INTEGRATION IN A CLONAL WOODY SHRUB, *VACCINIUM STAMINEUM*

by

WILLIAM L. DUVAL

(Under the Direction of Rebecca R. Sharitz)

ABSTRACT

The integration of clonal plants can have important consequences for plant interactions; however, there are relatively few studies that address this issue. In fact, many studies of plant interactions that include clonal plants have actually used individual ramets and have ignored integration completely. Most studies of clonality also involve herbaceous species, with many fewer studies utilizing woody species. Given the dearth of studies on woody species and clonal integration, it is important to address these issues. Clonal integration in *Vaccinium stamineum* was examined through a series of field experiments that addressed the following questions: 1) Does resource sharing actively occur? 2) Does clonal integration alter the competitive ability of the study species? 3) Does clonal integration alter the study species' ability to utilize resources? 4) Does clonal integration increase the ability of *V. stamineum* to respond to simulated disturbance? The findings from these experiments suggest that integration plays an important role in growth and reproduction in *Vaccinium stamineum* in the sandhills of the southeastern United States.

INDEX WORDS: Integration, Clonal, Vegetative, Nitrogen, Competition, Disturbance, Sandhills, *Vaccinium stamineum,* Long-leaf pine

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WILLIAM L. DUVAL

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WILLIAM DUVAL

Major Professor:	Rebecca Sharitz
Committee:	Beverly Collins Ron Hendrick David Porter Gregory Schmidt

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

This dissertation is dedicated to my wife, Elizabeth; my parents, Thomas and Susan; and my brothers, Mani, Luke, and Zac.

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Chapter One

Introduction

Integrated clonal plants have the potential to share resources, such as water, nutrients, and carbohydrates (Alpert, 1996; Stuefer et al, 1994; Wijesinghe and Hutchings, 1997). This ability to share resources may improve the growth and survivorship of both individual ramets and whole genets (Brewer and Bertness, 1996; Jonsdottir and Watson, 1997). Integration may alter competitive outcomes (Hartnett and Bazzaz, 1985; Schmid and Harper, 1985), mortality risk (Cook, 1983), and plant fitness (Caraco and Kelly, 1991). It can provide a method by which clonal plants conserve and recycle resources (Jonsdottir et al., 1996), provide a way in which they might buffer against resource heterogeneity in low-resource environments (Jonsdottir and Watson, 1997), and improve the capacity of the clonal plant to resist invasion by other species and maintain its presence in the environment (Bradbury, 1981; Lovett-Doust, 1981). With these potential advantages, there may also be several disadvantages. More rapid disease transmission among ramets may occur (Stuefer et al., 1994). Transporting resources and the costs associated with the maintenance of the physical connections among ramets have inherent costs as well, some estimated to be quite high (Hutchings and Bradbury, 1986; Jurik, 1983).

Clonal plants are quite common through the world in a variety of habitats (e.g. Jonsdottir and Watson, 1997; Pitelka and Ashmun, 1985). Two reviews provide an excellent history of the research on clonal woody species over the past decades (Jenik, 1994; Peterson and Jones, 1997). Woody species, however, remain largely unincorporated into clonal theory, with only limited studies specifically examining woody species’ effects (Peltzer, 2002). Furthermore, the herbaceous species that have been studied intensively are limited to a few model species in a limited number of habitats. This study seeks to address this shortcoming by examining a clonal woody species, *Vaccinium stamineum* L. in the sandhills of the southeastern United States, an ecosystem with few studies of clonal plants.

The initial examination of *V. stamineum* involved three pilot studies of the integration of the ramets, the extent of possible translocation of resources, and mortality of ramets due to creating non-integrated clones. These studies were necessary to determine that actual movement occurred among ramets and that these physical connections were active. An acid fuchsin dye and an herbicide were used to test for actual resource transport among ramets in the spring of 2004. The herbicide, Round-Up®, was applied to individual ramets which had been isolated from other ramets. A bag was a placed around each ramet that was to receive the herbicide treatment and, during application of the herbicide, other ramets were temporarily covered with a plastic material so as to prevent accidental exposure. Six clones were used for this experiment, with evidence of herbicide movement, as shown by ramet tissue mortality, exhibited in all six clones. Round-Up® is a phloem and xylem-mobile, non-selective herbicide whose movement in the soil is minimal (McNabb, 1996).

The acid fuchsin dye was applied in the method described by Price and Hutchings (1992). In each of six clones, multiple leaves on one ramet were selected as the injection point. The leaf tips were severed and the leaves then dipped into the 2% aqueous solution of acid fuchsin dye. Movement was then observed by creating cross-sections of both above-ground and below-ground plant tissues. This movement of the acid fuchsin dye was both acropetal (from older parental ramets to younger daughter ramets) and basipetal (from younger ramets to older ramets) in all six clones. This dye moves through the xylem, indicating that water and soluble mineral nutrients have the potential to be shared among ramets in the same way the acid fuchsin dye was.

A third pilot study was conducted to determine mortality due to initial trenching of ramets. Twelve clones, ranging from sixty to one hundred ramets in size, were chosen at random. In six clones, the ramets were mapped and the rhizome connections between all ramets were severed, so as to create six non-integrated clones. The six other clones remained integrated. Ramets were observed every two months for a six month period to determine if the creation of non-integrated clones via trenching might cause ramet survivorship to decrease. There was no significant difference in mortality at any time period between integrated and non-integrated clones. This allows for the comparison of integrated and non-integrated clones without any confounding mortality due to rhizome severing.

From observations of the transportation of both the herbicide and the acid fuchsin dye among ramets, it was determined that resource sharing among *V. stamineum* ramets can and most likely does occur (Table 1.1). Mortality due to rhizome severing was negligible, paving the way for integration treatments to be compared (Table 1.2). The goal of this dissertation is to examine the effects of clonal integration, with the potential sharing of resources, in *V. stamineum* under several different environmental conditions. The dissertation comprises three parts.

Chapter two examines integration under different disturbance regimes. It addresses the issue of whether integration affects the ability of *V. stamineum* to respond to a simulated herbivory disturbance. Thirty clones were examined in the Fall-Line sandhills of the Savannah River Site in SC. Control, intermediate, and extreme disturbance levels were established with both integrated (intact rhizome connections) and non-integrated (severed rhizome connections) clones of *V. stamineum*. The results showed that asexual reproduction increased most under intermediate levels of disturbance, and that integrated clones produced significantly more new ramets than non-integrated clones. Rhizome length was shortened by all disturbance treatments as well as in all non-integrated *V. stamineum* clones. Ramet length was unaffected by either integration or disturbance treatments.

Chapter three addresses the effect of integration under natural and reduced competition intensities. *Vaccinium stamineum* was examined in two different locations. An Athens, GA site was compared to clones at the Savannah River Site. No significant differences were found between clones at the two locations in their responses. Asexual reproduction and ramet weight were both significantly greater in integrated clones and in clones with reduced competition. Ramet length and rhizome length showed no significant differences under integration or competition treatments.

The fourth chapter examines integration under differing levels of soil nitrogen. This study was conducted in the sandhills at the Savannah River Site, and *V. stamineum* was subjected to two levels of nitrogen: natural and elevated. Clonal integration and nitrogen treatments had no effect on asexual reproduction in this experiment, contrary to the results from chapters two and three. Rhizome length was also not significantly different among treatments. Above-ground biomass, as measured by ramet length and weight, was greater in integrated clones and elevated nitrogen treatments. Non-integrated clones under natural levels of nitrogen produced the smallest ramets, both in weight and length.

This dissertation research fulfills the two important criteria set by Pitelka and Ashmun (1985) for the demonstration of clonal integration and its advantages or disadvantages. The first is that the experimental results show a statistically significant effect on ramet and/or genet survivorship or growth. The second is that there are data showing the movement of at least some small percentage of material from one ramet to another ramet. The experimental results clearly show that clonal integration has a significant effect on various aspects of ramet and genet growth. The findings represented in this dissertation have broad implications for clonal theory, both for herbaceous and woody species. They reflect the need for current theories to consider differences in plant structure, in order to accurately quantify and model clonal plant interactions and dynamics.

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	Round-Up® Treatment (average # of ramets the substance was transported)	**Dye Treatment (average # of ramets the substance was transported)**
Acropetal movement (older to younger ramets)	**4.2 ± 1.7**	**3.7 ± 0.8**
Basipetal movement (younger to older ramets)	**1.6 ± 1.4**	**1.3 ± 1.1**

Table 1.1: Results of two pilot studies that examined the movement of Round-Up® and dye treatments. Numbers reported are the average number of ramets that the substance was transported, either acro or basipetally.

	Two Months (average # of ramets dead)	**Four Months (average # of ramets dead)**	**Six Months (average # of ramets dead)**
Integrated	**0.75 ± 0.60**	**0.86 ± 0.55**	**0.98 ± 0.78**
Non-integrated	**0.65 ± 0.31**	**0.85 ± 0.54**	**0.92 ± 0.63**

Table 1.2: Pilot study results of mortality in six integrated and six non-integrated clones during a six month period.

Chapter Two

Clonal integration and disturbance in *Vaccinium stamineum*

Introduction

Many plants have the capacity to reproduce both sexually and asexually. Most current ecological theories, however, automatically assume that all plants considered are non-clonal. This assumption excludes both the potential benefits and costs of clonality, and can be a misleading representation of community and ecological processes given the vast numbers of plants with a potential for clonal reproduction.

Clonal plants have been postulated to have advantages and disadvantages that non-clonal plants do not. Buffering against resource heterogeneity in low availability environments, increasing overall genet survival, and acquiring resources more effectively than non-clonal plants, are all potential advantages for clonal plants (Hutchings and Bradbury, 1986). The inherent cost in maintaining these connections and transporting resources across them also exists, which may put clonal plants at a disadvantage against non-clonal plants without these resource drains (Pitelka and Ashmun, 1985; Alpert and Stuefer, 1997). There is also the possibility of more rapid disease transmission in clonal plants due to their physiological connections (Stuefer et al., 2004; Wennstrom, 1999; Wennstrom and Ericson, 1992). Genetic consequences, such as reduced genetic variation coupled with inbreeding depression, may also exist for populations that reproduce mainly through asexual means. These differences between clonal and non-clonal plants, combined with the widespread frequency of vegetative reproduction, emphasize the need for studies on clonal integration and fragmentation.

Many estimates exist, mostly in central Europe, of the percentage of plants in an ecosystem that have the potential to reproduce clonally. One study puts the estimate of clonal angiosperms in Great Britain at 46% (Salisbury, 1942), while two more recent studies of central European flora estimate the number to be closer to 70% (Klimes et al., 1997; van Groenendael and de Kroon, 1990). Since a potentially large percentage of a given habitat's flora may be clonal (Prach and Pysek, 1994), it is important to recognize ecological distinctions between clonal plants and their non-clonal equivalents.

Most studies that examine clonal plants in the context of disturbance do so solely with a few, exclusively herbaceous, model species. Such a bias is reasonable, since herbaceous species tend to have shorter generation periods and are generally more logistically tractable than their woody counterparts. Due to the exclusivity of study plants and their herbaceous nature, computer modeling has been done solely with herbaceous species' parameters. However, there are many obvious, potentially large differences between how clonal woody species and clonal herbaceous species might differ in their growth, survival, reproduction, and response to disturbance. For example, given the vast differences in biomass material, architecture, and lifespan, an aspen is not expected to grow and reproduce in the same way a strawberry might.

Disturbances have been shown to have a wide range of effects on clonal plants, both in the degree and pattern of the disturbance (Brewer and Bertness, 1996; Tobler et al., 2006; Price and Hutchings, 1992a). Responses also vary with the extent of physiological integration and asexual versus sexual reproduction within the clonal plants. The wide variety and overwhelming numbers of woody species in various ecosystems throughout the world make it important to examine how clonal woody species are affected by various disturbances.

The Fall-Line sandhills communities of the southeastern United States contain several clonal woody species and are vulnerable to a wide range of disturbances. Typical sandhills communities are a mix of oak and pine scrublands, as well as long leaf pine forests. Fires, both prescribed and natural, are common, as well as herbivory. Fires in the sandhills can range from small, low temperature ground fires which generally remove only herbs, forbs, and shrubs, to high-intensity fires that cause extensive mortality among all flora types (Breininger and Schmaltzer, 1990). The white-tail deer, *Odocoileus virginianus* Boddaert, is also another potential source of disturbance commonly found in the sandhills. Deer browsing affects a variety of community processes and this species is considered by some to be a keystone herbivore (Russell et al., 2001; Waller and Alverson, 1997). Herbivore species may also express preferences in the species on which they feed, potentially affecting some plant populations more than others (Miller et al., 1992) and affecting community composition.

The clonal woody shrub, *Vaccinium stamineum* L., is commonly found throughout the Fall-Line sandhills habitat. This shrub may act as litter traps in a rather sparse understory, providing habitat and safe refuges for fauna, but also making them susceptible to fire. The shrub's abundant production of berries may also make them a desirable food source for animals. Disturbances, such as fire, herbivory, or clear-cut logging of sandhills pine forests, may be particularly important for the spread of *V. stamineum*. It has been observed to re-sprout much more quickly than other plants in sandhills areas that have been recently burned or cut (Duval, personal observation). This may be due to its primarily clonal means of growth, which might allow for integrated ramets within a genet to utilize already existing resources that have been stored in the rhizome mass. In low resource environments, such as the sandhills, clonal plants may have advantages over their non-clonal counterparts due to this ability to store and transport resources. On a smaller scale, the loss of some biomass on individual ramets due to herbivory may alter the overall genet's ability to survive, grow, and reproduce. Defoliation from herbivory also may cause decreased growth and reproduction, or could lead to fragmentation, the disintegration of physical connections, within the clones.

Many studies of herbaceous species have shown that the defoliation of a given ramet increases the transfer of photoassimilates from other, non-defoliated ramets (Pitelka and Ashmun, 1985; Alpert and Mooney, 1986). For a sedge in the Icelandic ranges, movement of photoassimilates increased up to six-fold from parent ramets to daughter ramets, that is to say acropetally, after defoliation of the daughter ramets (Jonsdottir and Callaghan, 1989). If ramets were repeatedly defoliated, the parent ramets continued to increase resource transport immediately following the first and second defoliations, but not the third defoliation (Jonsdottir and Callaghan, 1989). This suggests that the genet engages in a form of risk aversion, recognizing a difference between ramets that experience a lower frequency of disturbance and those that experience a higher frequency of disturbance. As mentioned earlier, these effects of disturbance, specifically defoliation, have been examined using clonal herbaceous species, but not necessarily in an explicitly clonally woody species context (Price and Hutchings, 1992b).

We examined the effects of simulated herbivory on integrated clones of *V. stamineum* which had intact rhizome connections and on non-integrated clones in which rhizome connections between ramets had been severed. We hypothesized that integrated clones of *V. stamineum* would experience more growth and asexual reproduction (new ramet production), and would produce longer rhizomes, than non-integrated, fragmented clones in response to differing levels of a simulated herbivory disturbance. We also hypothesized that disturbed clones would experience less growth and asexual reproduction, and would produce shorter rhizomes than non-disturbed clones.

Materials and Methods

The study organism, *Vaccinium stamineum* L., is a shrub whose range extends from southern Ontario and New York to Minnesota, and south to Florida and Louisiana (Gleason, 1952). Unconfirmed reports also list the species as far west as Montana and south into Mexico. There is, however, considerable disparity in its abundance from region to region. It is listed as threatened in northern Ontario, as well as in at least one mid-western state, Illinois. Throughout its range it has great morphological variability, as evidenced by its originally being classified as twenty-one different species (Ashe, 1931). In the southeastern Coastal Plain, it is considered a characteristic shrub of the region (Godfrey, 1988). It grows up to five meters in height in some places, but in the Fall-line sandhills of South Carolina it does not typically grow taller than one-half meter (Foote & Jones, 1989).

Vaccinium stamineum forms mono-specific patches, a growth form known as "phalanx" (Lovett-Doust, 1981). Other species are rarely present in the interior of these patches. The patches tend to be spatially discrete from one another and from other species at younger ages, but as these plants become older and larger, intermingling can and does occur. In most of these cases, one is likely to observe *V. stamineum* actively growing around a sapling of another species as the sapling declines in health. Throughout its range, *V. stamineum* reproduces vegetatively through rhizomes (Yakimowski and Eckert, 2007; Vander Kloet and Cabilio, 1996), although evidence of genetic diversity within patches suggests some sexual reproduction and seedling recruitment (Kreher et al. 2000). Sexual reproduction in this species requires pollination by insects, particularly bees (Cane et al., 1985). Its berries are a green to purple color and are listed as appearing from April to October (Foote & Jones, 1989), although in the sandhills this period is generally restricted to the middle and late summer months.

Early pilot studies with an acid fuchsin dye, which moves through the xylem, and an herbicide revealed clear evidence of resource movement among *V. stamineum* ramets within a genet (Duval personal observation). Most movement was acropetal (from older to younger ramets), however some basipetal movement (from younger to older ramets) also occurred. This indicates that the physical integration of ramets in this species allows active sharing of resources. The movement of potential resources, both acro and basipetal, indicates that the resources taken up by individual ramets have the potential to be shared with other ramets within the genet; thus there is a potential for differential performance between integrated and non-integrated clones.

The field site was located in the Fall-Line sandhills, a narrow strip situated between the Coastal Plain and Piedmont regions of the southeastern United States. These particular sandhills were located on the Savannah River Site (SRS), a federal facility near Aiken, South Carolina and have undergone prescribed burning and other forest management practices for over fifty years. The sandhills environment is characterized by a sparse canopy, low nutrient resources, and deep, sandy soils. In these communities, *Pinus palustris* and *Quercus laevis* are the two most frequently encountered tree species, with an array of other oaks, such as *Q. margaretta* and *Q. incana*, also commonly found. Shrubs such as *V. stamineum* and *Gaylussacia dumosa* are abundant. The understory contains grasses of the genera *Aristida* and *Andropogon,* the fern *Pteridum aquilinum*, and a wide variety of herbaceous species (Workman and McLeod, 1990). Fire is a common occurrence, with some sandhills in the southeast being actively managed through prescribed burning. It is frequently cited that longleaf pine communities in the sandhills should experience fire every two to three years, however it is generally thought that this interval is too short (Mattoon, 1922 from Frost, 1993).

Clones of *V. stamineum* chosen for this study were restricted to a size class between one meter by one meter and two meters by two meters. Clones of this size tend to be younger, more actively growing plants that are logistically tractable for a study of this magnitude (Duval, personal observation). These were further selected so that no other plant species were growing in the interior of the clones. This restriction was necessary to remove a possible competition effect. Thirty clones were randomly chosen from a pool of over 120 within these limits during the spring of 2006. These thirty clones were randomly assigned to the integrated and disturbance treatments. These chosen clones were spread over a one square mile region on SRS property. All clones were mapped so that each individual ramet had a given X/Y coordinate. The total number of ramets and the location of each were noted at the beginning of the study, at six months, and at the one year conclusion of the experiment. The length of each individual ramet was measured at all three time periods as well. While most ramets did not grow fully perpendicular to the ground, the ramet length is a reasonable proxy measurement for ramet height. At the end of the experiment the length of each rhizome was measured via destructive harvesting. This harvesting also allowed for the connections between ramets to be determined.

Response to Disturbance

To test whether clonal integration may provide a benefit for *V. stamineum* under simulated herbivory disturbances, integrated and non-integrated clones were examined. Since the plants are naturally integrated, no changes needed to be made for the clones within the integrated treatment (figure 2.1). For non-integrated clones, the severing of rhizomes was necessary and was accomplished by 360^{o} trenching around each individual ramet (figure 2.2). This treatment had been determined previously to have a negligible effect on ramet morality through an early pilot study with *V. stamineum* (Duval, personal observation). The soil adjacent to integrated ramets, however, was disturbed in a similar manner to that around non-integrated clones without the actual severing of rhizome connections. This allowed for the control of any soil disturbance effect.

Three treatment levels of a simulated herbivory disturbance were established, with varying percentages of the ramets (0%, 25%, 75%) removed. This permitted an examination of response of the clones (both integrated and non-integrated) across a gradient of simulated herbivory. The ramet removal was done by clipping the above-ground biomass of the selected ramets at ground level (figure 2.3). This removal of above-ground biomass was meant to simulate the disturbance experienced by *V. stamineum* due to deer herbivory. To avoid disease infection and possibly speed wound closure, a fungicide was applied and a thin sheet of plastic was temporarily placed over the site of ramet removal. Of the thirty clones, ten were assigned to each percentage disturbance category (0%, 25%, 75%). Half of these clones were clonally integrated and half were not. The ten clones that had 0% ramet removal (five integrated, five non-integrated) were used for controls for this experiment. A completely randomized design was used to assign each of the treatments to the selected clones.

Statistical analyses were conducted using the SAS statistical package in 2008 (release 6.12, 1997, SAS Institute, Cary, N.C.). A repeated measures ANCOVA was used to examine the effects of integration and simulated disturbance on *V. stamineum*, with the starting ramet length and ramet number combined as one measurement and used as the covariate.

Results

Ramet Production

Integrated clones produced significantly more new ramets than non-integrated clones, after both six months and one year, in all but one of the disturbance treatment levels. At six months, the 75% treatment level showed no significant difference between integrated and non-integrated clones. At this time, there were also significant differences in new ramet production between integrated clones at every disturbance treatment level (figure 2.4). That is, the 0% ramet removal was significantly greater than the 25% ramet removal, which was significantly greater than the 75% ramet removal. The non-disturbed, integrated clones averaged 7.1 +/- 0.8 new ramets; this was the largest number of new ramets produced during the first six months for both integrated and non-integrated clones. The largest number of new ramets produced by the non-integrated clones, 3.3 +/- 0.7, was also in the 0% ramet removal group. This was significantly greater than in the 25% and 75% ramet removal treatments. The 25% and 75% non-integrated, disturbance treatments, however, were not significantly different from one another.

After one year, similar differences existed, but with a different pattern emerging (figure 2.5). Integrated clones again produced significantly greater numbers of new ramets at all disturbance treatment levels—0%, 25%, and 75% ramet removal. For both integrated and non-integrated clones, however, the greatest numbers produced were no longer in the non-disturbed group, but rather in the intermediate disturbance level, at 25% ramet removal. The integrated clones produced 18.6 +/- 1.2 new ramets and the non-integrated clones produced 6.9 +/- 1.1 new ramets. These numbers were significantly greater than their integrated/non-integrated counterparts in the 0% ramet removal treatments, which in turn were significantly greater than in the 75% ramet removal clones.

Ramet length

There were no significant differences in ramet length between integrated and non-integrated clones or between disturbance treatment groups. Initially, a significant difference was detected between integrated and non-integrated clones in the one year, 25% ramet removal group (F=5.37, p=.012). However, this result was driven by the difference between the two groups in the production of new ramets. When the differences in lengths of new ramets and of pre-existing ramets were examined in a separate analysis that also controlled for initial ramet size, no significant difference existed at any time period or between any disturbance treatment groups (F=0.32, p>.811).

Rhizome length

The integrated clones generally produced longer rhizomes than their non-integrated equivalents (figure 2.6). Non-disturbed clones also produced significantly longer rhizomes than their disturbed equivalents, with the greatest amount of disturbance corresponding to the smallest rhizome lengths. After one year, in both integrated and non-integrated clones, the control treatments with 0% ramet removal produced significantly longer rhizomes than the 25% removal treatments, which in turn produced significantly longer rhizomes than the 75% removal treatments (figure 2.6). The integrated clones produced significantly longer rhizomes than non-integrated in both the control and 25% disturbance groups. At 75% ramet removal, the highest level of disturbance, no significant difference existed between integrated and non-integrated clones.

Discussion

We found that clonal integration in *V. stamineum* is important for its recovery from herbivory disturbance in both new ramet production (asexual reproduction) and rhizome length. There was no effect of integration on above-ground ramet length. This suggests that the physiological integration of ramets plays an important role in asexual reproduction and horizontal expansion, but not in the vertical growth of either individual ramets or the clone as a whole. These results also concur with the long-held idea that integration can buffer the effects of local defoliation (Gifford and Marshall, 1973; review by Pitelka and Ashmun, 1985), shown by the near-ubiquitous differences between integrated and non-integrated clones. However, at the most extreme level of disturbance (75% removal treatment), in almost all metrics and time periods, the effect of integration was non-significant. These results are similar to those of other studies of herbaceous plants and suggest that high levels of defoliation may be too extreme for integration to maintain the survivability and growth of ramets in clonal plants (Wang et al., 2004; Brock et al., 1988; Seker et al., 2003). Differences between the intermediate and severe levels of defoliation in this experiment, however, indicate that integration can and does alter the growth and survival of the plants at less extreme disturbance intensities.

Measurements taken at six months indicate that disturbance of any magnitude is a detriment to the asexual reproduction of *V. stamineum*. The control groups produced significantly greater numbers of new ramets than treatments with any level of disturbance. Integrated clones with intermediate levels of defoliation experienced less negative effects than those with high levels of the disturbance. In both integrated and non-integrated clones, there was a linear response during the period immediately following the disturbance, with the most disturbed treatments producing the fewest ramets. In the non-integrated clones, the lack of a significant difference between the 25% and the 75% removal groups suggests that a disturbance at any level of severity reduces the ability of the clones to reproduce. In both control and ramet removal groups, non-integrated clones produced significantly fewer new ramets than integrated clones. This finding is contrary to studies of certain herbaceous species where it has been suggested that local rhizome reserves may serve as a buffer against defoliation in plants with severed rhizomes (Suzuki and Stuefer, 1999; Dong and de Kroon, 1994). The possibility exists that additional natural disturbances are acting on the plants, beyond the simulated herbivory. Studies examining multiple disturbances, including herbivory, have shown that a second disturbance may mask the effects of an original herbivore treatment (Strauss, 1991; Bach, 1998). The potential for the transport of resources among integrated ramets may allow intact ramets to support their damaged counterparts through these physiological connections, thus somewhat counteracting the negative effects of the simulated disturbance. However, at the most severe level of disturbance, the lack of significant differences between integrated and non-integrated clones suggests that intact ramets cannot initially support damaged counterparts under such extreme conditions.

After a year, new ramet production continued to show that integration is important for the recovery from disturbance. At this time, there was a significant difference between integrated and non-integrated clones in the severe disturbance treatment, contrary to the six month measurements. Furthermore, the greatest numbers of new ramets in both integrated and non-integrated clones occurred at intermediate levels of disturbance. These results fall more in line with other studies that show the stimulation of clonal plant growth in response to a simulated disturbance (Westley, 1993). However, the heavy defoliation experienced by the most severely disturbed clones still resulted in significantly lower numbers of new ramets in both integrated and non-integrated clones. These results suggest that, for asexual reproduction, *V. stamineum* is best suited to respond to small to medium-level disturbances, such as those commonly occurring in the sandhills. The concept of a positive response to an intermediate level of disturbance, originally applied to community species richness (Connell, 1978), might be applicable to the response dynamics of clonal plant reproduction, given the high levels of asexual reproduction in the 25% removal treatment.

These results suggest that, for asexual reproduction in woody clonal plants, the initial response to a disturbance of any magnitude is a decrease in production. The simulated disturbance treatments were administered during the spring of 2006, so it is possible that the disturbances, combined with the stress of summer temperatures and limited precipitation, dampened any initial response. During the moderate winter temperatures in South Carolina between the six month and one year measurements, new ramet production was highest in the intermediate treatment group. The final measurements suggest that a mid-level disturbance might activate dormant resources in the rhizome mass of the plants, allowing these new resources to be used for new ramet production. Extreme levels of defoliation, at least initially, may result in too many ramets in need of resources, thus diluting the resources distributed from the storage rhizomes among too many integrated ramets. Control clones, with no disturbance, may not have a signal for the mobilization of resources from storage, and thus do not experience the reproductive benefits of a resource influx. Rapid regeneration after a disturbance is a common characteristic of clonal plants, though it is usually clonal plants that grow in the "guerilla" form that experience this most strongly (Fahrig et al., 1994). Another potential benefit of clonality, the intraclonal regulation of competition (Jonsdottir and Watson, 1997), may also be evident here. This intraclonal competition may play a dominant role in the spatial pattern and reproductive processes of clonal plants (Lovett-Doust, 1981).

The integrated clones produced significantly longer rhizomes than their non-integrated counterparts in both the control and 25% ramet removal treatments. At high levels of disturbance, however, there was no significant difference between rhizome lengths. This finding is unique, in that most studies that find significant differences in rhizome length between integrated and non-integrated ramets relate these differences to nutrient, light, or water status (Evans, 1992; Cain and Damman, 1997, Dong and Alaten, 1999; Qing et al., 2004; Ikegami et al., 2007), rather than to a disturbance. A study of the herbaceous species *Leymus chinensis* showed that defoliation affected rhizome growth, but that no difference in response existed between low and high levels of defoliation or integration status of the plant (Wang et al., 2004). This was suggested by the authors to be due to the short time period (50 days) in which their study was conducted. The longer time-span of our study may have allowed a measureable effect to be detected, even given the differences in life-span between *Leymus chinensis* and *V. stamineum*.

The decrease in rhizome length by non-integrated clones in both undisturbed and disturbed treatments suggests that *V. stamineum* may exhibit apical dominance in rhizome growth. Damage to the apical meristem has been shown to increase lateral ramet proliferation with shorter rhizomes and greater branching intensity (Callaghan et al., 1986). Rhizome severance in non-integrated treatments and the subsequent damage to the apical meristems of the rhizome may release other nodes from apical inhibition. This would cause shorter rhizomes to be produced, as the interior nodes, no longer inhibited, produced new ramets. The resulting rhizome lengths would exhibit a pattern similar to that observed. Increasing disturbance intensity also decreases rhizome length in both integrated and non-integrated clones. This may be a mechanism for the maintenance of the phalanx growth form which *V. stamineum* exhibits. As above-ground biomass of the ramets is removed from the interior of the clone, it is possible that the plant senses the gap created by the simulated disturbance and produces new ramets to fill these gaps. On the interior of the clone, pre-existing nodes are present, thus negating the need for long rhizomes. Without disturbance, longer rhizomes are produced as new ramet production is focused on the exterior of the clone, necessitating longer rhizome lengths. This consolidation of interior space is consistent with the finding that the phalanx, monoculture growth form more commonly occurs in communities with low nitrogen and water availability (Song et al., 2002).

For ramet growth, no significant differences between integrated and non-integrated clones were observed at any point during this experiment. This suggests that integration is not important for individual ramet height growth. Given the high light environment of the Fall-Line sandhills, with the accompanying high soil temperatures, it may be that vertical growth is not an important component of overall clonal growth. This reflects the hypothesized tradeoff between spatial expansion and shoot competition (Sakai, 1995). The low availability of soil nutrients and water in the sandhills environment may create the need for plants to emphasize the acquisition of these resources through horizontal growth of rhizomes and increased asexual reproduction, rather than compete for a highly abundant light resource through stem elongation (Stalter, 1984).

Other habitats, such as grassland and wetland communities, have experienced a similar shift from above-ground to below-ground production due to grazing of clonal plants (Tobler et al., 2006; Milchunas et al., 1992). In a grassland community, with many clonal species, defoliation due to grazing has been shown to shift the emphasis of plant competition from above-ground to below-ground (Casper and Jackson, 1997), while other studies show grassland competition to be mainly below-ground even without disturbance (Milchunas et al., 1992; Wilson and Tilman, 1991, 1995). In these communities, above-ground competition may occur only if nearby plants grow sufficiently high to cause shading and thus induce above-ground competition for light (Peltzer and Wilson, 2001). Much like grasslands, the sandhills habitat is a high-light environment where woody shrubs may not experience much of a shading effect, reducing competition for light. It has further been hypothesized that plants in stressful habitats, like the sandhills, may be more subject to the impact of herbivory than plants in more productive ones due to the lower availability of resources for plant response (Grime, 1979). This could further shift the plants' response away from above-ground, shoot growth and towards below-ground, rhizome growth.

Disturbance recovery for clonal plants may vary with several different factors. The ability of the plant to quickly activate stockpiled resources, the extent of physiological integration, and the severity of the disturbance all can alter recovery (Pitelka and Ashmun, 1985). However, simulated disturbance may not elicit the same result from plants as actual herbivory (Hartley and Lawton, 1987). Significant differences between artificial and natural defoliation responses have been shown, although it is unknown if any such differences exist with clonal species (Capinera and Roltsch, 1980). This study's results suggest that integration is important in clonal woody shrubs of the southeastern Fall-Line sandhills under normal environmental conditions, as well as under conditions with intermediate levels of disturbance.

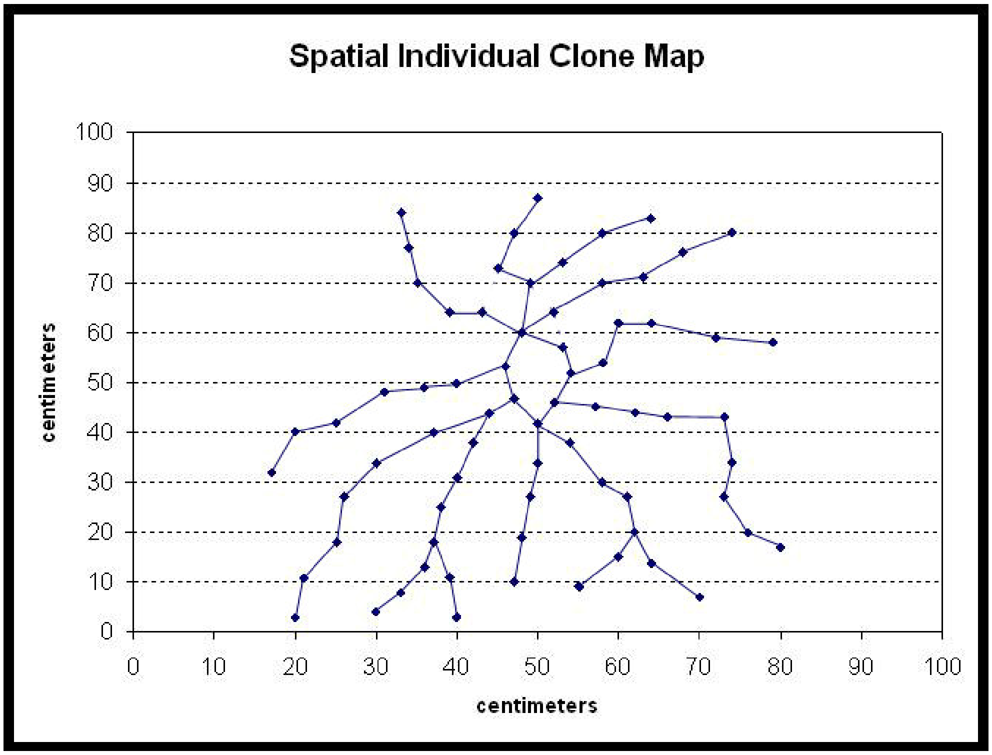

Figure 2.1: Example of an integrated clone mapped after the final measurements. Each diamond represents a ramet while the lines connecting the diamonds represent rhizomes.

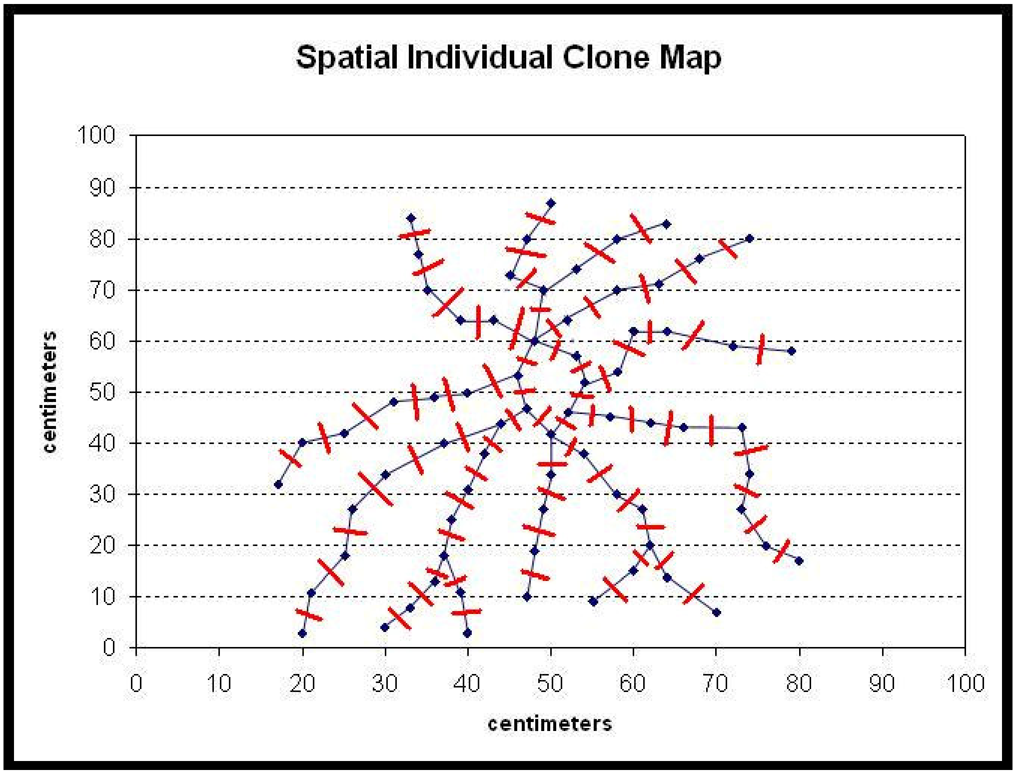

Figure 2.2: Example of a non-integrated clone mapped after the final measurements. Each diamond represents a ramet while the lines connecting the diamonds represent rhizomes. Red lines represent each rhizome being trenched, though in reality all trenching occurred for the full 360° surrounding each ramet.

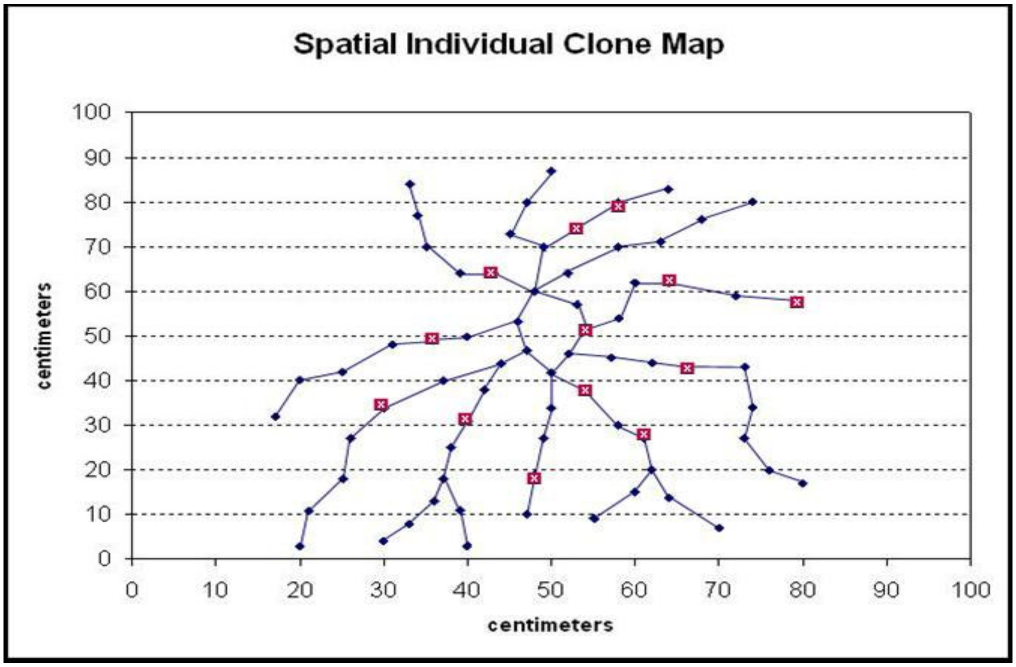

Figure 2.3: Example of an integrated clone mapped after final measurements. Each diamond represents a ramet while the lines connecting the diamonds represent rhizomes. Red X's represent ramets with above-ground biomass removed. Simulated herbivory treatments varied in the percentage of aboveground biomass removed (i.e. with 25% disturbance, 25% of ramets and their aboveground biomass was removed).

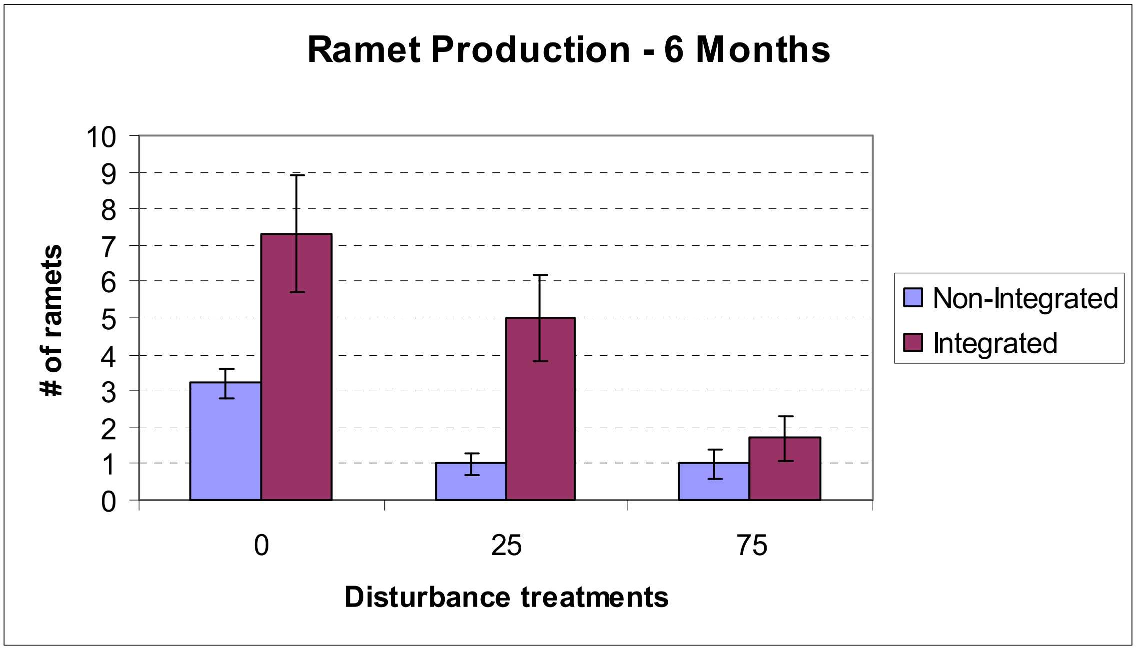

Figure 2.4: Ramet production at six months in integrated clones versus non-integrated clones in the 0, 25, and 75% ramet removal treatments

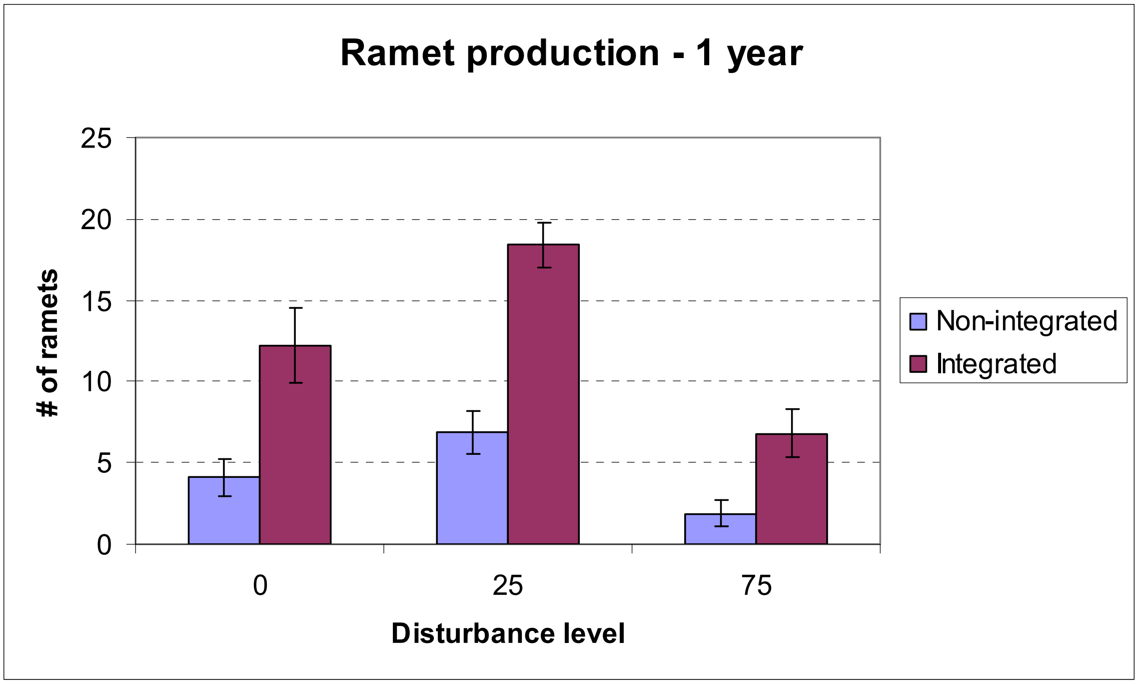

Figure 2.5: Ramet production at one year in integrated clones versus non-integrated clones in the 0, 25, and 75% ramet removal treatments

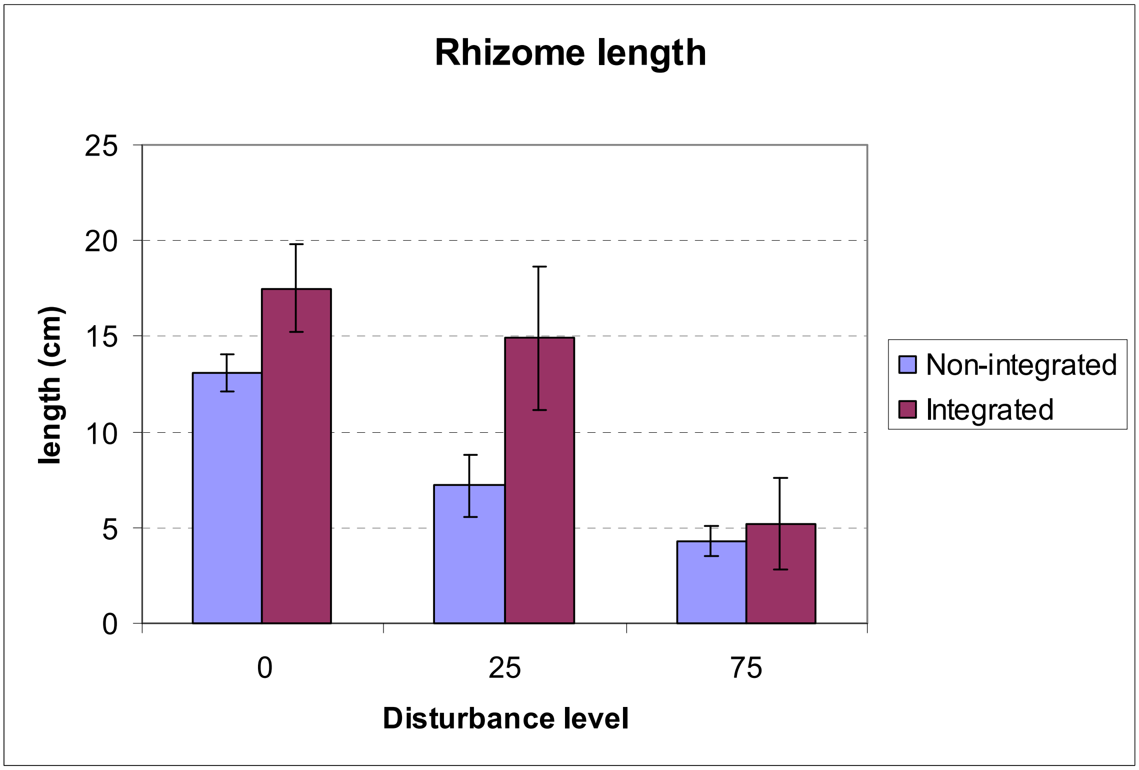

Figure 2.6: Rhizome length at one year in integrated clones versus non-integrated clones in the 0, 25, and 75% ramet removal treatments

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Chapter Three

Clonal integration and competition intensity in *Vaccinium stamineum*

Introduction

Clonal growth has been mainly ignored when considering competition among plants; many competition models do not incorporate the clonal aspect of plants (Tilman, 1982; Grime, 1977). Even most studies of habitats with typically high percentages of clonal plants, such as grasslands, do not consider the differences between clonal and non-clonal plants (Tilman, 1982; Monson et al., 1983). In fact, many studies of plant interactions that include clonal plants have actually used individual ramets and have ignored clonal integration completely (Wilson and Tilman, 1993; Goldberg, 1987). Given the possible repercussions of clonality, it is surprising that, as recently as 1997, clonal plant competition had not been thoroughly studied (Herben & Hara, 1997). Of the few studies that have examined competition in a clonal context, most have done so primarily with shoot competition and have not considered the below-ground aspects of competition (Suzuki and Hutchings, 1997). Even now, there are few papers that explicitly deal with the topic of integration in clonal plants (Gough et al., 2002; Oborny et al., 2000; Oborny & Kun, 2001; Hartnett & Bazzaz, 1985) in a competition context, and most studies that address this topic focus on herbaceous species.

The results of integration, sharing of resources among connected ramets, as understood from studies of herbaceous plants, may not be extrapolated to woody plants since herbaceous clonal plants differ greatly from their woody counterparts. The woody species are typically longer-lived, larger, and more durable (Jenik, 1994), and root grafting and root sprouting are also more likely in woody plants (Peterson & Jones, 1997). The residence time of physical connections also differs between herbaceous and woody species, with herbaceous species varying from less than a growing season to several years, while woody species' connections may be maintained for decades (Pitelka and Ashmun, 1985).

Due to these differences, clonal woody and herbaceous plants should not be expected to behave equally, and integration may not convey the same advantages and disadvantages. Integration can provide many benefits, such as the buffering of heterogeneous environments, regulation of competition with other ramets of the same clone, and risk aversion (Jonsdottir and Watson, 1997). A greater buffering against resource heterogeneity in low availability environments, increased overall genet survival, and acquiring resources more effectively than non-clonal plants are all potential advantages for clonal plants (Hutchings and Bradbury, 1996). The possible specialization of ramets may also be an added benefit of clonal integration (Alpert and Stuefer, 1997; Charpentier and Stuefer, 1999). Such specialization of ramets allows a clone to take advantage of and more fully exploit whatever resources are locally abundant in order to share resources that might be scarce in a heterogeneous environment. Furthermore, clonal plants can retain control of their own occupied area through multiple years, especially if under phalanx growth form (Suzuki and Hutchings, 1997; Midorikawa, 1959), and thus have a competitive advantage. The use of belowground storage organs, such as rhizomes, allows clonal plants to rapidly produce new above-ground material at the start of a growing season, thus deterring invasion by other species. Costs of integration range from possibly increasing disease transmission to reduction of sexual reproduction (Stuefer et al., 2004; Wennstrom, 1999; Klimeš et al., 1997). The cost associated with both the transport of resources and the maintenance of the physical connections among ramets may also be disadvantageous to clonal plants (Alpert and Stuefer, 1997).

The advantages of clonal integration may be more pronounced in extremely harsh environments or in areas of low plant productivity (Stuefer et al., 1996; Brewer and Bertness, 1996; Evans and Cain, 1995; Jonsdottir and Watson, 1997). In the Arctic vascular flora, for instance, over 99.9% of species exhibit clonal growth (Jonsdottir et al., 1996). The Fall-Line sandhills of the southeastern United States is a high-light environment with low nutrient and water availability, a habitat where clonal advantages may also be more pronounced. *Vaccinium stamineum* L. is a clonally woody shrub commonly found in these sandhills, and is an ideal species for the study of competition and integration in woody plants.

We hypothesized that integrated clones of *V. stamineum* would experience greater survivorship, more asexual reproduction, and greater ramet growth than clones in which ramets were separated (non-integrated). The absence of competition was hypothesized to have a beneficial effect on clones, with comparable results for both integrated and non-integrated clones.

Materials and Methods

Vaccinium stamineum is one of the more common woody shrubs found in the Fall-Line sandhills, and is considered characteristic of the region (Godfrey, 1988). This species is closely related to *V. corymbosum*, blueberries, and *V. macrocarpon*, cranberries, which are highly valued for their food crop value. *Vaccinium stamineum*'s primary growth form is characterized as "phalanx", creating mono-specific patches where few, if any, other species occur (Lovett-Doust, 1981). As younger, less-established plants, they form spatially discrete patches, and clonal growth appears to occur at the spatially-separated, patch level (Kreher, et al., 2000). Intermingling between different patches may occur as they become older and larger. High genetic diversity can occur within intermingled patches (Kreher et al., 2000), though as these plant populations become older, this may decrease due to increased vegetative reproduction (Erikkson, 1993).

Vaccinium stamineum's range includes middle and southern Canada and extends down to the northern region of Florida (Gleason, 1952). It stretches west to Texas and Minnesota, though unconfirmed reports put the species as far south as Mexico and as far west as Montana. Throughout this vast range, great variability is exhibited in both the species' morphology and its abundance. When originally classified, it was grouped as twenty-one different species (Ashe, 1931). Regional abundance also varies, with *V. stamineum* listed as a threatened plant species in northern Ontario, as well as in at least one mid-western state in the United States, Illinois. Throughout its' extensive range, *V. stamineum* reproduces almost exclusively through clonal means, with rhizomes producing new ramets (Vander Kloet and Cabilio, 1996; Yakimowski and Eckert 2007). It is not known to what degree sexual reproduction may occur and how it might influence the genetic diversity of this species. When sexual reproduction does occur, it requires insect pollination, particularly by bees (Cane et al., 1985).

The study was conducted in Fall-Line sandhills habitat at two locations. One site was an approximately two and a half square kilometer area located in Athens, Georgia and the other was an approximately five-square kilometer area on the Savannah River Site (SRS), a federal facility near Aiken, South Carolina. Given the variability in *V. stamineum*'s morphology, this was designed to determine if different locations yielded different results. The Fall-Line sandhills are a transition zone between the Coastal Plain and Piedmont regions of the southeastern US. These sandhills are characterized by high light levels due to an open canopy, low nutrient resources and sandy deep soils with low water holding capacity. Soil temperatures may exceed 60^{o} C in the hottest summer months (Wells and Shunk, 1931). Oaks, including *Quercus laevis*, *Q. margaretta*, and *Q. incana* are common sandhills tree species. In addition to *V. stamineum*, *Gaylussacia dumosa* is an abundant clonal shrub, and a wide variety of herbaceous species including the fern *Pteridum aquilinum* commonly occur (Workman and McLeod, 1990). Longleaf pine communities, with *Pinus palustris* and *Aristida stricta* as the dominant canopy and ground cover species also occur in sandhills areas.

Competition Experiment

Evidence of resource movement among ramets of *V. stamineum* was found in an early pilot study utilizing an acid fuchsin dye, which moves through the xylem, and an herbicide (Duval, personal observation). Movement was mostly from older to younger ramets (acropetal), however some movement from younger to older ramets (basipetal) did occur. This evidence, from both the dye and the herbicide, indicates that the physical connection of ramets allows for potential sharing of resources. Thus, resources taken up by individual ramets may be shared with other ramets within the genets, and there is a potential for differential performance between integrated (connected) and non-integrated (unconnected) clones.

While *V. stamineum* patches may grow to cover tens of meters, this study used plants that were approximately 1m x 1m to 2m x 2m. These clones tend to be younger, more recently established, and are more logistically tractable than their older, larger counterparts. These were further restricted so that no other woody species were growing in the interior of the clones, to control for any extraneous competition effect other than the prescribed treatments. A pool of over 120 clones fit within these parameters. Thirty-six were selected at random, 18 in Athens and 18 at the SRS, and then randomly assigned to a competition and integration treatment. Half at each location were designated as competition absent, while half were designated as those with natural levels of competition (competition present). Within each of these 18, half were integrated and half were non-integrated

To test the effects of competition and integration, clones of *V. stamineum* were exposed to four different treatments, with nine clones per treatment combination: 1) competition absent, non-integrated; 2) competition absent, integrated; 3) competition present, non-integrated; and 4) competition present, integrated. The competition absent treatments were created by establishing a 2m buffer zone around each *V. stamineum* patch in which all above ground biomass (both woody and herbaceous) was clipped, and the herbicide Round-Up® applied to the remaining stems. Round-up® was chosen because of its' short half-life and low mobility in the soil, thus minimizing the possibility of adverse effects on *V. stamineum* itself. At the 2m border of the buffer zone, trenching was conducted to minimize underground competition as well. The clones in the competition present treatments were simply left as they naturally occurred, coexisting with ordinary levels of woody and herbaceous species nearby. The number and type of competitors surrounding the clones in this treatment were fairly consistent.

For the clones in the non-integrated treatments (without and with competition), the rhizome connections between ramets were severed, as this species naturally grows with these connections intact. This was achieved by trenching 360° around each individual ramet. This treatment has been determined to have a negligible effect on ramet mortality (Duval, personal observation). Given that the clones are naturally integrated, no severing of rhizomes was done to plants in this treatment. However, soil was disturbed in a manner similar to the non-integrated treatments without the actual severing of rhizomes. This allowed for the control of any effect on clone performance by soil disturbance during the rhizome severance.

This experiment was started in spring 2005 and continued for two years. Measurements were taken at the beginning, as well as at six month intervals until the completion of the experiment. All clones in the study were mapped to the centimeter so that each ramet within the clone had a given coordinate on an X/Y map. This was repeated at the six month intervals. Above-ground length was also measured to determine the overall size of each ramet. Many ramets, however, do not grow vertically, but rather at an angle. Ramet weight was examined in a separate study to determine if this could serve as a proxy for ramet length; however the relationship between ramet weight and length was not sufficiently strong to use ramet weight solely (r^2~.50; Duval, personal observation). Thus, ramet length was measured at all time intervals and ramet weight was subsequently measured at the final destructive harvesting of the clones. The length of each individual rhizome was also measured during the final harvesting. This allowed for a component of the underground biomass to be measured.

The SAS statistical package was used to conduct the statistical analysis for this experiment (release 6.12, 1997, SAS Institute, Cary, N.C.). The repeated measures ANCOVA procedure was used to examine the effects of competition and integration on the performance of *V. stamineum*, thus the means reported are adjusted means. A combined measurement, the total number of initial ramets and the total length of initial ramets, was used as the covariate for this procedure.

Results

There were no significant differences between *V. stamineum* clones in Athens and those at the SRS ($F= 0.07$, $p<0.79$). This included all measurements and time periods. Thus, the Athens and SRS treatments were pooled and examined as one larger group for greater statistical power when examining the effects of competition and integration. Throughout the experiment, rhizome length was also not significantly different in any of the integration or competition treatments.

Ramet production

Integrated versus non-integrated

When simply grouped into integrated and non-integrated treatments, integrated clones produced significantly greater numbers of new ramets than non-integrated clones throughout the course of the experiment (figure 3.1). At every time interval there were significant differences between integration treatment levels (F=5.78, p=0.027). During the first six months, integrated clones produced an average of six new ramets per clone; was the largest increase of either integrated or non-integrated treatments in the experiment. In these integrated clones, four more new ramets, on average, were produced in the second six months, and approximately one and two new ramets were produced in the final two six-month periods, respectively. This totaled to slightly more thirteen new ramets per clone during the experiment's twenty-four month duration. Non-integrated clones produced approximately two new ramets during each of the first two six-month periods, and about one new ramet during each of the last six month periods. Thus, the total ramet production of non-integrated clones was, on average, six new ramets per clone. The final difference in ramet production between integrated and non-integrated treatments was approximately seven ramets.

Competition present versus competition absent

When simply grouped into competition present versus competition absent treatments, the clones with competition removed produced significantly greater numbers of new ramets at all measurement times (figure 3.1; F=4.96, p=0.033). The single greatest increase occurred in the first six months in the clones without competition, with an average of six new ramets produced per clone. Four new ramets were produced by the clones with competition absent in the second six month period, declining to approximately one new ramet in the following two six-month periods. Thus, the clones without competition produced approximately thirteen total new ramets during the course of the experiment. For clones with competition present, two new ramets, on average, were produced per clone in the first two six-month periods. One new ramet was added in each of the final two six-month periods, totaling slightly under six new ramets added per clone Thus, clones with competition removed produced approximately seven more new ramets during the two years than clones with competition present. There was little growth of competitors into the created buffer zones surrounding the competition absent treatments during the experiment.

Combined integration and competition treatment contrasts

Clones in the integrated, competition-absent treatment produced the greatest number of new ramets throughout the experiment (figure 3.2). These numbers were significantly different from all other treatment levels at all time periods. Ten new ramets were produced by these clones in the first six months of the experiment, the greatest increase in new ramets of any individual treatment during any time period. This increase declined in magnitude to four new ramets produced in the second six-month time period, and approximately one and two new ramets in the final two six-month periods. Thus, a total of slightly under seventeen new ramets were produced by integrated clones in the competition-absent treatment; this was the most extreme level of new ramet production.

Two treatment groups were similar to one another in their intermediate responses with respect to new ramet production. Integrated clones with competition present and non-integrated clones with competition absent produced essentially the same number of new ramets during all time periods (figure 3.2). Slightly over three new ramets were produced in the first six months, and three more from six to twelve months, totaling slightly over six new ramets for the first year of the experiment. This declined in the second year, as slightly over one new ramet was produced in each of the final two six-month periods. These treatements did not differ significantly from one another at any time period, but were significantly different from both the high magnitude response of the clonally integrated, competition-absent clones and significantly more than non-integrated clones with competition present.

The non-integrated clones with competition produced the lowest numbers of new ramets at every time period, with approximately two new ramets per clone at the end of the experiment (figure 3.2). In each six-month period, approximately 0.5 new ramets per clone were produced. The interaction factor between integration and competition was not significant ($F=0.55$, $p>0.465$).

Ramet length

No significant differences in ramet length (original and new ramets) existed between the competition or integration groupings during the experiment (figure 3.3). Furthermore, the four individual treatments also showed no significant differences (figure 3.4). In all treatments there was an increase in ramet length between three to five centimeters in the first six months. Ramet length continued a general linear increase throughout the experiment, ending with a cumulative eight to nine centimeter increase at the end of the experiment. It is interesting to note that the greatest increase in ramet length occurred in the initial response to the treatments and the magnitude of the increase declined thereafter.

Ramet weight

The weight of ramets (original and new), measured via destructive sampling at the end of the experiment, showed significant differences between the competition and integration treatments (figure 3.5). *Vaccinium stamineum* clones in the competition absent treatment had significantly heavier ramets than clones with competition present (F=9.79, p=0.004). Without competition, clones had, on average, ramets that weighed approximately 37g; these were roughly 13g heavier than those with competition present. In addition, integrated clones had significantly heavier ramets than their non-integrated counterparts, with a difference of slightly under 10g. Integrated clones had an average ramet weight of 35g; ramets of non-integrated clones weighed an average of about 25g.

The individual treatments showed variation from one another. Integrated clones with competition absent produced the heaviest ramets, significantly greater than all other treatments (figure 3.6). This treatment group had an average ramet weight of 43g, contrasting with the average ramet weight of 26g in integrated clones with competition present. Non-integrated, competition present clones produced the smallest average ramets weighing slightly over 20g. Non-integrated clones with competition absent fared better with ramets weighing an average of 31g, a difference of over 10g between the two treatments. The non-integrated, competition present treatment was significantly lower than all other treatments, and the integrated, competition absent treatment was significantly higher. The integrated, competition present and non-integrated, competition absent treatments were not significantly different from one another.

Discussion

Many studies have examined clonal integration and how this affects plant growth under differing competition regimes in herbaceous plants (Price and Hutchings, 1996; Hartnett and Bazzaz, 1985; Schmid and Bazzaz, 1987; Pennings and Callaway, 2000; Amsberry et al., 2000; de Kroon and Kalliola, 1995). For woody species, competitive ability and integration has been little studied (Peltzer, 2002). The competitive ability of clonal plants, defined as the ability to invade stands of other plants through ramet growth and reproductive means (Herben, 2004), can alter a variety of ecosystem processes. However studies of both herbaceous and woody plants show contradictory evidence with no clear patterns emerging. In herbaceous species, rhizome severing has often produced an initial increase in plant growth and asexual reproduction (Schmid and Bazzaz, 1987; Pauliukonis and Gough, 2004), though this was not found in our study. Integration in herbaceous species has been shown to convey an advantage with respect to photosynthetic activity, buffering plants against competition stress (Wang et al., 2008; Roiloa and Retuerto, 2006). The effects of clonal integration also have been found to vary in importance from habitat to habitat, suggesting that the various results may be location-specific (Pennings and Callaway, 2000). In our study, however, the lack of significant differences between clones from Athens, GA and the SRS in SC suggests that this may not be the case with *V. stamineum* in the southeastern US.

Our results show that *V. stamineum*'s new ramet production and ramet weight responses are affected by both integration and levels of competition. These results support our hypothesis that integration is important for asexual reproduction and ramet growth as measured by weight. Competition had a similar effect on the performance of *V. stamineum* as well, altering the same characteristics. The treatment contrasts show a definite increase in new ramet production in both integrated and non-integrated clones when competing plants are removed. Asexual reproduction was lowest in non-integrated clones when competition was present and highest in integrated clones when competition was removed. The lack of a significant difference between competition absent, non-integrated clones and competition present, integrated clones suggests that integration and competition have similar effects on clones of *V. stamineum* with regards to asexual reproduction.

These results agree with results from chapter two of this dissertation, supporting the hypothesis that integration is important for asexual reproduction and horizontal growth in *V. stamineum*, but not for vertical growth. These results also agree with many studies which suggest that in heterogeneous environments, such as the Fall-Line sandhills, integration in clonal plants increases both their survival and growth (Pitelka and Ashmun, 1985; Wijesinghe and Handel, 1994). Furthermore, in habitats with low resource availability, the advantages of clonal integration are more often thought to outweigh the disadvantages (Stuefer et al., 1994; Evans, 1992). Integration also has been hypothesized to be more highly employed in high stress environments (Pennings and Callaway, 2000). The Fall-Line sandhills are high stress habitats with low nutrient resources that are subject to normal environmental heterogeneity. These characteristics suggest that clonal integration may be more beneficial for *V. stamineum* in the sandhills than in other habitats where it occurs. In addition, other studies have shown that in "phalanx" type clonal plants, such as *V. stamineum*, integration may be more important for ramet growth than for their "guerilla" life-form counterparts (Schmid and Bazzaz, 1987).

This study indicates that competition is a significant factor in the asexual reproduction of *V. stamineum* (figures 3.1 and 3.2). Most asexual reproduction occurred in the first months following competition removal. This quick, initial response is a common characteristic of clonal plants (Fahrig et al., 1994), and the rapid activation of resources by storage rhizomes is hypothesized to be a strong advantage for them (see chapter two; Klimes et al., 1993; Fahrig et al., 1994). In this experiment, the differences in asexual reproduction between clones with and without competition declined over time. This declining trend suggests that if the experiment were to run for a longer period of time, eventually there might be no difference in new ramet production between competition treatments.

In this study, like many others, integration seems to have a positive effect on asexual reproduction (Brezina et al., 2006; Hartnett and Bazzaz, 1985; Dong, 1999). It is interesting to note that the interaction between competition level and integration was non-significant, as found in another study of a clonal woody species (Peltzer, 2002). This means that, regardless of the integration status of the plant, removal of competitors elicited the same response, and the competitive ability of *V. stamineum* was unaffected. Thus, integration improved the growth and reproduction of the plant but not its competitive ability. This reinforces the hypothesis that integration in clonal plants may be more important for exploiting open space and tolerating stressful environments (Peltzer, 2002; Pennings and Callaway, 2000) than for increasing their competitive ability.

Computer modeling has suggested that when the cost of resource transportat among ramets is low, integrated plants should have greater competitive ability than non-integrated, non-resource transporting plants (Herben, 2004). The lack of a significant effect on competitive ability in *V. stamineum* suggests that resource transportation, while found to occur, may incur such a high cost that it is inhibited. If the cost of resource transportation among ramets were prohibitively high, one might expect any plant response to be due more to the local mobilization of rhizome resources. This, however, would not explain the differences in asexual reproduction found in this experiment. The greater production of new ramets by integrated clones would suggest that resource transportation through the rhizomes is occurring.

No significant differences in rhizome length were found with respect to either integration or competition treatments. Most other studies that have found significant differences in rhizome length do so with varying nutrient conditions (Slade and Hutchings, 1987; Cain et al., 1996; Evans, 1992; Turkington and Klein, 1991). While some studies have examined rhizome length relative to competition, these results are conflicting and lead to no clear generalizations, varying with both plant genotype and environment (Price and Hutchings, 1996; Schmid and Bazzaz, 1992; Cheplick, 1997). In the sandhills, high-resource patches may be too low in frequency to offset the cost of longer rhizomes, as suggested in studies of some grasses (Humphrey and Pyke, 2001). The sandhills environment may be simply too resource-poor for *V. stamineum* plants to invest in longer rhizomes under non-disturbance conditions.

Ramet lengths were not significantly different from one another in the various treatments throughout the experiment, suggesting that neither integration nor competition affect this aspect of *V. stamineum*'s growth. Computer modeling has suggested that clonal integration may be most important in heterogeneous environments (Oborny et al., 2000). This has been backed up with empirical data demonstrating that the more heterogeneously distributed a resource is, the more important integration is to clonal plants (Pennings and Callaway, 2000; Stuefer et al., 1994). In the sandhills, light is not only a homogeneous resource, but an abundant one, as there is limited shading. Thus, integration does not provide an advantage with regards to ramet length. Greater ramet length, with the accompanying increase in vertical growth, may not be a growth priority when there is no scarcity of light.

While there were no significant differences in ramet length, both competition and integration were found to have a significant effect on ramet weight. This difference between ramet length and weight is a surprising one and has not been found in other studies. This seems to indicate that, while individual ramets do not experience differential height growth, they do produce different amounts of branches and leaf biomass under various conditions. Though the clones of *V. stamineum* grow in a monoculture, phalanx-type growth form, these results would indicate that room exists on the interior of the clone for above-ground biomass expansion. It could also be that the plant does not regulate intraclonal competition, which has been suggested as an advantage of clonal plants (Lovett-Doust, 1981).

As with asexual reproduction, there was no significant interaction between integration and competition. Thus, competitive ability is not affected by integration treatments in any of the metrics used in this experiment. If competitive ability of *V. stamineum* had been altered by integration, the slope of the asexual reproduction responses would have been altered under the two differing integration treatments (figure 3.2). Integration alters the reproductive and ramet growth capabilities of the plant, but does not make the clones more able to compete with neighbors. Clonal plants have varied greatly in their response to competition treatments (Hartnett and Bazzaz, 1983; Dong, 1999; Hartnett and Bazzaz, 1985; Pennings and Callaway, 2000). A similar study with a clonal tree species showed that integration did not change competitive ability (Peltzer, 2002). This same study suggested that competition had more influence than integration on ramet performance, something not found in our study, where both seemed to have an equal impact on asexual reproduction and ramet weight. Our findings indicate that integration is most important for both asexual reproduction and ramet weight in *V. stamineum*.

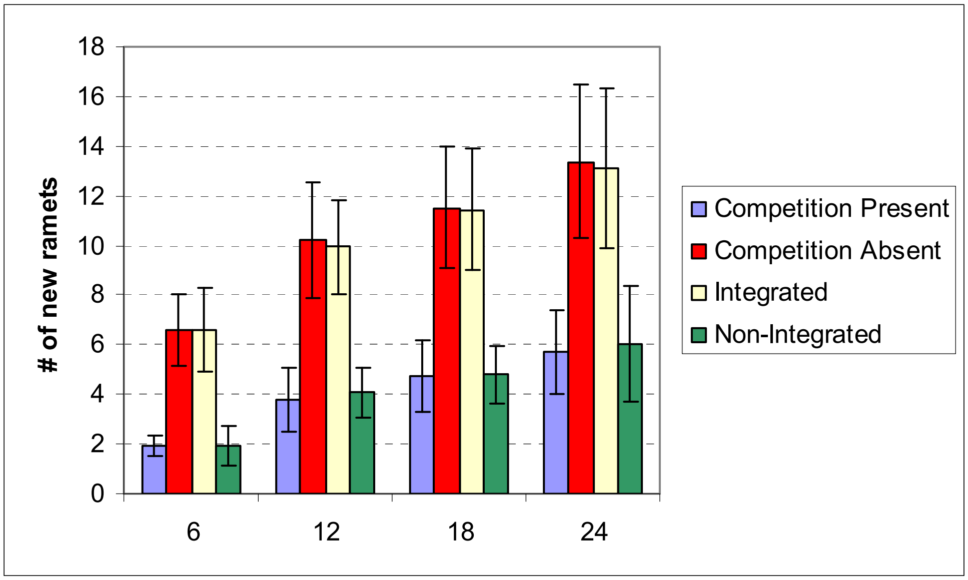

Figure 3.1: New ramet production of clones grouped into two contrasts: competition present versus competition absent and integrated versus non-integrated clones.

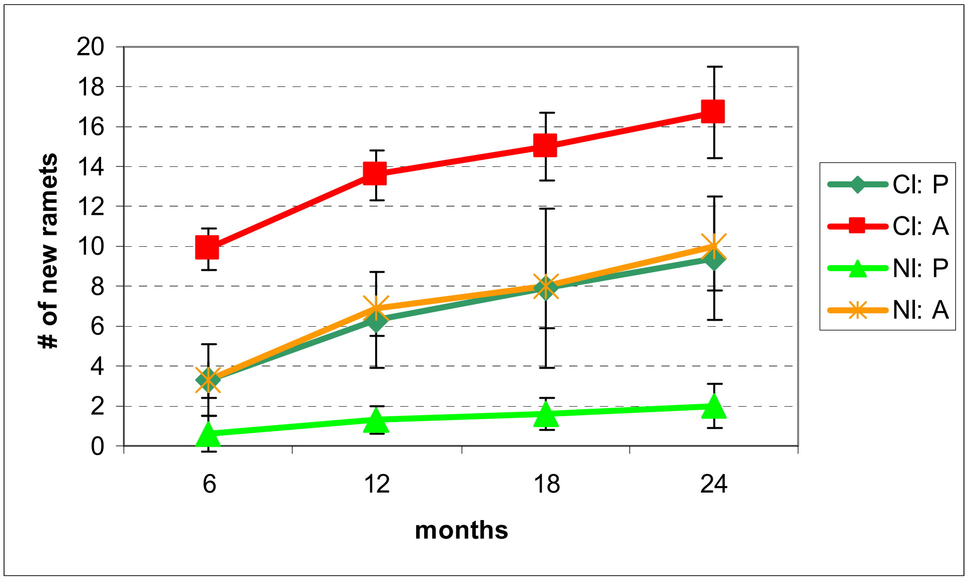

Figure 3.2: New ramet production with individual treatment contrasts.
(CI: P = Clonally integrated with competition present; CI: A = Clonally integrated with competition absent; NI: P = Non-integrated with competition present; NI:A = Non-integrated with competition absent)

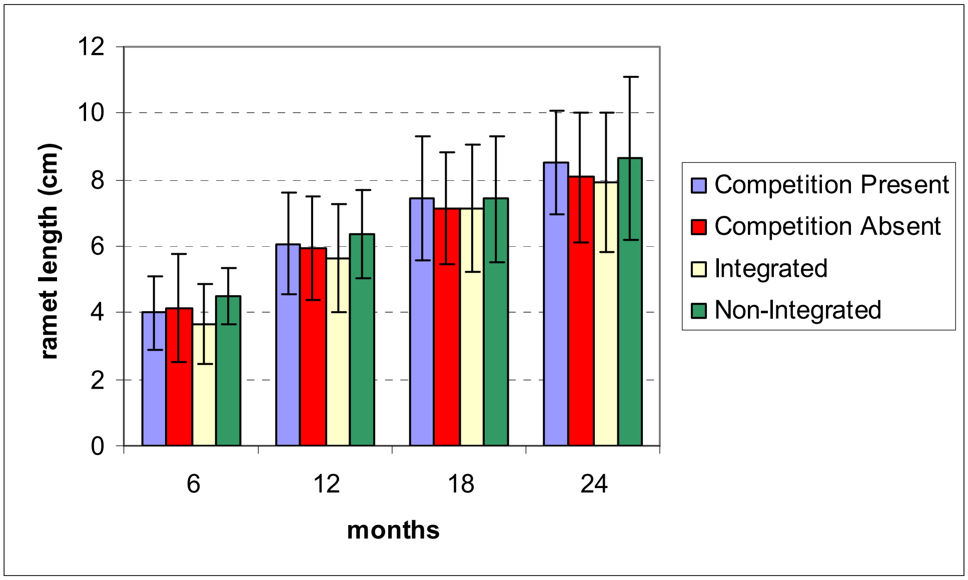

Figure 3.3: Ramet length with clones grouped into two contrasts: competition present versus competition absent and integrated versus non-integrated clones.

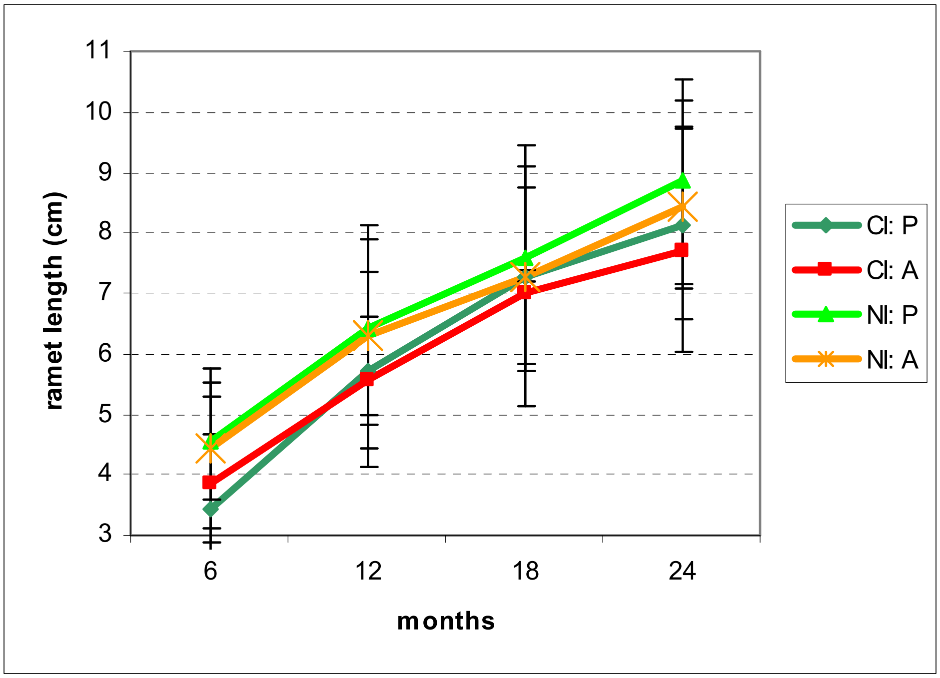

Figure 3.4: ramet length with individual treatment contrasts
(CI: P = Clonally integrated with competition present; CI: A = Clonally integrated with competition absent; NI: P = Non-integrated with competition present; NI:A = Non-integrated with competition absent)

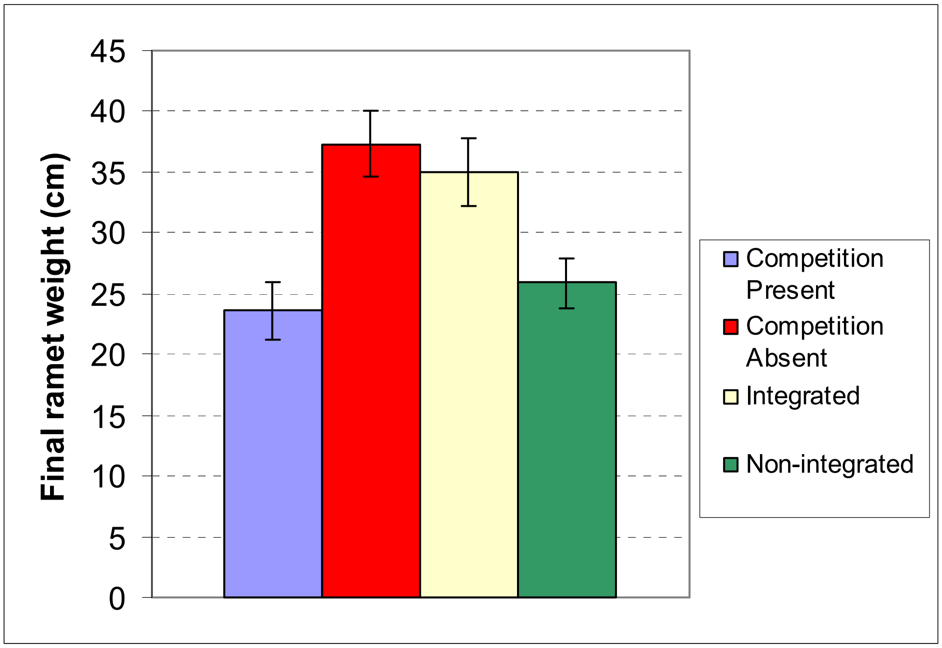

Figure 3.5: Average ramet weight of clones at the final, destructive sampling grouped into two contrasts: competition present versus competition absent and integrated versus non-integrated clones.

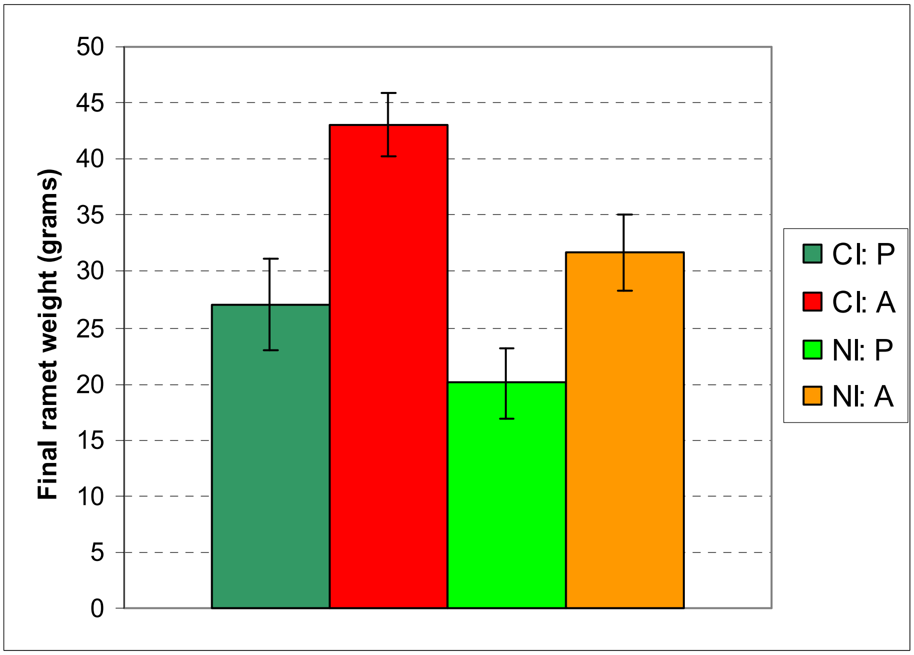

Figure 3.6: Ramet weight with individual treatment contrasts at the final destructive sampling (CI: P = Clonally integrated with competition present; CI: A = Clonally integrated with competition absent; NI: P = Non-integrated with competition present; NI:A = Non-integrated with competition absent)

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Chapter Four

Nitrogen addition and integration in *Vaccinium stamineum*

Introduction

Clonal growth is a very common means of reproduction in plants throughout the world. The angiosperm flora in Europe is estimated to be made up of almost seventy percent clonal organisms (Klimes et al., 1997). Various other environments, from Arctic tundra to temperate and tropical wetlands, also contain high numbers of clonal species, and quite often a large percentage of the flora of a habitat may be clonal (Prach and Pysek, 1994). One of the main characteristics of clonal plants is the potential for physiological integration between ramets within a genet. This integration may provide several advantages. First and foremost, integration allows the sharing of resources among ramets (Pitelka and Ashmun, 1985). This sharing of resources by transportation through rhizomes and stolons may buffer against environmental heterogeneity (Jonsdottir and Watson, 1997). Resource sharing can also alter growth and survivorship of the plant, possibly allowing it to exploit better habitats (Lovell and Lovell, 1985) or speed defensive signaling against herbivore damage (Stuefer et al., 2004). Integration among ramets may also allow for the division of labor among them (Alpert and Stuefer, 1997) or directional foraging for resources (Oborny and Cain, 1997; Stuefer et al., 1996).

While the hypothesized advantages are many, there are also disadvantages due to clonal growth. Both maintenance of the physical connections and resource transportation may be major costs for clonal plants (Hutchings and Bradbury, 1986). For example, a study of two herbaceous wild strawberry species found the maintenance of physical connections to be a significant cost (Jurik, 1983). These physical connections may also speed disease transmission among ramets

(Stuefer, 2004). Since vegetative reproduction produces a genetically identical daughter ramet from a parent ramet, decreased genetic variation may also occur in species with a high proportion of clonal growth.

While the topic of clonal plant performance under various resource conditions has been studied quite well in herbaceous species, studies of woody species are conspicuously absent. Herbaceous plant studies have shown a variety of results and have advanced several hypotheses on the differences in performance under various resource conditions (Birch and Hutchings, 1994; de Kroon et al., 1991; D'Hertefeldt and Jonsdottir, 1994; Evans, 1992). While these studies of herbaceous species have pushed the boundaries of integration and resource research, they have left behind a gap in the study of woody species. Woody species are quite different from herbaceous species in many cases, although it is easy to understand why herbaceous species have been the focal point of clonal research and woody species have remained largely unstudied. Herbaceous plants tend to be smaller, have shorter generation times, and are typically easier to manipulate experimentally. Woody plants may have greater biomass, varying clone architecture, and much longer lifespans than their herbaceous counterparts. Many herbaceous species have life spans that last for one growing season or less (Cook, 1985), though some may maintain active underground connections for several years (Jonsdottir and Callaghan, 1988; Pitelka and Ashmun, 1985). In woody species, both life span and connection time tend to be much longer, with one species, *Populus tremuloides* potentially living for over one hundred years (Barnes, 1966).

Furthermore, the number of ecosystems in which integration in clonal plants have been studied remains rather small (Jonsdottir & Watson, 1997). Given that highly disturbed and more stressful, nutrient poor environments are more likely to contain greater numbers of clonal plants

(Klimes et al., 1997), this is not surprising. While generalizations are being drawn from a few, model clonal organisms in a rather limited number of habitats, these studies often do show that different habitats may produce different results regarding the importance of integration (Pennings and Callaway, 2000; Oborny and Kun, 2001). Thus, it is not known to what extent these generalizations hold true in various habitats, under differing conditions.

This study seeks to expand the field of clonal research by examining a woody plant species and also by studying this species in a habitat that has been largely ignored with regards to clonality. It examines how a clonal woody shrub, *Vaccinium stamineum* L., is affected by integration under differing nitrogen regimes in the Fall-Line sandhills of the southeastern United States. Many of the current hypotheses on clonal development focus on homogenous and heterogeneous distributions of resources, since at the scale of both the ramet and genet, resources are rarely homogeneous. While heterogeneity versus homogeneity in resource availability may be an important factor for the growth of both individual ramets and clones as a whole, it was not explicitly examined in this study. Instead, we hypothesized that integrated clones would produce significantly greater numbers of new ramets (asexual reproduction), as well as longer and heavier ramets, than non-integrated clones in which rhizome connections no longer existed. Integrated clones were also hypothesized to produce significantly longer rhizomes than non-integrated clones. We also hypothesized that clones growing under elevated levels of nitrogen would have significantly greater asexual reproduction, with heavier and longer ramets, than clones growing under lower nitrogen levels. Finally, because of the concept that foraging for resources may affect clonal development, we hypothesized that clones in elevated soil nitrogen treatments would produce significantly shorter rhizomes so as to extend the plants' residence time in the favorable habitat, than clones in areas of lower nitrogen levels.

Materials and Methods

This study's materials and methods are very similar to those in both chapters two and three of this dissertation. *Vaccinium stamineum* was studied in the southeastern sandhills and measurements were taken similar to those in the first two experiments during the same time period.

Our field site was located on the Fall-Line sandhills of the southeastern United States, on the Savannah River Site (SRS), a federally managed facility near Aiken, South Carolina. These sandhills form the boundary between the Coastal Plain and the Piedmont regions. Fire suppression post-European colonization has altered the sandhills plant community in many places (Christensen, 1988). The areas at the SRS have undergone prescribed burning and other forest management practices for over fifty years, and currently are burned every five to ten years to prevent the invasion of hardwoods into the habitat. These sandhills may be one of the oldest fire-adapted communities in the southeastern US (Wharton, 1978), and contain a suite of characteristic flora, both rare and common (Collins et al., 2006). Many of the management practices are also aimed at preserving endangered animal species in the area, including the red-cockaded woodpecker and the gopher tortoise.

The sandhills have deep sandy soils, low nutrient resources, low water availability, and elevated light levels with very little shade. A sparse canopy is dominated by *Pinus palustris* and *Quercus laevis*, along with *Q. margaretta* and *Q. incana*. A variety of herbaceous species are common, from grasses such as *Andropogon* and *Aristida* spp. to ferns such as *Pteridum aquilinum* (Workman and McLeod, 1990).

The study species, *Vaccinium stamineum* L., is a woody shrub commonly found in these sandhills. It is considered a characteristic shrub of the southeastern Coastal Plain (Godfrey, 1988), and is common throughout the eastern United States. Its range extends from northern Florida into southern Ontario and as far west as Minnesota and Louisiana. Throughout this extensive range, *V. stamineum* exhibits great variability in its morphological characteristics. It was originally characterized as twenty-one different species (Ashe, 1931). This variability is also reflected in the variability in species abundance from region to region. In northern Ontario and at least one state, Illinois, *V. stamineum* is listed as a threatened species. Throughout the rest of its range, the species is very abundant.

Vaccinium stamineum grows in the "phalanx" growth form, characterized by mono-specific patches with few, if any, other species co-occurring within these patches (Lovett-Doust, 1981). Ramets may grow up to five meters in height, though in sandhills, typical growth is closer to one-half meter (Foote and Jones, 1989). Younger plants tend to be spatially separated from one another, with older patches more likely to intermingle. These older patches can extend for tens of meters and most likely contain several different genets. They also tend to contain high amounts of genetic diversity, possibly due to sexual reproduction or somatic mutation (Kreher et al., 2000), though we observed no evidence of sexual reproduction resulting in viable offspring. Insect pollination is required for sexual reproduction, particularly buzz pollination by bees (Cane et al., 1985). *Melitta americana* is the primary pollinator for *V. stamineum* in New York (Cane et al., 1985), but it is not known what the primary pollinator is in the southeastern US. Flowers appear in late April and early May (Duval, personal observation). Ripe berries of the species are a purplish color and are recorded as appearing from April to October, though in the sandhills this period is generally restricted to the late summer months (Foote and Jones, 1989). Species in the *Vaccinium* genus are mostly self-incompatible (review by Vander Kloet, 1988), with an added barrier of floral morphology in *V. stamineum*, which reduces intrafloral pollination (Reader, 1977). Given these impediments to sexual reproduction, it is not surprising that *V. stamineum* reproduces almost exclusively by means of rhizomes throughout its range (Yakimowski and Eckert, 2007; Vander Kloet and Cabilio, 1996).

Early studies with this species confirmed that resource transport does occur in the rhizomes through the xylem. An acid fuchsin dye and an herbicide, Round-Up®, were used in a pilot study. The results revealed clear evidence of resource transport among ramets of *V. stamineum*. Some basipetal movement occurred, from younger to older ramets, but most movement was acropetal, from older to younger ramets. This movement, both basi and acropetal, shows that resources taken up by a single ramet have the potential to be shared with other connected ramets in a genet. This active sharing of resources allows for potential differential performance between integrated clones with intact rhizome connections and non-integrated clones in which rhizome connections are broken.

This study was conducted over a two year period beginning in the spring of 2005. The study site was a sandhills habitat on the SRS approximately five square miles in size. An initial pool of *V. stamineum* clones was identified. Clones chosen for study were restricted in size to one to four square meters. These smaller clones tend to be younger and are more feasible for experimental study (Duval, personal observation). A second restriction was that no competing plants should be growing on the interior of the clones. Most clones do not have competitors inside their patch perimeters and the few that did were excluded to control for any effect of competition. From the initial pool of *V. stamineum* clones, over 120 met these criteria. Twenty-four clones were randomly chosen for the study and then randomly assigned to the various treatments.

To test whether integration provided a benefit to *V. stamineum*, integrated and non-integrated treatments were created. Clones are naturally integrated with ramets connected by rhizomes, thus nothing was needed for this treatment. To create non-integrated clones, the rhizomes between each of the ramets were severed by trenching. Each of the non-integrated clones had each individual ramet severed by 360° trenching around its base. A negligible effect of such trenching on ramet mortality was found in an early pilot study on this species (Duval, personal observation). In integrated clones, the soil around each ramet was disturbed in a manner similar to trenching without actually severing rhizomes, so as to control for any effect of soil disturbance.

To test the effects of nitrogen addition on clones of *V. stamineum*, two treatments were established. Half of the clones (twelve) were randomly assigned to remain under natural levels of nitrogen. Soils in the sandhills have been found to contain approximately 0.02% total nitrogen in the topmost twenty centimeters (Sharitz, personal communication). The other clones were randomly assigned to treatment with elevated levels of nitrogen. A commercially available, urea ammonium nitrate-based fertilizer was utilized through a water and nitrogen fertilizer mixture at approximately twenty-five kilograms per hectare. An equal amount of water also was applied to the treatment with natural levels of nitrogen. This controlled for any effect the water addition might have on growth and/or survivorship. At the end of the first year, the experiment was reevaluated and it was determined that it was likely that the first fertilizer treatment was lost due to leaching into the sandy soils. Subsequently, in spring 2006, additional fertilizer was added at approximately 34 kilograms per hectare. This fertilizer was not applied with water, but was applied in granule form. Soil cores were taken beneath the clones for several weeks following this second application of nitrogen fertilizer. These soil cores showed the new fertilizer's residence time in the soil to be around six to eight weeks (Duval, personal observation).

All twenty-four clones, regardless of treatment, were mapped so that every ramet in the clones was given X/Y coordinates. Individual ramet lengths were also measured, and ramet weights were determined following harvesting at the end of the study. Though ramets in *V. stamineum* do not typically grow perpendicular to the ground, the combined length and weight measurements give an accurate estimate of the overall size of each ramet. Each clones' ramets were also counted, so that asexual reproduction between time periods could be determined. Each of these measurements except ramet weight was taken at the outset of the experiment, as well as at six month intervals until the final set of measurements at the two year mark. To examine the below-ground component, the lengths of rhizomes were also measured through destructive sampling at the experiment's completion.

The SAS statistical package was used to analyze the data (release 6.12, 1997, SAS Institute, Cary, N.C.). To examine the effects of integration and nutrient status, repeated measures ANCOVA was performed, with the initial length and number of ramets combined as one measurement and used as the covariate.

Results

Asexual Reproduction

Contrary to findings of chapters two and three of this dissertation, there were no significant differences in new ramet numbers between integrated and non-integrated clones at any point during the two-year experiment (figure 4.1). There was also no significant difference in ramet production between clones in soils with natural levels of nitrogen and clones grown under elevated levels of nitrogen. At the end of the experiment, there was the possibility of a trend towards differential performance between high nitrogen and natural nitrogen levels, though this difference was not significant (F= 2.597, p>0.12). At this same time, the greatest difference between the integrated and non-integrated clones was also found, though it was even less than that found in the nitrogen treatments (F=1.522, p>0.25). During the first year of the experiment there also was no difference between any of the individual treatment contrasts (figure 4.2); differences begin to appear with the final measurements, though they are non-significant. The interaction factor for nutrient level and integration status was non-significant as well (F=0.02, p>0.889). In three of the treatments, three to four new ramets were produced within the first six months following the initial fertilizer application. Ramet production slowly declined in these treatments to one to two new ramets produced in the final six months (figure 4.2). The exception was the integrated clones with elevated levels of nitrogen. In the first six months these plants produced slightly less than four new ramets, and production declined in the second six months to about one new ramet per clone. Following the second nitrogen addition, there was an increase in the third six month period to slightly over four new ramets which decreased to about two new ramets per clone produced in the final six months.

Ramet Length

Clones in the elevated nitrogen treatment produced significantly longer ramets than those under natural nitrogen conditions (figure 4.3). This occurred throughout the experiment. The interaction factor between integration and soil nutrient status was significant (F=7.06, p=0.013), indicating that the integration state of *V. stamineum* alters the ability of the plant to exploit added nitrogen. There is a greater effect of nutrient addition on ramet growth when clones are integrated than non-integrated. Thus, when clones are integrated, they are better able to exploit added soil nitrogen than when they are non-integrated. Integrated clones with added soil nitrogen produced significantly longer ramets than the other treatments (figure 4.4). There was an increase in ramet length following the second fertilizer addition, similar to the increase found in new ramet production, though this difference was non-significant. Non-integrated clones grown under natural levels of soil nitrogen produced significantly smaller ramets than the remaining three treatments. The integrated clones under natural nitrogen conditions and the non-integrated clones under elevated nitrogen conditions did not differ significantly from one another, but did differ significantly from the other two treatments.

Ramet weight

Ramet weight differences were evident between treatments (figure 4.5), and the individual treatment contrasts exhibited a response similar to that found with ramet length (figure 4.6). Integrated clones produced significantly heavier ramets than their non-integrated counterparts (F= 20.73, p<0.0001, figure 4.5). Clones grown under elevated levels of soil nitrogen also had significantly greater ramet weight than clones under natural levels of nitrogen. The interaction factor between integration and nutrient status was also significant, indicating that integration affected the ability of the plant to grow more above-ground biomass under different nitrogen conditions. While the difference between integrated clones in natural and elevated levels of nitrogen was highly significant, the difference between non-integrated clones in these treatments was less so (figure 4.6). Integrated clones grown under elevated nitrogen levels produced significantly heavier ramets than in all other treatments. Non-integrated clones under natural nitrogen levels produced ramets weighing significantly less than the other treatments.

Non-integrated clones under elevated nitrogen levels and integrated clones under natural nitrogen levels produced ramets that were not significantly different from one another, but were significantly different from the other two treatments.

Rhizome length

Rhizome length did not differ significantly among treatments, indicating that integration and soil nitrogen levels are factors that do not affect rhizome length in *V. stamineum*. This result was surprising. Neither integration nor nitrogen treatments caused any significant differences at any time point. The interaction factor between integration and nitrogen was also non-significant (F= 0.29, p>0.832).

Discussion

The results refuted our hypothesis that clonal integration and nitrogen levels are factors in the asexual reproduction of *V. stamineum*. This is in direct contrast to chapters two and three of this dissertation, which found clear indications that integration is important for asexual reproduction. Ramet length and weight, however, were moderately affected by both integration and nitrogen levels, with an interaction effect between the two treatments causing differing results in individual treatment contrasts. Rhizome length was, surprisingly, unaffected by either integration or nitrogen levels.

In other studies, the effects of nutrient additions on ramet growth and asexual reproduction have varied with both habitat and species. Some studies have found that increased nutrient supply does not increase ramet growth and/or asexual reproduction (Ikegami et al., 2007; Hartnett, 1993). Others have shown altogether different results—that increased nitrogen does not increase ramet growth but does increase asexual reproduction (Wilson and Tilman, 1993). In chapters two and three, asexual reproduction of *V. stamineum* was found to be affected by the integration status of the plant. Integrated clones produced approximately seven more new ramets per clone than their non-integrated counterparts by the end of each study. It is not known why the integration treatments were not significantly different from one another in this study. It has been suggested that the importance of integration in clonal plants may be location-specific, even within the same species (Pennings and Callaway, 2000). The clones of *V. stamineum* in Athens, GA and the Savannah River Site in SC were not significantly different from one another in their responses to the treatments in chapter three, yet the difference in results to the same integration treatments in this experiment seem to support this idea.

This study suggests that nitrogen is an important factor for ramet growth, as measured by ramet length and weight. The differential performance of integrated clones and non-integrated clones under the same nitrogen treatments is not surprising. Many studies have shown that elevated nutrient levels increase plant above-ground biomass in various habitats, both ones with naturally high and naturally low resource availability (Slade and Hutchings, 1987a; Hartnett and Bazzaz 1983; 1985). It is hypothesized that the benefits due to integration are increased in naturally low resource environments (Pitelka and Ashmun, 1985), such as the sandhills. That is, integration may increase the nutrient uptake ability of clonal plants in these resource-poor environments. The significant interaction between integration and soil nitrogen level supports this hypothesis. The greater difference in ramet response, both in length and weight, in integrated clones suggests that they are better able to exploit the increase in available resources than their non-integrated counterparts. Integration and increased soil nitrogen levels seem to have similar effects on *V. stamineum*’s ramet growth, both in length and weight.

The lack of significant difference in rhizome lengths among all clones of *V. stamineum* in the experiments is in direct contrast to the hypothesis of clonal foraging. Clonal foraging is the concept that plants may selectively place ramets in habitats favorable for resource acquisition and away from unfavorable habitats (Slade and Hutchings, 1987b; Kelly, 1992; de Kroon and Hutchings, 1995; Oborny and Cain, 1997). Studies have suggested that this is manifested through variations in branching intensity, branching angle, and spacer length. Spacer length (length of rhizomes or stolons) is considered the primary indicator of a foraging response, as computer modeling suggests that branching angle has only a minor effect on the placement of ramets and is not a significantly variable plant characteristic (Sutherland and Stillman, 1988). Spacer length is expected to decrease in habitats with increased nutrients and this has been shown in some herbaceous species (Dong, 1993; Evans, 1992). This is thought to increase the clonal plant's residence time in favorable habitats while simultaneously avoiding habitats with low resource availability (Hutchings and Bradbury, 1986; Hutchings, 1988).

A null model of clonal foraging has been proposed that suggests that increased resource availability will affect only the plant growth and not the associated foraging characteristics, such as rhizome length (de Kroon and Hutchings, 1995). The lack of significantly shorter rhizomes in *V. stamineum* in the elevated nitrogen treatment does not refute this null model, and seems to indicate that the clonal foraging theory does not apply for this species in the Fall-Line sandhills. This increase in above-ground biomass without an associated difference in spacer length is not unique (Hutchings and Slade, 1988). In fact, the lack of a spacer length response is not uncommon, as de Kroon and Hutchings found that only two of sixteen species had shortened spacer lengths in response to elevated nutrient conditions (de Kroon and Hutchings, 1995). Thus, the clonal foraging response is a highly variable one. Plant response has been shown to vary with conditions, such as neighboring plant species identity, root system morphology, and competitive ability (Huber-Sannwald, 1998; Evans and Cain, 1995). Integration in clonal plants has also been shown to alter the foraging response (Evans, 1992; Evans and Cain, 1995), although this was not reflected in our results. This study and others suggest that many factors may constrain the optimization of clonal plant foraging and that one, clear set of generalizations may not exist (Oborny and Cain, 1997).

The lack of significant differences in rhizome lengths between nitrogen treatments may be due to the relatively short residence times of the nitrogen fertilizer in the soil. While it is not known how long elevated levels of nitrogen were maintained after the first fertilizer treatment, soil cores taken for eight weeks after the second fertilizer application showed that nitrogen remained elevated through this period. While this is a substantial amount of time, it may be that this is simply not long enough for *V. stamineum* to alter its clonal morphology. In the grass species *Cynodon dactylon*, it has been suggested that the lack of significant morphological differences is due to the rhizomes' primary function, that of storage (Dong and de Kroon, 1994). That is, if the main function of a plant's rhizomes is storage, this might negate the need for changes in morphology with varying resource status. The general growth and morphology of *V. stamineum* supports the hypothesis that its rhizomes' chief purpose is storage. While each plant grows in a phalanx growth style, large rhizome masses are present on the interior of the clone (Duval, personal observation). *Vaccinium stamineum* is also one of the first sandhills species to reappear after disturbances such as burning and clear-cutting, probably due to the stored resources available in the rhizomes.

This suggests that clonal plants use rhizomes to smooth over variation in the environment temporally, not necessarily spatially. By using rhizomes as storage, the plant does not need to respond to resource variation by varying its spatial configuration. Thus, a lack of evidence of morphological and spatial heterogeneity may simply mean that spatial variation of resources is shielded by a plant's ability to store resources temporally.

These results introduce some uncertainty as to whether nitrogen is the limiting resource for *V. stamineum* in the sandhills. While above-ground biomass was affected by nitrogen levels, asexual reproduction and rhizome length were not affected. Thus, above-ground biomass growth may be limited by nitrogen, but below-ground growth of rhizomes and associated new ramets may be limited by other factors. The Fall-Line sandhills have limited water availability with accompanying high temperatures, both in the air and soil. Other species have been shown to produce significantly longer rhizomes in areas of low soil moisture to avoid similar adverse conditions (de Kroon and Schieving, 1991). Increased moisture availability has been shown to increase asexual reproduction and ramet size in species of bamboo, thus indicating that it is the limiting resource (Qing et al., 2004). If water were the limiting resource for *V. stamineum*, one might have expected to see differential responses in growth, reproduction, rhizome length between years one and two of the experiment, as water was added along with nitrogen in year one but not in year two. No significant difference between years was found, perhaps because of the limited, one-time application of water. Thus, while nitrogen did have some effect on various plant measurements, it cannot be ruled out that other resources are limiting *V. stamineum*'s growth in the sandhills.

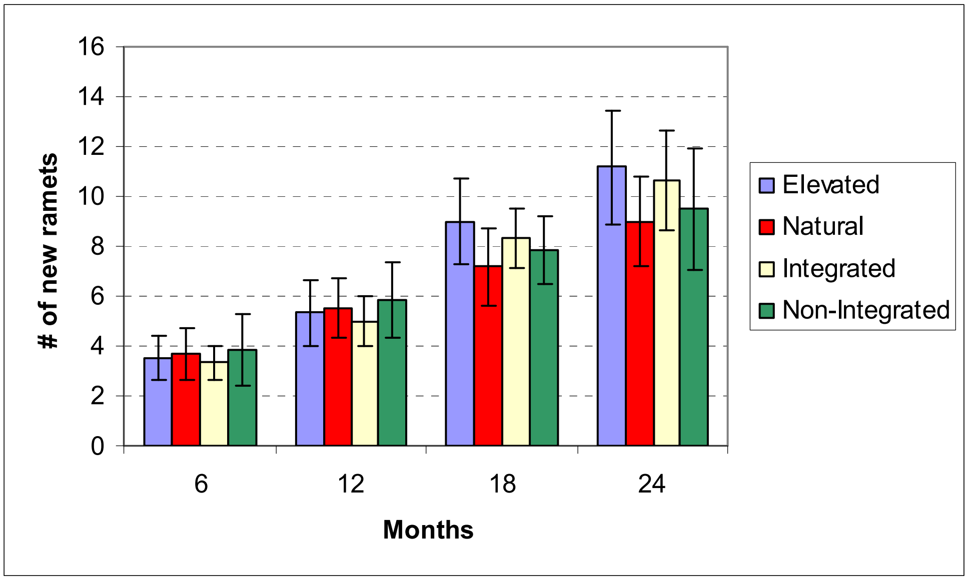

Figure 4.1: New ramet production of clones grouped into two contrasts: integrated versus non-integrated and elevated levels of nitrogen versus natural levels.

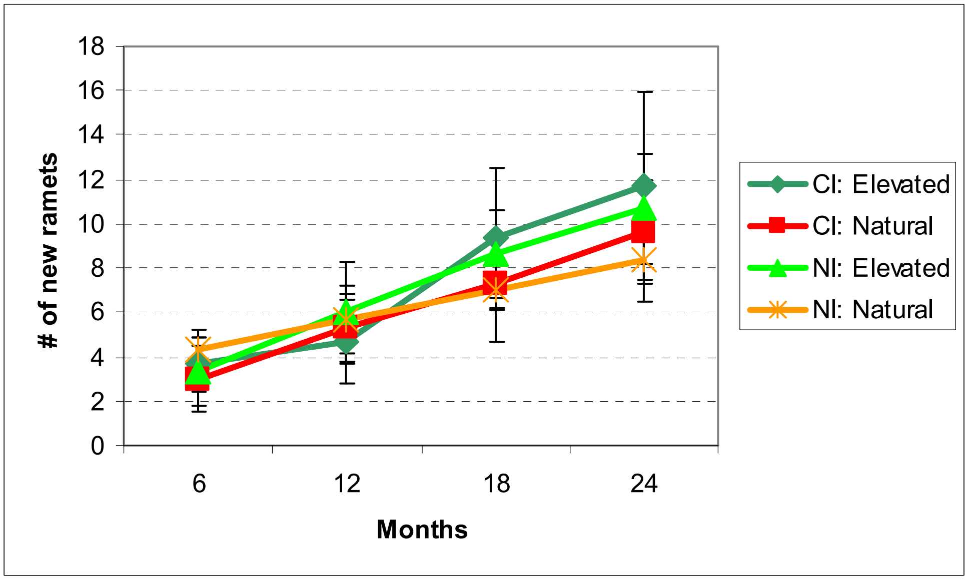

Figure 4.2: New ramet production with individual treatment contrast
(CI: Elevated = clonally integrated with elevated levels of nitrogen; CI: Natural = clonally integrated with natural levels of nitrogen; NI: Elevated = Non-integrated with elevated levels of nitrogen; NI: Natural = Non-integrated with natural levels of nitrogen)

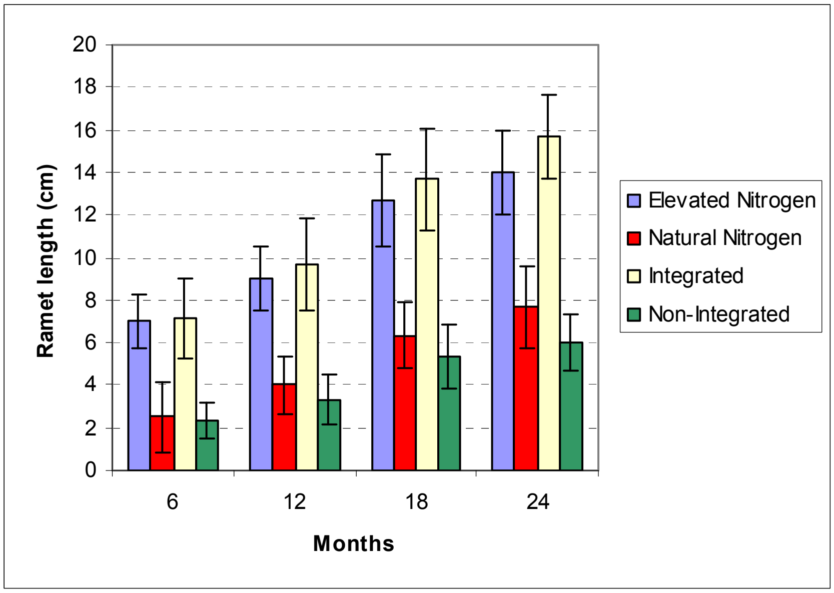

Figure 4.3: Mean ramet length of clones grouped into two contrasts: integrated versus non-integrated and elevated levels of nitrogen versus natural levels.

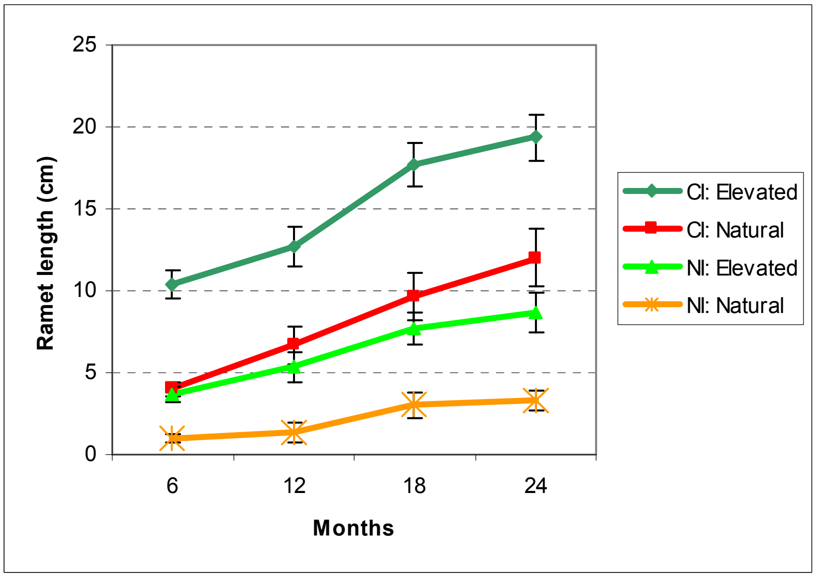

Figure 4.4: Mean ramet length of clones grouped into individual treatment contrasts (CI: Elevated = clonally integrated with elevated levels of nitrogen; CI: Natural = clonally integrated with natural levels of nitrogen; NI: Elevated = Non-integrated with elevated levels of nitrogen; NI: Natural = Non-integrated with natural levels of nitrogen)

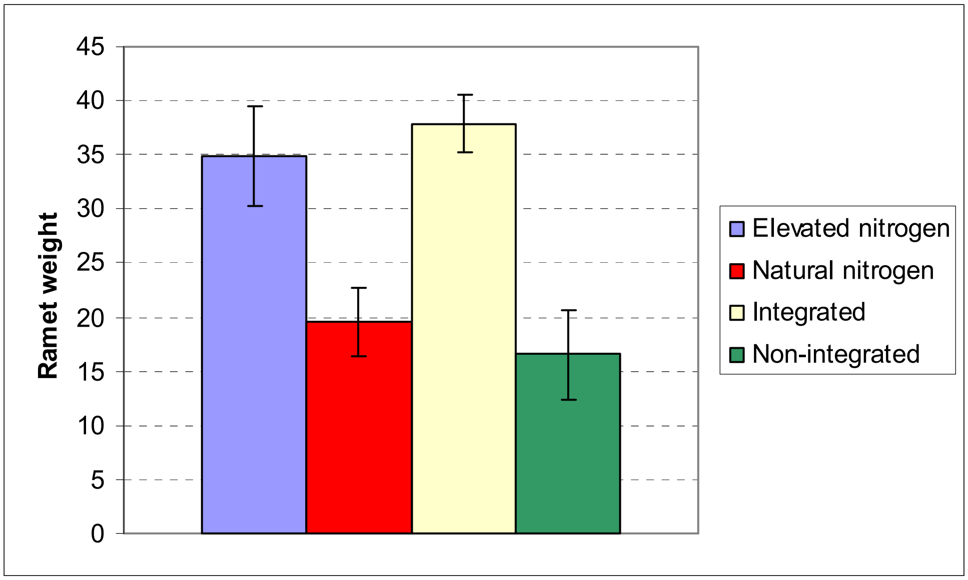

Figure 4.5: Average ramet weight of clones grouped into two contrasts: integrated versus non-integrated and elevated levels of nitrogen versus natural levels.

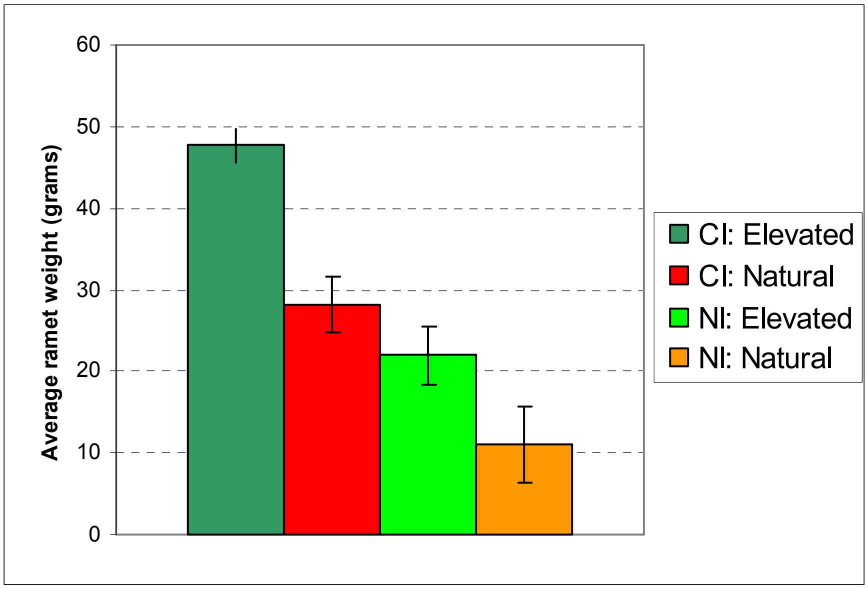

Figure 4.6: Average ramet weight with individual treatment contrasts.
(CI: Elevated = clonally integrated with elevated levels of nitrogen; CI: Natural = clonally integrated with natural levels of nitrogen; NI: Elevated = Non-integrated with elevated levels of nitrogen; NI: Natural = Non-integrated with natural levels of nitrogen)

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Chapter Five

Conclusion

This dissertation work has addressed the need to add to the knowledge of integration in clonal plants, specifically woody ones, and how clonal integration might alter various plant characteristics under different conditions. The differences in size and architecture suggest that woody species may not experience the same benefits of integration that herbaceous species do. Woody species can differ from their herbaceous counterparts in the increased longevity of both the above-ground biomass and the below-ground connections (Jenik, 1994). Longevity of above-ground biomass can last over one hundred years in some woody species, with the below-ground connections potentially lasting even longer (Barnes, 1966). These differences may alter the advantages and disadvantages that herbaceous and woody species experience with respect to clonal integration.

Chapter two examined whether integration altered the ability of *Vaccinium stamineum* to respond to differing simulated disturbance regimes. Integration increased asexual reproduction and rhizome length in both the control and intermediate disturbance treatments. The extreme disturbance treatment showed significant differences in asexual reproduction between integration treatments, but not with respect to rhizome length. Ramet length was unaffected.

Chapter three examined the response of integrated and non-integrate clones of *Vaccinium stamineum* in both natural and decreased levels of competition. Rhizome length and ramet length were not significantly different among the various treatments, while asexual reproduction and ramet weight were increased in integrated clones and in the absence of competition.

The fourth chapter addressed the effects of integration and the possible limiting nutrient in the sandhills soils – nitrogen. Asexual reproduction was not significantly different among treatments while both ramet weight and ramet length were significantly greater under conditions of increased soil nitrogen when clonal integration was maintained.

The measured effects of clonal integration on plant processes were not particularly consistent among these experiments. Chapters two and three of the dissertation showed that integration increased asexual reproduction under natural conditions. In chapter four, however, contradictory results were found, as asexual reproduction was not significantly greater in integrated clones under natural conditions. Similar contradictory results between chapters were also found with ramet length and rhizome length. Study of the effects of integration on clones in Athens, GA and the Savannah River Site, SC showed no significant differences among locations. While this shows a lack of location effect, given the dissimilar results among chapters, the effects of integration may be location-specific to a limited degree. Other studies also have shown that the effects of integration may be location-specific (Pennings and Callway, 2000). In both experiments where it was examined, clonal integration showed the same effect on *V. stamineum* ramet weight. Also, two of the three experiments showed similar results of the effect of integration on ramet length, rhizome length, and asexual reproduction. These indicate that there is a generally consistent effect of physiological integration in *Vaccinium stamineum*, yet location may play a role in the effect of integration. The significant interaction factors between competitive intensity and integration, as well as between nitrogen levels and integration, suggest that both environmental conditions and the surrounding neighborhood will dictate how integration effects plant processes.

This dissertation shows that integration is a significant factor in the growth and survivorship of *Vaccinium stamineum*. Integration affects both above-ground and below-ground individual ramet growth, as well as the asexual reproduction of the clone as a whole. Thus, integration improves the overall ability of *V. stamineum* to respond to a disturbance, compete with neighboring plants, and exploit a sudden influx of resources.

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