...tribution of *Monarda* on City of Boulder Open Space and Mountain Parks Lands

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Introduction

The genus *Monarda* (Lamiaceae) has sixteen species found only on the North American Continent, distributed north to south from Canada to central Mexico and east to west from the Atlantic to the Rocky Mountains (Prather et al. 2002). Mostly perennials, the genus also includes a few annuals and one shrub-forming species (Prather et al. 2002).

Two members of the genus, *M. fistulosa* L. var. *menthifolia* (Graham) Fernald and *M. pectinata* Nuttall, occur in Colorado (Weber and Wittmann 1996). Collection records from the University of Colorado Herbarium for both species reveal locations for *M. fistulosa* throughout the western half of the state and *M. pectinata* sites throughout much of the lower elevation areas of the state, including locations on the eastern plains and in the San Louis valley.

This report details work completed during the 2003 field season on City of Boulder Open Space and Mountain Parks (OSMP) lands with respect to research permit proposal entitled *Chemical Ecology of Monarda in Colorado*. The research included the collection and gas chromatograph analysis of leaf samples from *M. fistulosa* and *M. pectinata* individuals from OSMP lands to determine distribution of essential oil chemotypes.

As with most species in the Lamiaceae, all species of *Monarda* produce essential oil composed mostly of monoterpenes in glandular trichomes located on the surface of leaves, sepals, flower pedals and to some extent on stems (Keefover-Ring, unpublished data). In addition, like many other mints, the essential oil of most *Monarda* species is dominated by a single monoterpene which by definition identifies the plant's chemical type, or chemotype (Scora 1967).

Scora (1967) conducted essential oil analysis on half of the species in the genus *Monarda*, including three identified varieties of *fistulosa*; *menthifolia*, *brevis* and *mollis*. He also analyzed a fourth group of *M. fistulosa*, which he called "Arkansan Segregate". Chemical analysis revealed that the three known varieties all contained the phenolic monoterpene thymol as their dominant component, whereas the Arkansan Segregate contained mostly *p*-cymene. The presence of such a large amount of *p*-cymene and no detectable levels of thymol prompted Scora (1967) to designate the Arkansan Segregate as a possible new *p*-cymene chemotype. Zamureenko et al. (1989) also reported an analysis of *M. fistulosa* where *p*-cymene was the main constituent of extracted essential oil at 32.5%. However, unlike the *p*-cymene plants reported earlier these individuals contained relatively high amounts of thymol (12.6%) and carvacrol (23.9%), another phenolic monoterpene.

Subsequent studies of greenhouse grown plants also reported thymol as the main constituent of *M. fistulosa* individuals (Heinrich 1973). Weaver et al. (1995) collected *M. fistulosa* plants approximately 8 km northeast of Bozeman, Montana for use as a deterrent

to insect herbivores of stored food products. These plants contained essential oil dominated by carvacrol.

Another chemotype of *M. fistulosa* was formally announced in by Marshall and Scora (1972) from a limited area in southern Manitoba, Canada. The new chemotype contained the monoterpene geraniol as its main constituent. Individuals of the new chemotype grew within populations of thymol plants. To date, Marshall and Scora's (1972) study represents the only spatial examination of *M. fistulosa* chemotypes.

M. pectinata has received much less attention. Except for a single qualitative study by Burt (1936), which only confirmed the presence of phenolic monoterpenes (either thymol or carvacrol) as the only identified components, no rigorous analysis of the essential oil chemistry, much less chemotype distribution of *M. pectinata* has been done.

Plants that produce thymol and carvacrol usually also contain varying amounts of two other monoterpenes, γ-terpinene and *p*-cymene (Vokou et al. 1988, 1993, Thompson et al. 2003). Poulose and Croteau (1978) elucidated the pathway for the production of thymol and carvacrol and determined that both are made by the hydroxylation of *p*-cymene, which is initially synthesized from γ-terpinene (Fig 1.) These two precursors were detected in all *M. fistulosa* and *M. pectinata* samples in this study (Fig 2).

Figure 1. Biosynthetic pathway of carvacrol and thymol

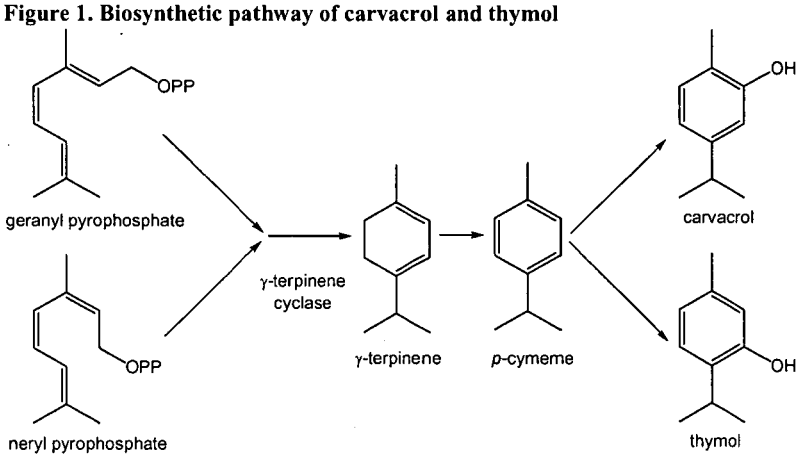
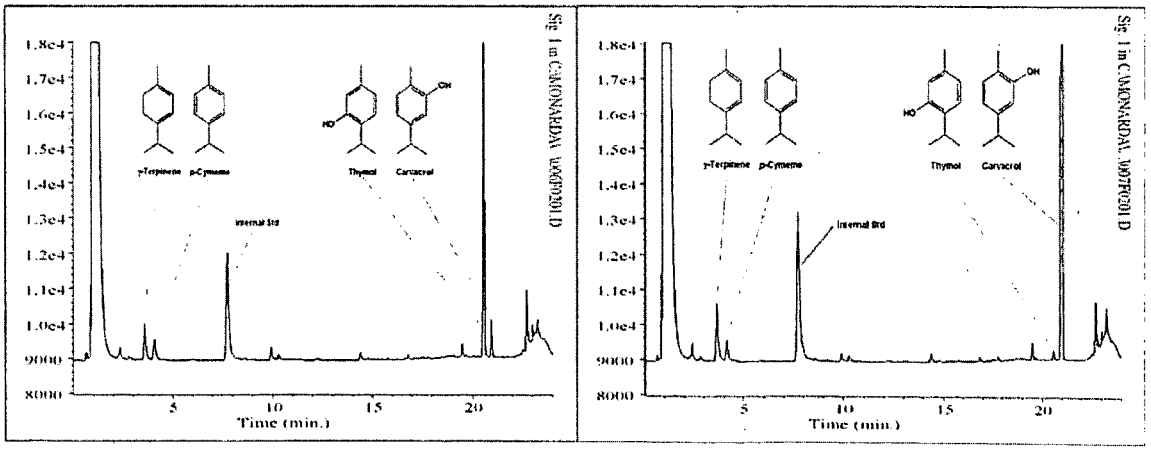


Figure 2. Typical gas chromatograms for *M. fistulosa*, thymol and carvacrol chemotypes, respectively



Methods and Materials

OSMP Sites

Leaf samples of *Monarda fistulosa* used in chemical analysis was collected from ten sites located on OSMP Lands. These sites included two sites each at Bear Canyon, Shadow Canyon, and Gregory Canyon, and one each at Daman (collected by OSMP personnel), Dowdy Draw, Shanahan Ridge, and Mount Sanitas. Samples of *Monarda pectinata* were collected at a single location, White Rocks.

Sample Collection

Single leaves from individual plants were collected from mid-May until mid-July 2003. Typically, one of the two leaves in the second position down from the sepal was chosen. Samples were placed in small re-sealable bags after collection and stored in an iced cooler or solvent soaked immediately in the field. Most samples that were kept on ice were extracted within approximately four hours after collection, except the Daman samples which were refrigerated overnight and extracted the following day. Extraction involved fully submerging *M. fistulosa* leaves in 1.5 mL and *M. pectinata* leaves in 1.0 mL of internal standard solution (1 μL/mL of fenchone in ethanol) for seven days. Prior to analysis, 100 μL of *M. fistulosa* samples were diluted with 100 μL of internal standard solution. *M. pectinata* samples were analyzed without further dilution.

Chemical Analysis

Analysis of monoterpenes from plant samples was carried out with a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. The injector temperature was set at 260°C and the detector at 255°C. The oven profile for each run was set as follows: 50°C for 5 minutes followed by a ramp of 3°C/minute to 75°C, then a final ramp of 10°C/minute to 160°C. Two μL of both samples and standard were injected via a spilt injection with a spilt ratio of approximately 100:1. Helium was used as the carrier gas and the flow rate was approximately 1 mL/minute.

Identification and quaintitation of individual terpenes was accomplished using retention times and peak areas, respectively, of injections of pure compounds diluted into the internal standard solution. Plant chemotypes were determined based on the identity of the compound that contributed the highest percentage to the total essential oil composition. In most cases the chemotype monoterpene constituted >60% of the total.

Results

Two chemotypes, thymol and carvacrol, were identified in the ten *M. fistulosa* populations sampled on OSMP Lands (Table 1, Figure 3). Due to proximity, the two collection sites at both Gregory Canyon and Shadow Canyon were combined as single populations within each respective site.

The carvacrol chemotype dominated the majority of the *M. fistulosa* populations sampled. All plants at the Bear Canyon 2, Dowdy Draw and Shanahan Ridge sites were carvacrol. In addition, out of the 40 plants analyzed from Shadow Canyon only one was thymol. The Bear Canyon 1 site contained only four thymol plants out of 20 measured. While the Daman site had a majority of carvacrol individuals, the area was the only site close to an even number of the two chemotypes (17 C: 13 T). Both the Gregory Canyon

and Mount Sanitas locations had thymol chemotypes as the greatest percentage of the total, with 81 and 61% thymol, respectively.

All 24 of the *M. pectinata* individuals collected from White Rocks Open Space were carvacrol chemotypes.

Table 1: Number of Thymol and Carvacrol Chemotypes for *M. fistulosa* and *M. pectinata* on OSMP Lands

Site	Thymol Plants	Carvacrol Plants
M. fistulosa		
Bear Canyon 1	4	16
Bear Canyon 2	0	20
Daman	13	17
Dowdy Draw	0	26
Gregory Canyon	25	6
Mount Sanitas	11	7
Shadow Canyon	1	39
Shanahan Ridge	0	15
M. pectinata		
White Rocks	0	24

Discussion

Multiple chemotypes in populations of a single plant species must be maintained by some external selection pressure, either biotic, abiotic or both. If a certain chemotype confers much greater fitness on individual plants then other chemotypes, we would expect populations to eventually become fixed for this beneficial chemotype. A classic case study of how these polymorphisms are maintained is *Thymus vulgaris* or common thyme in the South of France (Vernet et al. 1986, Thompson et al. 1998). In this area common thyme is known to have six separate chemotypes arranged over the landscape. Some of these chemotypes have been shown to be more repellent to herbivores than others; however, individuals of these repellent chemotypes are unable to survive in colder microclimates then the more palatable ones. Thus, through differential selection a mosaic of different thyme chemotypes persists over time.

The present study describes the chemotype distribution of two native mint species on OSMP lands. Understanding how different chemotypes are arranged spatially is the first step in attempting to determine how these patterns arise and are subsequently maintained. The chemotypes of both *Monarda fistulosa* and *Thymus vulgaris* appear to be strongly dictated by the plant's genetics (Scora 1967, Vernet et al. 1986). Common thyme also contains carvacrol and thymol chemotypes and controlled crosses have shown that the carvacrol chemotype has complete dominance over thymol (Vernet et al. 1986). While this relation has not been demonstrated for these chemotypes in *M. fistulosa*, Scora (1967) showed that the geraniol chemotype is dominant to thymol in populations in *M. fistulosa* populations in Manitoba; a relationship consistent with the dominance patterns of geraniol and thymol chemotypes in common thyme.

Pollination in *Monarda* is facilitated primarily by bumblebees of the genus *Bombus* (Cruden et al. 1984, Cresswell 1990). Bumblebees are relatively strong fliers and thus any *Monarda* population with a bumblebee's flight of another should share genetic material. While *M. fistulosa* is widespread on OSMP lands, populations can be isolated from one another due to the plant's preference for moister habitats. Thus,

populations are usually found in canyon bottoms or wet meadows separated by the dryer uplands. This spatial structure may account for the observed pattern of chemotypes. However, an alternate explanation for the chemotype differences between populations may be local adaptation of particular chemotypes to particular locations. In the case of *M. fistulosa* on OSMP lands and other populations in the state (Ken Keefover-Ring, unpublished data), it appears that thymol populations may be more common at higher elevation or colder locations, such as narrower canyons (Gregory Canyon for example). Currently, this theory is only anecdotal and additional data, namely temperature measurements, are needed.

The single *M. pectinata* population examined in this study represents the first thorough description of this species' chemistry. This population appears to monomorphic for the carvacrol chemotype, as was the case for two other *M. pectinata* populations from the San Louis valley in southern Colorado (Ken Keefover-Ring, unpublished data). Additional populations need to be assayed to determine if the thymol chemotype exists in *M. pectinata*.

The management implications of this study are that particular populations of plants can have unique genetic attributes, in this case differences in essential oil chemistry between populations. While a less obvious difference than many genetic polymorphisms, this must be taken into consideration when *M. fistulosa* (or any other native plant) is used in any restoration project. Moving plants of different chemotypes into areas where that chemotype is uncommon or not present could alter local populations by disrupting the natural chemotype distribution.

Acknowledgements

I would like to thank Lynn Riedel and Paulette Robles for assistance with the permit process and Ann Armstrong for collecting *M. fistulosa* samples at the Daman site.

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Figure 3. ***M. fistulosa*** **chemotypes on OSMP lands**