Correlations Between Environmental Factors and Wild Bee Behavior on Alfalfa (*Medicago sativa*) in Northwestern China

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ABSTRACT

To discover the effect of environmental factors on pollinator visitation to flowering *Medicago sativa*, several field experiments were designed to examine the diurnal movement patterns of wild bee species in the Hexi Corridor of northwestern China. Our study results showed that *Megachile abluta*, *M. spissula*, and *Xylocopa valga* showed unimodal diurnal foraging behavior, whereas *Andrena parvula* and *Anthophora melanognatha* showed bimodal diurnal foraging behavior. Correlation analysis indicated that diurnal foraging activities of pollinators were significantly correlated with environmental factors. Correlations of foraging activities versus environmental factors for *M. abluta*, *M. spissula*, and *X. valga* best fit a linear model, whereas those of *A. parvula* and *A. melanognatha* best fit a parallel quadratic model. Results of this study indicated that solitary wild bees such as *M. abluta*, *M. spissula*, *X. valga*, *A. parvula*, and *A. melanognatha* are potential alfalfa pollinators in the Hexi Corridor. An understanding of the environmental factors that affect the behaviors of different wild bees foraging in alfalfa are basic to the utilization of solitary wild bees in a practical way for increased, or more consistent, pollination of alfalfa for seed production.

KEY WORDS environmental factors, *Medicago sativa* L., pollinator visitation, wild bee

Alfalfa (*Medicago sativa* L.) is one of the most important forage species worldwide (Sunberg et al. 1983, Barnes 1993, Mertens 2002). Alfalfa has a typical legume flower that is dependent on a process known as “tripping” to release the sexual column for pollination (Armstrong and White 1935, Brink and Cooper 1936, Viands et al. 1998). Because of the unique relationship between flower structure and its pollination requirements, much research has been focused on the pollination potential of honey bees (*Apis mellifera*) (Nabhan and Buchmann 1997, Cecen et al. 2008), alfalfa leafcutter bees (*Megachile rotundata*) (Bosch and Kemp 2002), and alkali bees (*Nomia melanderi*) (Alan and Wooten 1987), mainly regarding the speed with which each species visits flowers and the proportion of visited flowers that are tripped. The results showed that honey bees, leafcutter bees, and alkali bees, alone or in any combination, are of great value for alfalfa pollination. Pollination systems involving solitary bees and flowers are of particular interest because bee foraging behavior can be directly correlated with reproductive output of both the plant (through seed set) and the bees (through the quantity of stored pollen and nectar), as has been done for the alfalfa leafcutter bee (Strickler 1996, O’Neill et al. 2004, Bosch and Kemp 2005).

Environmental factors, such as temperature, relative humidity, and sunlight intensity, can affect pollination processes (Elgersma et al. 1989, Culley et al. 2002). The flight activity of *M. nana* is positively correlated with temperature, light intensity, solar radiation, and nectar-sugar concentration, but negatively correlated with relative humidity (Abrol 1998). High air temperature may restrict floral visitors, and some pollinators may be more sensitive to this factor than others (Totland 2001). Visitation rate of pollinators was not significantly affected by any environmental factor other than temperature in many experiments (reviewed in Alfonso et al. 2005).

However, our knowledge of how these environmental factors affect pollination in arid and semiarid regions is limited, and the mechanisms by which these factors exert their influence in foraging activities are poorly understood. Therefore, our objective was to study wild bee pollinators of alfalfa and the relationship between their foraging activities and the environmental factors in the Hexi Corridor of northwestern China. The Hexi Corridor, a narrow terrestrial ecosystem between the Badain Jaran Desert and the Tibetan Plateau, is an arid region and one of the most important seed production regions in northwest China.

Materials and Methods

**Study Area.** This study was carried out in Lanzhou University Ecological Research Area (Linze Experiment Station, 39°21′ N, 100°07′ E, altitude 1,367 m) in...
the middle of the Hexi Corridor, northwest China in 2006 and in 2007. A sample area of 100 by 100 m² in the middle of a 2-yr-old cultivated alfalfa (M. sativa L.) seed field of 60 ha was selected for the study. Ten subplots (2 by 5 m) established within the sample area were randomly selected for conducting experiments described below in each year of the study. A similar plant density was maintained in all subplots. In this site, flowering time of M. sativa was from mid-May to late July, with pods ripening in the autumn. Each stem and stem branch of alfalfa terminates in a raceme or cluster of 10–30 purple florets.

**Determinations of Visiting Pollinators’ Numbers and Environment Factors.** The number of insect visitors to M. sativa flowers in each of 10 subplots were counted from 0700 to 2000 hours over 4 consecutive d in each year (19–22 June 2006 and 20–23 June 2007) and recorded at 1-h intervals during that time to estimate the pollinators’ diurnal foraging activity. A representative sample of each pollinator species was captured with a sweep net and taken back to the laboratory for identification. Pollinators were distinguished from other floral visitors using the following criteria: the insect effectively tripped the floret, had pollen on their bodies, and had relatively abundant floral visits. The tripping frequency was estimated by comparing, for each pollinator, the number of visits to alfalfa flowers to the number of visits resulting in tripped flowers. The number of inflorescences visited per bee per minute was recorded with a stopwatch five times in each of the 10 subplots.

**Environmental Factors.** During the time bees were being observed, diurnal environmental factors including air temperature, relative humidity, and light intensity were monitored continuously at 3-min intervals by the automatic Meteorological Station, located ≈50 m away from the trial area.

**Statistical Analysis.** All data were found to not be significantly different from a normal distribution, so no data transformations were performed. The mean and SE of the estimates were calculated on the basis of the 4 d of results over both years of the study. One-way analysis of variance (ANOVA) was used to test differences among wild bee species for pollinator numbers at particular times of the day. Correlation analyses were performed to determine the nature of the relationship between foraging activity and environmental parameters (temperature, light intensity, and relative humidity) for bees showing unimodal or bimodal diurnal foraging activity patterns. Analyses were conducted by correlating the total number of bees showing unimodal or bimodal foraging activity for each hourly observation period against the corresponding environmental parameter value for that hour. Both linear and quadratic models were tested. Coefficients of determination ($r^2$) were used to test the correlations between the numbers each wild bee species and the environmental factors. All the analyses were conducted using the SPSS 12.0 statistical software package (SPSS, Chicago, IL).

### Results

**Diurnal Foraging Activities of Different Wild Bees Visiting Alfalfa.** At least 20 insect taxa, including 12 species of Hymenoptera, 5 species of Lepidoptera, and 3 species of Coleoptera, were observed to visit the flowers of M. sativa. Bees accounted for 85% of all visitors, representing 4 families and 11 species (Table 1). Of all the insects, only bees were identified as alfalfa pollinators based on their relative abundance and tripping behavior. Based on their high visitation frequency and tripping efficiency, five bee species, M. abulta, M. spissula, X. valga, A. parvula, and A. melanognatha, were considered to be “principal pollinators.”

**Megachile abulta,** M. spissula, and X. valga showed unimodal diurnal foraging activity, with one visiting peak from 1130 to 1530 hours (Fig. 1). However, the diurnal foraging activities of A. parvula and A. melanognatha were the bimodal type, with two visiting peaks: one from 0930 to 1130 hours and another from 1630 to 1830 hours (Fig. 1).

**Correlation Between Different Wild Bee Species and Environmental Factors.** For bees with univariate foraging behavior, the number of bees visiting flowers was significantly ($P < 0.01$) and linearly correlated to the measured environmental parameters. The number of bees visiting flowers increased with increasing light intensity (Fig. 2a) and temperature (Fig. 3a) but decreased with increasing relative humidity (Fig. 4a). For bees with bimodal diurnal foraging behavior, foraging activity was nonlinearly associated with the environmental factors (significant quadratic response, $P < 0.05$). The number of bees, initially increased with increasing light intensity, temperature, and relative humidity to a maximum value, and then decreased as the value of each environmental factor continued to increase (Figs. 2b, 3b, and 4b).

### Discussion

**Pollinators’ Community Structure.** Pollination is one of the most important biological factors in alfalfa seed production in both managed and natural ecosystems. Much research has shown that most plant taxa are visited by a diverse array of animals, suggesting that pollination systems may frequently be generalized (Moeller 2005). In the Hexi Corridor in north-
west China, the principle pollinators of *M. sativa* were bees including many rare, incidental visitors that are unlikely to contribute significantly to pollination in managed systems (Liu 2008). Although honey bees are often the primary pollinator used for commercial crop production (Nabhan and Buchmann 1997, Cecen et al. 2008), honey bee numbers are declining worldwide and are a cause for concern. Wild bees likely will become more important in pollination as the honey bee numbers continue to decline. In this study, 20 insect species were recorded visiting alfalfa under natural conditions in the Hexi Corridor of northwestern China, suggesting that some of these may be useful pollinators of alfalfa seed. We found that 11 species of solitary wild bees accounted for 85% of the total visitations, with 5 species predominating as the principal pollinating agents of *M. sativa* species in this arid region. Our results suggest that more attention should be paid to the high diversity of wild bee species pollinating alfalfa in an adverse habitat in the future.

**Behaviors of the Pollinators.** To promote outcrossing, plants use nectar to attract visitors, but they limit this reward so that pollinators will visit other plants of the same species. Nectar secretion per flower or per plant is carefully optimized and adapted to time of day, season, and the species of pollinators (Louis et al. 2005). Our study (Liu 2008) on diurnal nectar secretion per floret of alfalfa found a bimodal pattern of nectar production, which could partly explain the diurnal foraging pattern observed for some of the wild bees in this study. Potential reward (numbers of stamens and nectar glands) may be important traits for explaining differences between plants in visitation rates (Totland et al. 1998, Alfonso et al. 2005). In addition, plant characteristics also influence pollinator visitation rates (Thompson 2001, Mitchell et al. 2004). The phenological patterns of alfalfa populations seem to differ considerably in the Hexi Corridor and warrant further study.

Although the aims of pollinators are the same, different insects seem to use different strategies to get the benefit from the plants. For example, *A. parvula* and *A. melanognatha* foraged primarily during the morning and late afternoon on either side of midday peaks in light intensity and temperature, whereas *M. abluta*, *M. spissula*, and *X. valga* foraged primarily

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**Fig. 1.** Diurnal foraging activities of different bee species visiting alfalfa (*M. sativa*) in the Hexi Corridor of northwestern China.

**Fig. 2.** Correlations between foraging activities and light intensity for wild bee species showing unimodal (a) and bimodal (b) patterns of diurnal foraging activity.

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y = 0.0028x - 0.4022 \\
R^2 = 0.7426 \\
p < 0.01
\]
during the midday when light intensity and temperature were highest. The different pollinating patterns (unimodal type versus bimodal type) among these wild bees may indicate a possible management strategy to overcome the problem of supplying enough pollinators to large acreages of alfalfa in this region.

**Correlation Between Bees and Environment Factors.** The value of different bee species as pollinators has been widely shown based on the speed with which they visit flowers and the proportion of visited flowers tripped (Klein et al. 2004). Thus, differences in the single-visit pollination efficiencies of the various bee species are entirely attributable to the proportion of visited flowers that they tripped. In this study, environmental factors were shown to be strongly correlated to environmental factors. None of the environmental factors considered in this study were significant predictors of visitation rates in another experiments conducted by Alfonso et al. (2005), despite the variability of these factors at the spatial scale analyzed in that study. Our results showed that environmental factors may be one of the factors responsible for the changing visitation rates of the pollinators of *M. sativa* in the Hexi Corridor. Although the visitation rate of unimodal type pollinators was linearly associated with all three environmental factors measured, the rate declined with increasing relative humidity, whereas increasing with temperature and light intensity. The probably reason for this is that relative humidity is known to vary with temperature and light intensity, all else being equal, being higher at low temperatures and low light intensities. Therefore, all three traits are likely showing the same pattern.

We found that alfalfa flowers that open early in the morning were visited mainly by bees with bimodal activity patterns, such as *A. parvula* and *A. melanogerratha*. However, as the temperature rose during the day, the bees with unimodal foraging activity, such as *M. abluta*, *M. spissula*, and *X. valga*, became active. The differences of diurnal foraging pattern among these wild bees likely relates to their ability to regulate body temperature (Liu 2008). The type of pollinator and the timing of their visitation may be related to the variability in sugar composition, concentration, and caloric content of the nectar or on the effect that environmental factors have on nectar secretion and dessication (Hocking 1953, Pedersen 1953, Shuel 1955). Therefore, plant or floral-related intrinsic fac-

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**Fig. 3.** Correlations between foraging activities and temperature for wild bee species showing unimodal (a) and bimodal (b) patterns of diurnal foraging activity.

**Fig. 4.** Correlations between foraging activities and relative humidity for wild bee species showing unimodal (a) and bimodal (b) patterns of diurnal foraging activity.
tors and site or environment-related extrinsic factors may both play a role in explaining differences among pollinators and should be studied further.

Acknowledgments

We thank C. Z. Liu for assistance in insect identification. The manuscript benefited greatly from the comments of E. C. Brummer and two anonymous reviewers. This study was supported by National Basic Research Program of China (973 Program) (2007CB108904) and Natural Science and Technology Program of Lanzhou University (582402).

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Received 12 April 2008; accepted 14 May 2009.