stakes to prevent removal. Fruit were removed from surrounding plants to minimize the chance of fruit getting into the trays from other sources. Weekly counts were made noting the number of fruit remaining on plant, fruit recovered from the tray, and fruit that were missing. In addition to damaged fruit, other observations such as feathers, regurgitated seeds, weather conditions and flowering were noted. One plant died in mid-June so the data were only analyzed from the remaining nine plants.

By mid-July, 67% of the plants had lost all of their fruit either by fruit fall or removal, and by mid-September all remaining fruit was gone. By the end of the study, the percentages of fruit recovered in the trays ranged from 50% to 96% with an average of 76% that would potentially remain under the plant to germinate. Thus, 24% of fruit were unaccounted for and

these may have fallen outside the trays, been removed and carried away from the plant, or may have fallen in the trays but were secondarily removed from them (Fig. 24).

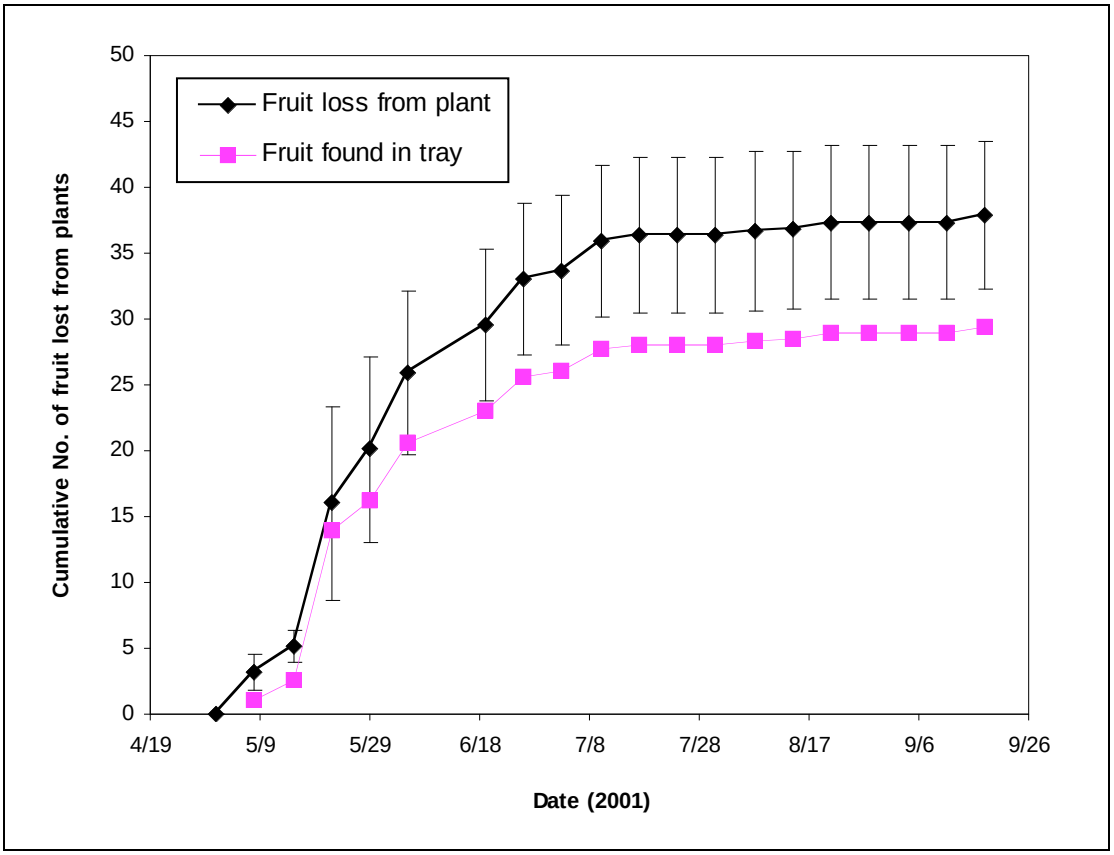


Fig. 24. Cumulative number of fruit lost from *A. creanta* plants (average for 9 plants) with the cumulative number of fruit recovered from the trays. Error bars = standard error for loss from the plant only (SE not shown for fruit accumulation in the tray).

During the first 4 weeks of the study, dry conditions caused some fruit to shrivel and drop, this was also the period in which the highest occurrence of animal damage was observed. Fruit that were recovered showed various degrees of damage, from small excised areas to total pulp removal. In some cases, entire petioles were torn off and the fruit removed. Clumps of regurgitated seeds were observed and in two instances, fruit were deposited in the tray from an outside source. In early June rain returned to the area and by mid-June new flowers were observed.

This study was initiated late in the year when a large proportion of fruit had already disappeared from the plants. A duplicate study has been set up in October 2001 to follow a new crop of fruit.

4. Germination of seeds shed from *A. crenata* plants in the field

The fate of seeds lost from *A. crenata* plants in the field has been investigated by tracking the appearance of fruit and seedling on the ground beneath isolated plants.[2, 3] This has been studied both for natural fruit fall and for fruit purposely placed under surrogate plants.

These data are still being collected but it does appear that there are someseasonal differences in the percentage germination of seeds placed under surrogate plants. This may be due to periods with inappropriate conditions for germination, or reduced viability of seeds harvested at certain times. These alternatives will be investigated. Preliminary estimations of the average proportion of fruit that germinate after falling off the plant are between 27% and 62% (estimates for different sites). These are fairly high proportions for reproductive success, and only include those seeds falling under the plant and not those that are dispersed and may germinate further away. It appears likely that one of the reasons that *A. crenata* is invasive in north Florida is because it is capable of producing many seeds, a large proportion of which become seedlings that can survive for many years.

5. Regrowth of *A. crenata* following various control methods applied in the field

There is no published recommended herbicide treatment for *A. crenata* but for *A. elliptica* it is basal bark treatment with 10% Garlon 4 (triclopyr) or cut stump application of 50% Garlon 3A and hand pulling seedlings (Langeland and Stocker 1997 – Control of non-native plants in natural areas of Florida UF/IFAS publication SP242).

A study of the fate of *A. crenata* plants treated in San Felasco State Preserve, Gainesville, with Garlon 4, was not very satisfactory due to incomplete treatments and failure to re-locate plots.[2, 3] All plants treated with Garlon 4 in June 2000 appeared to be dead by October 2000. By December 2000, only 4 out of 206 plants had any signs of re-sprouting, and there were only 3 new seedlings in the three treated plots. Only 1 out of 253 plants was observed to re-sprout in the three plots where plants were manually dug up, and 15 seedling were found in December.[3]

6. Influence of the seasonality of cutting treatments on the recovery of *A. crenata* plants

A study was initiated in September 2000 to investigate the influence of stem cutting on root carbohydrate storage and re-sprouting.[3] Plants between 0.3 and 0.6 m in height were marked in the NATL in September 2000 and randomly assigned to the following treatments (5 plants per treatment).

Control = intact plants to be harvested in September, December, March, June, and following September.
Cut once = shoots were cut at the base in September 2000 and monitored for shoot regrowth and change in root TNC 1 year later.
Cut twice = shoots were cut as “cut once” plants and again six months later to measure the shoot regrowth and change in root TNC 1 year later.

Very little shoot regrowth was observed between September and March (shoot length mean ± s.d. = 7.8 ± 4.0 cm). The majority of regrowth took place between March and September (33 ± 21 cm), coinciding with the seasonal shoot extension of control plants (July-August). Two out of the 15 cut plants did not resprout at all for 1 year after cutting treatment, although small shoot meristems were observed and the roots of these plants were alive and had high TNC concentration similar to control plants.

*Ardisia* roots have one of the highest starch concentration recorded for shrubs (up to 60% of root dry mass). Seasonal changes in carbohydrate reserves in roots were determined every three months for plants between 0.3 and 0.6 m in height. The root crown (c.f., tuber) and lateral roots were dried and analyzed separately for total non-structural carbohydrate (TNC, total of simple sugars and starch).

Root TNC concentration is the lowest in September after the annual shoot extension and leaf development in late July-August (Fig 25). These concentrations increase and are maximal in March-June, immediately before flowering and new shoot development occur. This pattern suggests the significant contribution of photosynthesis in winter months and role of carbon storage for construction of new shoots and leaves.

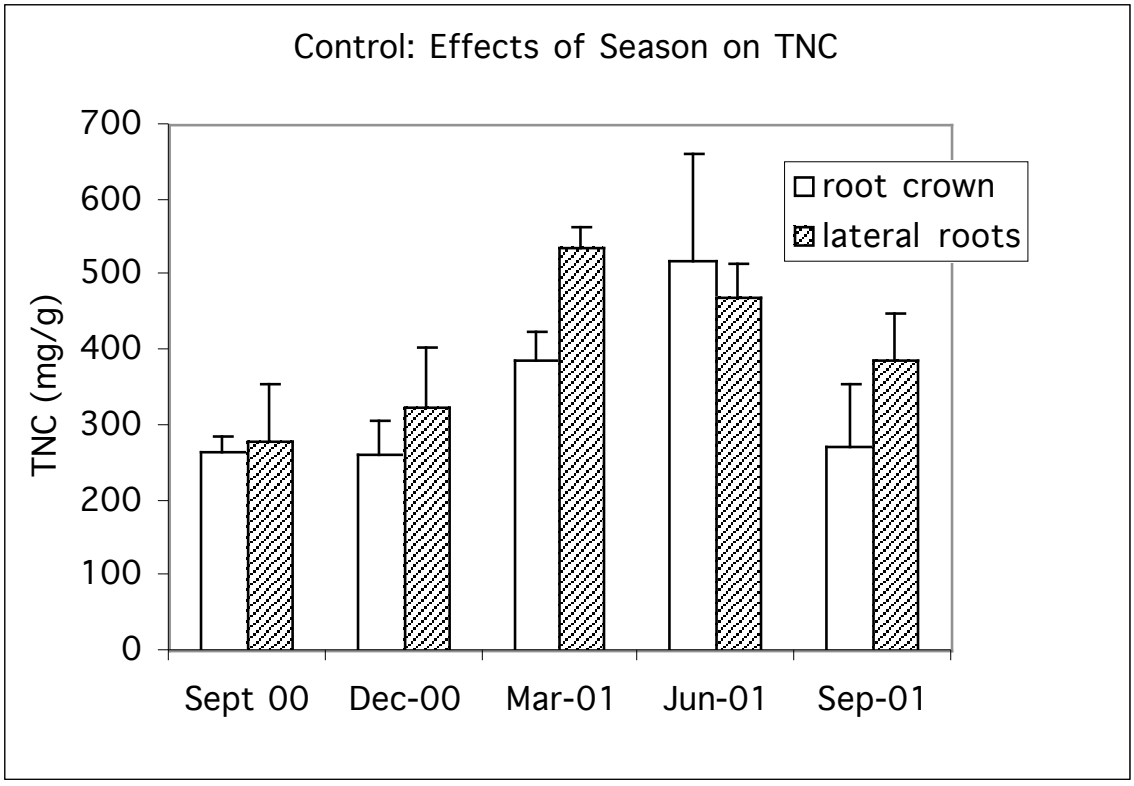


Fig. 25. Seasonal change of TNC concentration in root crown and lateral roots, measured milligram glucose equivalent per gram dry mass for intact plants (30-60 cm tall). Mean ± s.d. of five plants.

The seasonal pattern was even more marked for total root TNC per plant (Fig. 26). The leaf area reaches a maximum in September, but total root TNC does not increase until after December, probably because photosynthate was allocated to fruit maturation through December.

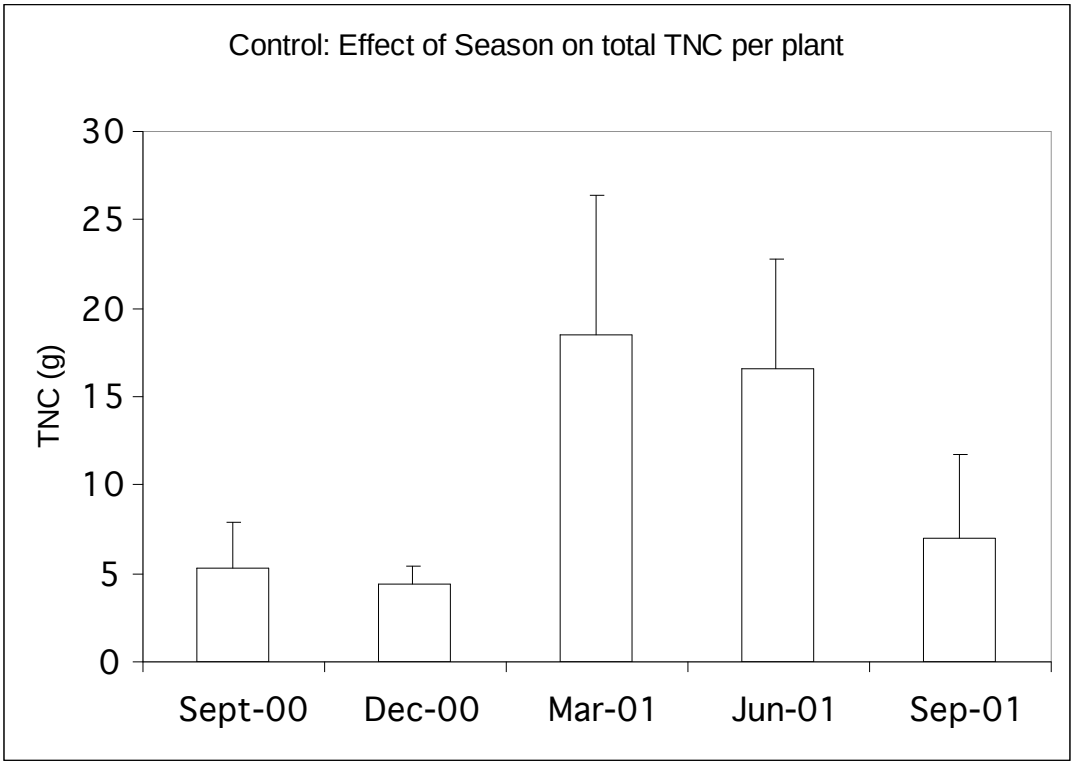


Fig. 26. Effects of season on total root TNC per plant for randomly selected 30-60 cm plant in the field.

These patterns along with observed patterns of vegetative and reproductive growth suggest the following seasonality of carbon balance.

July-August. New shoot and leaf development using carbohydrate stored over the winter months. Flowering on tips of the 1-yr old branches.

September-December. Leaf area is maximal, with a new cohort of leaves and 1-year old leaves that are gradually senesced and lost. Fruit development is paid for by the current photosynthetic income. Fruit maturation is complete in December.

December-March. Photosynthesis in winter months under partially deciduous overstory canopy contributes to increase in root mass and root carbohydrate storage.

March-June. Flowering in May-July, depending on water availability etc. Leaves older than 1 year are almost all lost. Photosynthetic income dwindles as overstory canopy become closed.

The effects of cutting on root carbohydrate storage is significant both in March and September (Fig. 27). A small number of plants that remained dormant after cutting treatment maintained high TNC levels. In March, the difference between cut and control plants reflects lack of photosynthetic income in cut plants, rather than the allocation to shoot regrowth which was minimal. The low root TNC in cut plants in September reflects further depletion of root TNC by the shoot extension growth in summer.

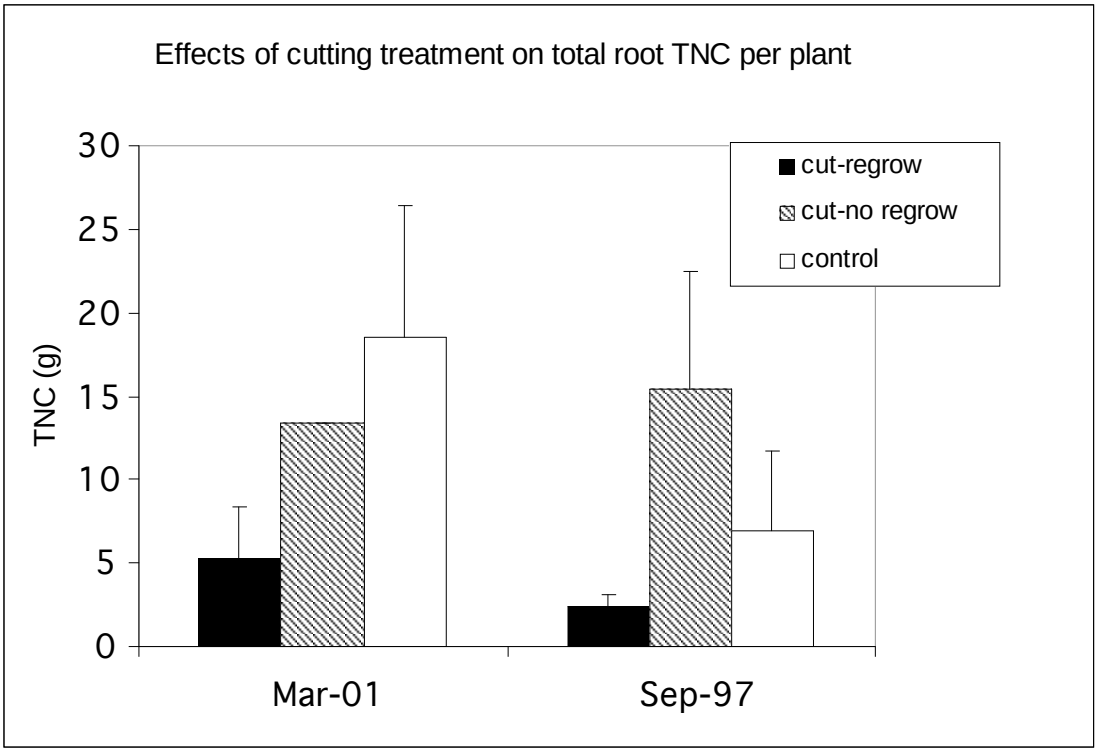


Fig. 27. Effects of shoot cutting treatment in September 2000 on TNC per plant 6 and 12 months later (March and September 2001, respectively). Cut plants with and without shoot regrowth were analyzed separately.

The above results suggest that the optimal timing for Ardisia control by shoot cutting or mowing is September when root TNC reserve is minimal. This is also a good time as it is before the new cohort of fruits and seeds mature on the plants. Repeated mowing or cutting one year later should further deplete the root TNC reserves and suppress vegetative and reproductive growth of the population. Although it is difficult to kill the plants with stem cutting and mowing alone, cutting or mowing are perhaps good methods to suppress the further growth of existing invading populations. It is much less expensive and causes less perturbation of soil and surrounding native plants than manual excavation or herbicide treatments.

7. Regrowth of *A. crenata* from isolated root sections

To examine whether plants may regenerate from fragments of lateral roots, lateral root segments of different length (1, 3, 6 cm) were planted in pots filled with moist potting soil (eight replicates per length). They were watered as needed and monitored from November 2000. None of the roots produced a new shoot and all roots appeared dead when they were examined in July 2001 (8 months later).

This result is consistent with our observation of revisiting reproductive plants removed by manual excavation of the root crown in the NATL area one year later. Very few (two out of hundreds of plants excavated) exhibited shoot regrowth that could be from lateral roots. Thus, we can conclude that shoot regrowth of *A. crenata* is restricted to be from the root crown not lateral roots. Excavation of the root crown manually is an effective measure to kill the plant and avoid future re-sprouting, although the population is likely to recover from viable seeds and small seedlings overlooked during the removal efforts.

## IV. Mapping the invasion dynamics of *Ardisia crenata* in a mesic hardwood

In the NATL, we have removed and mapped spatial distribution of individual stems of *A. crenata* at the precision of ± 1m in a 200 x 350 m area encompassing the most heavily infested area (mixed hardwood in the South East corner adjacent to apartments see Fig. 28 - attached .pdf file). Stems were measured for height to the nearest 5 cm (5, 10, 15, 20, etc.) and mapped for their locations (Fig. 29 – attached .pdf file).

The pattern reveals very strong aggregation and a strong association of juvenile plants (< 20 cm) to reproductive-sized plants (> 20 cm). This spatial pattern indicates that dispersal over 5 m is relatively infrequent, but must be important in the expansion of populations. Once a plant is successfully established as mature plant, it produces locally extremely dense population. This is a snap shot of an actively invading population, with extremely leptokurtic distribution of stem length.

## Conclusions and Recommendations

### Reproduction and dispersal of *Ardisia crenata*

Although *A. crenata* seeds may survive for at least two years in artificial conditions (e.g., moist but too cold for germination), there is no evidence that this species can form a soil seed bank. With some fruit surviving on the plant for up to a year, seed rain is almost continuous, but this does not constitute an on-the-plant seed bank that accumulates over time. The number of seeds available on the plants diminishes most rapidly in the spring and early summer and the new crop is not fully mature until December. The possibility that there is a seedling bank (with some seedlings showing little growth, remaining essentially dormant after germination, until optimal growth conditions occur) could be investigated further.

Management strategies that kill all above-ground parts and root crowns of *A. crenata* plants should be successful in the long-term, provided that the area is checked and re-treated for seedling regrowth at least two months later. Long-term monitoring should not be necessary unless material is being re-introduced from other sites. If management methods do not include removal of plants with fruits from the sites (including all stages of fruit development except the hardest, immature green fruit), the site will need to be revisited for seedling growth for several months after all the fruit on the killed plants are shed.

*Ardisia crenata* is unlikely to invade dry upland areas such as scrub and sand-hills because its seeds do no tolerate moisture loss, particularly if exposed without fruit skin and pulp (as might occur if fruit are damaged by birds or mammals). Data from a preliminary study indicate that 20 to 30% of fruit are lost from the area directly under a parent plant but it is not known what proportion of this loss is caused seed predation or dispersal by animals. Maps of plant distribution in a mesic hardwood show that most seedlings and juvenile plants occur close to the larger, parent plants, but a few longer-distance dispersal events must occur to initiate new clumps that are over 5m from larger stands. Future studies could focus on the role of birds and mammals in the dispersal of *A. crenata* seeds to determine how frequently this species is likely to invade new and remote sites.

### Growth and survival of *A. crenata* plants

Young *A. crenata* plants have poor tolerance to full sunlight and in irrigated, common garden studies show highest growth rates at moderate light levels (18% – 45% of full sunlight). This species may be well adapted to survive with low growth rates in very shaded conditions but is also able to grow rapidly to exploit greater light levels in treefall gaps. Measured growth rates in the field were much lower than in the common garden studies indicating that factors in the field other than light are probably limiting plant growth. The measured growth rates indicated that *A. crenata* plant in the field may be much older than was previously assumed (e.g., 10 years old for a 20 cm tall stem), but these estimates are complicated by the difficulty of distinguishing between single and multiple stems from the root crowns of larger plants. This aspect of the development of multiple stems on *A. crenata* plants needs to be investigated more thoroughly.

While severed roots do not appear capable of regrowth, *A. crenata* plants can only be considered dead when the root crown has been killed or removed so that no further stems can be produced. This is important in evaluating the use of mechanical or manual control methods, and dictates the use of systemic over contact herbicides for long-term control. Photosynthetic production and accumulation of carbohydrates in root tissues is maximal in winter when more light is available under the semi-deciduous tree canopy. Carbohydrate storage is minimal in the fall after most stem elongation and leaf and flower production in the late spring and summer. Consequently, regrowth from cut stems is least during September.

The proportion of seeds that become seedlings in the field is relatively high in *A. crenata* (25% – 65%). While mortality is proportionately the highest for young seedlings and the tallest stems, the densities of seedlings (up to 10 cm tall there are 100-200 plants $m^{-2}$) and reproductive plants (over 20 cm tall there are up to 3 plants $m^{-2}$) are sufficiently high to explain the ability of this species to dominate the ground cover of suitable habitats.

**Ecological impacts of *A. crenata***

Data from studies of the plant communities in and around infestations of *A. crenata* indicate that there is a negative correlation of the abundance of native understory plants with *A. crenata*. More detailed analyses are needed to identify any covariate species or conditions in this relationship but the consistency in the pattern of reduced numbers or coverage of native plant species with increasing coverage or density of *A. crenata* is an important quantitative indicator of how this non-native shrub is negatively impacting invaded habitats.

## APPENDIX

**Community survey.**

Sites:
Dr. Brian McNabb's property in Micanopy.
Dr. Jack Putz's property near Newnan's Lake in Rochelle.
Dr. Perran Ross's property near Payne's Prairie in Gainesville.
San Felasco State Preserve in Gainesville.
Coclough Pond in Gainesville.

Species (under – and overstory in both seasons all sites) 114 species

*Acer negundo* L.
*Acer rubrum* L.
*Acer saccharum* subsp. *floridanum* (Chapm.) Desmarais
*Peltandra virginica*
*Verbesina virginica*
*Ageritina aromaticum*
*Ambrosia artemisifolia* L.
*Ampelopsis arborea* (L.) Koehne
*Ardisia crenata* Sims
*Arisaema dracontium* (L.) Schott
*Arisaema triphyllum* (L.) Schott
*Aristolochia serpentaria* L.
*Asclepias* sp.
*Asplenium platyneuron* (L.) Britton et al.
*Baccharis glomerulifolia* Pers.
*Bambusa* sp.
*Betula nigra* L.
*Bignonia capreolata* L.
*Botrychium biternatum* (Savigny) Underw.
*Callicarpa americana* L.
*Campsis radicans* (L.) Seeman ex Bureau
*Carex digitalis* Willd.
*Carex nigromarginata* var. *floridana* (Schwein.) Kuk
*Carex willdenowii* Schkuhr ex Willd.
*Carpinus caroliniana* Walter
*Carya aquatica* (F.Michx.) Nutt.
*Carya glabra* (Mill.) Sweet
*Celtis laevigata* Willd.
*Cercis canadensis* L.
*Chasmanthium laxum* (L.) Yates
*Cinnamomum camphora* (L.) J. Presl
*Cornus florida* L.
*Cornus foemina*
*Dichanthelium* sp.

*Digitaria* sp.
*Dioscorea floridana* Bartlett
*Diospyros virginiana* L.
*Distichum* sp. {moss}
*Dryopteris ludoviciana* (Kunze) Small
*Elephantopus elatus* Bertol.
*Entodon* sp. {moss}
*Erythrina herbacea* L.
*Euonymus americanus* L.
*Eupatorium* sp.
*Fraxinus caroliniana* Mill.
*Galactia volubilis* (L.) Britton
*Galium pilosum* Aiton
*Gelsemium sempervirens* (L.) W.T. Aiton
*Gordonia lasianthus* (L.) J.Ellis
*Hedera helix* L.
*Hypericum* sp.
*Ilex opaca* Aiton
*Ilex vomitoria* Aiton
*Juglans nigra* L.
*Krigia virginica* (L.) Willd.
*Lamium amplexicaule* L.
*Liquidambar styraciflua* L.
*Lonicera sempervirens* L.
*Lyonia lucida* (Lam.) K. Koch
*Magnolia grandiflora* L.
*Matelea* sp.
*Melothria pendula*
*Mitchella repens* L.
*Mnium* sp. {moss}
*Morus rubra* L.
*Nyssa sylvatica* var. *biflora* (Walter) Sarg.
*Oplismenus hirtellus* (L.) P. Beauv.
*Osmanthus americanus* (L.) Benth. & Hook. f.ex A. Gray
*Osmunda cinnamomea* L.
*Ostrya virginiana* (Mill.) K. Koch
*Oxalis* sps.
*Parthenocissus quinquefolia* (L.) Planch.
*Persea borbonia* (L.) Spreng.
*Persea palustris* (Raf.) Sarg.
*Petiveria alliacea* L.
*Physalis* sp.
*Pinus elliottii* Engelm.
*Pinus glabra* Walter
*Pinus palustris* Mill.
*Pinus taeda* L.
*Poinsettia heterophylla*
*Polygonum* sp.

*Prunus caroliniana* (Mill.) Aiton
*Prunus serotina* Ehrh.
*Quercus hemisphaerica* W. Bartram ex Willd.
*Quercus michauxii* Nutt
*Quercus minima* (Sarg.) Small
*Quercus nigra* L.
*Quercus pumila* Walter
*Quercus virginiana* Mill.
*Rhapidophyllum hystrix* (Pursh) H. Wendl. & Drude ex Drude
*Rhododendron* sp.
*Rubus argutus* Link
*Ruellia caroliniensis* (J.F. Gmel.) Steud.
*Rumex hastatulus* Baldwin
*Sabal palmetto* (Walter) Lodd. Ex Schult. &Schult. f.
*Salvia coccinea* Buc'hoz ex Etl.
*Sanicula canadensis* L.
*Saururus cernuus* L.
*Serenoa repens* (W. Bartram) Small
*Smilax* sps.
*Soncus asper* (L.) Hill
*Stachys floridana* Shuttlew. ex Benth.
*Stellaria media* L. Vill.
*Tilia americana* L.
*Toxicodendron radicans* (L.) Kuntze
*Tradescantia fluminensis* Vell.
*Ulmus alata* Michx.
*Ulmus americana* L.
*Vaccinium* sp. L.
*Viburnum nudum* L.
*Viola sororia* Willd.
*Viola walteri* House
*Vitus rotundifolia* Michx.