

**Effects of road proximity on pollinator visitation
and reproductive success of *Ipomopsis aggregata***

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Abstract

Increasing urbanization has led to the expansion of human infrastructure, including road networks. Current research has shown that roads have important ecological effects, ranging from habitat fragmentation to contamination of water runoff. This study examined the potential effects of roads on the reproductive success of subalpine wildflower populations in two meadows at the Rocky Mountain Biological Laboratory in Gothic, CO. Based on studies conducted in previous years, we predicted that distance from a road would result in differences in pollinator visitation, pollen loads, and seed sets of *Ipomopsis aggregata*. Additionally, this study evaluated the road environment in terms of human traffic disturbance. In our characterization, we highlight road noise as a possible interpretation of observed differences near and far from the road. We found trends suggesting visitation rates were generally higher for plants near the road versus farther away. However, we did find that events of visitation are often longer in terms of number of flowers visited on plants further from the unpaved road, which suggests pollinators may have been interrupted during visits to plants near the road. Measurements of traffic volume and frequency along with paired decibel level measurements also indicated that the noise produced by the road is significantly higher than ambient noise levels, implicating that human disturbance, particularly in the form of motorized vehicles, directly alter the noise environment at these sites. Data continue to be analyzed to relate visitation rates, pollen loads, and seed sets obtained from this experiment. For now, we conclude that the effects of roads, particularly in terms of increased noise pollution, must continue to be studied in order to further our understanding of how the expansion of human infrastructure is potentially altering the surrounding natural environment.

Introduction

Evidence of human presence and activity can be found in nearly all ecosystems. Humans are overwhelmingly the dominant species on Earth and have dramatically altered the planet (Vitousek 1997). Human interactions with the environment take many forms, but many of the transformations made by humans are due in large part to urbanization, the expansion of human infrastructure. Recent studies have reported drastic effects of this expansion across ecosystems such as loss of species diversity, modification of animal behavior, and climate change (Ditchkoff et. al. 2006, McKinney et. al. 2006, Kalnay et. al. 2003). These studies, among many others, highlight urban areas as sites where humans both directly and indirectly affect the environment.

One could think of the vast transportation network of roads and highways as an example of a site where this interaction occurs. This network continues to grow along with increasing urbanization. An estimated 19% of the area of the United States is ecologically affected by roads (Forman et. al. 1998). Current research points to the important ecological effects of roads on both terrestrial and aquatic ecosystems. Roads also affect plant and animal communities within the “road-effect zone,” the area which is affected by the presence of the road and often spans an area many times wider than the road itself (Forman et. al. 1998). Recent research has suggested that this “zone” may be encouraging the invasion and promotion of exotic plant species (Christen et. al. 2008, Harrison et. al. 2002). Along those same lines, there may differences between unpaved and paved roads, suggesting levels of road improvement may also contribute to the abundance and propagation of invasive species (Gelbard et. al. 2003). The presence of motorized vehicles on roads has also had an influence on the dispersal of plant species (Zwaenepoel et. al. 2006), highlighting the important interactions between humans and environmental processes which take place on roads.

As a result of this interaction, there are a wide range of potential effects on natural communities existing along roads. Recent studies have shown that populations closer to the road experience habitat fragmentation (Keller et. al. 2003) and reduced gene flow in animal and insect populations (Riley et. al. 2006, Keller et. al. 2003). Effects also include increased exposure to dust (Thompson et. al. 1984, Walker et. al. 1987), and contamination of water runoff by harmful chemicals (Forman et. al. 1998, Faulkner 2004, Wheeler et. al. 2005). Less is known about the effects of noise produced by roads. Research has investigated the effects of increased noise pollution on certain ecosystems, particularly on animal communication and behavior (Wood et. al. 2006, Bee et. al. 2007). Francis et. al (2012) found that pollination of flowers was actually enhanced in a more isolated environment altered by constant noise from continuously powered generators. This investigation examined a similar relationship between noise and pollinator behavior, thinking specifically about roads and their associated sound levels. Gothic, Colorado, where this study was conducted, and the surrounding area have experienced increased tourism in recent years, specifically during the summer season. Tourism to this area attracts large amounts of people and their transportation vehicles (e.g. trucks, trailers, motorcycles), making this place particularly interesting to study noise produced by roads. This study also contributes to knowledge of our place by taking into account the differences in acoustic environment and how sound affects pollinators here versus other places (Warren et. al. 2005).

Previous reports have suggested that humans may be “indirectly responsible for potentially large, but poorly understood, perturbations” within regional and global contexts (Field et. al. 2010). This study examined these perturbations in terms of the indirect effects of human infrastructure. We examined the rate of pollinator visitation and overall reproductive success in terms of pollen count and seed sets of scarlet gilia (*Ipomopsis aggregata*) near and far from

Gothic Road at the Rocky Mountain Biological Laboratory in Gothic, Colorado. In this study, we tested the hypothesis that proximity to the road would influence wildflower pollination and reproduction, expecting to observe differences in pollinator visitation rates, pollen count, and seed production for plants near versus far from the road.

Materials & Methods

Study Sites

This experiment was conducted at two field sites, lower Maxfield Meadow (MFM) and at the northern boundary of RMBL land along the Gothic Road, referred to hereafter as Beyond Billy Barr's, or "BBB" (Figure 1). Both sites are open, dry meadows and share a similar subalpine climate. These two sites are also located along the same unpaved road, Country Road 317, also known as Gothic Road. The portion of road which runs through MFM, however, was sprayed with a dust suppressant (magnesium chloride) before the experiment began in late June. The decreased dust at this site provided a control for the potential effects of road dust on the experimental population. During the summer season, this area experiences higher human traffic due to the opening of the laboratory for students, scientists, and employees, and tourism.

Experimental Population

This experimental population utilized in this study was *Ipomopsis aggregata*, a native wildflower species which typically grows in patches in subalpine meadows. *I. aggregata*, commonly known as scarlet gilia, is an annual plant, flowering only once before dying. This species may take years to flower, growing and remaining in rosette stage, but typically begins budding mid-summer (late June, early July) and can continue budding throughout the season depending on availability of resources. When damaged (e.g. deer herbivory) in rosette stage, scarlet gilia grows side stalks which may also flower. Plants exhibiting this growth of side stalks were excluded from this study. *I. aggregata* is pollinated by a variety of animals, including two hummingbird species, *S. platycercus* and *S. rufus*, and certain species of Sphingidae moths and butterflies.

Potted Arrays

Plants used in this experiment were potted experimentally in order to control for environmental factors which may have affected the natural history and physical characteristics of each individual. 60 total plants were potted from our two sites. We found plants within wildflower patches and selected ones which we believed would flower during the timeframe of our experiment and which did not show signs of previous damage such as growth of side shoots. We created a system to assign a unique identifying code to each plant, which would allow us to return these plants to their original locations after the experiment. This code included the site from which plants were taken (B=BBB, M=MFM) and a number 1-30. A stake with this code, along with a flag, was inserted into the hole from which the plant was dug up. Once potted, another stake with this same code was inserted into the pot and remained with the plant throughout the duration of the experiment. Plants were watered immediately upon potting. Plants were also rinsed with a spray bottle to eliminate any dust which may have been affecting plants previously. While on a shaded potting bench, plants were watered twice a day — once in the morning and in the afternoon. After six days, they were transferred into a WeatherPort to allow individuals to receive sunlight. In preparation for the experiment, watering was reduced to once a day. Some individuals were bent during pre-experiment care and required a time-tape splint, which provided support for the stalk for the remainder of the care period and the actual experiment. Some plants experienced this loss of turgor during the actual experiment, at which point a splint was applied (in some cases, on top of an existing one). Plants that died were replaced immediately by another from the same site.

Individuals were paired and randomly assigned to near or far arrays within their respective sites. Plants were first placed into tentative pairs based on approximate height. We then measured stalk height (cm) from the base of the plant just above the point of previous growths to the end of the tallest pedicel. These measurements were used to more accurately pair individuals. Stalk diameters (mm) were also measured using electronic calipers as additional information about individuals. After measurements were entered into an Excel spreadsheet, a random number generator was used to assign individuals to a near or far array. This function assigned a zero or one to a given pair. A zero signified that the first individual in the pair would be assigned to the near array and the other to the far array. Once paired, we assigned sets a number 1-15 and painted this number large enough so it could be seen during pollinator observation. Individuals assigned to the far array were denoted with an additional painted dot below the painted number. Plants continued to be watered and monitored in the WeatherPort after pairing in preparation for the experiment.

At both sites, we set up two 0.5m x 4m arrays. Near arrays were placed 0m-2m away from the road whereas far arrays were set up 45m-50m away. Within these arrays, plants were arranged in a staggered fashion in order from 1-15. This staggered arrangement was inverted at the opposite array within sites in order to control for a potential position effect. Plants were then reordered when it was determined plants had been unknowingly arranged in height order. Corners of each array were marked with a flag. In order to deter deer from eating plants within our arrays we inserted tall wire stakes which on average were slightly taller than individuals. Some plants were bent during array set-up and required time-tape splints. All plants received water after being arranged in arrays.

We observed pollinator visitation at both sites for 4-5 days after setting up potted arrays. Near and far arrays at a site were observed at the same time by two observers. When possible, four observers watched all four arrays at the same time. Observers were also rotated between the four arrays to account for observer bias. Observation periods lasted approximately one hour and were distributed throughout the day, accounting for potential differences in pollinator activity at different times of the day. Before each observation period, we watered the arrays and counted the number of open flowers on each plant. We then sat far enough away from arrays for observation so as not to deter or disturb pollinators from visiting the plants. During this hour, we recorded any events of visitation to the array, including details such as number of plants visited and the number of flowers visited on each individual. We also recorded the time of the bout and the type of pollinator, identifying the species and gender when possible. As additional anecdotal information, we noted instances when a disturbance occurred, such as a car passing by, while a pollinator was visiting the array or when pollinators were observed visiting plants outside our arrays.

Potted arrays were collected following the fifth day of pollinator observation due to a large amount of deer herbivory at BBB in both near and far arrays. We attempted to pot more plants to replace those which were eaten, however we found that plants did not respond well to experimental potting during flowering and did not survive the process. Consequently, we decided to collect all four arrays from the sites and collect all the data possible from these plants.

Natural Arrays

In response to the deer herbivory at BBB, we decided to set up natural arrays in order to continue observing pollinator observation while *I. aggregata* was still flowering at the same

sites. We found patches of *I. aggregata* plants near (0-5m) and far (>40m) from the road at both sites. Patches varied in size but shared similar abundances of flowering plants. We marked the four plots with flags and wrapped string around them as a loose boundary. Soon after we rolled the flags up so as not to draw attention to pollinators to our arrays.

Observations for natural arrays were conducted similarly to those for the potted arrays. We observed natural arrays for four days in total, again distributing observation periods throughout the day — morning, afternoon, and evening. Observers were again rotated between the four arrays. Though we did not water these arrays, we began each observation period by estimating the total number of flowers in the plots. While observing, we recorded any visits to the arrays, including the number of plants visited and the number of flowers visited on each plant. For each bout of visitation, we noted the time and type of pollinator, identifying the species and gender when possible. We also recorded foraging behavior at patches near arrays as well as anecdotal information on behavior as cars passed pollinators visiting flowers near the road.

Pollen Loads & Seed Sets

After the deer herbivory at BBB, we opted to collect the potted arrays from both sites. We then collected up to 3 stigmas from each individual, depending on the number available after herbivory. We collected stigmas which were older, indicating that these flowers had been sufficiently exposed to the associated treatment. In order to find these same flowers again later for seed set counts, we marked the calyces of each flower with up to 3 different colors. The stigmas were stained with fuchsin gel and examined under a compound microscope to determine individual pollen counts. After stigma collection, plants were placed in the sun and watered until

fruits were produced. Flowers which opened during this time were removed in order to prevent further pollination of individuals.

In natural arrays, we collected stigmas after two weeks of observation. Within the arrays, we randomly selected 15 flowering plants and 3 fresh stigmas on each individual, indicating that these flowers had been open during our observation periods. Again, calyces were marked using three colors for later seed set counts. Stigmas collected were placed in glassine envelopes and labeled according to array, individual, and calyx color. We then stained the stigmas with fuchsin gel within 24 hours of collection. Stained stigmas were then examined to determine pollen counts.

Disturbance & Noise Data Collection

In order to characterize the road environment, I collected disturbance and noise data following the end of pollinator observations in late July through early August. Traffic data were collected by recording the number of human disturbances which passed the near arrays and timing them from when the disturbance passed the first set of flags on the sides of arrays to when it passed the second set of flags. Disturbances were divided into eight general categories based on the most common ones seen on the roads (Table 1). Initially, observers at near arrays recorded the incidence of disturbance throughout the pollinator observation period. However, we stopped collecting disturbance data during observations as it required much attention, which could have resulted in observers missing visitation bouts to arrays. The data which were collected initially were later used to determine the length of observation periods for disturbance data collection later in the summer. Based on this data, I determined that ten minute observation periods yielded a reasonable estimate of the average rate of traffic within a full hour at both sites. Traffic data

were then collected in ten minute periods at both sites. During observation, I recorded the time it took for the particular disturbance to pass a five meter stretch marked by flags. I also noted the type of disturbance and the time at which it passed. I observed traffic at five different time periods throughout the day — early morning, late morning, early afternoon, late afternoon, and evening — to account for changes in the daily cycle of traffic. Data were taken for two low/moderate-traffic days and one high-traffic day.

Noise data were collected for all disturbance types following traffic observation. I used the traffic data to determine when the rate of traffic was highest. This allowed me to replicate noise measurements for each disturbance type and obtain an average decibel level for each type. Decibel levels were measured using the free iPhone application My Decibel Meter - Free Version 1.0 at a 60dB reference level. This application allows the user to measure the decibel level over a desired period of time. It then produces an output listing the maximum peak and an average decibel range during that period of time. Decibel level measurements were collected near and far from the road at the same time by two iPhones as the disturbance passed a ten meter stretch marked by flags. The observer near the road signaled the start of a decibel level measurement to the observer far from the road by raising a flag, and then lowering it to signal the end of the measurement. The observer near the road then noted the type of disturbance and both observers recorded the time of the measurement as displayed in the application file.

Analysis

Data collected during this investigation were analyzed in the statistical program JMP, a product of SAS. Pollinator visitation rate was calculated in terms of total visits to an array or plant per flower-hour of observation, which quantified the number of hours spent observing the total flowers on the plant or in the array. Pollen counts were determined by averaging pollen

loads across three stigmas for a given plant and on an array level. Seed sets were averaged over the same three fruits from which we collected stigmas for pollen loads. Again, these data were averaged across fruits on a plant and on the array level. We conducted a series of analysis of variance (ANOVA) tests to determine differences in means for these three components of plant reproduction by site, array, and a site-array interaction.

Traffic data were used to determine an average frequency in terms of average number of human disturbances per minute over an observation period. These frequencies were then compared over the cycle of one day. In order to analyze noise data, we used matched pair analyses to understand differences in distance. We then weighted traffic data observed on the road by the noise levels associated with the particular disturbance to obtain an average weighted decibel level per minute and compared those values over the course of the traffic cycle of one day.

Results

Pollinator Visitation

General trends for average visitation rates appeared to be lower for far arrays than for near arrays across potted and natural experiments (Figure 2). Further analyses showed that for potted arrays, overall visitation rates to near and far arrays were not significantly different at either site ($F(3,18)=0.1697$, $p=0.9155$). The probability of zero visitation was higher for far arrays than near arrays (). Excluding periods where no visitation events were observed, visitation rates were higher at far than at near arrays, though this difference was not statistically significant ($F(3,7)=0.4281$, $p=0.7392$). Overall visitation rates on the individual plant level, though not significantly different, were generally higher for plants in the far arrays than for those in near arrays at both sites ($t(1)=1.72$, $p=0.0906$). Additionally, the number of flowers visited during a bout were higher on average for far arrays than for near arrays, though marginally significant ($F(1)=4.0613$, $p=0.0747$).

In natural arrays, overall visitation rates were higher for the far array at BBB, where differences in site and array interaction terms were marginally significant ($t(3)=2.01$, $p=0.0563$). When periods during which no visitation was observed were excluded from analyses, visitation rates were significantly higher for the far array at BBB ($t(3)=2.17$, $p<0.05$).

Pollen Counts & Seed Sets

Average pollen counts in potted arrays did not significantly differ between near and far arrays at either site ($F(3,45)=1.4105$, $p=0.2521$). Pollen loads appeared to be higher on stigmas near the road at BBB and far from the road at MFM, though differences between sites and arrays were not significant ($t(3)=-1.60$, $p=0.1176$, Figure 3). Similarly, pollen counts observed in

natural arrays were non-significant for distance ($t(57)=0.20$, $p=0.8405$) and marginally significant for site ($t(57)=1.71$, $p=0.0927$) comparisons, higher on average at BBB than MFM. The general trends across experiments indicated higher pollen counts for natural arrays than for potted arrays (Figure 3).

For the potted experiment, average seed sets were not significantly different between arrays at either site ($F(3,41)=0.0302$, $p=0.9928$). The probability for plants in potted arrays of producing zero seeds was not significantly different in array comparison at both sites ($\chi^2=4.675$, $df=3$, $p=0.1972$). Subsequent analyses where individuals which did not produce any seeds were excluded again yielded no significant differences between arrays at either site ($F(3,30)=0.4816$, $p=0.6976$). In natural arrays, array comparisons of seed sets at both sites were non-significant ($F(3,53)=0.7065$, $p=0.5524$). The probability of producing zero seeds in natural plants was significantly higher for far arrays ($\chi^2=3.886$, $df=1$, $p<0.05$). When cases of zero seed set were excluded, trends were also not found to be significantly different between arrays at either site ($F(3,50)=0.4024$, $p=0.7519$). Marginally significant trends near and far from the road indicate that plants in the potted experiment appeared to produce less seeds on average than plants in the natural experiment (Figure 4).

Road Environment

Overall traffic volume was comparable at both sites, showing no differences between BBB and MFM. Both sites experienced high levels of traffic during observation periods. Traffic frequency was highest in the early afternoon at both sites, which follow a similar trend throughout the day (Figure 5). The most common human disturbance at both sites was sport-utility vehicles (BBB=70; MFM=70), followed by pick-up trucks (BBB=41; MFM=42).

Weighted noise levels differed significantly near and far from the road ($t(4)=-49.937$, $p<0.05$, Figure 6). These levels also showed higher weighted decibel levels during early afternoon observation periods (Figure 6). For late morning, early afternoon, and late afternoon observation periods, weighted decibel levels were higher than ambient decibel levels (Figure 6).

Discussion

Pollinator Visitation

Though we did not observe significant differences between near and far arrays, there does appear to be a general trend suggesting that average visitation to far arrays was lower than near arrays. This is a surprising result as we expected road proximity to increase visitation to plants further away from the road. This was echoed in the higher probability of zero visitation to the far arrays than for the near arrays. On the plant level, we see this same trend with higher visitation for plants in the far arrays. This lower visitation to far arrays as a general trend may be due to the stress of plants particularly at BBB, which needed to be splinted often at more than one place in the stalk. A few plants in the far array at MFM also did not flower during the treatment, effectively reducing the amount of flowers which could have been visited. To supplement these results, we found that bout length was higher for far arrays, suggesting that although the incidence of visitation events were on average lower at far arrays, when a visit did occur, pollinators spent more time at the array to visit more flowers than they did at near arrays. This could be attributed to the quality of nectar between arrays, which may have discouraged pollinators from foraging in the near arrays for a longer period of time. The difference in bout length may also suggest that pollinators are being interrupted while foraging, particularly near the road compared to farther away.

Natural arrays exhibited higher visitation at far arrays compared to near arrays, which aligns with our original hypothesis. Excluding instances of zero visitation, average rates were higher, especially for the far array at BBB. It is possible that pollinators were more abundant at BBB at this time of the summer, increasing the incidence of visitation. Site differences, including proximity to trees or bushes, may also explain this higher visitation rate. However, this result still

suggests that the presence of the road may have caused pollinators to forage further away. Overall, potted and natural arrays showed similar trends, but it appeared that natural arrays experienced a higher visitation rate on average, indicating that there may be differences in visitation between experiments. This could be attributed to the nature of the experiments, where plants were more stressed in potted arrays which could have decreased the attractiveness of the plants, allocating resources to survival rather than attracting pollinators. This could have also been due to the time of the migratory season, where the natural experiment took place later, when the abundance of hummingbirds was higher.

Pollen Counts & Seed Set

Similar to visitation rates, we did not find differences in average pollen counts between arrays at either site. General trends for potted arrays suggest that pollen loads were higher near the road at BBB than farther away. This could have been due to the heavy deer herbivory at the far array, which reduced the number of stigmas we were able to collect. However, in line with visitation trends, it could be suggesting that pollinators were visiting the near array more frequently and subsequently depositing more pollen on those stigmas. At MFM, pollen loads were higher far from the road. While this contrasts with observed visitation rate, which indicated higher visitation to near arrays, it echoes our finding that pollinators were spending more time at the far array at MFM and thus depositing more pollen on stigmas. In natural arrays, pollen counts were higher on average at BBB. As the dustier site, we expected BBB to have less pollen than MFM, as dust has been shown to have detrimental effects on pollen adhesion to stigmas. Trends between potted and natural experiments also appeared to be different, where natural arrays had more pollen on stigmas than in potted arrays (Figure 3). This suggests that potting plants may

have an effect on the attractiveness of the plant and how much of its resources the plant uses to attract pollinators.

Average seeds sets were not significantly different between near and far arrays, however mean seed set appeared to be slightly higher for plants far from the road at MFM, while there were no obvious trends at BBB. The trend at MFM also aligns with higher bout length for far arrays as well as higher pollen counts for potted arrays. It appears that for this site, there may be a relationship between average bout length and subsequent pollen counts and seed set. Between experiments, there does appear to be a difference, echoing the difference observed in pollen counts as well. Again, this may suggest that the potting experiment may have had an unknown effect on the investigation compared to the natural experiment.

Traffic & Noise

Both sites experienced heavy traffic during observation periods, though the sites themselves did not differ much in the average frequency of volume. The daily cycle of traffic appeared to peak in the late morning, between 10:30 and noon, followed by the highest peak in the early afternoon, between 1:30PM and 3:00PM. This trend is echoed in terms of noise produced by the road. Decibel levels associated with motorized vehicles were very different near and far from the road consistently throughout the day at both sites. These levels were well above the average ambient decibel levels at either site. This difference can be attributed to the way in which sound waves travel and degrade over distance and topographic elements, together known as the acoustic environment. Sites themselves differed for this reason and are thus difficult to compare directly. The differences between noise levels near and far, however, align well with

what we might expect. The human presence associated with a road is what changes the noise environment at both sites.

The question remains whether this change in noise level of the environment is affecting pollinator behavior and resulting in less reproductive success. Visitation rates compared over the cycle of a day showed that visitation rates were highest during evening observation periods. Compared to the daily cycle of traffic and associated increases in noise levels, overall pollinator visitation increased when traffic was at its lowest. When we look specifically at daylight hours, visitation appeared higher in the afternoon compared to the morning. According to this comparison, pollinators are in fact visiting arrays during peak times of traffic. This was a counterintuitive result, where we might expect pollinators to be visiting more frequently earlier in the morning, but they appear to be doing the opposite. This suggests two potential explanations. The first is that pollinators may not be affected by noise produced by the road and instead are foraging during peak daylight hours or when flowers *I. aggregata* generally open. Another potential explanation might be that pollinators are intentionally foraging during peak traffic times, when the noise is loudest. The increased noise levels may not deter the pollinators, but their predators instead. This would be similar to results from a study completed in 2012, which reported that continuous noise much louder than ambient levels actually enhanced pollination of *I. aggregata* (Francis et. al). The effect size of our investigation in comparison to this study is much lower as noise was not continuous nor was it nearly as amplified, but the trend remains similar.

Bout length may also be a means of measuring reactions by pollinators to traffic and the associated noise levels. As stated earlier, bout length differed between arrays for the potted experiment, with pollinators spending more time at flowers in the far array compared to the near

array. However, overall visitation rates were higher to near arrays. This contradiction suggests that pollinators may have been interrupted while foraging at near arrays. During pollinator observation periods, we also described instances when hummingbirds, in particular, were disturbed and fled a few meters away or completely out of our sight. I will continue to look back on this anecdotal observation data to see if there are any consistent patterns that may help us explain the data we have obtained on bout length. Data on bout length remains to be analyzed for natural arrays and this additional analysis may also contribute to our understanding of observations performed this summer.

Larger Implication & Future Directions

The results of this study have potentially larger implications for our urbanizing world. First, roads appear to have some effects on pollination processes. Though we are not entirely sure of the mechanism behind these effects, it is clear that something is happening to systems near roads that we may not see in more pristine areas. Second, the road increases noise in the environment, which means there are likely implications for plant and animal communities near them. As the field of road ecology grows and the effects of roads become better studied and understood, studies such as this one could consult the future construction of road systems, particularly in rural areas and in areas continuing urbanization and improving roads.

As is often the case, the results of this study lead to many more questions. A further step in understanding the effects of noise on pollinator behavior would be to investigate the direct correlation between the two, rather than the indirect approach of this study. Measuring traffic and noise levels during pollinator observation periods would provide an example of the direct effects of human disturbance on pollinators. It would then be interesting to see if these effects on

pollinators translate into differences in seed set. A second question which remains to be answered is whether the auditory cues are more influential for pollinators than visual cues. This would help to understand whether increased noise pollution actually do affect pollinators and investigate whether pollinators respond differently to seeing a disturbance, such as a large red truck, versus hearing the same disturbance. Lastly, it would be beneficial to repeat this study in a more urban environment, particularly on a paved road to determine whether differences observed in the study would be paralleled. This could contribute to our understanding of how improved roads affect these same processes in terms of noise.

Appendix

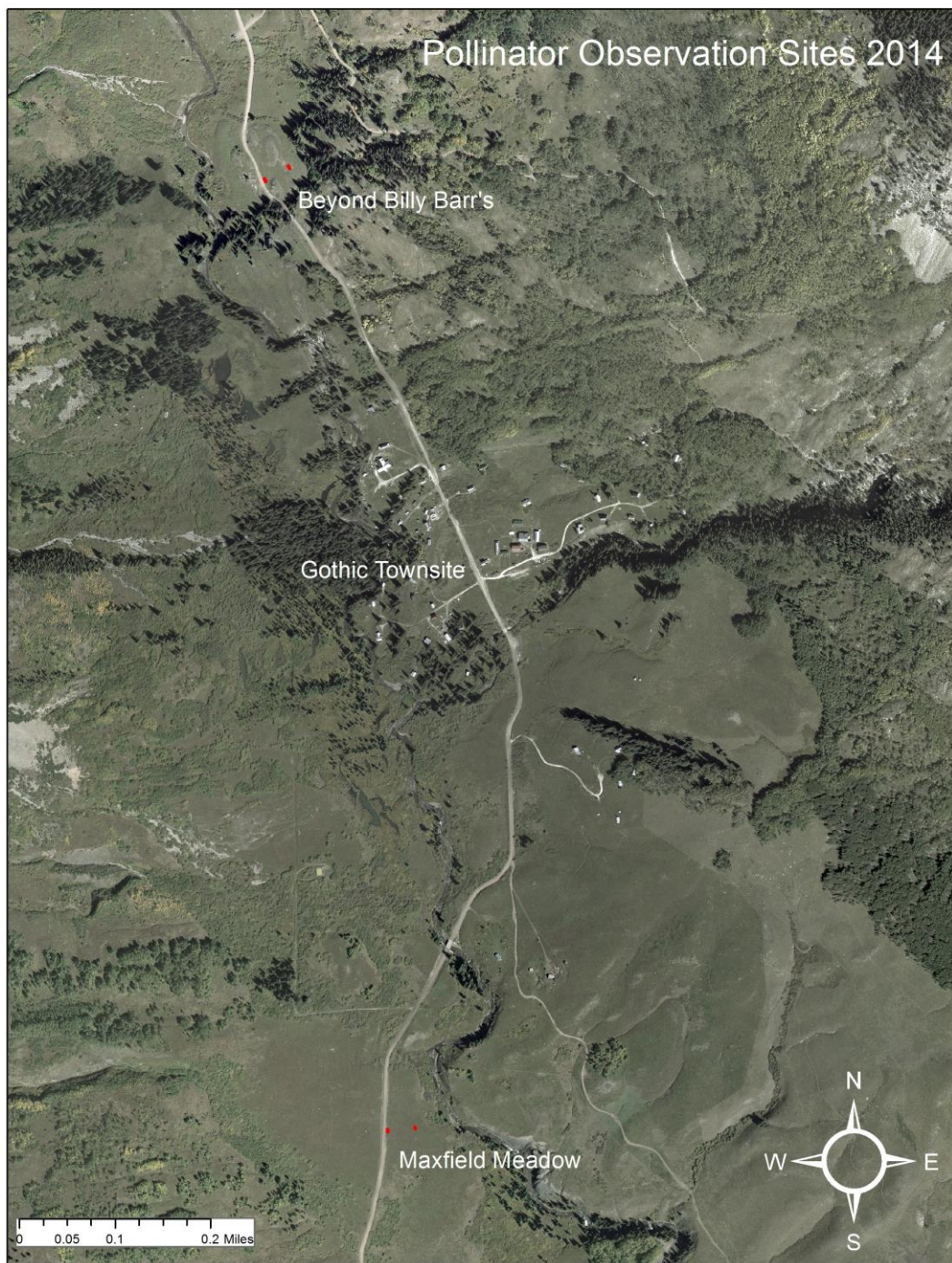


Figure 1. GIS map of research sites used in the investigation for pollinator observation.

Table 1. Types of disturbances observed on the road during traffic data collection. Descriptions were used as criteria for categorizing disturbances.

Disturbance Type	Type Description
CAR	smaller vehicles (e.g. sedans, station wagons)
SUV	sports utility vehicle, larger in size
JEEP	vehicles used for off-roading activities
TRUCK	vehicles with a truck bed
RIG	oversized trucks (e.g. trucks with horse trailers, construction trucks)
MOTORCYCLE	motorized vehicles with two wheels
BIKE	bicycles
HUMAN	pedestrians

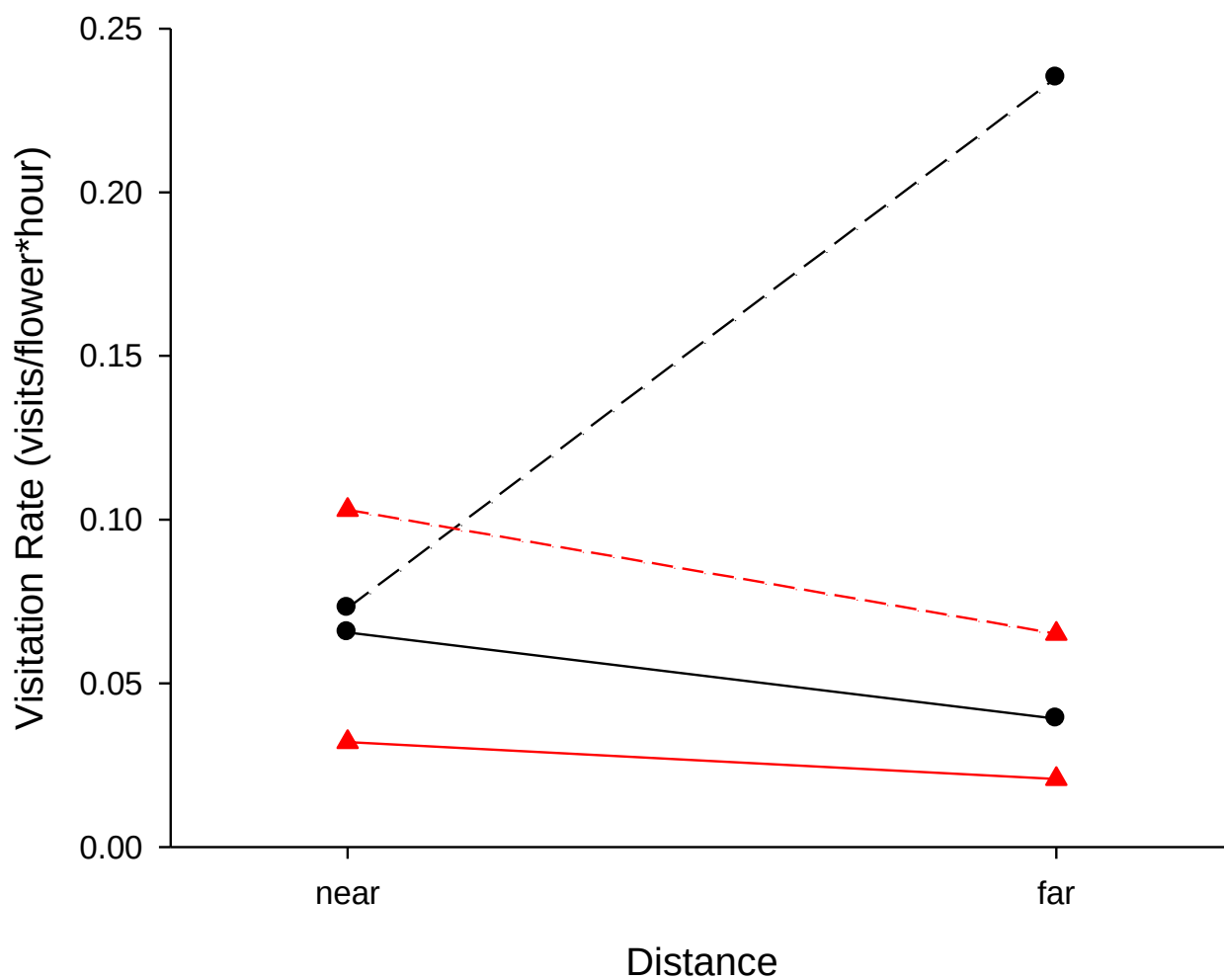


Figure 2. Visitation rates to potted (solid) and natural (dashed) arrays near and far from CR 317 at Maxfield Meadow (red) and Beyond Billy Barr's (black). Least squares means calculated over observation periods per array ($N_{\text{potted}}=22$; $N_{\text{natural}}=28$).

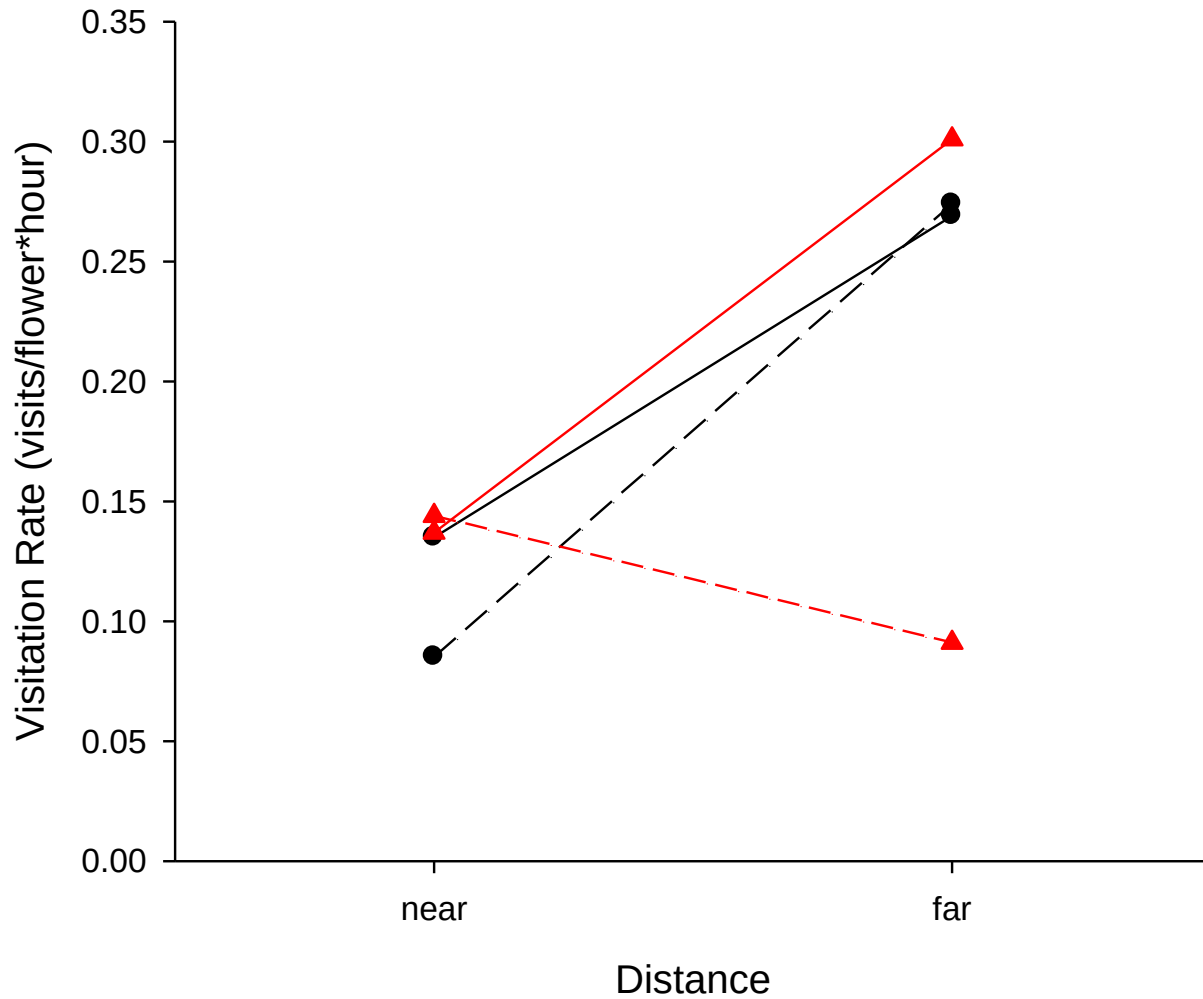


Figure 3. Visitation rates (excluding instances of zero visitation) to potted (solid) and natural (dashed) arrays near and far from CR 317 at Maxfield Meadow (red) and Beyond Billy Barr's (black). Least squares means calculated over observation periods per array ($N_{\text{potted}} = 11$; $N_{\text{natural}} = 22$).

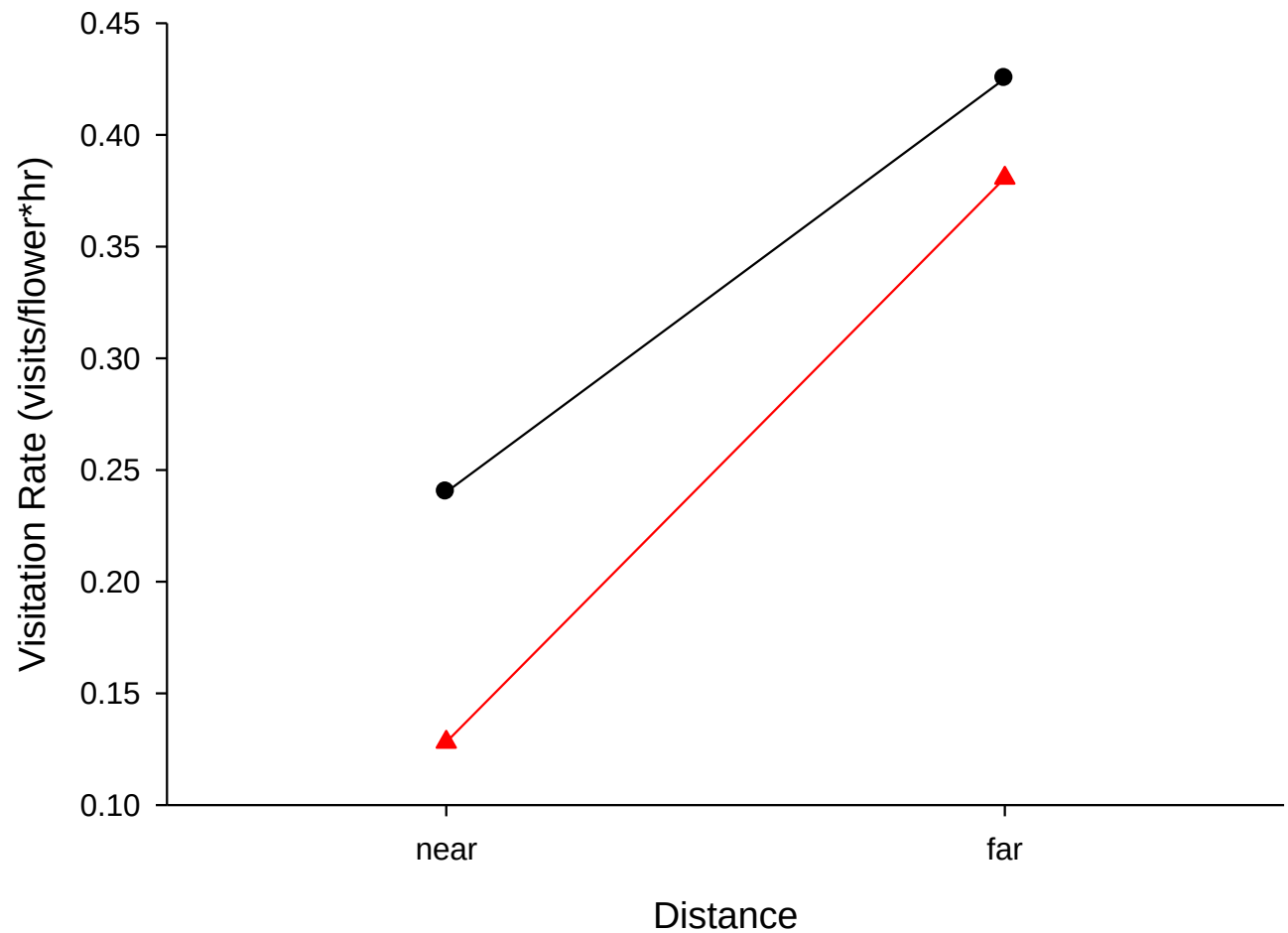


Figure 4. Visitation rates to potted arrays near and far from CR 317 at Maxfield Meadow (red) and Beyond Billy Barr's (black). Least squares means calculated for individual plants within arrays ($N_{\text{potted}}=59$).

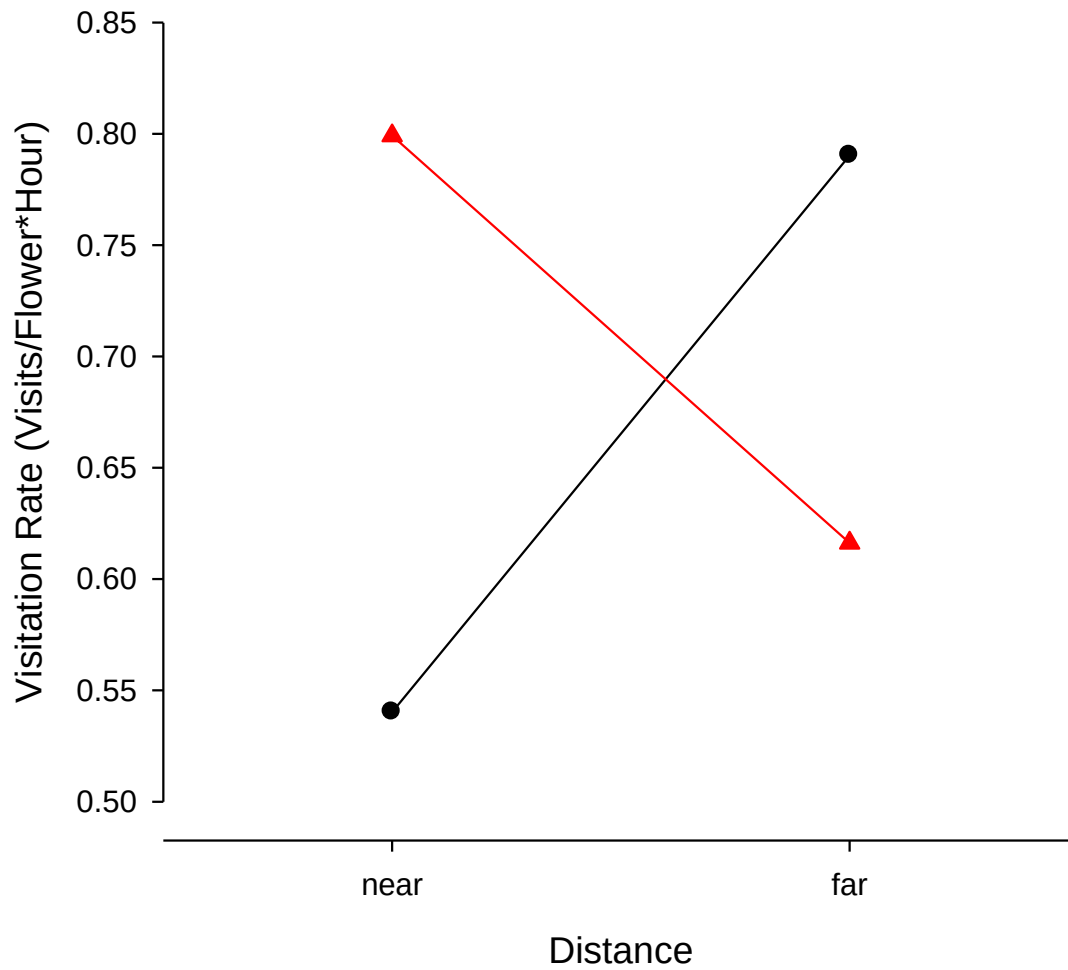


Figure 5. Visitation rates (excluding instances of zero visitation) to potted arrays near and far from CR 317 at Maxfield Meadow (red) and Beyond Billy Barr's (black). Least squares means calculated for individual plants within arrays ($N_{\text{potted}} = 38$).

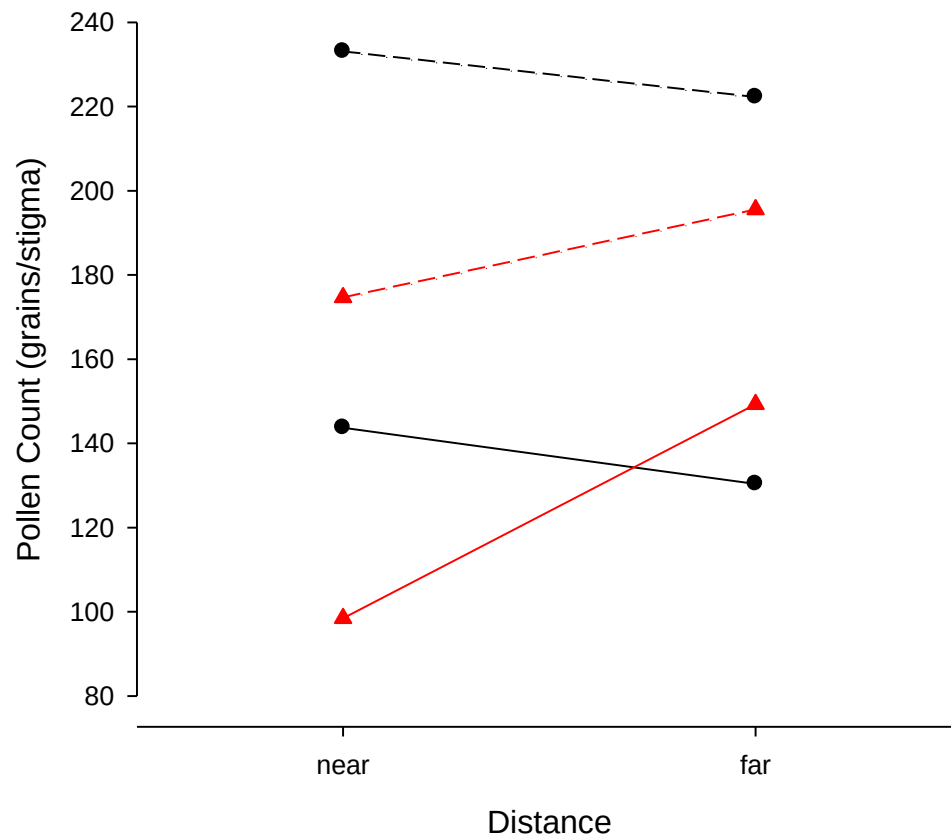


Figure 6. Pollen counts for potted (solid) and natural (dashed) arrays near and far from CR 317 at Maxfield Meadow (red) and Beyond Billy Barr's (black). Least squares means calculated over average pollen counts per plant in an array ($N_{\text{potted}}=49$; $N_{\text{natural}}=58$).

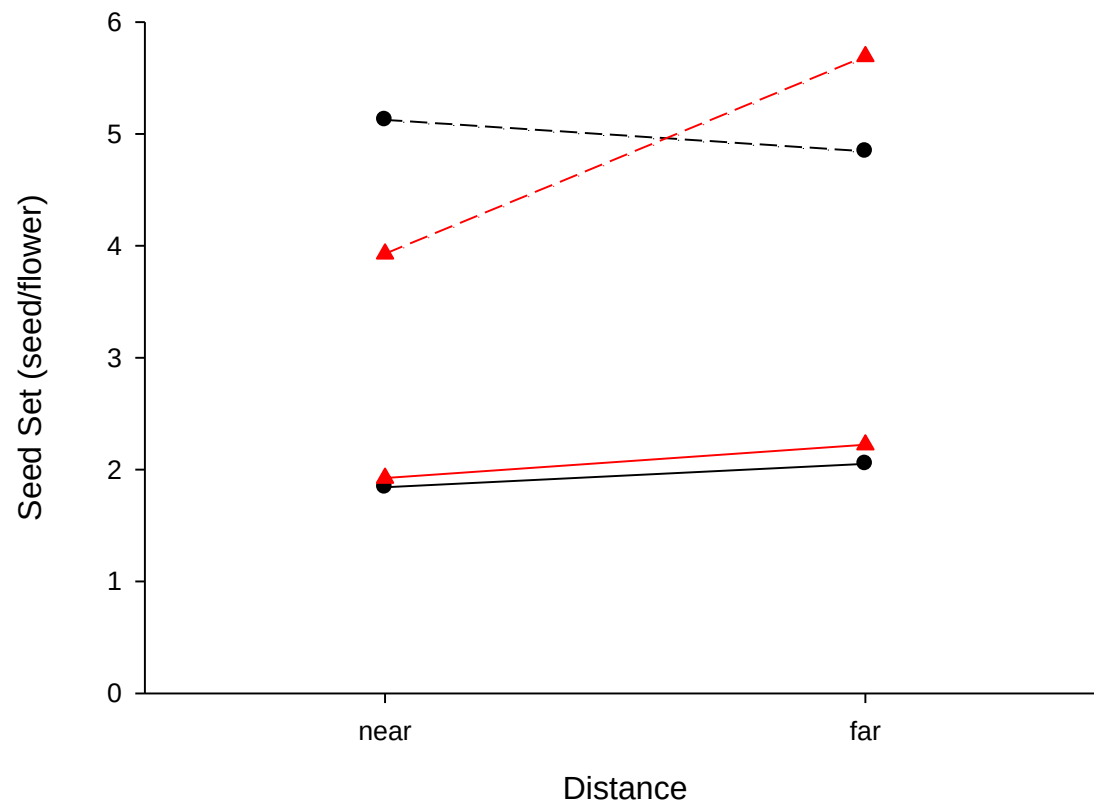


Figure 7. Average seed sets for potted (solid) and natural (dashed) arrays near and far from CR 317 at Maxfield Meadow (red) and Beyond Billy Barr's (black). Least squares means calculated over average seed sets per plant in an array ($N_{\text{potted}}=45$; $N_{\text{natural}}=57$).

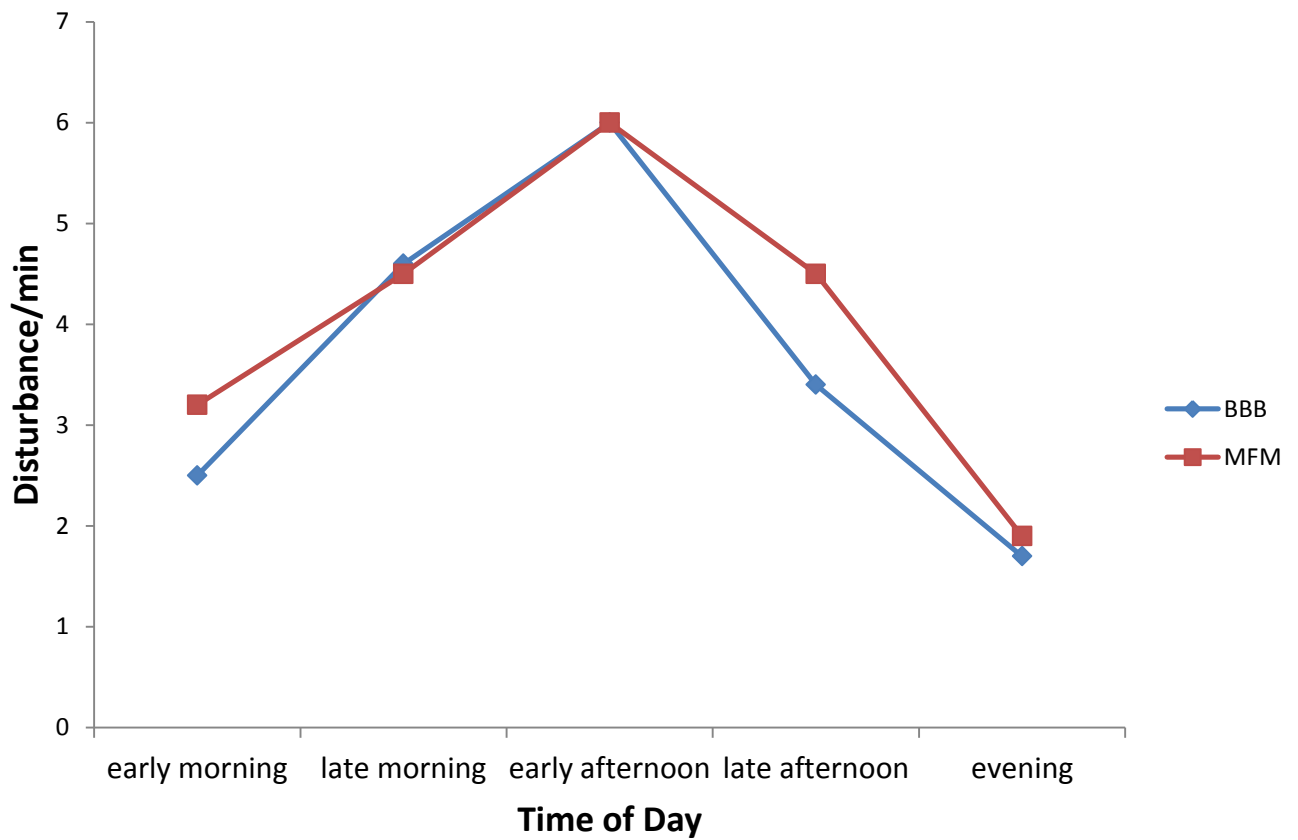


Figure 8. Disturbance frequencies throughout the day in terms of average disturbance per minute over a ten minute observation period. Average frequencies did not differ significantly between sites.

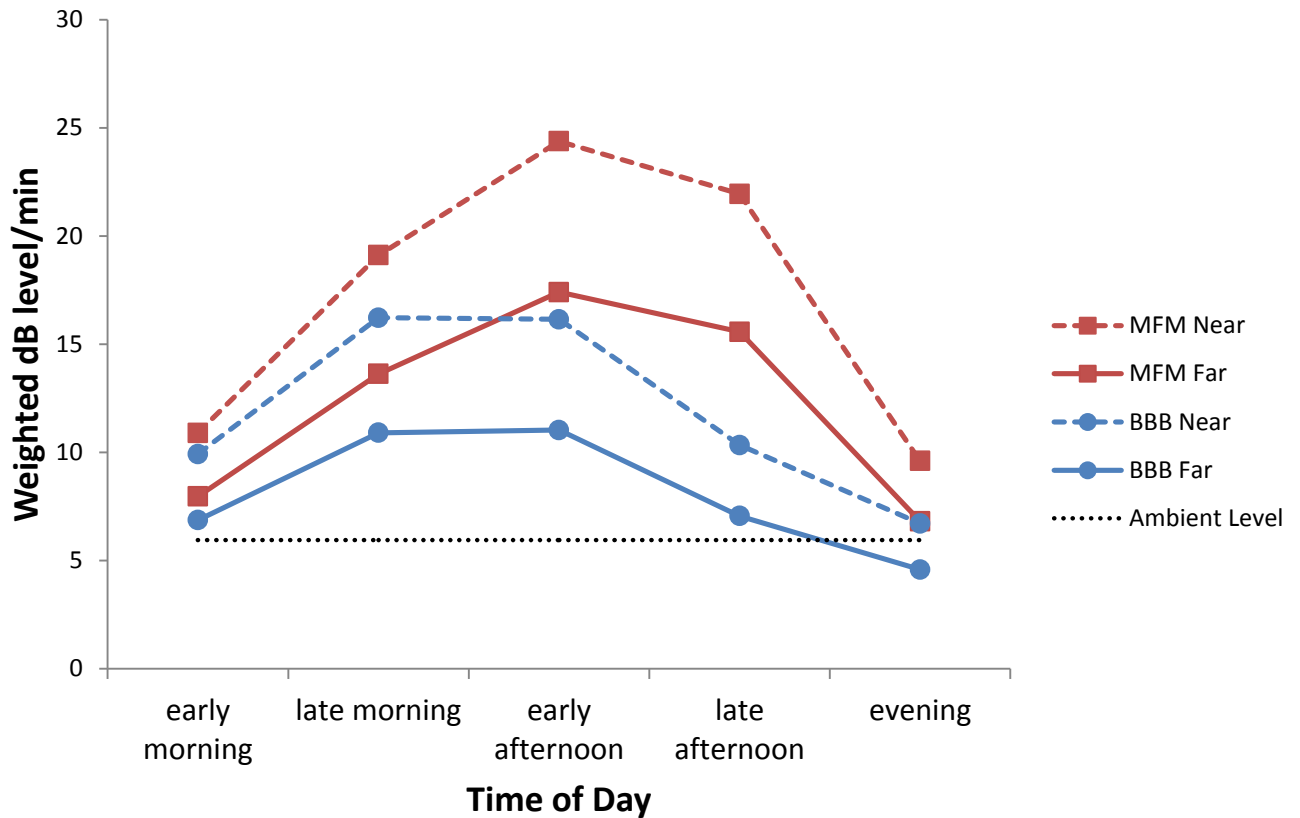


Figure 9. Average decibel levels at different times of the day. Trends appear different where decibel levels are higher near the road versus far from the road at Beyond Billy Barr's consistently throughout the day. Weighted dB/min = (total number of one type of disturbance * associated average midpoint dB level)/30 total minutes of observation at each time of day.

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