ECOLOGY OF THREE POPULATIONS OF THE RARE WOODLAND PERENNIAL, *TRILLIUM PUSILLUM* MICHAUX (LILIACEAE) IN SOUTHWESTERN MISSOURI

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Trillium pusillum Michx. (incl. var. *ozarkanum* (E.J. Palmer & Steyerm.) Steyerm.), the Ozark wake robin, is a perennial, woodland spring ephemeral that blooms in Missouri in late March or early April and fruits and senesces in early July. The species was formerly considered federally for listing as an endangered species, is listed as category S2 of conservation concern in Missouri by the Missouri Natural Heritage Program (Missouri Natural Heritage Program, 2006), and is considered to be imperiled in all five of the states where it grows. It is believed to be limited to 17 sites in Missouri.

As a member of the spring ephemeral guild, *T. pusillum* is a potentially important component of Ozark woodlands. Spring ephemerals provide nectar and pollen to pollinators early in their above ground season when few other plants are in bloom, and they function as a "vernal dam" by temporarily sequestering nutrients and preventing leaching from forest systems during a period of high nutrient availability (Vitousek and Reiners, 1975; Bormann and Likens, 1979; Blank et al., 1980; Hedin et al., 1995; Anderson and Eickmeier, 2000; Anderson, 2004).

Trillium pusillum habitat in Missouri has only been described in general terms, such as "mesic to dry-mesic upland forests on gentle slopes, usually in cherty soils on calcareous substrates" or

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as occurring on a "gentle west-facing slope [with] shallow cherty soils" (Yatskievych, 1999; Morgan and Wallace, 1987). A site in Barry County in southwestern Missouri studied by Morgan and Wallace (1987) was described as an oak-hickory woodland with flowering dogwood (*Cornus florida*) as the dominant subcanopy species; the dominant spring-flowering herbaceous species included *Anemonella thalictroides, Podophyllum peltatum, Dentaria laciniata, Erythronium albidum,* and *Viola* sp.

The growth of *Trillium* consists of three major stages: one-leaved, three-leaved, and flowering (Ohara and Kawano, 1986; Kawano et al., 1986; Ohara and Utech, l986). The stage structure that would result in a stable, viable population was described by Morgan and Wallace (1987) for a *T. pusillum* population in Barry County, Missouri. Morgan and Wallace's stable population, (determined by counting all plants in one 25 m^2 plot), was 159 one-leaved stems, 89 three-leaved stems and 14 flowering stems (see Fig. 1). The number of flowering stems increased from 14 to 48 during the three year period of the study and the authors concluded that this population would probably increase in size or at least remain stable.

Our study was undertaken to survey the current and possible future status of *T. pusillum* in southwestern Missouri. We surveyed three populations in 2001 and 2002, hereafter referred to as the *Baker, Heckmaster*, and *Hoover Woods* sites. Population size and stage structure, along with pollinator visitation and seed dispersal, were determined to assess population viability. In addition, plant community structure (species composition, species diversity, cover) and general habitat characteristics (canopy light penetration, soil moisture and pH) were related to population viability. This study contributes to a general biological and ecological knowledge of this rare species in Missouri and identifies some habitat conditions that vary at sites where the *T. pusillum* population is viable vs. contracting; and therefore, contributes to future conservation and/or restoration efforts on behalf of this species.

METHODS

GENERAL DESCRIPTION OF *T. PUSILLUM*.—*Trillium pusillum* has been described by Freeman (1996), Timmerman-Erskine (1999), Timmerman-Erskine et al. (2002a, b, 2003), and Yatskievych

(1999) as a perennial with one or more glabrous, erect, aerial stems arising from a short, horizontal rhizome. Each stem when mature has one terminal, actinomorphic flower, which is borne above the whorl of three leaves on a pedicel up to five centimeters long. The flower has three, free, oblong, white petals turning pink to rose as the flower ages. There are three herbaceous green sepals and three styles, these united at the base and elongating through the six stamens as the flower matures, with the stigmatic branches slowly separating and recurving. The ovary is superior, with three locules, each producing numerous ovules. The fruit is an erect, green berry that abscises basally from the receptacle to disperse eventually into the leaf litter, sometimes with all seeds remaining inside. The seeds are shiny, dark brown, and relatively large, each with a white fleshy aril developing from the cells at the upper part of the raphe and at maturity covering the hilum.

REPRESENTATIVE SPECIMENS.—**Barry County.** Hoover Woods site in woods at base of slope under city of Cassville water towers. 36˚40.52'; 93˚52.31', elevation 414 m, 21 April 2002, Andre *128* (SMS 65838). **Barry County.** Baker site east of Cassville. 36˚41.39'; 93˚50.58', elevation 414 m, 21 April 2002, *Andre 129* (SMS 65827). **Lawrence County.** Heckmaster site east of Friestatt. 36˚00.53'; 93˚50.32', elevation 396 m, 24 April 2002, *Andre 130* (SMS 65826).

STUDY SITES**.—**Study sites were found by examining Element Occurrence Records of the Missouri Department of Conservation's Natural Heritage Database, contacting botanists and landowners, and driving surveys in Barry and Lawrence counties. Of the 16 populations of *T. pusillum* known to the authors at the time, at least 8 were already extirpated or reduced to only a few flowering stems in 1999 when the study began. The three sites where populations were examined were named generically or with reference to historic names in the Heritage Database.

The Baker site is believed to be "Source 7" of the seven Wildwood North populations (sources) in the records of the Missouri Natural Heritage Database and is the largest of the three sites selected for this study. There are no historical data on population size for Source 7. The site is open woodland on a west-facing slope located in eastern Cassville in Barry County. The site is an irregularly shaped area of ca.13,500 m^2, which is fenced on three sides and bordered by woods on all four sides. There are two soil types within the site. The upper half of the slope (9–14%

incline) is Clarksville-Noark and is described as well drained and very friable, very gravelly silt loam, subsoil to 152.4 cm (Aldrich and Meinert, 1994). The soil type on the lower half of the slope (ca. 35% incline) is Clarksville, which is described as somewhat excessively well drained. There has been no harvesting of timber or major disturbance to this site during the last 58 years (Barbara McClure, pers. comm.), although cattle have had constant access to this area throughout that time. Cattle were evident at the site during the first year of the study, when their trampling in the area resulted in the loss of some plants near the top of the slope. *Trillium pusillum* was found scattered throughout this site, but primarily on the mid- to lower portions of the slope. The most abundant growth was located at the northwestern corner in the lower third of the slope.

The Hoover Woods site is also in Barry County. It is within the city limits of Cassville. The site has a north-facing slope with an incline ranging from 14% at the bottom to 35% near the top. It has been undisturbed since 1945 (Gerry Hoover, pers. comm.). History prior to 1945 was not available. The site is ca. 4,000 m^2 and the soil type is classified as Clarksville (Aldrich and Meinert, 1994). Numerous signs of deer were noted on this site and one group of eight deer was observed during one visit. An invasive exotic evergreen vine, *Euonymus fortunei*, covers much of the ground and tree trunks at this site. Heritage Database occurrence records indicate there were approximately 2,000 plants in 1981 and 300-400 scattered stems (flowering and sterile) in 1994. We found *T. pusillum* only in ca.144 m^2 area of the lower eastern portion of the site in 2001 and 2002. Flowering stems were found scattered in the sampling area in April 2001, but could not be located to be counted when the site was visited again in early May. In 2002, 87 stems (30 flowering) were found in a ca.9 m^2 area at the base of the slope under some shrubs, but only three flowering plants were found scattered in the rest of the area.

The Heckmaster site is the northernmost known population for this taxon and is located in Lawrence County, ca. 30 miles north of Cassville. The soil type at the site is Clarksville-Nixa, a cherty silt loam, 5 to 14 percent slopes (Hughes, 1982). There has been no disturbance to this land for about 80 years (Donald Heckmaster, pers. comm.). This site may be the "Spring River Tributary" site noted in Heritage Database records. In 1984, the

population of *T. pusillum* at the Spring River Tributary site was described as "130 and 50 plants in two separate patches". In 2002, we found *T. pusillum* throughout the 1,000 m^2 site, with the most abundant growth, approximately 500–600 flowering stems, occurring within 100 m^2 at the bottom of the slope (14% incline).

DEMOGRAPHY AND REPRODUCTIVE POTENTIAL.—Population sizes, density and dispersion were estimated from surveying the entire area of each site, where the area of the site was bounded by the spread of the species across the site, and by estimating from counts within permanent 1 m^2 plots (see below). Stage structure at each site was determined by using transects and permanent points along the transects. A transect was run across each site either above or below the slope and perpendicular to it. At random points along each transect, perpendicular transects were run up or down the slope (Baker n=5; Heckmaster n=4; Hoover Woods n=3). Permanent points were then established by random selection along each transect on the slopes and marked using metal spikes and flags (Baker n=45; Heckmaster n=20; Hoover Woods n=15). Stems in each stage—unifoliate, trifoliate, and flowering—were counted within 1 m^2 plots around each point.

Preliminary observations of floral visitors of *T. pusillum* were made at each site in early April 2002. Sites were observed for one hour in mid-morning, one hour in mid-afternoon and for one hour during early evening.

To assess the number of seeds per fruit, berries were randomly gathered at Baker and Heckmaster in 2001 and 2002 and at Hoover Woods in 2002 (Baker n=10; Heckmaster n=11; Hoover Woods n=5). Seeds were counted and seeds and elaiosomes were weighed to the nearest 0.0000 grams (Baker=26; Heckmaster=25; Hoover Woods=30).

To assess dispersal, seed depots were created (Baker n=11; Heckmaster n= 6; Hoover Woods n=2) by clearing the leaf litter from 100 cm^2 areas. Ten seeds were placed in each depot. Each seed depot was observed for one hour during the late morning or early afternoon.

COMMUNITY AND HABITAT CHARACTERISTICS.—Tree species >5 cm dbh were surveyed in May 2001 using a point quarter method (Barbour et al., 1999). Trees were also surveyed together with

shrubs and woody vines in May 2002 in randomly placed 25 m^2 plots. Herbaceous plant cover was surveyed in randomly placed 1 m^2 plots in April and May 2002. In addition, a species list was maintained of all plants observed at each site from late March through early July of 2001 and 2002 (see Appendix 1, 2).

We used leaf area index (LAI), which is the ratio of the total area of all leaves on a plant to the area of ground covered by the plant (Barbour et al., 1999), as a measurement of canopy light penetration (CLP). Based on the inverse relationship of LAI and CLP, we defined CLP as: CLP=12 – LAI.

CLP was determined by measuring the photosynthetically active radiation (PAR) above the tree canopy and at approximately 15 cm above the ground using an Accupar (Decagon Devices, Inc., PAR-80). Areas with a low CLP are shady; areas with a high CLP are sunny. Measurements were taken at each site in May 2001 (Baker n=36; Heckmaster n=20; Hoover Woods n=15), in March 2002 (Baker n=62; Heckmaster n=39; Hoover Woods n=15), and in June 2002 (Baker n=25; Heckmaster n=13; Hoover Woods n=15).

Soil samples were collected twice at a depth of ca. 6–8 cm, approximating the depth of the trillium rhizomes, using a stratified random sampling technique to gather samples from different areas on the slopes and across each site (Baker n=9; Heckmaster n=5; Hoover Woods n=6). Samples were sent to the University of Missouri Soil Laboratories for analysis of pH or dried at 100˚ C for gravimetric determination of soil moisture.

RESULTS and DISCUSSION

DEMOGRAPHY AND REPRODUCTIVE POTENTIAL.—Based on current estimated population sizes and density (Table 1), historical population sizes, and stage structures (Fig. 1) it appears that *T. pusillum* populations in southwestern Missouri have to be large and clumped to be viable in the long-term. Both the Baker and Heckmaster sites had populations greater than 1,000 stems, whereas the Hoover Woods site had a population of less than 100 stems (Table 1). The Heckmaster population is very dense within a relatively small area, but the Baker and Hoover Woods populations are sparse (Table 1), with the caveat that the Hoover Woods population is essentially one small clump (see Methods).

Table 1. Population characteristics for *Trillium pusillum* at three sites in southwestern Missouri and related community and habitat characteristics. Means are followed by standard deviations (±).

Population Variable	Baker	Heckmaster	Hoover Woods
Population size (n)	6,900	6,500	90
Total density/site (m^{-2})	0.51	6.5	0.02
Mean density/plot (m^{-2})	5.3±1.9	15.8±12.5	6.0±3.0
Sequential pollinator visitation per stem (%)	55	69	0
Mean seeds/capsule (#)	18.5±9.2	11.0±4.8	17.8±6.5
Mean seeds dispersed/depot (#)	7.0±2.9	6.0±4.0	10±0.0
Canopy tree density (#/hectare)	891	443	1391
Herbaceous cover (%)	41	29	82
Mean canopy light penetration	8.7±2.3	9.5±1.4	6.9±4.5
Mean soil moisture (%)	4.5±0.8	5.1±1.0	7.5±0.2
Soil pH	6.1	5.9	6.5

Based on previous unpublished census records it appears that the population at Heckmaster has grown substantially since recorded populations in 1984 (approximately 180 stems), but the population at Hoover Woods has declined since 1981 (2,000 stems) and 1994 (300–400 stems) censuses. The Heckmaster population has a stable stage structure and is predicted to expand, whereas both the Baker and Hoover Woods populations have unstable stage structures and are predicted to decline (Fig. 1) (Kawano et al., 1986). However, the population at Baker may increase in the short-term due to the high percentage of three-leaved stems. In fact, over the two year period the number of flowering individuals increased by only 6% at the Baker site but increased by 57% at the Heckmaster site.

The primary apparent pollinator observed at all three sites was honey bees (*Apis mellifera*), which, based on sequential visitation to stems, were effective pollinators at the Baker and Heckmaster sites, but not the Hoover Woods site (Table 1). Therefore, out-crossing is apparently limited at Hoover Woods. Few native insect pollinator species were observed sequentially visiting flowers. These observations indicate that if honey bees were to decline (e.g., due to tracheal and varroa mites) *T. pusillum* might effec-tively lose its ability to outcross at our study sites.

There was no strong indication that reproductive output varied among sites, as seed number per capsule (Table 1) and seed weight (data not shown) did not vary significantly between populations

(P>0.05, one-way Analysis of Variance). Nor was there apparent seed dispersal limitation at any site (Table 1). Lower dispersal at higher density sites is expected based on disperser satiation (Smith et al., 1989). However, seed predation may contribute to the low population size at Hoover Woods; at the Baker and Heckmaster sites ants dispersed 100% of the seeds, but at the Hoover Woods site harvestmen dispersed 30% of the seeds. This is significant because harvestmen may consume or damage the seed or leave it wherever the elaiosome is eaten; ant dispersed seeds, by contrast, are generally deposited in nests or nearby in the ants' refuse piles providing nutrients and/or protection from predators (Culver and Beattie, 1980: Beattie and Culver, 1983).

COMMUNITY AND HABITAT CHARACTERISTICS.—There appear to be some clear relationships between plant community characteristics and population size and density of *T. pusillum*. The density of *T. pusillum* was greatest at the site with the lowest density of canopy (and subcanopy) trees (Table 1), and lowest at the site with the highest density of canopy trees. In addition, both the Heckmaster and Baker sites are dominated by *Quercus velutina* and *Carya* spp., whereas Hoover Woods has less than 10% *Quercus* spp. with over 20% of the canopy dominated by *Juglans nigra*.

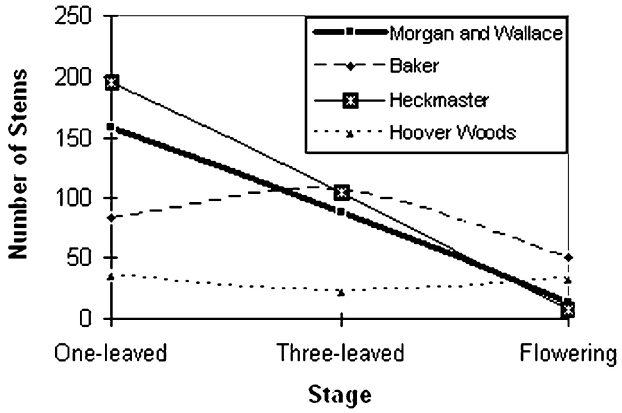

Figure 1. Comparison of *Trillium pusillum* stage structures at the Baker and Heckmaster sites in 2001 and Hoover Woods in 2002 to the stage structure of a viable *Trillium pusillum* population in Barry County, Missouri (Morgan and Wallace, 1987).

Herbaceous cover was two times greater at the Hoover Woods site than at the Baker or Heckmaster sites (Table 1), largely due to the invasion of *Euonymus fortunei* at that site. These data suggest that in Missouri *T. pusillum* is most expected in oak dominated habitats with a relatively low density of herbaceous cover. Lists of all tree and forb species at the sites are included in Appendices A and B.

In addition, a very dense overstory leading to low canopy light penetration is apparently not suitable for viable *T. pusillum* populations. Canopy light penetration averaged over the entire growing season was highest at the Heckmaster site and lowest at the Hoover Woods site (Table 1). However, during anthesis Hoover Woods had the greatest canopy light penetration, which might suggest that high light early in the season may have a negative effect on population growth—and that overstory species identity and phenology is an important characteristic of com-munities that can support viable *T. pusillum* populations.

The bulk soil analysis of each site indicated high levels of organic matter compared to other Ozark sites (Gaylord Moore, pers. comm..), with pH ranging between 5.9–6.5. Both soil moisture and pH were greatest at the site with a declining population (Hoover Woods) (Table 1). The range of optimal moisture levels has been suggested to be quite narrow for *T. pusillum* (Freeman, 1996), and Hoover Woods soil moisture may be higher than suitable due to the dense overstory canopy.

SUMMARY

This study indicates that *T. pusillum* populations may not be viable in habitats where there is a high soil pH, low light levels associated with high tree density, and the presence of an exotic invasive such as *Euonymus fortunei.* Other variables related to a contraction of *T. pusillum* include pollinator limitation, a change in seed dispersers, and increased herbivory by deer (Augustine and Frelich, 1998; Knight, 2004). Although the data reported here offer some clues to factors responsible for the decline of certain populations of *T. pusillum*, further data are needed to preserve this species in Missouri. In addition to expanding the study to other populations in the future, data are particularly needed on its breeding systems, seed predation, moisture requirements, and, if it outbreeds, its pollinators in non-fragmented forests. For the

remaining populations in Missouri, which largely grow on slopes unsuitable for development, the greatest threats to *T. pusillum* populations appear to be from changes in the habitat, including invasive exotics and, if it primarily outbreeds, overdependence on honey bees (*Apis mellifera*), whose populations are threatened by parasitic mites.

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Appendix A. Checklist of trees, shrubs and woody vines at Baker (Site 1), Hoover Woods (Site 2), and Heckmaster (Site 3).

Site #			Scientific Name
1			*Acer negundo* var. *negundo*
1			*Acer saccharum*
1			*Amelanchier arborea*
1			*Campsis radicans*
1	2	3	*Carya cordiformis*
1			*Carya texana*
		3	*Carya tomentosa*
1		3	*Celtis occidentalis*
1			*Cornus florida*
1	2	3	*Corylus americana*
	2		*Euonymus alatus*
1	2	3	*Euonymus atropurpureus*
1	2		*Euonymus fortunei*
1	2	3	*Juglans nigra*
1		3	*Juniperus virginiana*
1	2		*Lonicera japonica* var. *japonica*
1			*Morus rubra*
1			*Nyssa sylvatica* var. *sylvatica*
1	2	3	*Parthenocissus quinquefolia*
1		3	*Prunus serotina* ssp. *serotina*
1		3	*Quercus alba*
	2		*Quercus muehlenbergii*
1			*Quercus stellata*
1		3	*Quercus velutina*
1			*Rhamnus caroliniana*
1			*Rhus aromatica*
1			*Rhus copillina* var. *latifolia*
1	2	3	*Ribes missouriense*
1	2	3	*Rosa multiflora*
1	2	3	*Rubus* spp.
1	2	3	*Sassafras albidum*
1	2	3	*Smilax* spp.
1	2	3	*Symphoricarpos orbiculatus* f. *orbiculatus*
1	2		*Toxicodendron radicans* spp. *negundo*
1			*Ulmus alata*
1		3	*Ulmus rubra*
1			*Vaccinium* spp.
1	2	3	*Vitis* sp.
	2		*Vitis vulpina*

Appendix B. Checklist of spring and early summer forbs at Baker (Site 1), Hoover Woods (Site 2) and Heckmaster (Site 3).

Site #			Scientific Name
	2		*Adiantum pedatum* var. *pedatum*
1			*Asclepias quadrifolia*
1	2		*Asplenium platyneuron*
1		3	*Botrychium virginianum* var. *virginianum*
1			*Cimicifuga racemosa*
1			*Claytonia virginica*
1			*Dentaria laciniata*
1		3	*Desmodium* spp.
	2		*Dioscorea quaternata*
1			*Dodecatheon meadia*
1			*Erythronium albidum*
1			*Eupatorium rugosum* var. *rugosum*
1			*Galium arkansanum*
1		3	*Galium virgatum*
1	2	3	*Galium aparine*
1	2	3	*Geranium maculatum*
1		3	*Geum canadense* var. *camporum*
		3	*Ipomoea purpurea*
1		3	*Isopyrum biternatum*
1		3	*Lamium purpureum* var. *purpureum*
		3	*Lactuca serriola* f. *serriola*
	2		*Lonicera japonica* var. *japonica*
1			*Maianthemum racemosum* ssp. *racemosum*
1			*Monarda fistulosa*
	2		*Osmorhiza longistylis*
1			*Oxalis violacea*
1			*Parietaria pensylvanica* var. *pensylvanica*
1			*Perilla frutescens*
	2		*Phlox divaricata*
1		3	*Phryma leptostachya* var. *leptostachya*
1	2	3	*Podophyllum peltatum*
1	2		*Polygonatum biflorum* var. *commutatum*
	2		*Polemonium reptans* var. *reptans*
		3	*Potentilla simplex* var. *simplex*
1	2	3	*Ranunculus* sp.
	2		*Ranunculus recurvatus*
1			*Rudbeckia hirta*
1			*Silene virginica*
1	2		*Smilacina racemosa*
1	2	3	*Stellaria media*
		3	*Taraxacum officinale*
		3	*Tradescantia ernestiana* f. *ernestiana*
1	2	3	*Trillium pusillum* var. *ozarkanum*
	2		*Trillium viridescens*

		3	*Triodanis perfoliata*
		3	*Triosteum perfoliatum*
	2		*Uvularia grandiflora*
	2		*Veronica hederifolia*
1		3	*Viola triloba*
1	2	3	*Zizia aptera*