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Geographic distributions and physiological characteristics of co-existing *Flaveria* species in south-central Mexico

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Abstract

The genus *Flaveria* consists of 23 species with significant variation in photosynthetic physiologies. We tested whether photosynthetic pathway variation in seven co-existing *Flaveria* species corresponds to geographic distributions or physiological performance in C_3, C_4, and intermediate species growing under natural conditions in south-central Mexico. We found that *Flaveria pringlei* (C_3) was the most widely distributed species with multiple growth habits. Numerous populations of *Flaveria kochiana* (C_4), a recently described species with a previously unknown distribution, were located in the Mixtec region of Oaxaca. *Flaveria cronquistii* (C_3) and *Flaveria ramosissima* (C_3-C_4) were only located in the Tehuacán Valley region while *Flaveria trinervia* (C_4) was widely distributed. Only one population of *Flaveria angustifolia* (C_3-C_4) and *Flaveria vaginata* (C_4-like) were located near Izúcar de Matamoros. Midday leaf water potential differed significantly between *Flaveria* species, but did not vary according to growth habit or photosynthetic pathway. The quantum yield of photosystem II did not vary between species, despite large differences in leaf nitrogen content, leaf shape, plant size and life histories. We did not find a direct relationship between increasing C_4 cycle characteristics and physiological performance in the *Flaveria* populations examined. Furthermore, C_3 species were not found at higher elevation than C_4 species as expected. Our observations indicate that life history traits and disturbance regime may be the primary controllers of *Flaveria* distributions in south-central Mexico.
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Introduction

South-central Mexico and particularly the Tehuacán-Cuicatlán Valley contain an extraordinary diversity of plant species, including numerous Asteraceae species (Mendez-Larios et al., 2006; Toledo et al., 1997). Among the plants described from this region, species from the genus *Flaveria* Juss. (Asteraceae) are very well studied. Phylogenetic evidence combined with biogeography data suggests that the genus *Flaveria* arose in south-central Mexico and radiated as species migrated outward (McKown et al., 2005). The presence

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Table 1. The phylogenetic relationships, carbon isotope values, photosynthetic types, and growth forms of the *Flaveria* species studied in the states of Puebla and Oaxaca, Mexico

Species	Clade	$\delta^{13}C‰$	Photosynthetic type	Growth form
F. cronquistii Powell	Basal	-30.0 ± 0.3	C_3	Woody perennial
F. pringlei Gandoger	Basal	-28.8 ± 0.1	C_3	Woody perennial
F. ramosissima Klatt	A	-27.6 ± 0.5	C_3-C_4	Annual
F. angustifolia (Cav.) Persoon	B	Unknown	C_3-C_4	Herbaceous perennial
F. vaginata B.L. Robinson and Greenman	A	-15.4 ± 0.2	C_4-like	Herbaceous perennial
F. trinervia (Spreng.) C. Mohr	A	-16.0 ± 0.1	C_4	Annual
F. kochiana Turner	A	-15.2 ± 0.3	C_4	Herbaceous perennial

Species with the same clade designation are more closely related than species with different designations, according to phylogenetic analyses (from McKown et al. (2005)). The $\delta^{13}C$ values are the mean $\pm$ SE, with $n = 3$ (data from Sudderth et al. (2007)).

of C_4 photosynthesis in the genus was first described by Smith and Turner (1975), and numerous studies have since characterized the photosynthetic pathways of almost all *Flaveria* species (Apel et al., 1988; Cheng et al., 1988; Krall et al., 1991; Ku et al., 1983, 1991; Moore et al., 1989; Sudderth et al., 2007). *Flaveria* species utilize a remarkable diversity of carbon fixation syndromes, including C_3, C_4, and a variety of intermediate pathways (Table 1). The C_3 pathway is ancestral, and two independent origins of both C_3-C_4 intermediacy and C_4-like photosynthesis are present in the genus (McKown et al., 2005). The genus *Flaveria* is an important model for understanding C_4 evolution in dicots.

Non-optimal environmental conditions that enhance photorespiration, including low atmospheric CO_2 concentrations, warm temperatures, and aridity have likely promoted evolution of the C_4 cycle (Sage, 2001, 2004). Evolution of the C_4 cycle requires coordinated anatomical and biochemical modifications, including the development of Kranz anatomy and the localization of C_3 and C_4 cycle enzymes to mesophyll and bundle sheath cells (McKown et al., 2005). Species with intermediate C_3-C_4 cycles can have partial to complete Kranz anatomy and increased C_4 cycle enzyme activities compared to C_3 species, resulting in lower CO_2 compensation points and reduced photorespiration. C_4-like species demonstrate complete Kranz anatomy, CO_2 compensation points similar to C_4 species, and further enhanced PEP carboxylase activity. Current hypotheses suggest that the C_4 cycle evolved through the step-wise acquisition of C_4 traits, in which gene duplication and anatomical changes precede the localization of photosynthetic enzymes to specific organelles, followed by the enhancement of C_4 enzyme activity (McKown and Dengler, 2007; Monson, 2003; Sage, 2004).

Additional knowledge of the current distributions and specific habitat associations of basal and derived *Flaveria* species is necessary to better understand how environmental conditions drive the evolution of the C_4 photosynthetic cycle in this genus. While many intermediate *Flaveria* species have been described, physiological studies have concluded that the ability of intermediates to reduce photorespiration is only advantageous under environmental conditions such as high temperature and drought, where stomatal closure may limit internal CO_2 concentrations (Ku et al., 1983; Monson, 1989). A study of *F. floridana* under field conditions demonstrated that the intermediate syndrome conferred the ability to maintain a photosynthesis rate four times greater than a sympatric C_3 species at high leaf temperatures (Monson and Jaeger, 1991). With the exception of this study, previous research has not examined physiological characteristics of Flaverias growing under natural conditions. Therefore, we do not know if photosynthetic pathway variation corresponds to physiological performance in co-existing C_3, C_4, and intermediate *Flaveria* species growing under natural conditions.

The primary goal of this study was to record the current distributions of *Flaveria* species in south-central Mexico, and measure physiological characteristics of each species under field conditions, including midday leaf water potential and the quantum yield of photosystem II. A secondary goal of the study was to collect additional *Flaveria* seeds for the research collections. Most laboratory studies of the Mexican *Flaveria* species to date have used seeds or cuttings derived from the specimens collected during the 1970s. After being bred for many generations, some species in the current research collections are highly inbred and undocumented hybrids are present (McKown, personnel comment). The south-central region of Mexico was chosen as the study location for this work because it is the only area where examples of all photosynthetic types can be found growing in close proximity. In addition to determining if *Flaveria* populations still exist at reported localities, we present the first population distribution data for the recently characterized species, *Flaveria kochiana* (Sudderth et al., 2007). We also present qualitative descriptions of the habitats that each *Flaveria* species

were observed growing in. We expected to find a wider distribution of C_3 *Flaveria* species at higher elevations and of C_4 species in the warm Tehuacán Valley, the region at the lowest elevation in the study area.

Materials and methods

Study region

Documented locations of populations of the seven *Flaveria* species known to co-exist in south-central Mexico were plotted onto a map of the region. The selected study area was located in the states of Puebla and Oaxaca, between Izúcar de Matamoros, the Mixtec region of the Sierra Madre del Sur, and the Tehuacán-Cuicatlán Valley. The Tehuacán-Cuicatlán valley is part of the Mexican xerophytic phytogeographic region (Rzedowski, 1978). There are over 3000 plant species in this area, of which 30% are estimated to be endemics (Dávila et al., 1993; Villaseñor et al., 1990). The region is classified as semi-arid, with a summer rainy season and a medium annual precipitation of 400–600 mm. The mean annual temperature is 21 °C (Dávila et al., 2002; Esparza-Olguín et al., 2002). The minimum average temperature of the coldest month is 6.9 °C, and the maximum average temperature of the warmest month is 32.9 °C (Arias-Montes et al., 1997; Valiente-Banuet et al., 1991). The soil in this region is an aridosol, and is calcareous, rocky and shallow (Camargo-Ricalde et al., 2002; Zavala-Hurtado and Hernández-Cárdenas, 1998). Izúcar de Matamoros is located in the agricultural region of Matamoros valley. The climate is hot and subhumid while the mountains to the north and southwest are semi-warm (average temperatures vary between 18 and 22 °C). The Mixtec region of Oaxaca is located where the Sierra Madre del Sur region and the Sierra de Oaxaca meet, an area referred to as the Mixtec Knot. The region is characterized by its steep mountains, which reach 3000 m above sea level. The climate is arid with semi-desert conditions and the soils in the region are primarily calcic cambisols. Precipitation throughout the region is highly variable, ranging from 610 to 2180 mm per year (Instituto Nacional para el Federalismo y el Desarrollo Municipal, 2002). The topography, climate, and land degradation make farming difficult; therefore there is less disturbance due to agricultural activities in this region compared to Izúcar de Matamoros.

Data collection

During the study we drove between the towns of Izúcar de Matamoros, Tehuacán, Huajuapan de León, Santiago Juxtlahuaca, and the surrounding areas. All

established roads up to 50 km beyond the localities mapped based on herbarium records were searched for *Flaveria* populations (~1300 km of roads were surveyed). The *Flaveria* populations encountered were documented on topographic maps of the study region. *Flaveria* species are reported to occur primarily in roadside habitats, so our study focused on searching these areas. This survey likely omitted more remote *Flaveria* populations because the data were gathered by driving the passable roads. However, on numerous occasions, areas 200–2000 m away from the roads were also searched for *Flaveria* populations to verify that large populations did not exist in less-disturbed areas. *Flaveria* plants were generally not encountered in the areas away from the roads, with the occasional exception of *Flaveria pringlei*, the most widespread C_3 species. *Flaveria* populations were mapped during the afternoons and some of them were selected for detailed measurements, to be taken the following morning. This approach allowed us to effectively map *Flaveria* populations over a wide region, while restricting the eco-physiological measurements to mid-mornings.

Eight randomly selected target plants in one to five populations of each species were measured, depending on how many populations were found (four populations for *F. pringlei*, three for *Flaveria cronquistii*, *F. kochiana*, and *Flaveria trinervia*, *Flaveria ramosissima*, and one for *Flaveria vaginata* and *Flaveria angustifolia*). The eco-physiological measurements were completed in about 1 h per population and included determination of midday leaf water potential and leaf chlorophyll fluorescence, and sampling for leaf nitrogen content and soil moisture content. These measurements were chosen to give an indication of physiological performance while allowing many species and populations to be measured during the brief study period. The data were collected between 9 a.m. and noon on sunny days, with light levels ranging between 1000 and 1500 $\mu mol\,m^{-2}\,s^{-1}$. During the measurement periods average temperatures varied between 22 °C at the higher elevation sites and 28 °C at the lower elevation sites while relative humidity varied between 15% and 30%.

Midday leaf water potential was measured on terminal shoots with 4–5 leaves attached using a pressure chamber (PMS, Corvallis, Oregon, USA). Leaf chlorophyll fluorescence was recorded using a mini-Pam system (Walz, Effeltrich, Germany), and soil moisture was measured using a theta probe (Dynamax, Houston, Texas, USA). The effective quantum yield of PS II ($\Delta F/F_{m'}$) was calculated as $(F_{m'}-F)/F_{m'}$, where F is fluorescence yield of the light-adapted sample and $F_{m'}$ is the maximum light-adapted fluorescence yield when a saturating light pulse is superimposed on the prevailing environmental light levels (Schreiber and Bilger, 1993). Leaf nitrogen content was analyzed by the University of Georgia Soil Ecology Lab using a C/H/N analyzer

(NA1500, Carlo Erba Strumentazione, Milan, Italy). The field observations and physiological measurements were completed from November 5th to November 19th, 2005. Permits for the collecting activities, physiological measurements, and export of specimens were obtained from the appropriate Mexican agencies (Secretaría de Medio Ambiente y Recursos Naturales and Secretaría de Relaciones Exteriores). Voucher specimens from all populations measured were submitted to the Mexican National Herbarium (Universidad Nacional Autónoma de México, Ciudad Universitaria, México). The identities of all the *Flaveria* species were verified with molecular techniques according to the methods used to analyze the phylogeny of the genus *Flaveria* (McKown et al., 2005).

Data analysis

The geographic positioning coordinates of all *Flaveria* populations documented during the study period (data available upon request) were converted to decimal degrees and imported into ArcMap (ESRI, Danvers, Massachusetts, USA). The population locations were overlaid onto a 1:50,000 digital elevation model of the region (Instituto Nacional de Estadística Geografía e Informatica, Mexico). The population distribution function of ArcMap was used to generate the one standard deviation ellipses representing the average population distribution of each *Flaveria* species with sufficient data (Fig. 1). This function calculates the standard deviation of the x-coordinates and y-coordinates from the mean center to define the axes of the ellipse, and shows if the distribution of the points has a particular orientation. The ellipses do not indicate how evenly the populations are distributed through the study region, but they do differentiate species with localized versus broad distributions. The ArcMap software was used to export the elevation data from the digital elevation model for each population located in the field. The physiological measurements taken on a subset of populations (1–4) from each species were analyzed using ANOVA, with species (fixed) and population nested within species (random) included as the main effects (SAS Institute, Cary, North Carolina, USA).

Results

Population locations and habitat descriptions

F. pringlei was the most widely distributed species with the most abundant populations (Fig. 1). Many populations of *F. pringlei* were continuous for several kilometers. We encountered several *F. pringlei* populations growing in and along seasonal riverbeds and near gypsum mines, in soils with very little organic matter. Multiple growth habits of *F. pringlei* were observed in the field, including (1) woody shrubs up to 3 m tall, observed on steep slopes and often further away from the roadsides, (2) plants with broad leaves and non-woody stems, (3) plants with long narrow leaves, tough stems and abundant flowers and (4) plants with both kinds of stems (broad leaves and long narrow leaves) growing from the same root system. The stems with broad leaves were not flowering during this study, but developing inflorescences were observed at the stem apex. The plants with two stem morphologies were observed in roadside populations around Huajuapan de León. These populations had been cut back to the ground level by road crews using machetes (E. Sudderth, personal observation).

F. cronquistii, the other C$_3$ perennial species in the study region, was only found in the Tehuacán Valley region (Fig. 1). Like *F. pringlei*, this plant was found growing in gypseous soil along the roadsides but the populations occurred in areas with very diverse plant communities. While this species was generally not very abundant, a few large populations were observed to the southeast of Tehuacán. Although *F. pringlei* is reported to occur sympatrically with *F. cronquistii* (Powell, 1978), we only observed *F. ramosissima* growing at the same sites as *F. cronquistii*.

F. trinervia was found growing along the road, in empty lots, and on the edges of parking lots in almost every town in the study region. It was also very common in irrigation and waste-water ditches. *F. ramosissima* was the most ruderal species. The plants were generally very small (20–40 cm tall), and were flowering and producing seeds, even when they only had a few leaves. Populations were found in the Tehuacán-Cuicatlán valley (Fig. 1) growing on very rocky soils in abandoned fields and on the edges of sugar-cane fields. This species does co-occur with *F. trinervia*, although in this case *F. trinervia* tended to occupy the moist microsites with high organic matter content. A single population of *F. angustifolia* was located in a wet field near Izúcar de Matamoros. The population consisted of a few plants in an area that is mostly mowed; however the farmer who owns the land does not cut the *F. angustifolia* plants. *F. vaginata* is only known from a few herbarium specimens and has not been reported in the field for several years. We located two populations of *F. vaginata* growing close together along the edge of sugar-cane fields near a locality reported in 1978. *F. kochiana* populations were documented in the south-western part of the study region, in the Mixtec region of Oaxaca, and *F. pringlei* plants were found at almost all sites where *F. kochiana* grew. *F. kochiana* populations occurred in open, sunny sites with sparse shrub cover and some populations formed large, mono-specific stands. Although there are not many records of this

Fig. 1. Geographic distributions of *Flaveria* species in the states of Puebla and Oaxaca, Mexico. The symbols each represent one *Flaveria* population while the bold lines show the areas searched for *Flaveria*. For clarity, only populations of the same species that were found more than 2 km apart are shown. The directional distributions of the average population locations for each species with sufficient data are indicated by the dotted outlines.

species in the herbarium collections, (McKown, personnel comment), we found many large populations throughout this region.

The elevation data generated by overlaying the GPS coordinates of each population onto a digital elevation map of the region showed that two of the perennial species, *F. pringlei* (C_3) and *F. kochiana* (C_4), generally

grew at higher elevations than the annual species, *F. ramosissima* (C_3-C_4) and the C_4 *F. trinervia* (Fig. 2). We only found 1–3 populations of *F. angustifolia*, *F. cronquistii*, and *F. vaginata* so there were not sufficient data to properly analyze elevation differences for these species. The maps of the population locations show that *F. pringlei* (C_3) and *F. trinervia* (C_4) were the
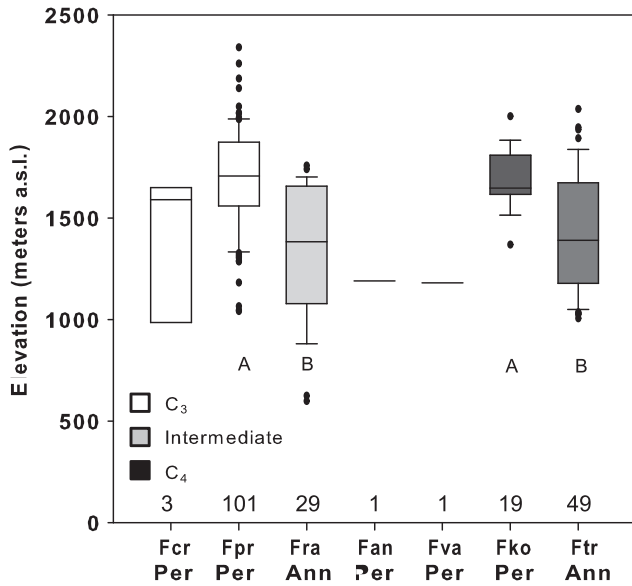
Fig. 2. Box-plot showing the average elevations (and 95% confidence intervals) where populations of each *Flaveria* species were found growing. The number of populations recorded for each species is indicated above the *x*-axis. In Figs. 2–5, species names along the *x*-axis are abbreviated using the first letter of the genus (F) followed by the first two letters of the species names. The life history of each species is also shown below the species abbreviations (Ann = Annual; Per = Perennial).

most widespread throughout the study region, and were often found growing in the same regions (Fig. 1). In the field however, these species were rarely observed growing at the same sites.

Physiological measurements

The random effect of population (nested in species) was highly significant for all of the physiological measurements (results not shown). In this study, we were primarily interested in whether the physiological traits of species differed according to photosynthetic pathway, after the population differences were accounted for in the ANOVA model. We found that the average midday leaf water potential of the *Flaveria* species differed significantly ($F = 7.92$, $p = 0.002$). *F. angustifolia*, *F. kochiana*, and *F. ramosissima* operated at leaf water potentials between -0.2 and -0.3 MPa, while the other species had more negative water potentials ranging from -0.3 to -0.4 MPa (Fig. 3). These values indicate that the plants were not suffering from high water stress. There was no correlation between leaf water potentials and soil moisture content (data not shown). The reported values for the physiological traits only include measurements made on the wide leaf morphology of *F. pringlei*, the most common leaf morphology observed. The water potential of
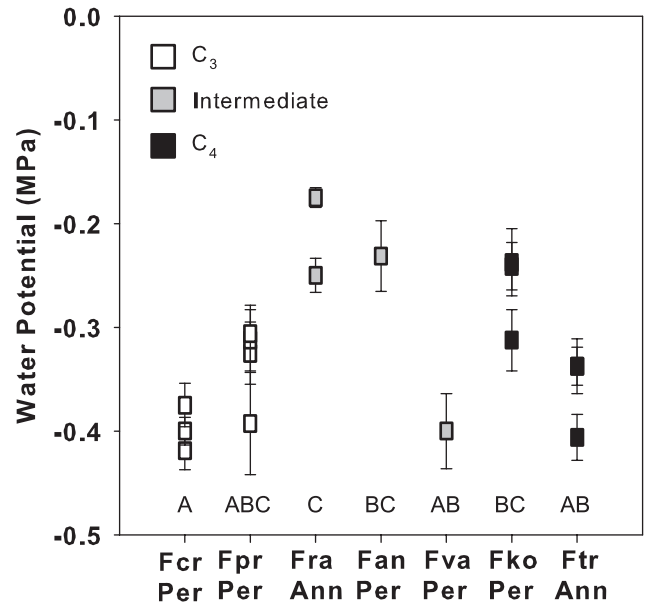

Fig. 3. Average midday water potential of each *Flaveria* population measured for each *Flaveria* species ($\pm$ standard error). Species with different letter labels are significantly different based on Tukey's post-hoc tests.
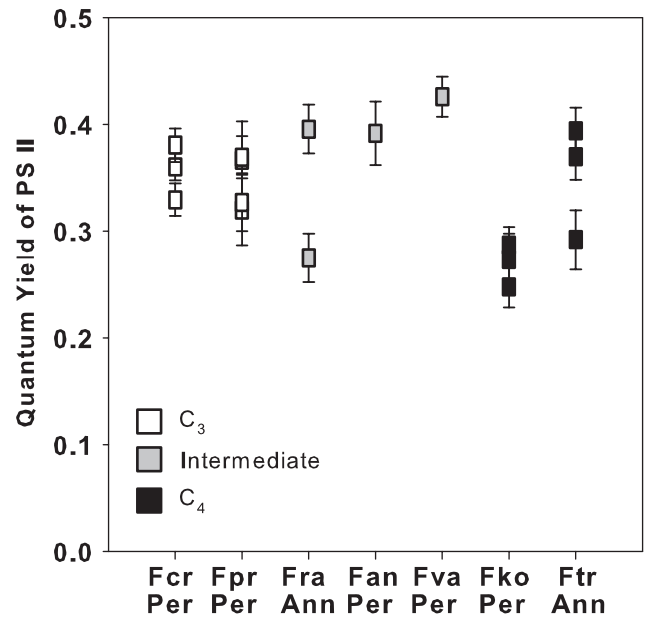

Fig. 4. Average quantum yield of PSII for the *Flaveria* populations of each species ($\pm$ standard error). There were no significant differences in the quantum yield values of any species.

narrow-leaved *Flaveria* stems was considerably lower than that of the wide leaf stems in *F. pringlei* individuals with both leaf types (-2.4 vs. -0.2 MPa). All species had a similar effective quantum yield of PS II ($F = 2.51$, $p = 0.094$), indicating that habitat conditions did not generally affect photosynthetic efficiency (Fig. 4).
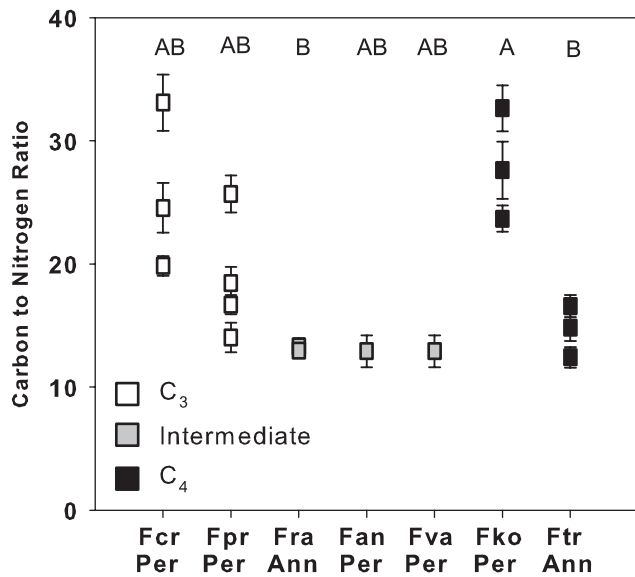
Fig. 5. Average carbon to nitrogen ratio of leaves from populations of each *Flaveria* species ($\pm$ standard error). The letter labels indicate significantly different values based on Tukey's post-hoc tests.

The leaf carbon to nitrogen ratios varied significantly between *Flaveria* species ($F = 5.09$, $p = 0.012$). C:N ratios are typically higher in C_4 compared to C_3 plants, but we did not observe this pattern for the *Flaveria* species in south central Mexico.

The species that were primarily associated with agricultural fields or very disturbed sites had a lower average C:N ratio than species growing in less-disturbed sites (Fig. 5). However, the species with higher leaf N content did not have higher quantum yield of PSII.

Discussion

All species of *Flaveria* that we observed in south-central Mexico grew in mildly to extremely disturbed areas, primarily on the edges of roads and agricultural fields, or in empty lots and abandoned fields. The *Flaveria* localities obtained from herbarium specimens were observed between 1890 and 1970. Many of these sites are now developed; therefore we did not find *Flaveria* populations at the majority of the reported localities. *F. angustifolia* and *F. vaginata* were particularly rare in the field, and we were only able to locate one population of each species, both associated with agricultural fields. These species possess unique C_4 cycle characteristics that provide important information for inferring the evolution of C_4 photosynthesis. With continued land development these *Flaveria* species could go extinct in this region of Mexico.

With the exception of coastal taxa, *Flaveria* species are rarely known to exist in continuous populations

(Powell, 1978). However, we observed many large populations of *F. pringlei*, which extended for several kilometers along the roads in the mountainous Mixtec region of Oaxaca. *F. pringlei* was the most abundant species with the largest populations, even though *F. trinervia* is a weedy species with a broad distribution throughout North America. The annual species primarily grow in more disturbed sites and have a higher leaf N content. Possibly, they may have a higher nitrogen requirement than the perennial species, a hypothesis that could be tested in a common garden experiment. The observed differences between current species distributions were not likely due to dispersal limitation as all species have numerous small seeds and grow in roadside habitats where they can be easily transported.

F. pringlei can grow as a large woody shrub, a plant with broad leaves and with a delayed flowering period, a plant with narrow leaves that flower early in the dry season, or as plants with both types of leaves and flowering periods when cut back. We observed these two discrete leaf shapes in field populations, rather than the continuous variation in leaf shape described by Powell (1978). Hybridization studies (Powell, 1978), examination of herbarium specimens, and molecular evidence (McKown et al., 2005) indicate that *F. pringlei* can hybridize with *F. angustifolia*. Based on these reports, we expected to find some hybridized populations in the study region. However, the molecular analysis of collected samples did not find evidence of hybridization in the populations of *F. pringlei* used for the physiological measurements, or in any of the plants with two leaf morphs (A.D. McKown, personal communication).

The growth habit of *F. pringlei* appeared to be related to the disturbance regime of each habitat. The plants with both types of leaves occurred in the more disturbed areas directly adjacent to the road that were cut back every year. In these plants our observations suggested that the stems with long narrow leaves were growing from the previous year's stems that had been cut back, while the large leaves grow on the newly sprouted stems. The populations of *F. pringlei* with the narrow leaves and earlier flowering period were observed very close to the roads, but in areas where they seemingly were not cut back. Typically, the woody shrubs occurred a few meters away from the edge of the roads. Both small and large plants of each growth form were observed. Therefore the differences in stem and leaf morphology were probably not related to plant age. Rather, the disturbance regime appears to have strong effects on the morphology of *F. pringlei*. After observing many populations of both species, we hypothesize that the flexible growth habit of *F. pringlei* allows this species to grow across a wide range of habitats.

Contrary to our expectations, the average elevation that each *Flaveria* species was growing at, is not correlated with its photosynthetic pathway. *F. kochiana*

was recently characterized as a perennial C_4 species (Sudderth et al., 2007). We found this species at higher elevations, growing in the same habitats but in different microsites than a C_3 species, *F. pringlei*. Where these species overlapped in occurrence, *F. kochiana* grew closer to the road in more disturbed areas. Only the woody shrub form of *F. pringlei* was found at these sites, growing further back from the road.

C_3 and C_4 *Flaveria* species commonly occur together in a variety of habitats. Originally, we did not expect significant overlap between closely related species with different photosynthetic pathways. Altitudinal gradients typically cause a shift from C_4 to C_3 grasses over a narrow temperature range (3 °C), while latitudinal gradients cause this shift over a much greater temperature range (15 °C) (Long, 1983). Common occurrence of C_3 and C_4 Flaverias indicates that climatic preferences of C_4 dicots can differ from those of C_4 grasses, as reported already for C_4 plants from Central Asia (Pyankov et al., 2000). While the relative abundance of C_4 grasses is strongly correlated with minimum daily temperatures during the growing season, C_4 dicots show strong correlations with water availability (Stowe and Teeri, 1978; Teeri and Stowe, 1976). Many C_4 dicots are found in arid regions with extreme cold and heat, such as Mongolia where 70% of the 80 C_4 species found are dicots (Pyankov et al., 2000). The extra energy required by the C_4 pathway is normally a disadvantage in low-temperature regions, but the C_4 pathway may be more efficient even at cool temperatures if high light, saline soils, or drought promote photorespiration in C_3 species (Long, 1983; Sage, 2004; Sage and Sage, 2002).

The C_4 species found at higher average elevations in our study, *F. kochiana*, is not a short-living plant, but a rhizomatous perennial species (Sudderth et al., 2007). Minimum temperatures at the highest points in the Mixtec region of Oaxaca are above freezing, and the summer maximum temperatures are warm enough to promote photorespiration. Furthermore, there is a pronounced dry season during which the higher water use efficiency of the C_4 pathway may be advantageous over the C_3 cycle. The herbaceous perennial growth habit of *F. kochiana* may allow this species to avoid the cold season, and grow in the summer when conditions are most favorable for C_4 photosynthesis.

The quantum yield of photosystem II, which provides an indication of photosynthetic efficiency, did not vary markedly between species, despite differences in leaf carbon to nitrogen ratios, leaf shape, plant size and life histories. The midday leaf water potential values indicate how water-stressed each *Flaveria* species tends to be during the day at the beginning of the dry season (November). *F. angustifolia*, *F. kochiana*, and *F. ramosissima* had the least negative water potentials of ~−0.2 MPa, while the other species had more negative values of ~−0.3 to 0.4 MPa (Fig. 3). While there were significant differences between several species, the difference did not correspond with life history, photosynthetic pathway, or growth habit. The species with the least negative water potential were in the late-flowering stage, while the other species were just beginning to develop flowers. Two species with more negative water potentials, *F. pringlei* and *F. trinervia* had the largest species distributions (Fig. 1). The ability to maintain PSII efficiency under greater water stress may contribute to the broader distributions of *F. pringlei* and *F. trinervia* across a range of habitats.

Krall et al. (1991) also found that PSII fluorescence activity is similar between different *Flaveria* species, regardless of their photosynthetic mechanism, but the partitioning of energy between photosynthesis and photorespiration varies dramatically. We did not measure photorespiration in the field; therefore our results only indicate how efficient the electron transport mechanism is in plants growing under natural conditions. The fluorescence values for plants measured in the field were lower than the values of 0.5–0.6 that have been found for greenhouse grown plants (Krall et al., 1991), indicating that plants growing in the field have less efficient photosynthetic cycles. Considering that almost all of the *Flaveria* studies to date have used greenhouse grown plants, this result highlights the importance of also examining the photosynthetic traits of plants growing under natural conditions.

Conclusions

Our research extends the knowledge of the current distributions of *Flaveria* species in south-central Mexico and provides valuable information for placing the numerous laboratory studies of these species (Apel, 1994; Dai et al., 1996; Dias and Brüggemann, 2007; Huxman and Monson, 2003; Krall et al., 1991; Ku et al., 1991; Leonardos and Grodzinski, 2000; McKown and Dengler, 2007; Monson, 1989) in an ecological context. We found that *F. pringlei* is the most abundant species and is an important component of roadside plant communities throughout south-central Mexico. Only a few populations of *F. cronquistii* were found but the populations were large and were located in the diverse Tehuacán Valley biosphere reserve. Large populations of the recently described perennial C_4 species, *F. kochiana*, were found at high elevations. *F. ramosissima* was abundant on the poorest habitats encountered, but had a similar physiological performance as the other *Flaveria* species. *F. angustifolia* and *F. vaginata* were only found as remnant populations and are likely to go extinct in this region. These observations of the habitats and growth habits of *Flaveria* species indicate that life history traits and disturbance regime

may be the primary controllers of *Flaveria* distributions in south-central Mexico. Our observations may solicit additional studies of the genus *Flaveria*. While species of this genus are well studied in the laboratory, it is still not known if the intermediate and C_4-like *Flaveria* species are in the process of evolving optimized C_4 cycles, or if these photosynthetic cycles are stable pathways, well adapted to their current habitats. Further field studies of population level variation in ecophysiological traits of *Flaveria* species will improve our understanding of the environmental conditions that promote the evolution of C_4 photosynthetic traits in dicot species.

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