

PLANT GROWTH AND CHEMICAL RESPONSES TO VARIATION IN SOIL
PROPERTIES BY A TEXAS SAND RIDGE ENDEMIC
MONARDA VIRIDISSIMA (LAMIACEAE)

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Chapter I

INTRODUCTION

Texas encompasses a wide range of diverse and unique habitats, which contributes to the large number of endemic species found there. With 379 endemic plant species, Texas ranks fourth in the United States behind California, Florida and Hawaii (United States Department of Agriculture, 2011). The vast size of Texas allows for drastic differences in climate and geography across the state which in turn creates a heterogeneous landscape with potential for the diversification of plant species. Edaphic factors refer to the substratum on which a plant grows, and from which it derives its mineral nutrients and a majority of its water supply (Rajakaruna, 2004). Second to climate, edaphic factors are the most important feature of a landscape that dictates a species' distribution (Rajakaruna, 2004). An edaphic endemic refers to a species that is restricted to a specific soil type. Edaphic endemics provide a unique opportunity to study plant speciation by means of natural selection. Comparisons of the edaphic endemic to its closest relative are useful for revealing factors that differ between the two species as well as the mechanisms that cause the congeners to retain distinct populations.

Volatile oil production is a tool used to determine relationships among species. Production of volatile oils can be influenced by biotic and abiotic factors, including edaphic factors. Edaphic endemics have shown significant differences in volatile oil

production compared to congeners (Scora, 1967a) as well as decreased seedling survival (Hart, 1980) and less efficient use of resources when grown in non-native soils (Baltzer et al., 2005). The genus *Monarda* is an excellent group to study the relationships between edaphic endemics and their widespread congeners because the plants produce large amounts of volatile oils and there are a number of edaphic endemics in the genus.

The distribution of the genus *Monarda* is limited to North America. Species of *Monarda* can be found as far north as the Canadian prairies and as far south as central Mexico and range from the Atlantic to the Pacific coast. *Monarda* consists of 19 species separated into two subgenera, *Monarda* and *Cheilyctis* (Scora, 1967a; Diggs et al., 1999). The subgenus *Cheilyctis* comprises two sections, *Cheilyctis* and *Aristatae*. There are a total of four taxa in *Monarda* that are narrowly distributed edaphic endemics found only in Texas: *M. fruticulosa* Epling, *M. maritima* (Cory) Correll, *M. stanfieldii* Small, and *M. viridissima* Correll (Prather and Keith, 2003). This study focused on two closely related species, *Monarda viridissima* (an endemic) and *Monarda punctata* (a geographically widespread species), both belonging to section *Cheilyctis* (McClintock and Epling, 1942; Scora, 1967a; Prather et al., 2002).

Study Species

Monarda viridissima Correll (Lamiaceae) is an aromatic suffrutescent perennial with multiple stems to 100 cm tall. It has linear to very narrowly linear-lanceolate, gland-dotted, opposite leaves ranging from 2 to 8 mm wide and up to 9 cm long but usually smaller, tapered to a petiole about 1 cm long and long-acuminate at the apex, usually finely pubescent especially on the abaxial surface, and an irregularly serrate

margin. The flowers are sessile and occur in up to 6 whorls per stem with conspicuous linear to ovate bracts up to 2 cm long with an acute to acuminate apex. Bract color varies from pink to lavender to whitish green. The calyces are tubular with a bearded orifice; the corolla two-lipped, white, with an arcuate upper lip with long hairs and a lower lip with purple maculations (Scora, 1966b; Diggs et al., 1999).

Monarda viridissima is restricted to the Carrizo sand formation in Texas where it has been collected in seven counties: Wilson, Caldwell, Lee, Gonzales, Bastrop, Guadalupe and Lavaca (fig. 1). The counties form a continuous block near the central region of the long narrow strip of the Carrizo formation. Many authors refer to this species as an edaphic endemic due to its limited habitat in certain regions of Eocene age Carrizo sands. These sands are named for the area of Carrizo Springs in Dimmit County (southwest Texas) where they begin. The Carrizo sands trend in a north-easterly direction crossing thirty six counties in a continuous strip, ranging from approximately 9 to 19 km in width (McBryde, 1933). The beds of the Carrizo formation consist of sandstone and vary in color, texture, and thickness. The soils are considered sandy because they contain less than 18% clay and more than 65% sand (Gardiner and Miller, 2004).

Monarda punctata is an aromatic herbaceous annual or short lived perennial, freely branching at the upper portion of the stem with a height of up to 100 cm tall. It is widely distributed throughout Texas and can form large stands due to a lack of grazing by cattle and other herbivores. It is characteristically found in sandy open woods or open ground and is an indicator of sandy soils. It flowers in late May through July and then sporadically until September (Diggs et al., 1999).

Monarda punctata var. *lasiodonta* has leaf blades 4 to 7 cm wide with 0.3 to 1mm long hairs along the midvein on the abaxial surface. The flowers are sessile and occur in up to 6 whorls per stem with conspicuous linear to ovate bracts up to 2 cm long with an acute to acuminate apex. Bract color varies from pink to lavender to green to yellowish whitish green. The corollas are white to cream with maroon maculations on the upper and lower lips. It occurs mostly in north-central Texas and shares more similar habitat with *M. viridissima* than with any other variety of *M. punctata* found in Texas. It occupies fifteen of the thirty-six counties that the Carrizo formation covers (Diggs et al., 1999) (fig. 2).

Monarda punctata var. *intermedia* has leaf blades 10 to 23 mm wide and 5 to 7 cm long with 0.1 to 0.2 mm long hairs on the adaxial and abaxial surfaces. The flowers are sessile and occur in up to 6 whorls per stem with conspicuous linear to ovate bracts up to 2 cm long with an acute acuminate apex. The bracts vary in color from green to yellowish green. The calyx teeth are narrow, elongate, acuminate often two times as long as broad. The corollas are yellow, white, cream or pink with reddish brown maculations. *Monarda punctata* var. *intermedia* occupies habitat mostly to the northwest of the Carrizo formation and does not occur in any of the six counties in which *M. viridissima* has been collected (Diggs et al., 1999) but has been collected in three counties in which the Carrizo formation occurs (fig. 3).

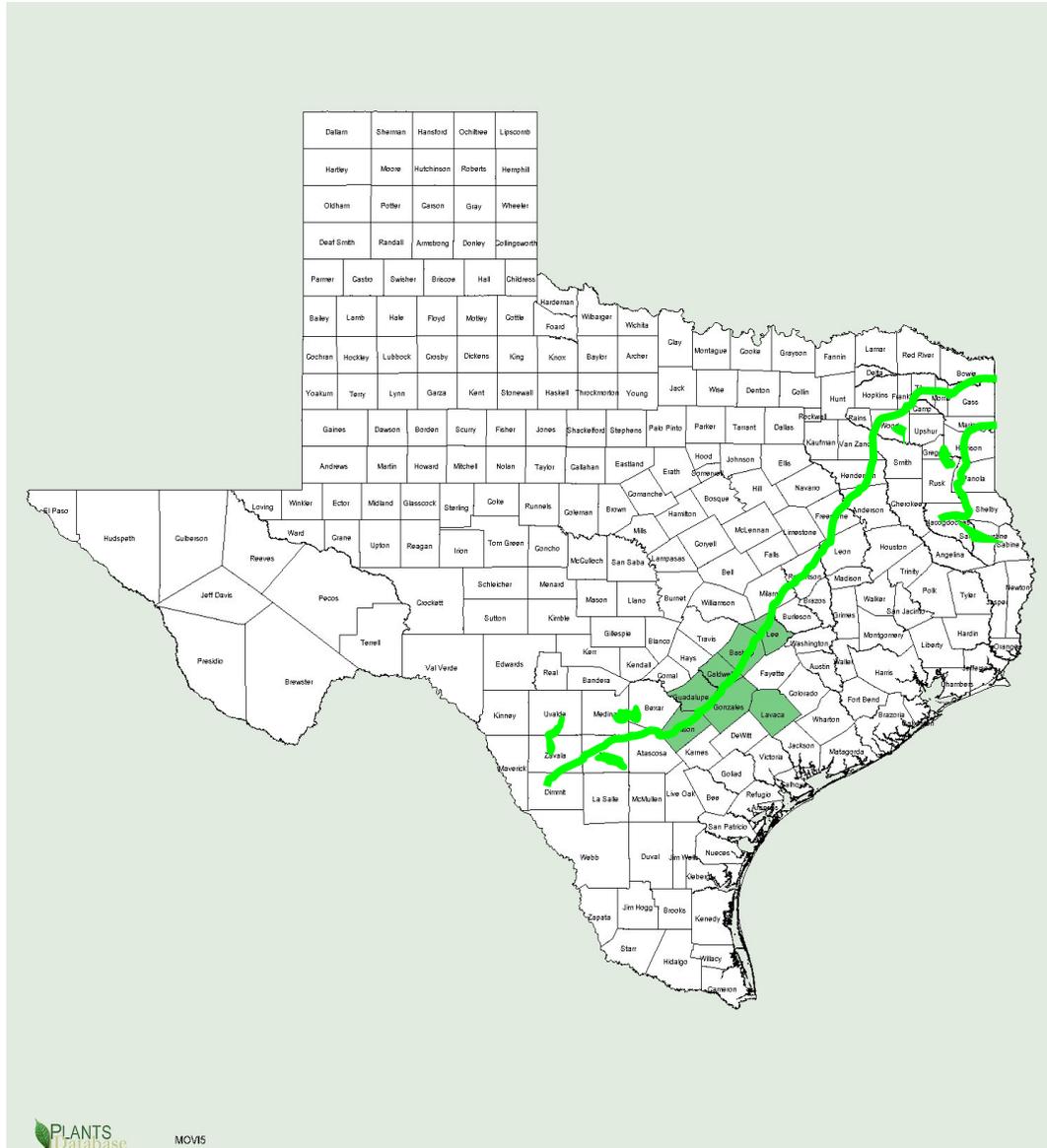


Figure 1. Distribution map of *Monarda viridissima*. Green indicates the seven counties (Lee, Bastrop, Caldwell, Guadalupe, Gonzales, Wilson, and Lavaca) from which *M. viridissima* has been collected. The location of the Carrizo formation is indicated by the bright green line.

Volatile Oil Production

Lamiaceae is one of the largest and most highly evolved plant families with a worldwide distribution (Hay and Svoboda, 1993). A number of labiates are well known for their production of volatile oils, which has led to their cultivation and use by humans since the beginning of civilization (Verlet, 1993). The most widely recognized mint species are used for culinary purposes, but they are also commonly used medicinally and industrially (pesticides, insecticides, preservatives). Volatile oils have also been useful tools in the fields of phylogenetics and chemotaxonomy (Scora, 1966a, 1966c, 1967a, 1967b).

The production of volatile oils (lower molecular weight terpenes) is not uncommon throughout the plant kingdom and was a feature of the earliest land plants (Hay and Svoboda, 1993). The synthesis of a number of biologically important molecules such as chlorophylls, carotenoids and gibberellins is very similar to that of terpenes. All of these compounds are built from the isoprene unit (Hay and Svoboda, 1993; Waterman, 1993). Volatile oils are composed almost entirely of two major groups of compounds. The larger group, terpenes, consists mostly of monoterpenes and sesquiterpenes. The second major group is the phenylpropenes, which are usually responsible for the majority of flavors and odors of plants (Waterman, 1993). The main site of synthesis and storage of volatile oils is located in glandular trichomes (peltate and capitate). In labiates this occurs in secretory cells at the base of glandular trichomes (Scora and Mann, 1967; Hay and Svoboda, 1993).

Plants can produce a variety of secondary compounds (McKey, 1974). Basil, for example, has been found to contain up to 100 different secondary compounds. This

diversity can be attributed to the need for defense against a variety of herbivores and pathogens (Keefover-Ring and Linhart, 2010) as well as pollinator attractants (Scora, 1964; Whitten, 1981; Deans and Waterman, 1993). Environmental factors such as light, temperature, nutrients, water, and soil, also influence plant chemistry. Numerous studies have demonstrated variation in terpene compounds across geographic regions, within populations, with season and maturity, and among different tissues of individuals (Scora, 1966c; Hay, 1993; Keefover-Ring and Linhart, 2010). A study of *Satureja douglasii* (Lamiaceae) showed two distinct chemotypes in their natural habitats but when the two chemotypes were grown under identical controlled conditions the differences disappeared (Hay, 1993).

Quantity of oil production can be influenced by flowering period in many labiate species with higher oil production during the flowering period (Hay, 1993). There are also studies that have shown an increase in oil yield before or after the flowering period (Hay, 1993). This inverse relationship between flower production and oil yield is seen in *Salvia* spp. (Lamiaceae) and *Rosmarinus officinalis* (Lamiaceae) and is a well studied exception to coincident oil production and flowering (Hay, 1993). The flowers of rosemary produce small amounts of oil and leaves show little seasonal variation in oil production. Yet another variation in oil production is the rise in particular compounds at the onset of flowering. For example, *Ocimum vulgare* (Lamiaceae) has shown a sharp rise in thymol content that coincides with the onset of flowering (Hay, 1993). Another complication in predicting oil composition is the effect of environmental conditions, especially in plants that are phenotypically plastic.

Edaphic Factors and the Niche Concept

A number of studies have looked at the presence of rare plants on rare substrates and have come to varied conclusions. One study, by Hart (1980) on serpentine endemics, showed an increase in biomass when plants were grown on nonserpentine soils but much higher seedling survival on serpentine soils. Other studies (Shelton, 1963; Mellinger, 1972; Miller, 1977) on serpentine endemics have shown normal growth on nonserpentine soils in the absence of competition (Baskin and Baskin, 1988). All of these studies are similar due to the fact they compare environmental aspects including resource needs, habitat requirements and environmental tolerance of endemic species to closely related congeners. This suite of ecological attributes makes up what Hutchinson (1957) referred to as the multidimensional niche.

The niche concept can be used to elucidate patterns of species distribution along environmental gradients. Recognizing the difference between a species' ecological (or realized) niche versus physiological (or fundamental) niche is important in understanding the mechanisms responsible for many species distributions (Ellenberg, 1958). A physiological niche represents the type of habitat that a species could potentially occupy in the absence of other competing species. The ecological niche represents the area of habitat that a species actually occupies in the presence of other plants and natural enemies (Barbour et al., 1998). The geographic range of a species can be thought of as a reflection of its ecological niche (Lomolino et al., 2006).

Ellenberg (1958) used six grass species grown in monoculture and in multispecies mixtures to illustrate the difference between an ecological and physiological niche. He grew the six grass species along a gradient of water table depth and found that when

grown in monoculture the five species all did best, in terms of dry weight, at the same water table depth. When grown in competition with one another they all did best at different areas along the gradient. The area along a gradient where species reach their maximum abundance is referred to as the ecological or physiological optimum depending on whether you are referring to the ecological or physiological niche. Ellenberg's experiments demonstrated how the ecological niche is narrowed by the presence of competitors and that the ecological and physiological optima rarely occur at the same point along the gradient (Aukland, 1978; Austin, 1985). Species can also be restricted to certain habitats due to special resource or habitat requirements; these species are referred to as habitat specialists. Specialists usually have a narrow ecological and physiological niche because they have adaptations that allow them to survive in certain environments but prevent them from living in others. Habitat generalists are those species with no "special" adaptations to a specific habitat (Baltzer et al., 2005).

Environmental gradients can be placed in to three categories according to the way they affect the plant or environment. Resource gradients involve factors such as light, water, and mineral nutrients that the plant must utilize to grow and that can be depleted from the local environment by the plant. Regulator gradients involve air temperature and soil pH, which affect the plant physiologically but are not actually consumed or depleted from the local environment. Complex gradients are factors such as latitude, altitude, and ocean depth, which do not directly affect plant growth or physiology but do affect resource and regulator gradients, which in turn affects the plant (Huston, 1994).

A number of studies have manipulated these gradients independently to help understand the mechanism behind patterns of species distributions. Edaphic

specialization has been studied by numerous botanists interested in plant speciation and the association between certain soil types and certain species or communities (Rajakaruna, 2004). Baltzer et al. (2005) compared tropical tree associations on a reserve in Borneo where soil types created two distinct edaphically specialized communities. One soil type was a nutrient poor sandstone and the other was an alluvial soil. He and his colleagues tested the resource use efficiency hypothesis, which states that species differ in their ability to withstand conditions of limiting resource availability that vary among soil types. If a specialist is highly adapted to its native edaphic conditions, it will have lower resource use efficiency when grown on non-native soils. By comparing edaphic specialists with generalists using survey and reciprocal transplants, they were able to determine what specific factors restricted the specialists to their native soil type. They concluded that the resource use efficiency of the specialists, when grown on their non-native soil, was unable to reach similar levels as the generalists. Edaphic factors may be one of the most important factors in determining species ranges (Clements, 2002).

The intent of this study is to determine the effects of edaphic factors on growth and volatile oil production of *Monarda viridissima*, a narrow endemic of the Carrizo sands of Texas and to compare volatile oil production and habitat characteristics with its closely related, geographically widespread congener, *Monarda punctata*. Specifically this study examines the germination rate, growth rate, and volatile oil production in *M. viridissima* across different edaphic conditions to identify characteristics that may contribute to its narrow habitat range and seeks to determine whether there are differences in volatile oil production between populations of *M. punctata* and *M. viridissima*. I hypothesize that (1) species assemblages will be similar at all field sites

due to similar edaphic conditions; (2) *Monarda viridissima* grown in a peat-based potting mixture will have the highest growth rate and germination percentage of all species-soil combinations due to the water holding capacity of the peat-based potting mixture and *Monarda*'s generalist adaptations; (3) the physiological niche of *M. punctata* is narrower than that of *M. viridissima* and does not include the sandy soils where *M. viridissima* can grow; (4) the ecological niche of *M. viridissima* is reflected by properties of its native soils that create drier conditions, reduce the growth of *M. punctata*, and allow *M. viridissima* to maintain populations free from competition by *M. punctata*; (5) the number and amount of volatile oils produced will be lowest in *M. viridissima* plants grown in the peat-based potting mix; and (6) *Monarda punctata* and *M. viridissima* growing sympatrically will produce different types of volatile oils.

To explore these hypotheses, I (1) determined whether selected soil properties (available water capacity and soil texture) and species assemblages differed between populations of *M. viridissima* and *M. punctata*; (2) experimentally determined if selected edaphic factors affect the growth rate, germination rate, and volatile oil production of *M. viridissima*; and (3) determined whether there are differences in volatile oil production between *M. punctata* and *M. viridissima* growing sympatrically and parapatrically.

Chapter II

METHODS

Prior to field work in June 2009, I used herbarium vouchers to locate populations of *M. viridissima* and *M. punctata*. *Monarda punctata* was absent from a number of places it had previously been collected. This may have been due to the extreme drought conditions in central Texas during summer 2009. I observed a greater number of individuals of *M. punctata*, especially on roadsides, during summer 2010, which was characterized by more precipitation and lower temperatures than summer 2009. I selected three field sites to represent a sympatric (Bastrop Co.), parapatric (Wilson Co.) and an allopatric (Llano Co.) habitat.

Vegetation and Soil Analysis

In order to determine whether there are differences in soil properties at field sites supporting populations of *M. viridissima* and *M. punctata* soil maps, available water capacity and particle size data were downloaded from the USDA-NRCS Web Soil Surveys for each County (United States Department of Agriculture, 2010).

In order to determine whether there are differences in species assemblages at field sites supporting populations of *M. viridissima* and *M. punctata*, percent cover, species richness and species evenness were determined by sampling five 1 m² quadrats along a 10 m transect, with one quadrat every other meter. For each quadrat every species was identified, counted, and the percent cover was visually estimated. Vegetation surveys

were conducted in November 2010, while *M. viridissima* was flowering, at the sympatric (Bastrop Co., 30.108009, -97.263508), parapatric (Wilson Co., 29.29082, -98.04088) and allopatric (Llano Co., 30.879465, -98.90811) field sites.

Seed and Soil Collection for Experimental Plantings

Seeds of *Monarda viridissima* were collected from two spatially separated populations and from five different individuals from each population to ensure sampling of more than one genotype. Seed collection entailed breaking off the dried inflorescence inside a paper bag and shaking the bag to remove the seeds from the calyx.

Soil samples used in the experimental plantings were collected in June 2009. Soil was taken from the uppermost 0.5 m with a shovel and put into a bucket for transport back to the Biology Department greenhouse at Texas State University-San Marcos where it was transferred into 4 liter pots. An artificial soil was created using a peat mixture consisting of 32 liters peat moss, 8 liters vermiculite, and 8 liters perlite.

Germination and Growth Rates of Experimental Plantings

In order to determine if edaphic factors affect the growth and germination rates of *Monarda viridissima*, seeds collected from the field were planted in the four different soil types described above and observed over a nine month period.

Monarda viridissima seeds collected in June 2009 were successfully germinated at the end of September 2009 in the Biology Department greenhouse at Texas State University-San Marcos. *Monarda punctata* seeds were ordered from the Native American Seeds Company and for unknown reasons did not germinate. Therefore they were not included in the experimental treatments. The germination percentage of *Monarda viridissima* was calculated based on ten pots per treatment with three seeds per

pot (n = 30). The growth rate of *M. viridissima* was calculated by measuring the height of each plant growing in each of the four experimental soils (n = 10). Height measurements were recorded after the cotyledons had fully opened, every five days over a nine month period. Starting in late November of 2009, in order to provide adequate irradiance, the plants were grown under a 1,000 watt high pressure sodium bulb until March 2010 when they were moved outside. Seedlings were given 100 ml of tap water every other day until they were well established and then 100 ml every three days.

Leaf Collection for Oil Analysis

Leaf samples of *M. punctata* and *M. viridissima* from Bastrop County were collected at Bastrop State Park near the north intersection of Lost Pines Trail and Roosevelt's Cutoff (30.108009, -97.263508). The Wilson County leaf samples of *M. viridissima* were collected from the roadside of County Road 333 in Sutherland Springs (29.29082, -98.04088) and *M. punctata* leaf samples were collected from the roadside of Farm to Market Road 1681 near Nockenut Cemetery (29.329509, -97.881317). One leaf was taken from the first node down from the apex, from five different individuals of both species at each field site. The leaves were stored in a plastic zip-lock bag, placed in a cool ice chest and brought back to the lab where they were stored in the refrigerator until they were prepared for GC/MS analysis. Samples were collected and processed within 24 hours to diminish the effects of storage on the results.

Leaf samples of *Monarda viridissima* from the experimental treatments were taken from the first node down from the apex and prepared for GC injection immediately after removal. One sample was taken from each of the ten plants for each of the four experimental soils.

Volatile Oil Analysis

In order to determine whether there are differences in volatile oil production between *Monarda punctata* and *M. viridissima* growing in the same habitat, I selected a sympatric habitat (Bastrop County) and a parapatric habitat where both species grow separately a short distance from one another (Wilson County).

Gas chromatography samples consisted of one leaf, measured (length and width), soaked in 1 ml of methanol mixed with naphthalene at a concentration of 10 µg/ml for 30 min as the internal standard. One µl of sample was injected using splitless mode into an Agilent 6890N GC with a capillary column of 30 m x 250 µm x .25 µm coupled with a 5975B mass selective detector. Helium was the carrier gas with a flow rate of 0.5 ml/min and the inlet temperature was 250°C. The initial oven temperature was 55°C for 4.5 min then ramped to 65°C at 2°/min and held for 2 min, then 3°/min to 75°C and 5°/min to 170°C. Concentrations of terpene compounds were calculated using peak areas. Identification of compounds was verified using published estimated retention times and the NIST library.

Statistical Analysis

The program R, version 2.8.1 (R Development Core Team, 2008) was used for all statistical analysis and to examine the distributions of data to insure assumptions of normality and homoscedasticity were met, making transformations as needed. The threshold of significance in all cases was $P = 0.05$. According to seed weight histograms the data violated normality so a Mann-Whitney test was used to determine if there were differences in seed weights between *Monarda viridissima* and *Monarda punctata* instead of a two sample t-test. A one-way ANOVA followed by a Tukey's HSD post hoc test for

multiple comparisons among means was used to test for germination differences among the four soil types. Growth rate data were homoscedastic so ladders of power were used to determine the best transformation for the data. The data were log transformed and a one-way ANOVA followed by a Tukey's HSD post hoc test for multiple comparisons among means was used to detect differences in growth rates among the four different soil types.

Separate ANOVAs were used to determine if cover and species richness differed among sites. Significant results were further explored using Tukey's HSD. Simple linear regressions were used to investigate relationships between total mean cover and species richness and total mean cover and *Monarda viridissima* cover.

To analyze the oil data I first calculated the absolute concentrations by dividing the percentage of the total peak area of each compound by the percentage of the total peak area of naphthalene. These data were used to make the graphs which automatically calculated the relative proportion of each compound. For the statistical analyses I calculated the relative concentrations, leaving out naphthalene, by summing the total relative concentration and dividing each relative concentration of each compound by the sum.

Of the twenty seven compounds identified, fifteen were tested for differences among treatments and between species using one-way ANOVAs. The compounds used for statistical analyses were 3-thujene, 1-octen-3-ol, myrcene, alpha-terpinene, O-cymene, sigma terpinene, terpineol cis-beta, linalol, borneol, thymoquinon, thymol, carvacrol, alpha-bourbonene, germacrene-D and farnesene. These compounds were chosen because they were present in relatively large proportions and consistently found in

each treatment. Significant results were followed up by a Tukey's HSD post hoc test to determine which sites and or species differed from one another.

Chapter III

RESULTS

Soil Characteristics

The allopatric *M. punctata* soil type from Llano County was Campair (CaB) sandy loam with parent material of residuum weathered from Cambrian sandstones (fig. 4). This soil had the lowest sand content and highest clay content as well as the highest water holding capacity of the three soil types. The soil type from the parapatric field site in Wilson County was the Aluf and Hitilo series (EPB) classified as deep sand with parent material of sandy eolian deposits derived from sandstones (fig. 5). The dominant soil type from the sympatric habitat in Bastrop County was Padina (PaE) deep, fine sand (fig. 6). The parent material consists of residuum weathered from Eocene sandstones of the Carrizo, Queen City, Simsboro and Sparta formations. Available water capacity and particle size analysis data were downloaded from the USDA-NRCS for each of the three counties and are shown in Table 1.

Table 1. Available water capacity (AWC) & soil texture for the three field sites. PaE (Padina) = Bastrop CO., CaB (Campair) = Llano CO., Aluf & Hitilo (EPB) = Wilson CO.

Habitat Type	Source Co.	Soil Type	AWC (cm/cm)	% Sand	% Silt	% Clay
Allopatric	Llano	CaB	0.12	70.4	11.7	17.9
Parapatric	Wilson	EPB	0.06	76.8	10.2	13
Sympatric	Bastrop	PaE	0.09	83.8	5.6	10.7

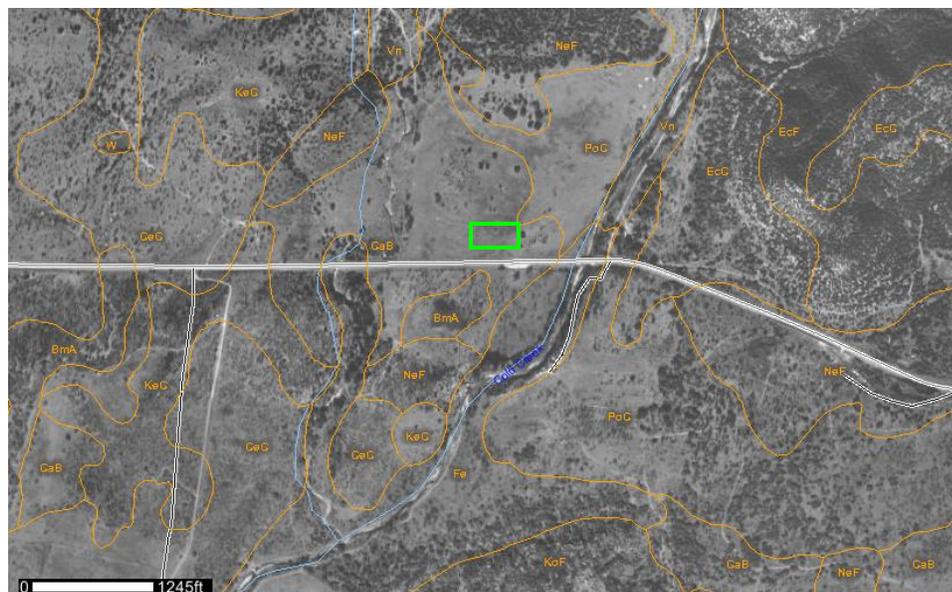


Figure 4. Soil map of the allopatric field site (Llano Co.) indicating where field work was done (green rectangle) and the distribution of the Campair soil (CaB).



Figure 5. Soil map of the parapatric field site (Wilson Co.) indicating where field work was done (green rectangle) and the distribution of the Aluf and Hitilo soils (EPB).

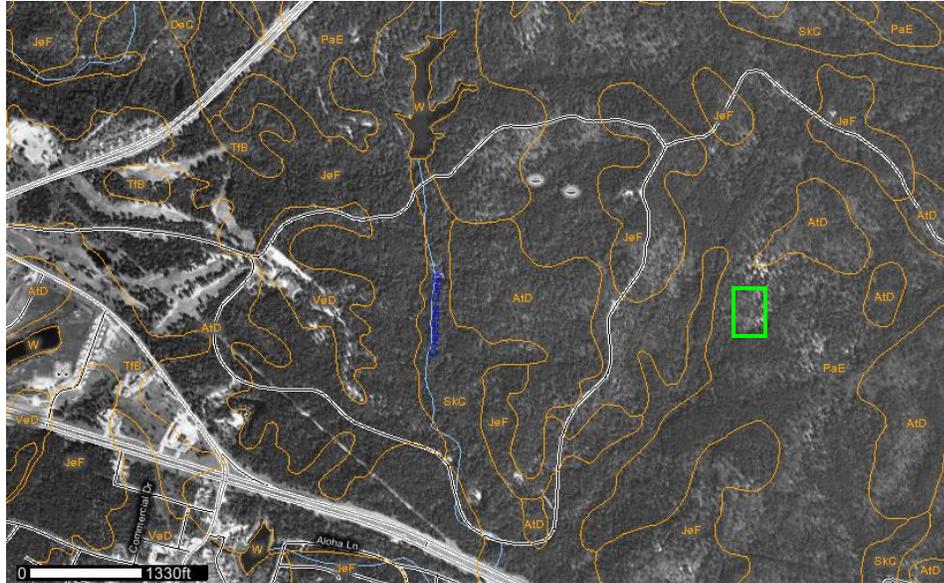


Figure 6. Soil map of the sympatric field site (Bastrop Co.) indicating where field work was done (green rectangle) and the distribution of the Padina soil (PaE).

Germination and Growth Rates of Experimental Plantings

The mean weights of *M. viridissima* and *M. punctata* seeds were 0.220 mg and 0.218 mg, respectively. Seed weight did not differ significantly between the two species ($n = 30$, $W = 485.5$, $P = 0.6048$). Germination percentages for *M. viridissima* differed significantly across all four soil types, $F_{3,36} = 4.80$, $P = 0.007$ (fig. 8). Tukey's post hoc comparisons indicated germination in the Wilson County soil, $P = 0.005$, and Bastrop County soil, $P = 0.039$, was significantly higher than germination in the allopatric Llano County soil. Germination in the peat-based mixture was also greater than germination in the Llano County soil but ($P = 0.07$) missed the threshold of the 0.05 significance level.

Growth rates differed significantly among the four soil types, $F_{3, 356} = 32.51$, $P = < 2.2 \times 10^{-16}$. Tukey's post hoc comparisons indicated the growth rate of plants in the peat-based soil mixture was much higher than all other treatments ($P = 0.000$ for all treatments compared to the peat-based soil mixture). Plants in Llano County soil (non-native *M. viridissima*) had a significantly higher growth rate than those in soil from Bastrop County, $P = 0.02$. Llano County plants also had a higher growth rate than Wilson County plants but with a 0.07 significance level (fig. 9). There was no significant difference between growth rates in the Wilson and Bastrop County soils.

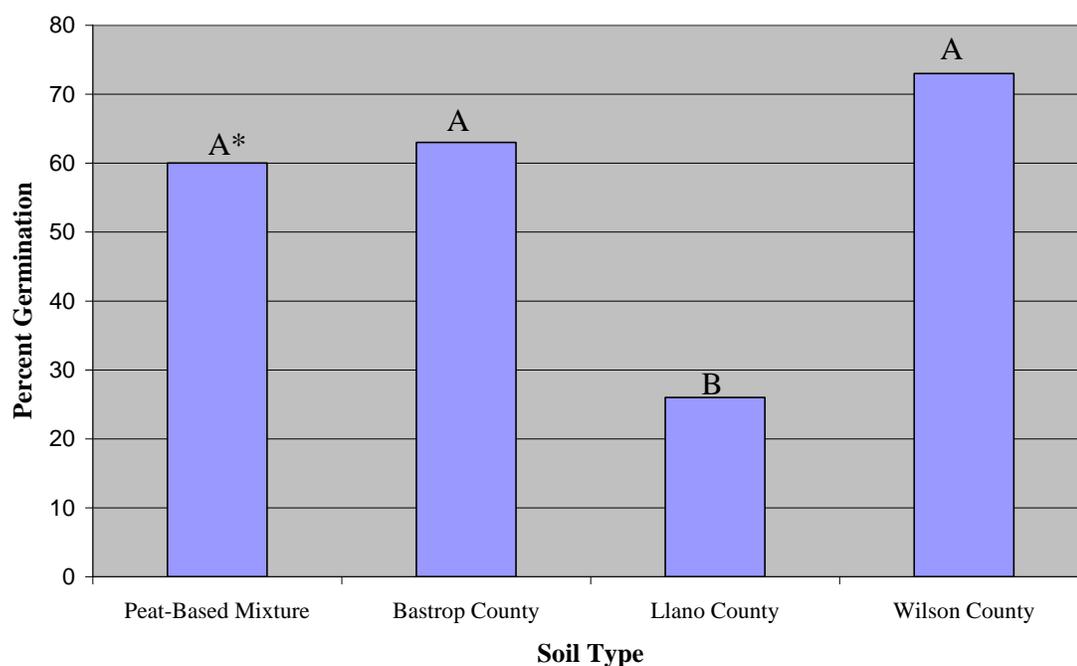


Figure 7. Percent germination of *Monarda viridissima* in four different soil types. Statistically homogenous groups based on Tukey's post hoc comparisons are represented by the same letter above the bars ($P = < 0.05$, * $P = 0.07$).

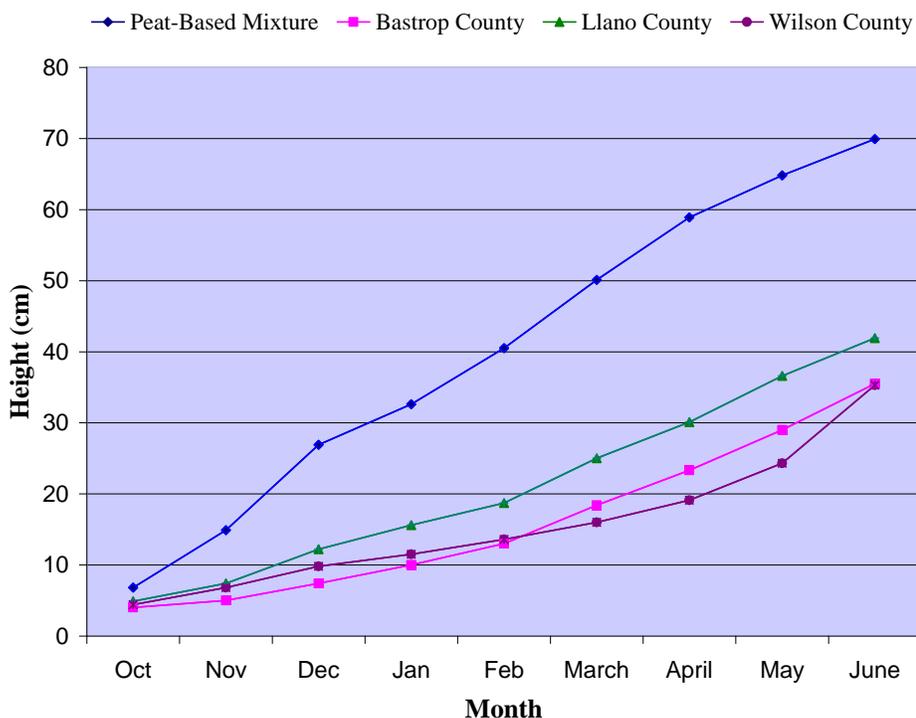


Figure 8. Growth rates of *M. viridissima* grown over nine months in four different soil types.

Vegetation Structure

The total percentage of cover differed at the three field sites (fig. 10 A and B).

The parapatric site had the highest total percentage of cover (61.2 %) which differed significantly from the sympatric field site, $P = 0.0143$ (Tukey HSD multiple comparisons following ANOVA, $F_{2,12} = 5.70$, $P = 0.0182$, $N = 15$). The total percentage cover did not differ between the parapatric and sympatric sites ($P = 0.426$). Figures 11 A and B show the species richness for the three field sites which was significantly higher at the parapatric site (Wilson Co.) than it was at the sympatric site (Bastrop Co.) ($P = 0.0328$) and significantly higher at the parapatric site (Wilson Co.) than it was at the allopatric site (Llano Co.) ($P = 0.0328$) (Tukey HSD multiple comparisons following ANOVA, $F_{2,12} = 5.63$, $P = 0.0189$, $N = 15$).

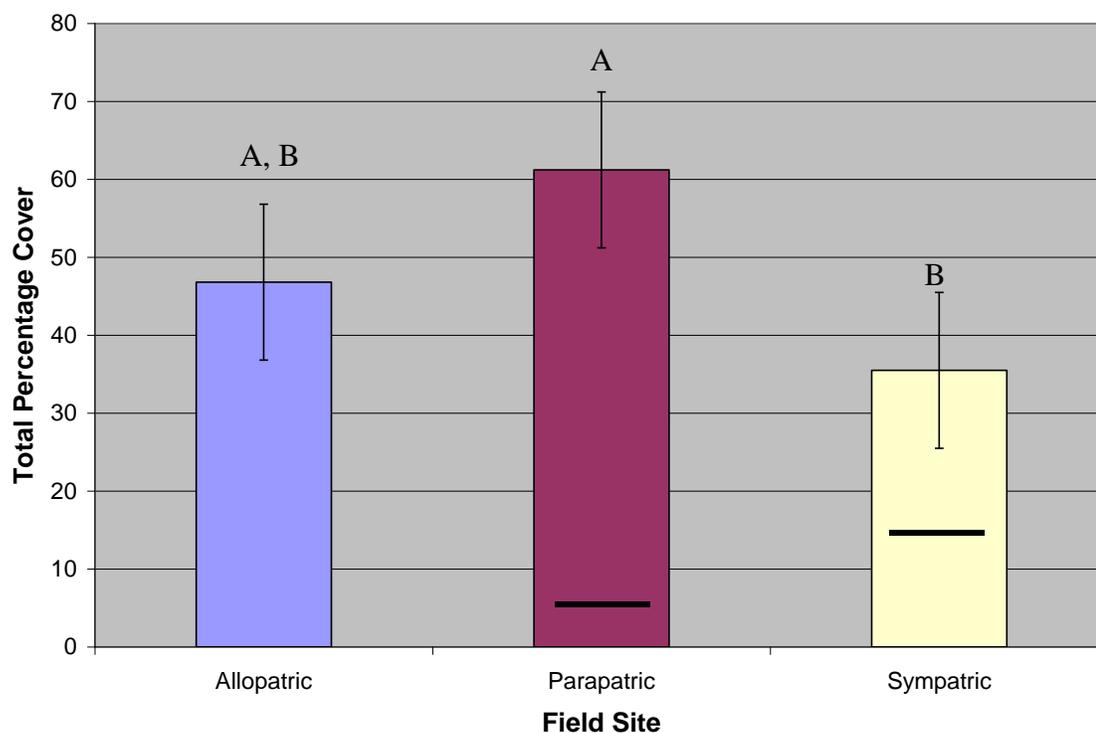


Figure 9 A. Total percentage cover at the three field sites, Llano Co. (allopatric), Wilson Co. (parapatric), Bastrop Co. (sympatric). Horizontal bars indicate mean cover of *M. viridissima*. Sites with different letters indicate significant differences. Error bars represent standard error.

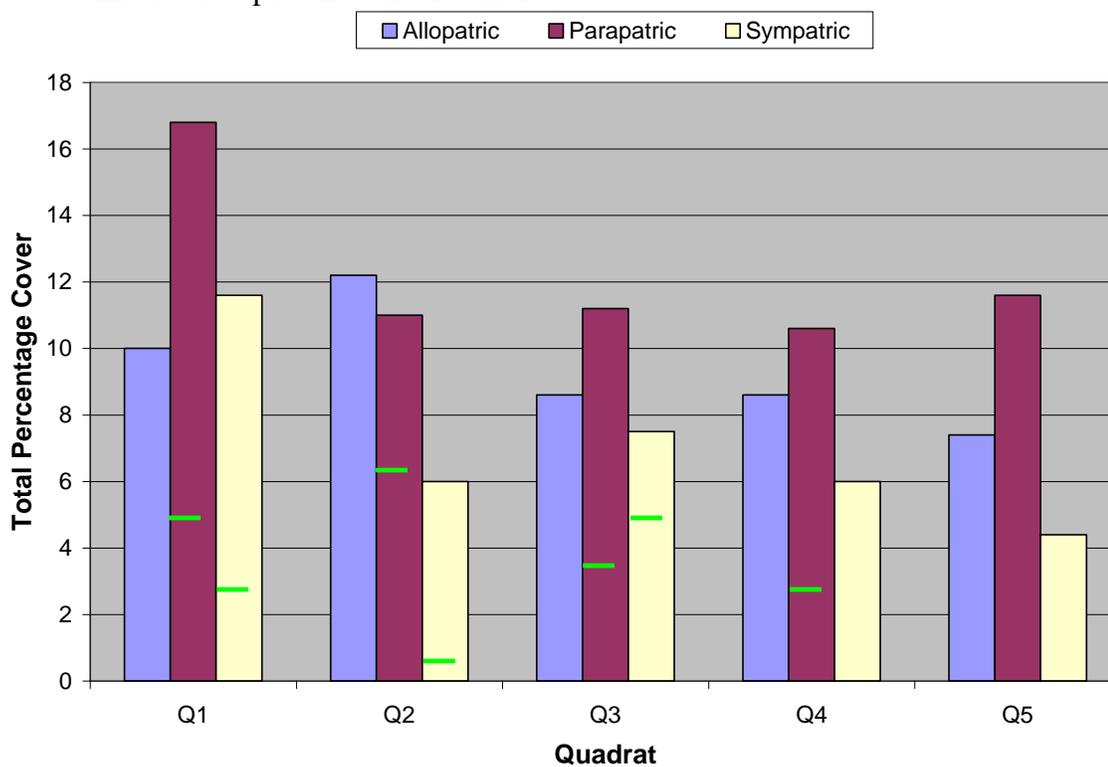


Figure 9 B. Total percentage cover by quadrat at the three field sites. Horizontal bars indicate mean cover of *M. viridissima* by quadrat. See figure 12.

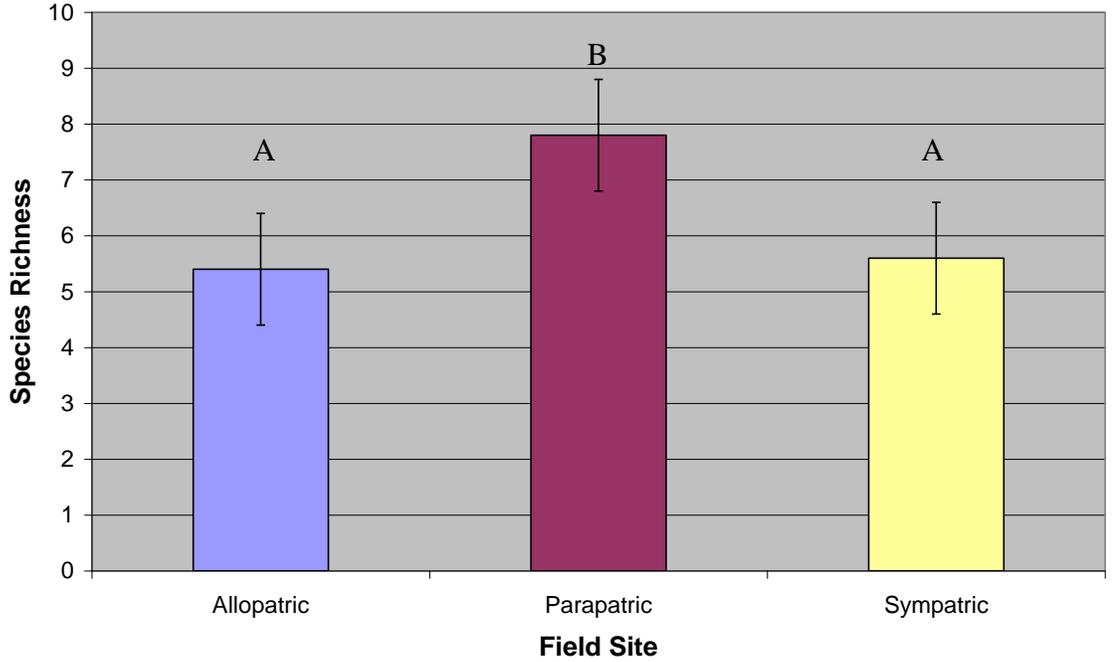


Figure 10 A. Species richness of the allopatric (Llano Co.), parapatric (Wilson Co.) and sympatric (Bastrop Co.) field sites. Sites with different letters indicate significant differences in species richness. Error bars represent standard error.

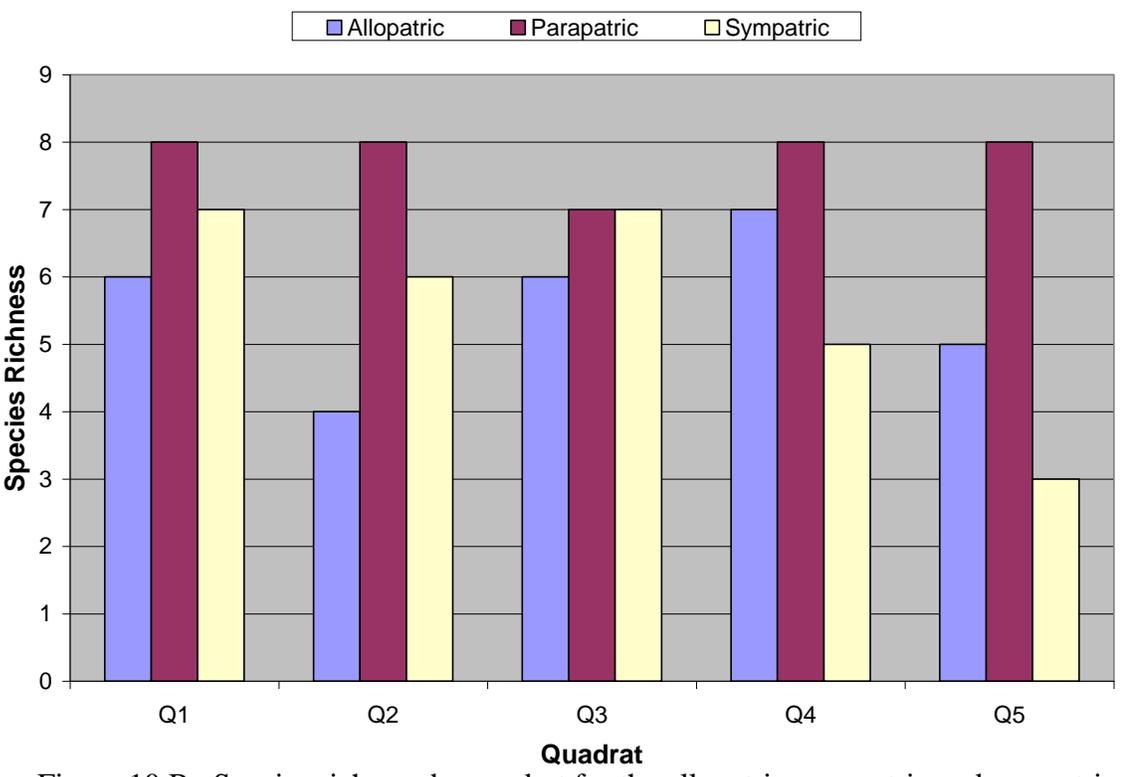


Figure 10 B. Species richness by quadrat for the allopatric, parapatric and sympatric field sites.

Total cover and species richness showed no significant relationship at any of the sites. The sympatric field site (Bastrop Co.) showed the strongest relationship between cover and species richness ($R^2 = 0.59$, $P = 0.1318$). Total cover and *Monarda viridissima* cover had a weak positive relationship at the sympatric (Bastrop Co.) field site ($R^2 = 0.60$, $P = 0.1244$) and a very weak relationship at the parapatric (Wilson Co.) site ($R^2 = 0.07$, $P = 0.6667$). There was no relationship between total cover and *M. viridissima* cover when the parapatric and sympatric field sites (Wilson and Bastrop County) data were combined in a single regression ($R^2 = 0.09$, $P = 0.72$) (fig. 11). However there was a distinct unimodal curve that revealed *M. viridissima* reaches its maximum cover at an intermediate level of total plant cover. There was no relationship found between total cover and species richness when all three sites were combined in a single regression ($R^2 = 0.40$, $P = 0.047$), although there was a distinct trend of increasing species richness with increasing cover (fig. 12).

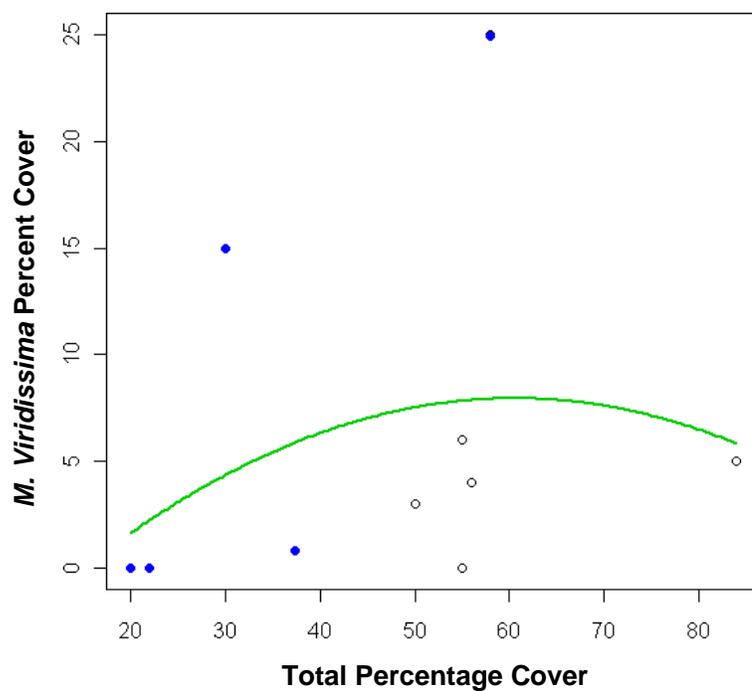


Figure 11. Relationship between total cover and *M. viridissima* cover combining the parapatric (open circles) and sympatric field sites (blue circles). ($R^2 = 0.09$, $P = 0.72$). Note the maximum cover has a unimodal response. *Monarda viridissima* reaches its maximum cover at an intermediate level of total plant cover.

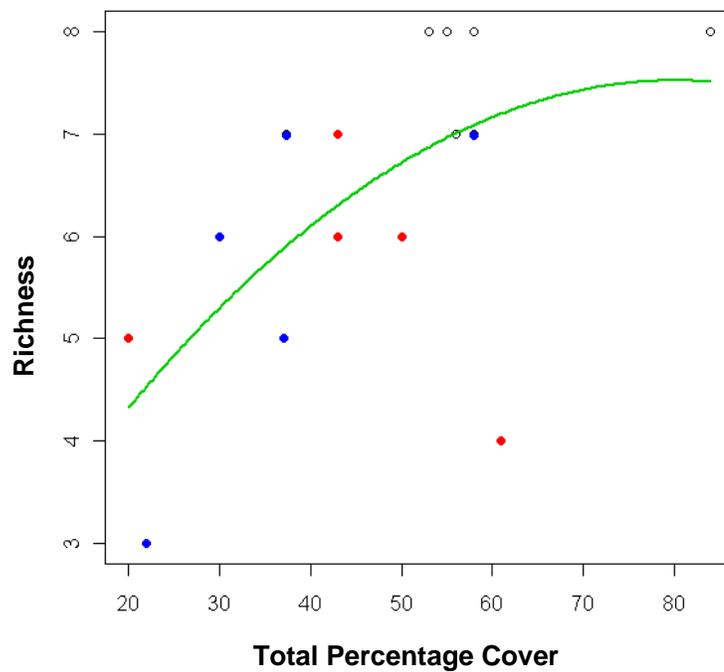


Figure 12. Relationship between total cover and species richness for the allopatric (red circles), parapatric (open circles) and sympatric (blue circles) field sites ($R^2 = 0.40$, $P = 0.047$). Note that species richness increases uniformly with increasing cover.

Table 2. List of the species identified at all three field sites. Measurements for each site are based on total percentage cover, number of individuals and species richness per quadrat.

Species Name	Allopatric Site (Llano County)		Parapatric Site (Wilson County)		Sympatric Site (Bastrop County)	
	Mean # of Individuals	Mean Cover	Mean # of Individuals	Mean Cover	Mean # of Individuals	Mean Cover
<i>Acalypha radians</i>	0	0	1.8	9.4	0	0
<i>Aristida oligantha</i>	8	8.4	0	0	4.6	6.6
<i>Bothriochloa laguroides</i>	2.4	8	2.2	7	0	0
<i>Cenchrus spinifex</i>	2	4.4	1	1.4	0	0
<i>Chloris cucullata</i>	0	0	1.7	1.6	0	0
<i>Chloris verticillata</i>	0.8	3.6	1.6	1.6	0	0
<i>Coelorachis cylindrica</i>	0	0	2.4	7.4	0	0
<i>Croton capitatus</i>	0	0	0.6	1.4	0	0
<i>Cynodon dactylon</i>	3	6.6	0	0	0	0
<i>Eragrostis secundiflora</i>	1.4	3	0	0	0	0
<i>Eriogonum multiflorum</i>	0	0	1	2.4	0	0
<i>Froelichia floridana</i>	0	0	0	0	0.6	1.6
<i>Heterotheca subaxillaris</i>	0	0	0.8	3.6	0.8	3
<i>Hymenopappus artemisiifolius</i>	0	0	1.6	1.6	0.2	0.7
<i>Ilex vomitoria</i>	0	0	0	0	0.2	1.6
<i>Liatris elegans</i>	0	0	0	0	0.6	1
<i>Monarda viridissima</i>	0	0	2	3.6	3.6	8.2
<i>Opuntia</i> spp.	0.4	2	0.2	0.6	0	0
<i>Panicum oligosanthos</i>	0	0	0	0	6.8	8.6
<i>Panicum virgatum</i>	2.2	4.6	6.8	15	0	0
<i>Quercus stellata</i>	0	0	0	0	0.4	1
<i>Schizachyrium scoparium</i>	0	0	0	0	0.2	2
<i>Selaginella arenicola</i>	0	0	3	4.6	0	0
<i>Sorghastrum nutans</i>	0.6	4.2	0	0	0	0
<i>Tradescantia subacaulis</i>	0	0	0	0	0.6	1.2
<i>Verbesina virginica</i>	0.4	2	0	0	0	0
Mean # of Individuals per Quadrat	11.8		9.1		8.5	
Total Mean Cover		46.8		61.2		35.5
Species Richness	5.4		7.8		5.6	

Volatile Oil Production

Gas chromatographic analyses of the volatile oils from the leaves of *Monarda viridissima* grown experimentally in a peat-based potting mixture and soils collected from the allopatric (Llano Co.), parapatric (Wilson Co.) and sympatric (Bastrop Co.) field sites showed significant differences in the absolute concentrations of O-cymene ($F_{3,36} = 3.64$, $P = 0.022$, $N = 40$), linalol ($F_{3,36} = 5.13$, $P = 0.0047$, $N = 40$), thymol ($F_{3,36} = 5.13$, $P = 0.018$, $N = 40$) and alpha-farnesene ($F_{3,36} = 3.919$, $P = 0.0161$, $N = 40$). Figure 15 shows the relative proportion of leaf volatile oil composition of ten individuals grown in a peat-based potting mixture. Thymol was the predominant compound in eight individuals and differed from the allopatric soil treatment ($P = 0.025$). The peat-mixture also differed from the allopatric treatment (fig. 16) in relative concentrations of O-cymene ($P = 0.071$) and linalol ($P = 0.012$). Phelladrene was present in two individuals in the allopatric treatment in trace amounts.

The allopatric soil treatment (fig. 16) did not share the characteristic of high concentrations of thymol in a majority of the individuals as was found in the other treatments. Relative concentrations of thymol in the allopatric treatment also differed from thymol concentrations in the parapatric ($P = 0.069$) and sympatric ($P = 0.051$) treatments. Although not significant, there were lower concentrations and less diversity of compounds in the allopatric soil treatment and the dominant compounds varied between thymol, carvacrol, O-cymene, alpha bourbonene and sigma terpinene. Linalol and alpha-farnesene were the most consistent minor compounds present in the majority of individuals grown in the allopatric soil. Alpha-farnesenene in the allopatric soil differed from the parapatric ($P = 0.039$) and sympatric ($P = 0.019$) treatments. Linalool was

significantly higher in the allopatric soil than the parapatric ($P = 0.035$) and sympatric ($P = 0.009$) soils (Tukey HSD multiple comparisons following ANOVA).

Thymol was the predominant compound in seven individuals of *Monarda viridissima* grown in the sympatric soil and carvacrol was dominant in three of the ten individuals (fig. 18). O-cymene was also present in relatively large amounts which differed between the allopatric soil and the parapatric ($P = 0.075$) and sympatric ($P = 0.027$) soil treatments. The parapatric soil treatment (fig. 17) was very similar to the sympatric and peat treatments with large amounts of thymol in a majority of the individuals. O-cymene was consistently present among individuals in all four treatments. The peat-based mixture had the highest average number of compounds, 17, followed by Bastrop, 16.4, Wilson, 14.4 and Llano, 12.6. However there was no difference between treatments in the mean absolute amounts of oils produced ($P = 0.1769$) (fig. 19).

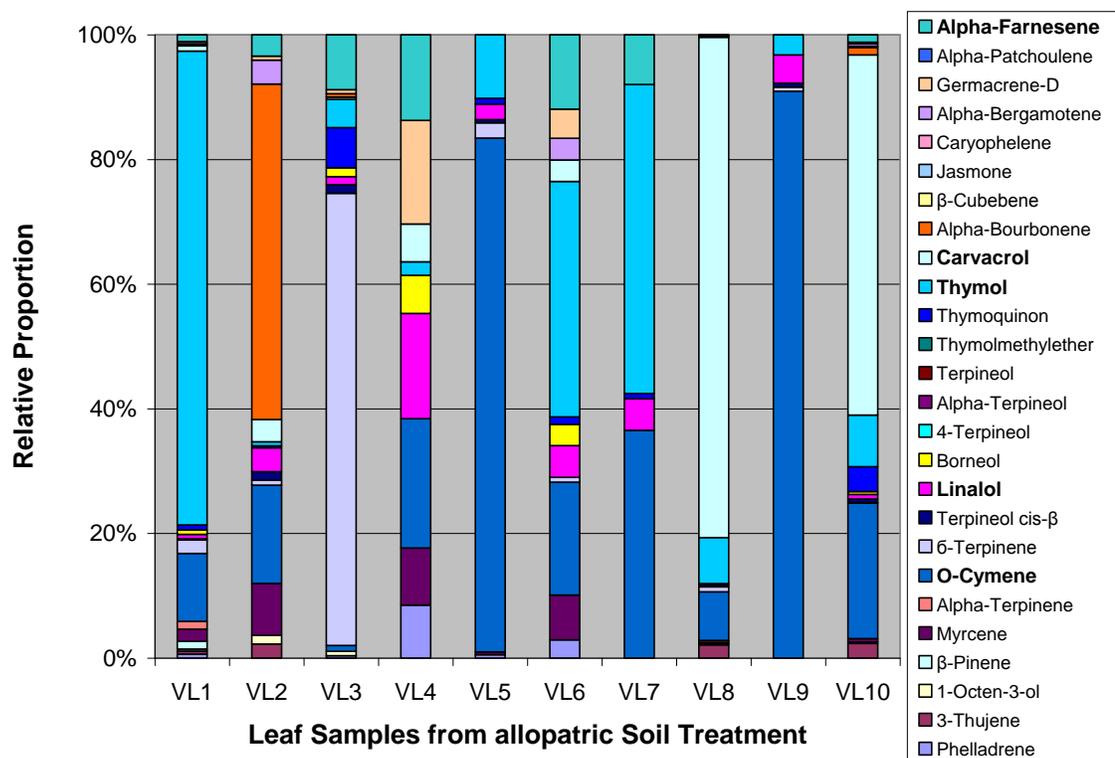


Figure 13. Volatile oils found in leaf samples of ten individuals of *M. viridissima* grown in allopatric soil (Llano Co.). Note the lack of a consistent pattern among individuals.

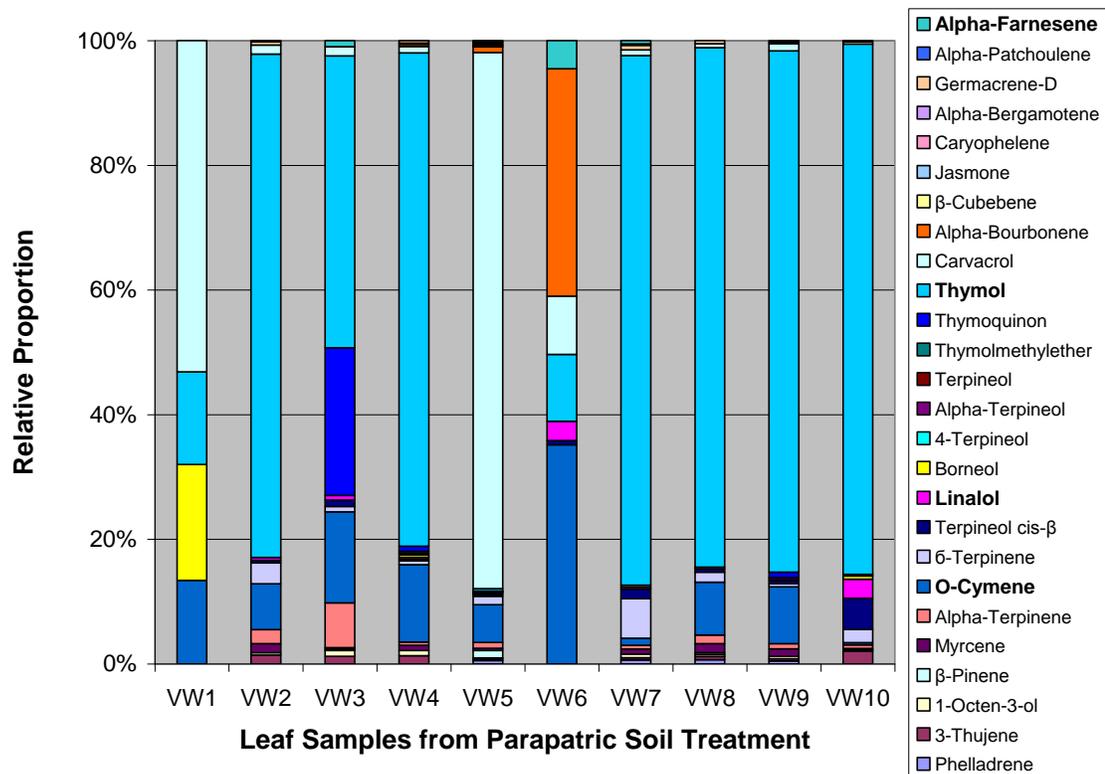


Figure 14. Volatile oils found in leaf samples of ten individuals of *M. viridissima* grown in the parapatric soil (Wilson Co.).

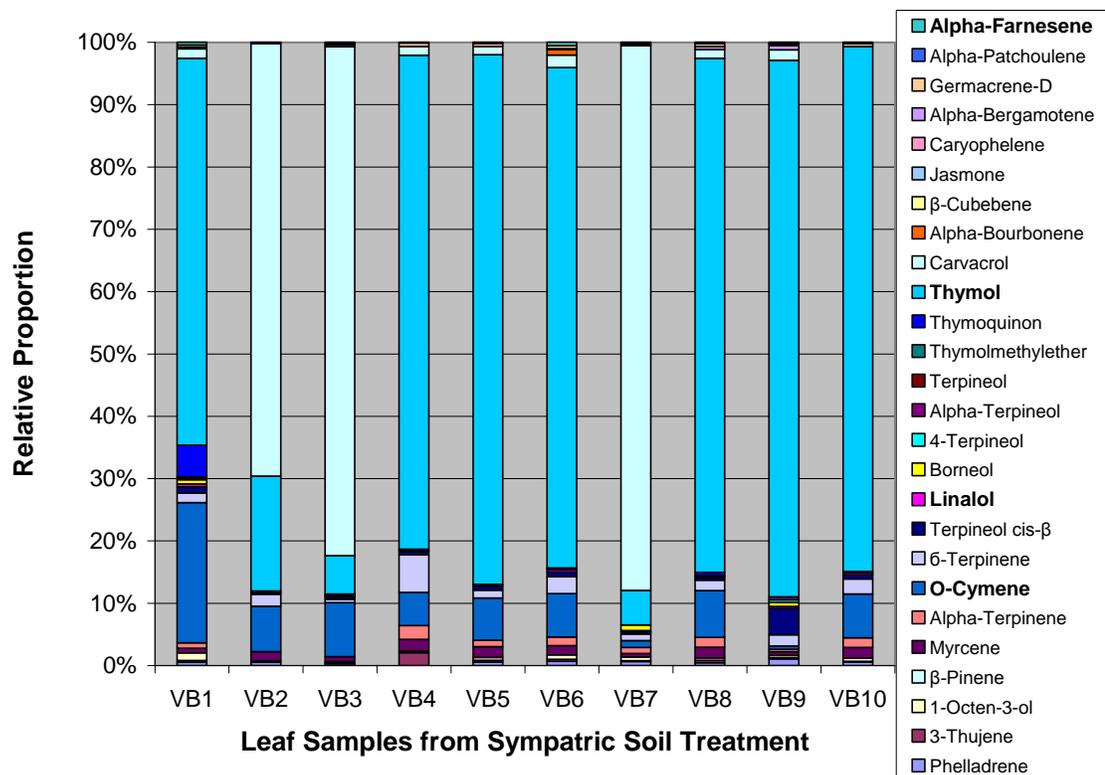


Figure 15. Volatile oils found in leaf samples of ten individuals of *M. viridissima* grown in the sympatric soil treatment (Bastrop Co.).

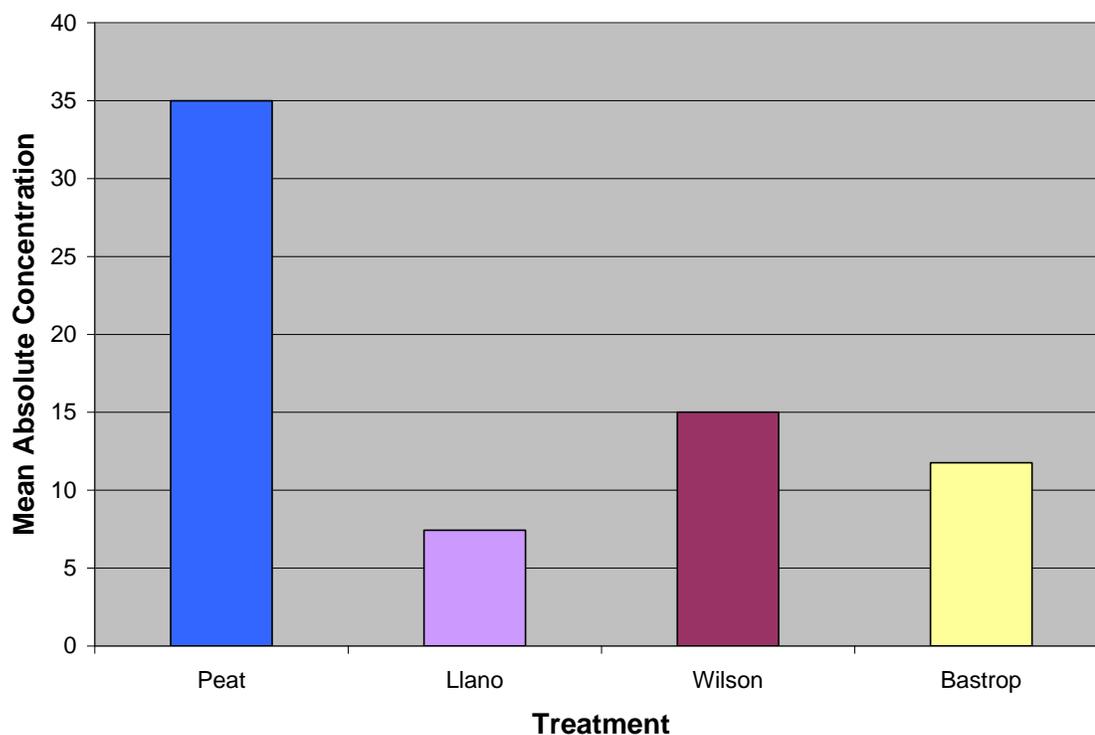


Figure 16. Mean absolute concentration of compounds detected in leaves of *M. viridissima* experimentally grown in four different soil types; a peat-mixture, Llano Co. (allopatric), Wilson Co. (parapatric) and Bastrop Co. (sympatric) ($F = 2.27$, $P = 0.1769$, $N = 40$).

Gas chromatographic analyses of the volatile oils from the leaves of *Monarda viridissima* and *Monarda punctata* collected from the parapatric and sympatric field sites showed significant differences between sites as well as species (figs. 20, 21, 22 and 23). Compounds that were found to differ significantly between sites and species included thymol ($F_{3,16} = 1837.2$, $P = 2.2 \times 10^{-16}$), carvacrol ($F_{3,16} = 752.77$, $P = 2.2 \times 10^{-16}$), 3-thujene ($F_{3,16} = 16.331$, $P = 3.98 \times 10^{-5}$), alpha-terpinene ($F_{3,16} = 12.890$, $P = 0.0001545$) and germacrene-D ($F_{3,16} = 25.916$, $P = 2.214 \times 10^{-6}$). At the parapatric field site (Wilson Co.) *Monarda punctata* and *M. viridissima* differed significantly in relative amounts of thymol ($P = 0.00000$), carvacrol ($P = 0.00000$), 3-thujene ($P = 0.0028$), alpha-terpinene ($P = 0.0067$) and germacrene-D ($P = 0.000009$) (figs. 20 and 21). At the sympatric field

site (Bastrop Co.) *Monarda punctata* and *M. viridissima* differed significantly in relative amounts of thymol ($P = 0.00000$), carvacrol ($P = 0.00000$), 3-thujene ($P = 0.0005$), alpha-terpinene ($P = 0.00095$) and germacrene-D ($P = 0.0008$) (figs. 22 and 23). There were also differences between *Monarda punctata* at the sympatric site and *M. viridissima* at the parapatric site in amounts of thymol ($P = 0.00000$), carvacrol ($P = 0.00000$), 3-thujene ($P = 0.0231$), alpha-terpinene ($P = 0.0059$) and germacrene-D ($P = 0.00007$). *Monarda punctata* at the parapatric field site and *M. viridissima* at the sympatric field site also differed in amounts of thymol ($P = 0.00000$), carvacrol ($P = 0.00000$), 3-thujene ($P = 0.00007$), alpha-terpinene ($P = 0.0010$) and germacrene-D ($P = 0.00008$) (figs. 21 and 22). Phellandrene was completely absent from *M. punctata* at both the parapatric and sympatric sites but was present in *M. viridissima* at both the parapatric and sympatric sites.

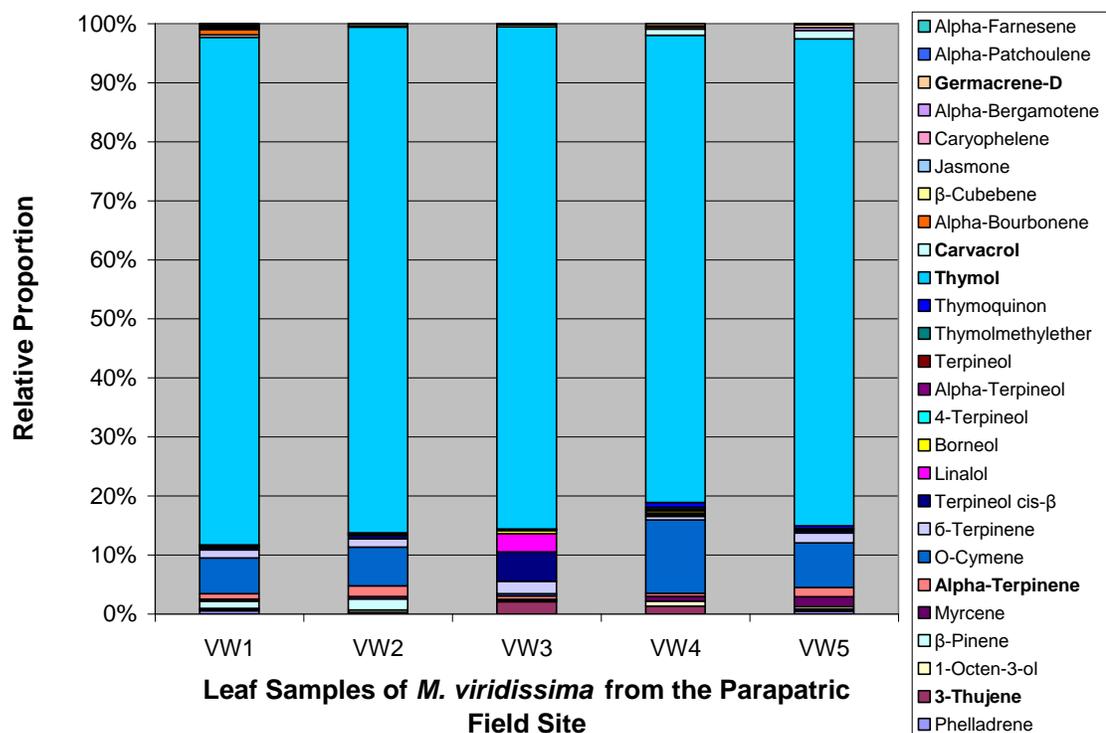


Figure 17. Relative proportion of leaf volatile oils from five individuals of *Monarda viridissima* collected from the parapatric (Wilson Co.) field site.

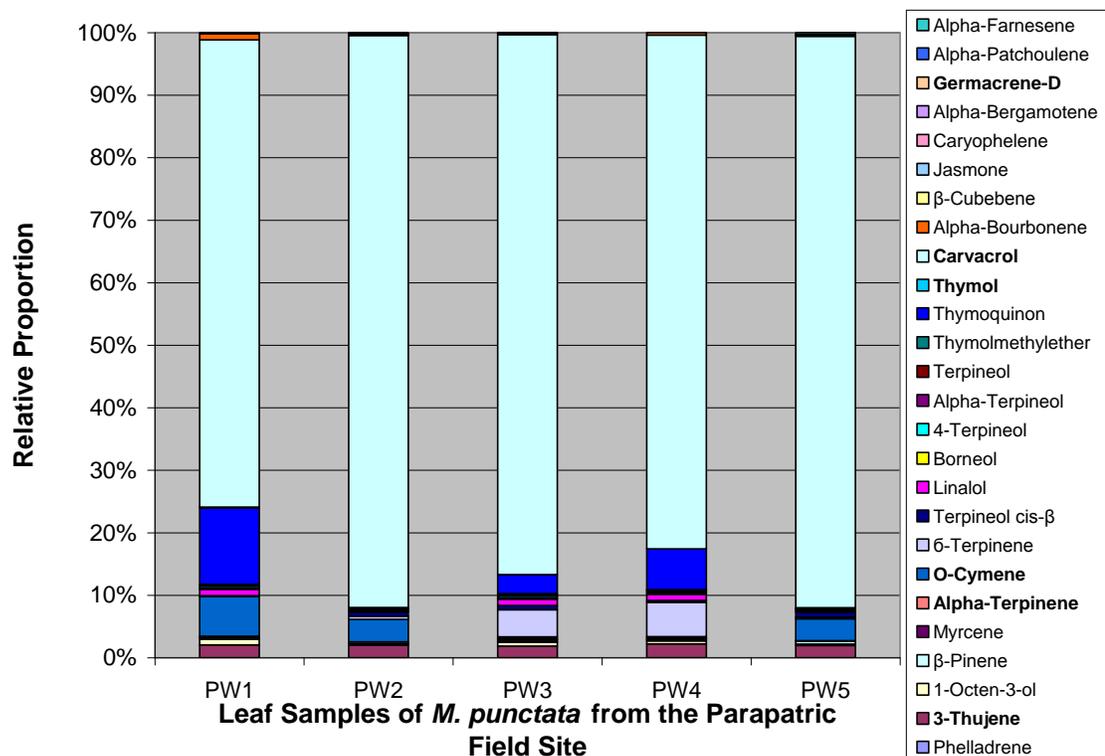


Figure 18. Relative proportion of leaf volatile oils from five individuals of *Monarda punctata* collected from the Wilson CO. field site.

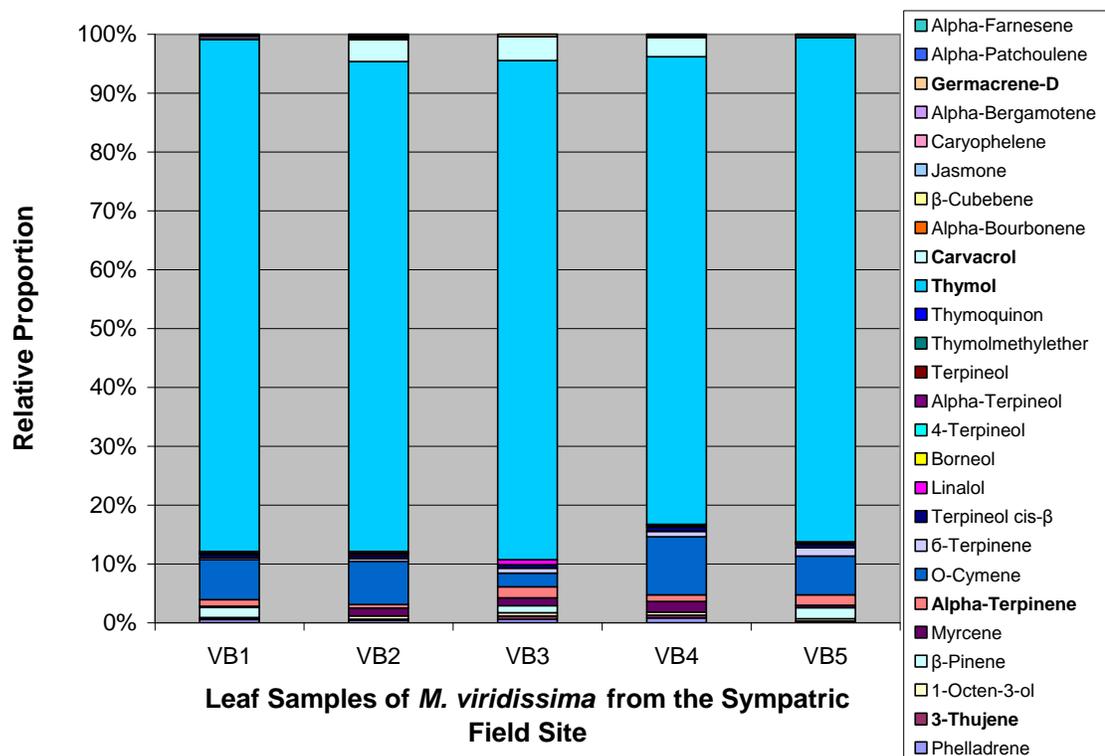


Figure 19. Relative proportion of leaf volatile oils from five individuals of *Monarda viridissima* collected from the sympatric (Bastrop Co.) field site.

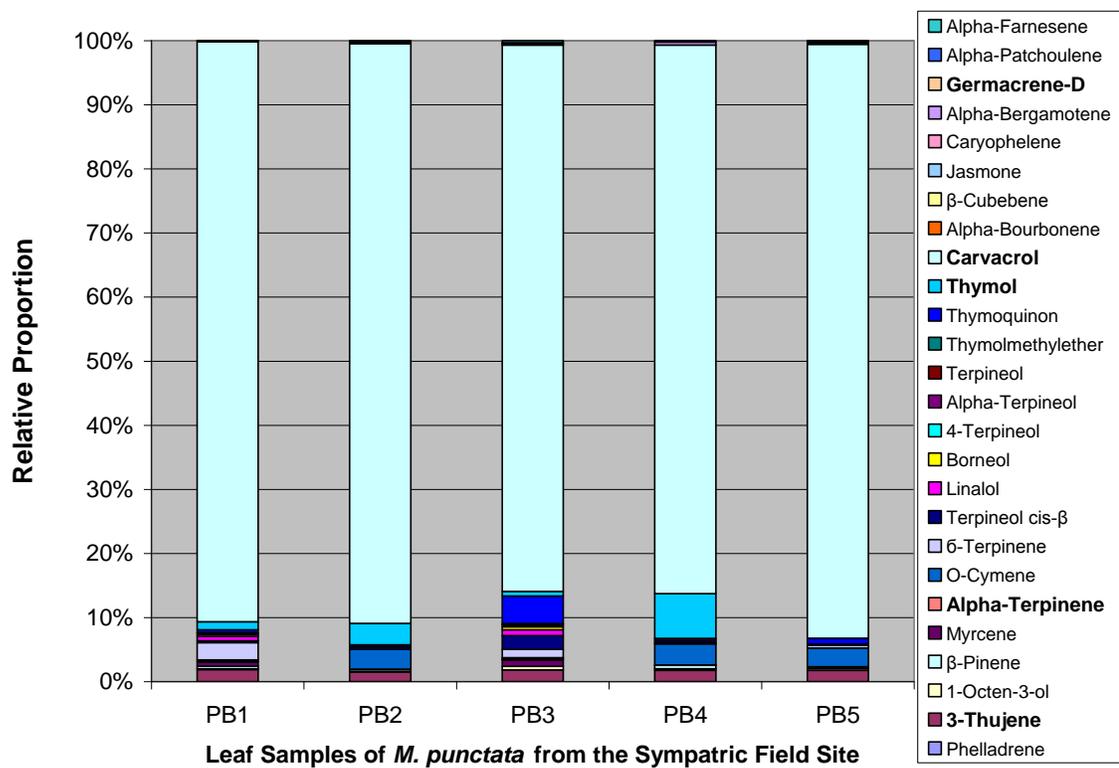


Figure 20. Relative proportion of leaf volatile oils from five individuals of *Monarda punctata* collected from the sympatric (Bastrop Co.) field site.

Chapter IV

DISCUSSION

Growth and Germination Rates of Experimental Plantings

Seed weight data showed no significant difference between *Monarda viridissima* and *Monarda punctata*. However *M. punctata* seeds were from a commercial source not from the same sites as *M. viridissima*. Related studies comparing endemics with widespread relatives found that seed weights of the endemic species were generally greater than seed weights of the widespread species (Kruckeberg and Rabinowitz, 1985; Cody and Overton, 1996). The hypothesis is that selection would favor fewer, larger seeds as opposed to more, smaller and widely dispersing seeds. The advantage of high dispersal ability is being able to reach a new habitat, but this is not an advantage at all if the plant is unable to establish populations in the new habitat. Oceanic islands are the extreme example of plants evolving lower dispersal abilities but the idea can also be applied to mainland habitats where edaphic endemics are restricted to a distinct soil type which acts as an island of suitable habitat (Cody and Overton, 1996; Harrison et al., 2000). Seed size is also related to seedling survival, with larger seeds often resulting in higher survival rates. K-selected species in resource poor habitats, such as sands, often have large seeds (Huston, 1994; Williamson et al., 1994). Therefore endemics often have lower dispersal abilities that keep the population restricted to smaller, suitable areas of

habitat. However, this was apparently not the case for *M. viridissima* and *M. punctata*, based on the seed sources used.

Germination data did not support the hypothesis that the peat-based mixture would have the highest germination rate. Instead, germination rates in the native *Monarda viridissima* Wilson County soil were the highest. The non-native, Llano County soil had lower germination rates than all other soil types (although germination in the peat-based mixture was not significantly lower). Hart (1980) found similar results for serpentine endemics which had higher seedling survival on their native soil. He attributed the difference in seedling survival to possible nutrient toxicity of the serpentine endemics when they are grown in more fertile soil and that seeds of serpentine endemics may be more susceptible to pathogens in non-native soil. The Llano County soil may contain microorganisms to which *M. viridissima* seeds are not resistant. These explanations are plausible for the results from the present study. The data may also indicate that soil water holding capacity does not have a significant impact on germination since the available water capacity for native soils was lower than the peat-based soil mixture and similar to the non-native.

Growth rate data did support the hypothesis that the peat-based mixture would have the highest growth rate among the different soil types. The fact that *M. viridissima* grew well on all soil types, and actually did not grow as well on its native soils, indicates its physiological niche is not restricted by special soil properties. However, in order to grow, seeds must germinate and the germination data confirm significantly higher germination rates in the native soils. The leaf morphology of *M. viridissima* in the peat mixture differed from the other soils by having larger, wider, weakly keeled leaves which

may suggest they had more water and nutrients available. This leaf morphology is similar to that of *M. punctata* and may be one reason plants in the peat mixture had the highest growth rate. *Monarda viridissima* grown on the other three soil types produced small, narrow, strongly keeled leaves which are characteristic of field grown plants in areas with low water and nutrient availability. While keeled leaves reduce the area for light absorption and therefore reduce photosynthesis, narrow leaves aid in cooling by convection which is advantageous in the hot, dry habitat of *M. viridissima*.

This type of leaf morphology is not found in *M. punctata*, which has wide non-keeled leaves, and may be a factor that prevents it from establishing large populations on the Carrizo sands where *M. viridissima* grows. These results demonstrate that the leaf morphology of *M. viridissima* is highly plastic, being xeromorphic under dry conditions and converging with the more mesomorphic morphology of the widespread *M. punctata* under more favorable, wetter conditions. Unfortunately I was not able to evaluate the leaf plasticity of *M. punctata*. In conclusion the ecological niche of *M. viridissima* may be determined by properties of its native soils that create drier conditions, reduce the growth of *M. punctata*, and allow *M. viridissima* to maintain populations free from competition by *M. punctata*, and other species in wetter and more fertile soils.

Vegetation Structure

The two native *M. viridissima* field sites, Bastrop and Wilson Counties, had a significantly different percentage of plant cover. The higher percentage cover in Wilson County, 61.2%, may help limit the growth of *M. punctata* in the same locations as *M. viridissima*. The generalist adaptations of *M. punctata* that allow it to survive in a wide range of habitats may prevent it from surviving and effectively competing with *M.*

viridissima in the slightly harsher environments that *M. viridissima* occupies. Since *M. viridissima* is a perennial it does not rely on open ground for seeds to sprout each year. This may be another feature that helps *M. viridissima* survive competition in areas that have more vegetation.

The sympatric field site (Bastrop County) had the lowest percentage cover, 35.5%, which may be one reason *M. viridissima* and *M. punctata* grow in such close proximity to one another. The sympatric site is located farther northeast than the parapatric site (Wilson County) which causes it to have a slightly wetter and cooler climate that may allow *M. punctata* to establish populations there and compete more effectively, or just survive, with *M. viridissima*.

The parapatric field site had significantly higher total and plot-level species richness compared to the sympatric and allopatric sites which may indicate that *M. viridissima* survives better and may be a better competitor in that particular habitat where its specialist adaptations allow it effectively compete with a wide range of species with similar adaptations. *Monarda punctata* does not compete well under dry conditions with numerous perennials and therefore *M. punctata* is restricted to certain areas with a slightly different community structure.

The soil map of the parapatric habitat shows it is much more homogenous than the other two sites which may also contribute to the success of *M. viridissima* in relation to *M. punctata*. An edaphically heterogeneous landscape such as the allopatric and sympatric sites creates more opportunity for *M. punctata* to find habitat that satisfies its physiological niche requirements. The allopatric and sympatric sites also have diverse

topographic features compared to the parapatric site which is flat. The drainage of water to the more level areas where *M. punctata* grows may help it survive and compete with *M. viridissima* at the sympatric habitat and may allow it to survive much better at the allopatric site even if *M. viridissima* were to grow there. It is likely that the higher clay content at the allopatric site also has a similar effect. Although *M. viridissima* grew well on allopatric soil, from Llano County, in the experimental treatments it may have difficulty competing effectively with the numerous fast growing annuals found at the actual field site, resulting in ecological constraints on its physiological niche. Perennials are often characteristic of drier or overall harsher environments where there are fewer species and therefore less competition.

Volatile oils

Scora's (1967a) study of leaf oils in *Monarda* found that most taxa possessed large amounts of thymol or its isomer carvacrol and members of the subgenus *Cheilyctis* section *Cheilyctis* were quite homogenous. The present study did not investigate as many species but it did find that the "finger prints" of *M. punctata* and *M. viridissima* were quite similar. There were both alternative (presence or absence of compounds) and quantitative differences found between the two species, between treatments and within treatments. Phelladrene, β -cubene, jasmone, and caryophelene were not found in any *M. punctata* leaf samples. *Monarda viridissima* had 21 individuals with Phelladrene present in the leaf oils, 2 with β -cubene, 5 containing jasmone and 1 individual which contained caryophelene.

Alternative variation is said to be controlled by one or only a few genes that are responsible for the expression of a compound (Franz, 1993). Quantitative variation is

thought to be controlled by multiple genes that express a certain compound and unlike alternative variation can be affected by the environment (Franz, 1993). In terms of intraspecific variation, emphasis is placed on major components and minor or trace amounts are assumed non characteristic. This study agreed with others in terms of the major components present in leaf samples of *M. punctata* and *M. viridissima*, namely thymol, carvacrol and cymene. Chemical diversity at the species level is said to be the rule rather than the exception which would also agree with the results from this study (Franz, 1993).

The hypothesis that the number and amount of volatile oils produced will be lowest in *M. viridissima* plants grown in the peat-based potting mix was not supported due to the highest average number of compounds occurring in the peat mixture, even though it was not statistically significant. *Monarda viridissima* grown in the other experimental soils may have experienced more water stress but they did not produce a greater number or larger amount of volatile oils. The average number and relative proportion of volatile oils produced by *M. viridissima* grown in the four experimental soils differed significantly between treatments and therefore water stress may be a factor that affects oil production. The greatest differences in oil production occurred between the allopatric soils and all others but there were no significant differences between the peat, parapatric or sympatric soils.

At field sites differences were found between the two species at the same site and at the two different sites. Thymol differed in the allopatric soil treatment compared to the other treatments and at the field sites it differed both between species and sites. Thymol is a phenylpropene known to be a very effective antimicrobial, antifungal and antibacterial

agent. It is also used as a pesticide and is particularly effective against mites. Thymol is an isomer of carvacrol which shares the same antibiotic properties and it is likely that *M. viridissima* and *M. punctata* share a physiological divergence that may explain the difference in thymol and carvacrol between the two species. Consistent differences in carvacrol concentrations were only found between the two species at the field sites, which may indicate that carvacrol is species-specific but further sampling is needed to determine the extent to which carvacrol occurs in *M. viridissima*.

O-cymene also differed in both the experimental treatments and at the field sites. Again, the differences were found between the allopatric soil treatments and all others and between sites and species. In addition to thymol and carvacrol, at the field sites, 3-thujene, alpha-terpinene and germacrene-D differed between sites and species. A majority of the compounds identified in leaf samples of *M. punctata* and *M. viridissima* have ecological and or biological importance. Germacrene-D, a sesquiterpene, is used by tobacco horn worms to locate their host plant, and a number of other insects use it in chemical communication. Unlike other compounds found in *M. viridissima* germacrene-D is not used as a food flavoring or fragrance. *Monarda punctata* and *M. viridissima* growing sympatrically did produce different types of volatile oils which may indicate chemical divergence was a factor in creating the two distinct species or in facilitating their coexistence. The monoterpene cymene is known to repel leaf cutter ants and the sesquiterpene caryophelene is known to act as an antifungal agent (Crawley, 1998). Linalol and alpha-terpinene are known to reduce digestive efficiency in ruminants due to their antibacterial properties (Crawley, 1998). Germacrene, thujene, pinene, phellandrene and terpinene are also known to have strong antibacterial properties

(Verlet, 1993). It seems likely that microbial activity in the environment would have an effect on volatile oil production.

Monarda viridissima grew well on a wide range of soils which may mean its physiological niche is not restricted by special soil properties and it is possible that its range may expand over time due to its generalist properties and phenotypic plasticity found in leaf morphology. The levels of the main components were not all consistent but with more extensive sampling it is very possible that distinct chemical races of *M. punctata* and *M. viridissima* could be identified. Keeping track of the individuals that *M. viridissima* seeds were collected from would also be useful in revealing genetic variation between individuals within a population, which was not done in this study. Volatile oil production of *Monarda viridissima* grown in the experimental soils did not increase with increased water stress and therefore may be more dependent on other environmental factors such as nutrient availability, pollinators, herbivores and plant-plant interactions (Mattson and Haack, 1987; Herms and Mattson, 1992).

There are numerous aspects of *M. viridissima* and *M. punctata* that need to be investigated. This study explored various aspects of growth and oil production but in order to understand specific causes of endemism in *Monarda viridissima* and how it relates to volatile oil production a more detailed study of differences among individuals is needed. Sampling of *M. viridissima* from its complete range in the Carrizo formation, and *M. punctata* that occurs sympatrically and parapatrically within that range, would also help reveal characteristics that may be species specific and related to the range restriction of *M. viridissima* and its coexistence with *M. punctata*. Experimental treatments of *M.*

punctata grown in different soil types and in competition with *M. viridissima* may give valuable insight to specific interactions between the two species. Anatomical studies of the leaves and trichomes of the two species and data pertaining to pollinator differences between the two species would also be valuable.

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VITA

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