2002

Diet for an endangered insect : what does the zayante band-winged grasshopper eat?

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DIET FOR AN ENDANGERED INSECT: WHAT DOES THE ZAYANTE BAND-WINGED GRASSHOPPER EAT?

A Thesis Presented to

The Faculty of the Department of Environmental Studies

San Jose State University

San Jose, California

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

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December 2002

UMI Number: 1411604

ProQuest Information and Learning Company
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ABSTRACT

TITLE: DIET FOR AN ENDANGERED INSECT: WHAT DOES THE ZAYANTE BAND-WINGED GRASSHOPPER EAT?

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This study used a combination of frass analysis, field observations, and habitat use correlations to infer host plant use and preference for adult Zayante band-winged grasshopper (ZBWG) *Trimerotropis infantilis* (Orthoptera: Acrididae) in Santa Cruz County, California. Of 128 frass samples collected, host plant identity could be clearly determined through microscopic analysis in 103 samples. The majority of host plants found were *Lupinus albifrons* (Fabaceae) and *Heterotheca sessiliflora* (Asteraceae), with occasional evidence of the use of species in Poaceae. Results from frass analysis corresponded with data obtained through 33 hours of field observation. Habitat choice did not correlate well with host plant choice, however. Suggesting that factors other than food source in the plant community, such as cover from predation or support of courtship and mating, may be important to ZBWG life history and fitness. Results will be used to identify critical plant species needed to protect the species.

Acknowledgements

This research was funded in part from a Section 6 grant of the Endangered Species Act, through the California Department of Fish and Game and the U.S. Fish and Wildlife Service.

Thank you to:

- Dr. Rachel O'Malley and Dr. Rod Myatt, San Jose State University
- Ms. Colleen Sculley, U.S. Fish and Wildlife Service
- Ms. Deb Hillyard California Department of Fish and Game
- Volunteers: Jennifer Arbitrario, Anne Crealock, Iona Cheng, Amy DeWeerd, Melinda Dorin, Sean Dreilinger, Judy Fenerty, Matt Finley, Kathy Fischer, Ingrid Graeve, Matt Johnston, Christinia Lau, Weston Miller, Michael Powers and Tom Robinson
- Granite Rock Corporation for allowing access to field sites
- Jodi McGraw and Suzanne Schettler for advice and knowledge about Sand Hills plants
- Cybertracker™ for the use of their free data collection software, available on the Internet: http://www.cybertracker.co.za/
- My parents and brother: Theodore and Virginia Chu and David Chu, for their support

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Background

In the last 35 years there has been a growing recognition of the importance of biological diversity on Earth. California is one of the most biologically diverse areas in the world, with habitats, plants, and animals found nowhere else in the world. California's diversity, however, is being lost in many important habitats around the state due to the growing human population and the impacts that come with development. In particular in the Zayante Sand Hills (Sand Hills), human-caused habitat loss from sand mining and urban development are the most significant factors putting species at risk (U.S. Fish and Wildlife Service 1998).

Regulatory Context

In 1966, as people began to realize that serious efforts were needed to be made to stop the wave of extinctions that had begun, the first Federal Endangered Species Act (FESA) was passed by the U.S. Congress. The FESA gives some degree of protection to plants and animals that are listed as endangered (in danger of extinction) or threatened (likely to become endangered), but it fails to explicitly protect habitats. The FESA as it stands today gives significantly more protection to animals than plants and carries more strength on federal lands than on private property. In fact, plants are protected only on federal lands. The FESA is designed to protect species, subspecies, and populations so that an organism that is likely to disappear from even part of its habitat range is protected.

Policy

The Zayante band-winged grasshopper was listed as endangered in 1997, under the Federal Endangered Species Act. The species carries a recovery priority of "8" which means it is viewed as a species with a moderate degree of threat and a high recovery potential (U.S. Fish and Wildlife Service 1998). Typical of many endangered species, its primary threat of extinction is human development and destruction of its habitat.

Listing of a species as endangered under the FESA (or "the Act") does provide some refuge to the species. Section 9 of the Act makes it unlawful for any person to "take" a listed species. "Take" is defined as "harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or attempt to engage in any such conduct" (definition from Section 3 (18) of the FESA). By prohibiting take of an endangered species, the FESA requires all people (federal, state or private) with endangered species on their land to conserve the species either through protection or some sort of agreement with the U.S. Fish and Wildlife Service (FWS) that would authorize take through an "incidental take permit."

For example, the Act requires that all federal agencies protect and preserve any listed species' habitats by making sure that agency actions do not jeopardize the continued existence of the listed species. Thus, any federal actions that could result in significant habitat modification or degradation, or actually killing, injuring, or impairing a listed species' essential breeding, feeding, or sheltering behavior patterns are included in this regulation. The FWS and the National Marine Fisheries Services (NMFS) work with other federal agencies to plan or modify federal projects so that they have minimal impact on listed species and their habitat.

The Act encourages non-federal land owners (state or private) to work with the FWS to help protect species on their lands as well. Through Section 6 of the Act, the FWS works with states to encourage each state to develop conservation programs for listed species. Federal technical and financial assistance and other incentives are used to help attract state participation.

When working with private land owners, the FWS may help to protect listed species in part through "habitat conservation plans" (HCPs). In this case, private landowners develop an approved HCP that provides for some conservation of the species in exchange for an "incidental take permit" that allows for a development project to continue.

The Act requires that a recovery plan for each listed species be developed to outline the steps needed to restore the species to health with the goal of recovering the species and removing it from the endangered species list. The development of these plans often involves public and private institutions and agencies, which may help in research, land acquisition, and other protective measures.

Ecosystem level recovery plans and conservation plans began to be used in California in the early 1990s in San Diego, Orange, and Riverside Counties, and portions of Los Angeles County. Since then, in recent years the need for recovery plan updates has been discussed, in particular as new scientific information on the species or habitat is discovered. Federal ESA ecosystem plans were evaluated as part of a study done by

Boersma et al. (2001), to look at the effectiveness of endangered species recovery plans. The authors evaluated the use of revised plans, plans written by a group of authors versus those written solely by federal employees, plans with recovery criteria based on biology of the species versus those plans that were not, and the effectiveness of multiple species plans compared to single species plans.

They found that among all sampled recovery plans, species with revised recovery plans were significantly more likely to show a positive condition than were species for which plans remained unrevised. They also discovered that the use of nonfederal participation (academic scientists) in recovery plan development has a positive impact on the status trend of a species. An improving status was also seen when species biology and recovery goals were coupled in recovery plans.

Multiple species plans, however, were shown to be less effective than the single species plans. It was suggested that this might be due to the fact that efficiency could take away thoroughness and explicit science. The authors state that in ecosystem and multiple species plans, the same amount of time and money needs to be spent per species as would be with a single species plan. Finally the authors found that recovery actions are not monitored well, recovery plans take too long to complete, and longer plans are not more effective.

Boersma et al. suggest an adaptive management approach to creating recovery plans. They assert that when an opportunity to revise a plan with improved information arises, it should be used so as not to risk that an unrevised plan become irrelevant with time, as a species status might change. The need for improved information and understanding is also noted, including linking recovery criteria to species biology, as well as using quantitative data on the status of species through monitoring. The authors urge caution when using an ecosystem or multiple species recovery plan, citing the need for equal funding and effort by personnel as is used in individual species recovery plans, so as not to jeopardize the recovery of each species. Finally Boersma et al. highlight the importance of using diverse participants in recovery plan development. By ensuring public involvement and educating all involved, one can build support for the plan, getting greater investment and commitment to the species that can help to protect the most biodiversity.

Critical habitat designation is another part of the FESA. Through this process, geographic areas that contain physical or biological features upon which each listed species depends are identified. In these areas federal agencies may not take actions that jeopardize the species.

A similar state act was passed in 1970 in California. Like the federal law, the California Endangered Species Act (CESA) prohibits take of listed species, and it requires other state agencies to consult with the California Department of Fish and Game (CDFG) to ensure state projects do not further jeopardize the species. The state law, however, fails to protect habitats that are often the key to species survival and the maintenance of biodiversity, and importantly, it does not protect insects under any circumstances. CESA does, however, afford protection to listed plants on private as well as public property.

In California there has been an increasing emphasis on how to balance conflicts of economic activities and rare and sensitive species, as rapid urbanization has taken off around the state. Deciphering the best way to use federal Habitat Conservation Plans (HCP) and state Natural Community Conservations Plans (NCCPs) in conjunction with endangered species recovery plans in California is at the heart of the controversy in conservation planning under the ESA and the CESA. The California state NCCP Act of 1991 was intended to encourage voluntary participation in the development of regional or areawide protection plans (NCCPs). It was meant to improve upon the existing project-by-project regulatory system by creating large areas of protected wildlife habitat, while seeking to allow for more local goverenment participation, streamlining the land-use permiting process related to endangered species and reducing the need to list species in the future (Rolfe 2001).

The Santa Cruz Sand Hills' special status species and unique habitats are a case in point going through these planning processes. In fact the CDFG considers maritime coast range ponderosa pine forest (or sand parkland) a "rare and endangered natural community, and as such, this community is a high priority for conservation by the Department of Fish and Game" (Roxanne Bittman [CDFG], personal communication, 2002). With protection seen as being of great value for a variety of plant and animal species in one of the rarest habitat types on Earth, much debate has been exhibited around creating an ecosystem level (Santa Cruz regional or countywide) conservation plan.

Quail Hollow Quarry Habitat Conservation Plan

The Zayante Band-winged Grasshopper population at Quail Hollow Quarry is specifically protected under the FESA through a Habitat Conservation Plan prepared by the private land owners, and approved by the FWS, the California Department of Fish and Game and the County of Santa Cruz. In 1997, Granite Rock Corporation (GRC) applied for an incidental take permit from the FWS under section 10(a)(1)(B) of the FESA. GRC requested a permit authorizing take of the ZBWG, and three other federally endangered species (the Mount Hermon June beetle, (*Polyphylla barbata*), the Ben Lomond wallflower (*Erysimum teretifolium*), and the Ben Lomond spineflower (*Chorizanthe pungens var. hartwegiana*)) at the Quail Hollow Quarry site (QHQ) in Santa Cruz County, California. In support of this permit application, GRC prepared a HCP that provided for conservation and long-term management of the four listed species at QHQ. Also, the HCP identified and delinated areas authorized for take from mining activities. Furthermore, the HCP outlined the responsibilities of Granite rock in cooperation with the County of Santa Cruz, the FWS, and the CDFG. These three agencies reviewed and approved the HCP, and the FWS issued the incidental take permit to GRC in 1998.

The HCP indentifies requirements for the continued operation of existing sand mining operations in the "current mining area" (the area that GRC proposed to mine). The FWS's permit allows the incidental take of the species in this "current mining area" and the "future mining area" of Quail Hollow Quarry (Thomas Reid Associates 1997).

The "current mining area" is 7.6 hectares in size and is located on the south central portion of the QHQ site. To minimize and mitigate the take of species for in this area the HCP required GRC to grant a conservation easement in perpetuity to the County of Santa Cruz for 4.4 hectares on the North Ridge of the QHQ site (North Ridge Habitat Set Aside). In addition, the HCP required GRC to protect and manage into the long-term this area, by patrolling the set aside to ensure that illegal access is not occurring and providing fencing and signage, as necessary. The HCP also details how 0.8 hectares of disturbed sand parkland habitat on the site will be enhanced. The HCP states that GRC: must implement pertinent provisions to avoid disturbing the protected species in all areas of the quarry property except for areas within the current mining area and other designated overburden, stockpile and road access areas.

The "future mining area" as discussed in the Quail Hollow Quarry habitat conservation plan consists of an area of 33.2 hectares. In order to minimize and mitigate the take of plan species there, the HCP required that prior to habitat disturbance, GRC grant a conservation easement in perpetuity on the 8.24 hectares of the West Ridge Habitat Set Aside (and provide protection and long term management of that area), as well as supply funding for the long-term management of the 13.04 hectare South Ridge Habitat Set Aside which the County of Santa Cruz committed to purchasing at the fair market value. In addition, enhancement of 1.2 hectares of disturbed sand parkland habitat and 2.08 hectares of disturbed maritime chaparral habitat was required. A long-term management and maintenance program for these enhanced areas was also included to assure long-term survival.

The HCP also limits the quarry owners' development of the property in the "future mining area" by restricting the time of disruption in sensitive habitat areas to the months other than June, July and August (when the insect species are most likely to be breeding). Other specific measures to reduce impacts on insects of concern included prevention of erosion and runoff of loose materials into habitat areas, the removal of non-native invasive plant species that could reduce the extent and quality of the insect food resources, and limiting human use of habitat set asides by restricting people and pets and providing educational signage.

Finally the HCP requires the County of Santa Cruz to report annually to the FWS and the CDFG regarding compliance to the mitigation measures. Factors to be reported include, status and condition of the three habitat set asides (including controlling authorized use of the site), exotic pest control, monitoring of the endangered species. For the ZBWG, this includes monitoring surveys conducted every two years and the confirmation of habitat preference and food plants. The County of Santa Cruz was chosen to administer the HCP and to contract with knowledgeable firms to assist with carrying out the management activities, while the GRC was given the task of carrying out or providing funding for all of its habitat management activities to take place on the 88 hectare parcel as specified.

The Zayante Band-winged Grasshopper

Physiology

The Zayante band-winged grasshopper (ZBWG) (*Trimerotropis infantilis*) (Orthoptera: Oedipodinae) is federally listed as endangered under the FESA. The ZBWG is typical of the Oedipodinae (the band-winged grasshoppers) in that it frequents areas of sparse vegetation, often alighting on bare ground. Its hind wings are concealed at rest and its front wings blend in with the habitat background (Borror et al. 1976). It is a conspicuous flier with bright colored hind wings (pale yellow), blue hind tibiae (Appendix D) and a noticeable crackling stridulation sound, which is made by its wings during flight (Rentz and Weissman 1984).

Habitat

ZBWG lives in the harsh Zayante sand parkland habitat of the Santa Cruz Mountains (see Figure 1). The grasshopper has a limited distribution throughout its range in Santa Cruz County, California where it is restricted to the sand parkland habitat found on the ridges and hills within the Zayante Sand Hills ecosystem. It is estimated that up to 240 hectares of sand parkland existed historically, but now fewer than 80 hectares remain intact near Ben Lomond, California (U.S. Fish and Wildlife Service 1998).

ZBWG occurs in association with the Zayante soil series, which is part of the Santa Margarita Sandstone formation that is derived from consolidated marine sediments and sandstones dating from the Miocene era. Zayante series soils are found in the Ben Lomond, Mount Hermon, Felton, Zayante and Scott's Valley communities (USDA Soil Conservation Service 1980). These areas are made up of northern maritime chaparral and maritime coast range ponderosa pine (*Pinus ponderosa*). Ponderosa sand parkland is the mosaic of these two plant communities found in the Sand Hills. These areas contain open sandy areas with sparse low annual and perennial herbs on high ridges with ponderosa pine, as well as sparse chaparral, mixed with patches of grasses and forbs (Arnold 1999).

Major Threats

Undoubtedly the Zayante band-winged grasshopper was more widespread through the restricted Sand Hills region before human impact. The primary threat to ZBWG has been and continues to be loss of habitat. Intensive sand mining and urban development has altered much of its original range with over 50% of its habitat having been lost from soil removal and destruction of plants that they may depend on, thus jeopardizing the Zayante band-winged grasshoppers' survival. Other threats include recreational activities, agriculture and the disruption of natural landscape-level processes. Active fire suppression has resulted in the encroachment of mixed evergreen forest into ponderosa pine forest (Marangio 1985) which increases shading and thus may restrict the areas available for use by ZBWG .

Restoration Needs

In order for species recovery to occur, a number of different actions are needed. Protection of habitat through acquisition or establishment of conservation easements will be vital, as will development of Habitat Conservation Plans (HCPs) with the quarry owners and with the County of Santa Cruz to minimize the loss of habitat from sand mining and urban development. Development and implementation of a management plan for State and County-owned Quail Hollow Ranch County Park is also important.

No less important to the recovery of the species is continued research that focuses on the habitat requirements for long term survival of the species. This study was the first to identify the adult Zayante band-winged grasshopper's host plant range, microhabitat use and to observe the adult behaviors. With host plants and habitat needs established, these resources may finally be restored, especially in areas where intensive mining practices have left the landscape altered with steep slopes and compacted soils that tend to not hold plants.

Importance

Identifying host plants is important to understand the insect-plant interactions that are a part of the larger ecological and evolutionary relationships that have developed in an ecosystem. Because insects are often the most vulnerable indicators of habitat health, their restoration can reflect restoration of wider ecological functions.

Identifying rare insects' host plants is also of significance when deciding which intact areas of habitat to protect, how to manage such areas and finally how to best design the restoration of degraded habitat areas. The incorporation of a federal, regional Habitat Conservation Plan (HCP) and/or the use of the state's Natural Community Conservation Planning program (NCCP), in conjunction with conservation banking, land purchases, at the species and the general habitat level (Sand Hills) may be a vital way of insuring that important land areas are protected for the species. In such instances, accurate information on the natural history of the species is critical when making tradeoffs in development versus species/habitat protection, or when funds are limited.

Thus, determining ZBWG's host plant range is not only critical to understanding the species' biology, but also necessary to create a proper protection or restoration plan that involves protection of essential plant species and maintenance of this unique community of plant and animal species. This research will contribute background information, that could be used in future research projects focused on restoring or mimicking disturbance regimes in the Sand Hills ecosystem. The challenge remains of how to maintain the open habitat of sand parkland, (from encroaching non-native species and chaparral plants), that is needed by this grasshopper species, while maintaining sensitive plant species as well.

RELATED RESEARCH

Host Plant Theory

Plant-feeding, or phytophagous, insects make up over one-quarter of all macroscopic organisms on earth, while the green plants they eat make up another quarter (Bernays 1994). Insects are the major link between primary producers, green plants and a number of animals at higher trophic levels. Many vertebrates and insect parasitoids depend on insects for their livelihood. Some plants and insects also appear to have coevolved and therefore depend on each other for survival.

Determining a phytophagous insect's host plant range involves a number of techniques including observation of host-choice behavior and physical analysis of materials from the insect. Some insects are polyphagous, which means that they feed on a relatively large number of plants from different families, while others are more specific in their choice of food. Oligophagous insects are those that feed on a number of plants usually in different genera within one plant family, while monophagous insects are still more specific, feeding on only one species of plants or on plants from one genus. Grasshoppers are usually polyphagous, but monophagous species occur within the order Orthoptera as well (Bernays and Chapman 1994).

Identification of Host Plants

Methods for identifying host plants for grasshoppers vary from field observations in the natural environment to experiments and microscopic analysis in the laboratory. These methods are usually conducted in conjunction with studies of grasshopper behavior.

Eigenbrode and Bernays (1997) review how insects in general choose their host plant, including the use of factors unrelated to the host, such as microclimate, vegetational associations and presence of other insects. The authors advocate the use of direct observational studies of plant selection by insect herbivores, especially in the field. They state that in spite of the perceived difficulty and laboriousness of field observations, advantages exist, and manipulations can help with field observations. The advantages of field observations include the ability to document behaviors as they occur in the field and the identification of specific plant factors that may mediate behaviors. Experimental manipulations, done in the field, can help make observation easier. For example, insects can be marked with brightly colored powder for easier observation, while plants can be enclosed in large field enclosures for easier study while maintaining near normal conditions.

Field observation methodology can also be simplified with the use of video tracking and computer automation. Eigenbrode and Bernays suggest that direct observational techniques are not as formidable as one might think; and they recommend types of data to record and analyze, experimental design and statistical analyses tat can be used for particular behavior experiments.

Using a laboratory oriented approach, Blust and Hopkins (1990) describe an electronic monitoring device that they used to monitor the feeding activities of two grasshoppers: *Hypochlora alba*, a specialist, and *Melanoplus sanguinipes* a polyphagous species. The authors designed a feeding monitor to detect biting, using the varying resistance of grasshopper-plant contact. To record the feeding activity of each insect, a fine wire was implanted into the grasshopper, which was also connected through the top of a cage to a swivel, allowing the grasshopper to move without tension on the wire. The wire was also joined as input to an operational amplifier, while another wire was inserted into the stem or soil of the test plant. If the signal from the grasshopper exceeded the reference voltage an output voltage was released to trigger a transistor, which the event recorder could recognize. The event recorder thus provided a trace of when feeding was taking place, or "blocks of activity", and when feeding was not.

The number of feeding bouts was recorded (where feeding bouts were considered separate when at least four minutes of non-feed time elapsed between feedings). Then, the average minutes of feeding/hour, the frequency of feeding bouts and the length of feeding bouts were calculated for light and dark phases of the photoperiod. Duncan's multiple range test was used for the analysis of means to compare results.

The results of this study demonstrated that the specialist and generalist species achieved the same feeding time by different feeding patterns. It was not specifically designed to look at host plant choice (instead it examined feeding patterns on one plant type), but was of interest for the electronic monitoring of the plant-insect interaction. The electronic monitoring device was effective for their work but it is too invasive and too restrictive to be used in host plant choice experiments (especially for endangered species).

Capinera (1993) used two choice and four choice tests to find the preference of a grasshopper for 40 potential host plants relative to one known host plant, bahia grass (*Paspalum notatum*). Average preference index values were compared between small nymphs, large nymphs and adults using Pearson Correlation, while large nymph and adult index values were pooled and analyzed using nonparametric Kruskal Wallis analysis of variance (ANOVA). Mean index values were separated with a Tukey type nonparametric multiple comparison procedure.

This method, although much less invasive than that of Blust and Hopkins (1990), was flawed in the test design. It became apparent that there were significant differences in grasshopper host selection between two choice and four choice cases. Six additional tests were conducted to help distinguish the reasons behind these behavioral differences.

The results indicated that preferences were consistent through grasshopper growth stages and from generation to generation. Analysis was done to measure the effect of bahia grass in each test, as there could have been some weighting of the outcome from the bahia grass, but little effect was found. They did find that when other more preferred host material is present, bahia grass is abandoned.

Joern (1983) describes host plant use of 31 species of grasshoppers from a sandhill prairie in Nebraska. Gut analysis was used to determine the grasshopper diet, and involved killing adult grasshoppers immediately after collection, removing the foreguts and placing them in 70% ethanol within an hour of death. Plant fragments from the gut were compared using microscopes to plant material collected in the field.

Relative abundances of the plant materials in the diet were estimated and scored from most abundant to least. This information was then compared to the relative abundance of the food plants by each species. The author found that in the wide range of plants to be selected amongst, individual species of grasshoppers had a relatively restrictive diet. Seventy-seven food categories were found in 31 species of grasshoppers. which is 43% of the species in the dry prairie of which the grasshoppers live. He also found that relatively few plants make up most of the diet for all grasshopper species. and that the relative abundance of food plants in the environment appears to affect the overall utilization of the food plants. The study also indicated that coexisting species show a wide range of niches for feeding behavior and that some species were eating smaller insects.

This study is helpful in that it shows what grasshoppers are actually eating in the field (versus a laboratory) but the methodology is very invasive. as it requires the death of the subject to obtain the material from the gut for analysis.

In an article by Chambers. Sword. Angel. Behmer and Bernays (1996). field observations were conducted on the foraging activities of two cryptic species of generalist grasshoppers. The researchers sought to clarify feeding patterns observed in the laboratory with patterns viewed under natural conditions. as well as to determine how polyphagous grasshoppers mixed foods. for nutritional benefits.

Methods of observation involved watching single individuals continuously for up to 8 hours using a behavior–recording program on hand held computers. Grasshoppers were located in mid-morning and observed with binoculars for as long as possible. usually from a distance of 2-3 meters so as not to disturb the species. The authors occasionally also marked some individuals with a spot of red paint to help observers see and relocate the individuals. If the individual was lost within the first hour. a second individual was chosen. Most data from individuals were watched for four hours or more. with a few watched for 8 hours. Only individuals watched for at least 3 hours were used.

Behavior patterns examined included: locomotion, (the start and end times of all displacements, including short walks before or after feeding), feeding (start and end times of all feeding activity), and biting or palpating events that did not end in feeding. Researchers identified food plants eaten and rejected to species and noted young/old leaf, bud, flower or calyx. Plants were collected at the end of the observation periods for species verification. Line transects and meter quadrat sampling was used to estimate relative abundance of plant species.

Data were analyzed for proportion of time spent moving, time spent feeding, number and length of feeding bouts, and number and length of gaps between feeding bouts and rejections (bites not followed by feeding and feeding bouts of less than 10 seconds). Overall selectivity for foods among the available plants was examined by comparing the relative abundance of tissue of different plant species with the number of feeds seen on each plant species. Log survivor curves and gap lengths were used to examine the feeding patterns and two regression lines were fit to all possible combinations of points using a computer program, to end up with two best fit regression lines. Data of all individuals were pooled since large data sets for any one individual on a day were not taken.

The authors found that both species fed upon several plant species, but rejected others as well. The study found that little movement occurred between foods and that few feeding bouts occurred on one or two plants. They also studied the length of feeding bouts and the gaps between feedings and found that short gaps with feeding bouts close together made up a meal, while longer gaps represented periods between meals. It was also discovered that feeding bout length declined on a single plant with time and when individuals switched to new food plants long feeds occurred.

Sword et.al. (1996) found that similar feeding patterns occurred for the two different species they studied, as all showed restricted polyphagy, had little locomotion and low switching between plant species. This was considered similar to patterns found in the laboratory setting for these species. It was found that food mixing for nutrition probably occurred mostly within a plant species (eating different plant parts). These cryptic grasshoppers were compared to other aposematic generalist grasshoppers of other studies which showed very different strategies, suggesting that cryptic coloration and movement within and between plants is related.

Field observation in conjunction with a computerized recording device seemed to work well for the purposes of this study. With plant identification and relative abundance of plant species information, much was learned from field observation, which is a relatively non-invasive method of data collection, but is often considered too difficult and time consuming (Eigenbrode and Bernays, 1997). It seems that laboratory experiments can give similar results, but clarification can come from actually watching the insects in their natural environment.

Sword and Dopman (1999), examined host plant use and availability in nymph and early adult grasshoppers in Texas. They were interested in the geographic structure of host plant use since diets may vary with time (developmentally) and spatially (specialization). Fecal material was collected from nymphs and adults, by collecting the grasshoppers off all potential host plants and putting them into clean glass vials where at least three fecal pellets per individual where taken and analyzed.

Proportions of each plant type present in each pellet were examined microscopically and recorded. Proportions were added together and divided by the total number of pellets examined to estimate the percent of each plant in the population level diet. Percent cover of each plant available to the grasshopper was measured using random points and transects traversing the collection area. Specimens of plants were collected to serve as reference material for fecal matter identification. A chi-squared test for goodness of fit between the expected and observed frequencies of plants found in the diet was computed from the plant data.

To look at the diet breadth (population level) the Shannon-Weiner diversity index (H) was used. This value accounts for the total number of plant species eaten by the members of a population and their relative abundances in the diet.

Survivorship experiments were also done on reared first instar nymphs to see the acceptability and suitability of a number of host plants that were seen in the adult diet. Five treatments were used with 15 individuals per treatment; data was analyzed by Kaplan-Meier survival analysis.

It was found that nymphal population level diet breadths were less than the adults for all locations, indicating that nymphal diet is often restricted to specific plants. Survivorship experiments showed that two plant species supported 80 and 53% of nymph survival.

Sword and Dopman found that geographic and developmental specialization of host plant use is present for the grasshopper studied (*Schistocerca emarginata*), a geographic structure was observable and plants used by the populations were identified. They concluded that nymphal mobility cannot account for the observed specificity of nymphal host plant use in the grasshopper species, since eggs are deposited in the soil and are not restricted to a single host plant. Host plant availability was also not considered to account for the observed differences in host plant use from nymph to adult and different locations. Instead the authors suggest that host plant use differences are due to host-plant associated genetic variation.

Scholtens and Holland (1997) examined the distribution and habitat selection of the threatened Lake Huron Locust (*Trimerotropis huroniana*) of the northern Great Lake sand dunes in Michigan. As part of their study they sought to discover if the insect was restricted to shoreline dune habitat due to host plant specialization. To examine host plant use by the locusts, 25 nymphs were gathered, allowed to deposit frass pellets in vials, and then returned to the field.

Leaf samples from each of the major plant species in the habitat were taken and microscopically analyzed. Proportions of species found in the frass pellets were then compared to the proportions found in the transect data, to compare for feeding preferences. The results of frass examination showed that only four plant species were used as host plants, but surprisingly parts of insect exoskeletons were also found in some of the pellets.

Microscopic host plant identification analysis

Microscopic examination of the feces of wild-caught animals has been used successfully and applied to grasshoppers in the past by many, including Isley and Alexander (1949); Mulkern and Anderson (1959)and Gangwere (1961). This technique uses the detailed structure of the leaf epidermis and related structures, such as hairs, spines and serrations (Mulkern and Anderson 1959) to identify plant material found in frass.

Joern (1983) describes microscopic analysis of gut contents of grasshoppers which is similar to frass analysis. Plant fragments once mounted on slides were then compared to permanently mounted fragments from plants collected in the field. Slides were scanned for trichomes (hairs), cell wall structures and stomatal patterns in order to identify plants from the grasshoppers. Most fragments could be classified to species; however, some were unknown. Insect fragments were also noted, although unidentified. Joern also estimated relative abundances of the plant materials in the diet and compared to the abundance of the food plants in the field using an index of diet breath.

Population-level diets were estimated by Sword and Dopman (1999) using microscopic fecal analysis; nymphs were collected directly off plants and put into clean glass vials for feces collection. All potential host plants were systematically searched for nymphs to avoid sampling bias. Adults were flushed into flight, captured in nets and transferred into vials. An estimate of the percentage of each plant in the population level diet was obtained by summing the proportions of each plant species found in each pellet and dividing by the total number of pellets.

By sampling at ten random points, along ten randomly placed 30m transects across the collection area, plant availability was measured as a percentage of the total cover in the habitat. All green plants present directly beneath or above each point were recorded and specimens were collected for use as reference material. Diet selectivity at each location and population diet-breadths were quantified.

Sword and Chapman (1994) describe their use of fecal analysis in which three pellets were taken per individual and then teased apart on a microscope slide and covered with a glass slip and scanned under a compound microscope. Fragments were identified by comparing the epidermis of the plant specimen (from a reference collection) to the frass pellet. Reference samples were prepared by scraping away the parenchyma with a razor blade so that only the epidermis remained, leaving a thin layer that was mounted. Flower parts were also prepared when present. Most fragments were easily identified and usually no more than two different plants per pellet were found. Plant cover was assessed by using point transects and food available in the habitat was compared to the amounts of different foods eaten by the insects using the G test.

Scholtens and Holland (1997) ground collected plant samples in a blender and then retained them as a reference in 70% ethanol. These sample leaf fragments were found readily identifiable and were used to identify plant fragments in the frass pellets. Each frass pellet was broken and prepared in glycerin on a slide, where it was scanned in a set pattern and the first 25 fragments were identified. The proportions of species found from the frass pellets were then compared to plant habitat data from transects and point quarter quadrants.

On examination of the techniques discussed above to study the grasshopper host-plant interaction, clearly some methods of collecting data are more appropriate for certain studies than for others. The methods of Joern's (1983) gut analysis, and Sword and Dopman (1999) survivorship experiments both resulted in death of the grasshoppers being studied. Clearly this is not appropriate for all studies, especially when endangered species are involved or when one is looking at restoration of habitat or furthering the understanding of the species ecology.

Host plant identification using gut analysis can be replaced by fecal material analysis as done by Sword and Dopman (1999) and by Scholtens and Holland (1997) when a relatively non-invasive technique is desired. Frass analysis allows the insects to be released after fecal material is deposited and is relatively harmless. Blust and Hopkins (1990) electronic monitoring of grasshoppers and plants was a somewhat unique technique, but posed problems of limited mobility for host plant choice experiments and potential harm or death to the insect. Capinera's (1993) two choice and four choice tests, did find some valuable results for host plant preference and were relatively non-invasive to the subjects, but did require removal of the species from it's habitat which may not be appropriate for endangered species. They did, however, need more pre-test study to look for potential problems in the experiments. Chambers et al.'s (1996) field observation was the least invasive, yet likely the most laborious and difficult technique. It did, however, clarify laboratory experiments in that actual natural movements of the grasshoppers were observed. This seems to be ultimately invaluable and necessary as a way to truly understand the ecology of a species.

It seems that when possible laboratory experiments, used in combination with fecal material analysis and field observations, are the best way to learn about a host plant-insect interaction. If dealing with a species of concern where laboratory work is considered too disturbing, much can be learned from frass analysis in combination with behavioral field observations, allowing one to observe many developmental stages and the geographic structure of host plant feeding.

OBJECTIVES

The goal of this research was to identify the host plant use and preference of the endangered Zayante band-winged grasshopper (*Trimeritropis infantilis*) (Rentz and Weissman 1984).

The specific objectives of this study were (1) to assess the overall and site specific (South Ridge versus North Ridge) habitat needs for ZBWG by describing the microhabitat availabile to ZBWG and determining their location within it, (2) to determine overall host plant choice and site specific trends by examining microsite plant availability, feeding behavior and frass contents and any within-season or stage (green or senesced) variation, (3) to look at differences in behavior related to courtship and locomotion observed in the field. Finally patterns of habitat and host plant use are outlined and their implications discussed.

Results of this work are intended to assist in the knowledge base for the species' recovery plan, to illuminate restoration priorities for its critical habitat, and to identify essential areas for protection in a future regional HCP/ NCCP process.

METHODS

Study System

Zayante band-winged grasshopper natural history

The Zayante band-winged grasshopper (*Trimerotropis infantilis*: order Orthoptera, family Acrididae) was first described as a new species by Rentz and Weissman in 1984. It is described as a small sized species with blue hind tibia, yellow wings and a band through the eye, with its geographic distribution occurring within the Santa Cruz Mountains of California. Rentz and Weissman further note that this species of grasshopper is found on "sandy substrate sparsely covered with *Lotus* and grasses at the base of pines above a rock quarry". The authors initially observed individuals making flights of 1-2 meters, with a rapid buzz-like crepitation. The flight season of *T. infantilis* is from May through August with peak activity during July and August. The Zayante band winged grasshopper is typical of the band-winged grasshoppers (Oedipodinae) in that it is a conspicuous flier and frequents areas of sparse vegetation, often alighting on bare ground (Borror et al. 1976). Little information about its life cycle known (U.S. Fish and Wildlife Service. 1998) and questions remain regarding where it oviposits, what may parasitize the species and its major predators and competitors.

The Zayante band-winged grasshopper's population status at QHQ began being monitored in 1999, as one of the conditions of Granite Rock's permit. At Quail Hollow Quarry very little is known about the population status of the ZBWG. In 1999, the relative abundance of *Trimerotropis infantilis* at the South Ridge of Quail Hollow Quarry was estimated using a transect count method on three dates from August 28- September 25. A total of 394 ZBWGs were noted, with 62% of all sitings occurring on one transect (#8) located on the South Ridge habitat set aside (Arnold 1999).

Research site

Work was conducted at two locations, the South Ridge Habitat Set Aside (South Ridge) and the North Ridge Habitat Set Aside (North Ridge), within Quail Hollow Quarry (QHQ), Felton, CA (Figure 2). Quail Hollow Quarry is located in an unincorporated portion of Santa Cruz County near the communities of Ben Lomond and Felton (Figure 3). The quarry is a 88 hectare area comprised of a complex mix of intact protected areas containing natural vegetation, barren areas that are currently being mined, future mining areas with natural vegetation, and former hydraulically mined areas that have been invaded by native and non-native plants, but are currently undergoing restoration. QHQ also has an operations area that is comprised of the quarry offices, sedimentation ponds, processing facilities, product stockpiles and equipment storage areas.

Eight natural vegetation types occur within the quarry property boundaries: northern maritime chaparral (consisting of silverleaf manzanita chaparral, mixed chaparral and successional chaparral), successional scrub, central coast scrub, maritime coast range ponderosa pine forest, hardwood-conifer woodland, sand parkland, central coast live oak riparian forest and central coastal arroyo willow riparian forest (Thomas Reid Associates 1997). These vegetation types are distributed in a mosaic from the ridgelines, down the slopes to the bottomlands. The soil is comprised of ancient marine sandstone and mudstone formations, collectively called the Zayante series, derived from Santa Margarita sandstone. Zayante soils series are light colored, coarse, deep, well drained and erodible. They cover 3200 hectares or three percent of Santa Cruz County's 112,400 hectares (USDA Soil Conservation Service 1980). These unique soil conditions limit plant species to those that are well-adapted to drought, and, with the coastally-influenced summer climate, this has created a unique habitat as well as number of sensitive plant and wildlife species occurring within or near the quarry.

The 13.04 hectare South Ridge Habitat Set Aside is the most diverse area at QHQ with vegetation assemblages that contain sand parkland, northern maritime chaparral, central costal scrub and woodland plant communities. Several foot and horse trails also traverse this site and erosion and unmanaged horse traffic are the main management concerns for the South Ridge.

Work at the South Ridge site was done primarily along the ridge tops (and down the slopes on either side up to 15m from the ridge top). In these locations much of the habitat is either sand parkland or silverleaf manzanita chaparral and is typically sparsely vegetated over bare sand. Sand parkland is described as having ponderosa pines (*Pinus ponderosa*) in open stands, where they often grow in association with an under-story of small ephemeral, isolated populations of herbaceous species, generally with no shrub under-story. It is typically restricted to the tops and upper slopes of the highest hills and ridges of the Sand Hills, although it is occasionally found in small pockets at lower elevations. The ponderosa pines are widely spaced with only occasional Coast Live Oaks (*Quercus agrifolia*) and Manzanitas (*Arctostaphylos spp.*) encroaching from adjacent habitat (Marangio 1985). The herbaceous layer is an assortment of small annual and perennial flowering herbs and an irregular distribution of six semi-woody perennials (primarily, *Eriogonum nudum, Lupinus albifrons, Lotus scoparius, Adenostoma fasiculatum, Heterotheca sessiflora* and *Lessingia filaginifolia var. filaginifolia.*) Grasses are usually sparse and sometimes absent, often with the soil looking nearly bare. Ninety plant species have been determined as "sandhill specialty" plants (Morgan 1983) these plants in particular are associated with the hot, dry conditions on the south-facing slopes of the sand parkland habitat. South Ridge has 78 of the 90 "sandhill speciality" plant species, (Marangio 1985) with six of them listed by CNPS, the FWS or CDFG. They include *Erysimum teretifolium, Chorizanthe pungens var. hartwegiana, Arctostaphylos silvicola, Cupressus abramsiana, Monardella undulata, Mimulus rattanii ssp. decurtatus.* Other plant species found at the South Ridge site include *Lupinus albifrons, Heterotheca sessiflora*, and a variety of other shrubs, grasses, and forbs (Appendix A).

Surrounding the high quality sand parkland found along the top of South Ridge is vegetation primarily made up of Silverleaf Manzanita Chaparral. This vegetation type is characterized by dense stands of woody shrubs, of mostly the endemic *Arctostaphylos silvicola*. Associated shrub species include, *Ceanothus cuneatus, Ceonothus ramulosus, Adenostoma fasciculatum, Arctostaphylos crustacea, Arctostaphlyos sensitiva, Salvia mellifera, Eriodictyon californicum, and Mimulus aurantiacus*. It has been suggested that Silverleaf Manzanita develops into nearly pure stands of chaparral as a fire type replacement of ponderosa pine (McMinn 1939).

The 4.44 hectare North Ridge site is located between the Quarry and Quail Hollow Road and is made up primarily of sand parkland and northern maritime chaparral with 59 of the 90 "sand specialty" plants, but with many populations reduced (Marangio 1985). Foot and horse trails are also present on the site, in particular along the ridgeline. Data collection at the North Ridge site was done primarily along the middle to lower portion of the south-facing hillside, where the slope's habitat has more bare sand and sparsely vegetated areas. The North Ridge site has more ponderosa pine trees and coast live oaks and generally has smaller patches of sand than the South Ridge site.

Study Design

Research was conducted during the summer of 2001, with the majority of the fieldwork occurring from July-September 2001 (when the ZBWGs are adults) and with the laboratory work completed in the fall and winter months of 2001

A total area of 6600 m^2 at South Ridge and 5300 m^2 at North Ridge were each divided into smaller plots ranging in size of 4500m^2 to 1750m^2, that were further divided into 1m^2 circular quadrats that were selected randomly (Figure 4). Total approximate sampling area covered at both sites was 11,900 m^2. Areas sampled were known to have positive evidence of Zayante band-winged grasshopper populations from previous monitoring surveys (Arnold 1999).

Microhabitat Choice – Design

To assess the overall and site specific (South Ridge versus North Ridge) habitat needs for the ZBWG, microhabitat availability was measured by estimating the cover and abundance of the plants available to the grasshoppers during the summer months when ZBWGs are adults. Plant cover and abundance were recorded for 32 stratified-randomly placed 1m^2-quadrats (Random = R microsites) at each of two ZBWG sites (North Ridge and South Ridge). Plant cover and abundance were then recorded in 32-1m^2 quadrats where ZBWG were found (Grasshopper = G microsites) using different stratified random starting points (see hostplant choice below) on each of two dates (July-Aug and August-Sept) at the two different sites (North Ridge and South Ridge) for a total of 128 G microsites (32 G microsites x 2 dates x 2 sites = 128 G microsites total).

Analysis

Plant cover and species abundances were compared between the random microsites (habitat matrix) and the grasshopper-associated microsites (Grasshopper = G / Random = R), controlling for variability due to date sampled (DATE) and site sampled (North Ridge = N / South Ridge = S) and relevant interactions using the General Linear Model (GLM) procedure (Systat™ for Windows OS, 2001). The model used was: G/R+N/S+DATE+ G/RxN/S + N/SxDATE = plant variability.

Host Plant Choice-Design

Frass

Knowing diet breadth is important for endemic and endangered species. Grasshoppers are usually thought to be polyphagous (generalists), but monophagous (specialist) species occur within Orthoptera as well (Bernays and Chapman 1994). Often endangered species are specialists, and since there may have been coevolution of plants and animals, and it may be important to preserve these food related interactions.

In order to assess what plants and in what stage (green or senesced) ZBWG are choosing to eat (from frass evidence) from the selected quadrat locations. 128 frass samples were collected between July and September of 2001 from both sites.

Frass samples were collected using the following procedure. A ZBWG was sighted, caught in a fine mesh sweep net and placed in a 20ml vial. Vials were then covered with screen material and placed in a warm, dark place for up to 6 hours until individuals deposited at least 1 frass pellet. Individuals were then released and frass was stored dry for later microscopic analysis.

ZBWG frass was analyzed microscopically using a technique that involved comparing frass contents to type specimen plant material found in the field (Scholtens and Holland 1997). Frass pellets were mounted on slides with glycerin and examined microscopically for trichomes and other identifiable features. Frass samples were then compared with type reference plant material obtained in the field to confirm plant identity. For each plant found a photo record was taken (Figure 5) and the plant composition in the frass was compared to the data on plant species available to grasshoppers in their immediate vicinity.

To determine if ZBWG have some plasticity in feeding in response to host availability frass collected from each site (North Ridge and South Ridge) were compared. Within-season frass content patterns were also compared to see if physiological changes (green or senesced) in plant community would be reflected in the frass contents.

Analysis of frass contents

Raw frass data was compared qualitatively to see what the most frequently found plant species were compared to the random matrix in the field and to see if observation data upheld frass results.

Feeding Observations and Other Behavior

To determine if observation data supported frass evidence on foods eaten (plant species type and number) and to determine what foods were observed eaten compared to the ZBWG selected microsites. ZBWG feeding behavior was observed at South Ridge for a total of 33 hour-long samples. Fifteen assistants helped in recording behavioral data using Cybertracker™ (a behavior recording program) on hand-held computers. Volunteers recorded all observed hostplant palping, biting and ingestion.

In order to discover any other patterns of behavior in the field (including differences between males and females) observation data of host choice behavior related to locomotion, time idle, courting, mating and oviposition were also taken four times through the summer season, with two weeks between observation periods.

Analysis

A t-test was used to compare the number of plant species used from data on quadrats versus grasshopper frass versus observations. The nonparametric Mann-Whitney U rank test (Kruskal-Wallis two sample test) was used to compare observation data categories for any gender differences. Information was also gathered and compared qualitatively on the percent time an average adult grasshopper spent doing the various actions involved in reproduction, feeding and locomotion.

Procedure

Plant availability and proportion of plant species cover:

In order to sample the vegetation at each study site, a grid was developed and divided into subplots that were then randomly sampled using ($1 m^2$) circular quadrats for grasses, herbs and sub-shrubs, shrubs and trees. Sixty-two randomly stratified quadrats distributed in each collection area were measured for percent total cover, percent cover of each plant species and the number of individuals of each plant species. Specimens from each site were collected for identification and used as "type" reference material for fecal analysis. Plant stage (alive or senescing) was also noted upon collection of plant cover information to indicate whether host choice simply results from plant availability or whether the herbivore exhibits preference for particular species or stages of available plants in the community.

Collection of frass:

To collect frass, grasshoppers were chosen in the field using new random numbers that had been assigned to plots delineated (in meters) across each area of collection. Once a random number was assigned, the researcher started at that location, and began spiraling out from that point, watching to see the first grasshopper that was scared up or found.

Once a grasshopper was sited, its original location was flagged then the grasshopper was then in a fine mesh sweep net, identified and transferred immediately into a clean, medium sized vial (20ml). Vials were then covered with a fine screen (to allow air circulation) and placed in a warm, dark, safe place for up to 6 hours to allow individuals to deposit at least one frass pellet. After gathering the frass, the grasshopper was released to its original location, and the frass was stored dry until analysis. Analysis of *T. infantilis'* fecal materials included over 120 frass pellets obtained from adults of the species from the two sites at Quail Hollow Quarry.

Following release of the grasshopper, the data collector returned to the flagged spot and collected vegetation data on the original location of the grasshopper (where it is likely to have been eating and selecting from the plants in the immediate vicinity). To detrmine the plants actually available to the grasshopper, vegetation was sampled in $1 m^2$ circular quadrats (microsites) around the flagged location, measuring the same parameters as used above for to determine the overall vegetation make up of each site.

Microscopic analysis:

Bernays suggests using a good compound microscope and mounting the particles on slides for identification (Personal communication Dec. 2000). All frass pellets were examined in detail on slides for plant and insect materials under a compound microscope. Plant fragments from the frass pellets were mounted on slides and compared with type reference plant material, obtained from the field, that had been identified, photographed and scanned for trichomes (outgrowths such as a hair, scale or water vesicle on epidermal cells), cell wall structure, stomate patterns, and for floral parts such as pappus and ray flowers of composites, perianths, pollen grains and spores (Joern 1983, Isley and Alexander 1949) any other notable or characteristic features. These type-reference slides were made by chopping known sampled plant species to a particle size approximating that found in the grasshopper frass (Mulkern and Anderson 1959). All plants used had been collected in the plot areas of the Sand Hills habitat.

Each frass pellet was broken apart, prepared in glycerin for mounting and enclosed with a cover slip. The slide was then scanned by the same observer and compared to fragments from the reference samples. Plant fragments within the frass were observed, identified and photographed. Only one frass pellet per grasshopper collected was examined even if multiple pellets were collected. Proportions of each type of plant material present in each pellet were recorded and summed for each plant species and divided by the total number of pellets examined to obtain an estimate of the relative abundance of each plant in the individual diet and population-level diet (Chambers et al. 1996).

Field observation of host-choice behavior and preliminary observations of reproduction activities:

On each of four dates between June and September 2001, host-choice behavior was observed for two, hour-long periods on four different dates, using a behavior-recording program (Cybertracker™) on hand-held computers (Appendix C). Observations were taken only at the South Ridge site, with one continuous hour-long morning observation and one continuous hour-long afternoon observation. Each observation was on a new grasshopper, for a total of at least twelve individual insects per day (Raubenheimer and Bernays 1993). To better understand any possible correlation between behavior and diet, several aspects of the grasshoppers' behavior were observed, including the type and duration of movements involved in feeding.

Foraging periods (the time of day in which feedings occurred) were noted by using observations of locomotion (the start and end times of all displacements, including short walks before or after feeding), ingestion (start and end times of all feeding activity), and biting or palpating (events that do not end in feeding). Food plants eaten and rejected by the species were recorded and identified for species, plant part and life stage and specimens of each plant were collected at the end of the observation periods for identification (Raubenheimer and Bernays 1993). The type and duration of reproductive interactions (courtship, mating, males posturing and oviposition), locomotion type (walking, jumping flying) and idle behavior were also recorded.

The general micro-location (plants type, leaf litter or bare ground, and amount of shade present) of each grasshopper, along with time of day and weather conditions, before, during and after it feeds were recorded to identify any importance of sun, shade, vegetation associations or other microclimate conditions. Finally, the presence of other animal species were noted to better understand any potential interspecific interactions, such as mutualisms, competition or predatory influences on feeding and reproduction.

RESULTS

Microhabitat Choice

Throughout the range studied ZBWG were found in microsites (quadrats) with lower cover and fewer invasive non-natives than average for the plant community matrix.

In comparisons of plant cover of the random vegetation samples with those of grasshopper associated vegetation samples using the General Linear Model (GLM-ANOVA), the mean total cover of plants in the grasshopper-associated samples was less than the mean total cover in random samples (df = 1, $p \leq 0.05$) (Figure 6). Grasshoppers were associated with less total cover.

When individual plant species cover was compared, *Bromus diandrus*, *Lotus scoparius*, and *Hypochaeris glabra* were significantly negatively associated with grasshoppers (Figure 6). Whereas, *Calyptridium umbellatum* was significantly positively associated with adult ZBWGs. The grasshoppers located in areas with less *Bromus diandrus*, *Hypochaeris glabra* (both non-native) and *Lotus scoparius* (a native perennial herb, of small shrub size), and with more *Calyptridium umbellatum* (a native annual herb). Comparisons of the average number of individuals of plants sampled yield similar results. Greater abundances of *Bromus diandrus* and *Hypochaeris glabra* were found in randomly sampled plots than in the grasshopper associated plots (df = 1, $p \leq 0.05$) (Figure 7).

Microhabitat Choice Between Sites

Microhabitat choice varied between the two research sites. In comparisons of plant cover between the two sites sampled (North Ridge and South Ridge) there was not only a significant difference in the overall plant cover found at each site (with more total cover at North Ridge), but also in many of the individual species present (Figure 8). Nine different species of plant showed significant differences in cover between sites, with *Eriodictyon californicum* statistically absenct at North Ridge, while *Lessingia filaginifolia var.filaginifolia* showed statistically absenct at South Ridge.

No significant difference was found for the overall number of species found in random samples when compared to grasshopper associated samples. However there was a significant difference in the overall number of species found between sites, with more species found at South Ridge than North Ridge, indicating a greater diversity of plants at South Ridge (Figure 9). Statistical tests also showed a variety of significant differences for distinct species, at the site level, when comparing the number of individuals. Greater abundances for six species of plants were found at the South Ridge site, while greater abundances for four species of plants were found at the North Ridge site ($df = 1$, $p \leq 0.05$) (Figure 10). Two plants, *Adenostoma fasciculatum* and *Eriodictyon californicum*, showed a statistical absence at the North Ridge site .

In a comparison between sites, it was found from both cover data on the plant species that at the more pristine site (South Ridge) ZBWG was more negatively associated with non-natives than it was in the less intact plant community (North Ridge), indicating possible facultative use of non-natives on the North Ridge site (Figures 11).

Host Plant Choice

Adult Zayante band-winged grasshoppers are not generalists overall. ZBWG frass contained fewer plant species than are available in the plant community in the immediate vicinity of capture, indicating some selectivity in host plant choice. Similarly, feeding was observed on fewer plant species than were available in the plant community in the immediate vicinity of capture, also indicating some selectivity in host plant choice. Of 128 frass samples collected, host plant identity could be clearly determined through microscopic analysis in 103 samples. No insect remains were found in the frass contents although they were also looked for and a few fragments were unidentifiable as either plant or insect material. The majority of the plant fragments found in frass were *Lupinus albifrons* (Fabaceae) (61.16%), while *Heterotheca sessiliflora* (Asteraceae) made up 14.56%. Two species from Poacaeae (*Vulpia myuros* and *Aira caryophyllea*) made up 16.51% and two other Asteraceae family species *Filago californica* and *Hypochaeris glabra* contributed to 7.76% of the species found in frass (Figure 12).

ZBWG frass contained a higher percentage of the native plant species than were found in plant community in the immediate vicinity of capture, indicating use of native species. Some grasses were found in the frass but there use was less and may be facultative. It appears they have coevolved and prefer endemic plant species.

ZBWG had some plasticity in feeding in response to host availability. Frass samples from ZBWG collected at Northridge had a higher percentage of non-natives plants than did South Ridge frass (Figure 13).

Feeding Observations and Other Behavior: Observations - Food Related

Observation data showed grasshoppers feeding on only one species at a time and generally supported information obtained from frass analysis, with ingestion of *Lupinus albifrons* witnessed six times out of the 11 total cases of observed feeding that led to ingestion. In two instances feeding on *Heterotheca sessiliflora* was observed to have lead to ingestion of that species. In both cases above, the material eaten was from new, green leaves of the plants. The three other instances of feeding that led to ingestion were cases where unidentifiable dried or dead plant material was eaten off the ground.

Thirty-three usable observation points were obtained, from approximately 30 hours of data. In a few cases, where a grasshopper was lost early on, an observation point was either lost (if the time was too short), restarted (at a new randomly chosen point) or was used with less time than the optimal hour long designated period. Observations had to have elapsed at least 20 minutes to be considered a valid data point.

Observations corresponded with frass results as a higher percentage of native plant species were observed to be ingested than found in the average plant community or in the immediate vicinity of capture. Frass evidence indicated use of more plant species than did observation evidence alone. While observation and frass evidence indicated a narrow range of plant species used compared to the plant species available in ZBWG selected quadrats (Figure 14).

Total time observed where feeding led to ingestion was approximately 48 minutes out of the approximate 30 hours of observation time.

Feeding Observations and Other Behavior: Observations –Other Behaviors

On average, all grasshoppers spent most of its time resting (idle) (45.58%) or in the locomotive activities of walking, jumping or flying (44.74%). Reproduction (courtship, mating, oviposition, males posturing) (4.03%) and feeding (biting, palpitating, ingesting) (5.11%) occurred considerably less often.

Statistical analysis was run on the observation data using the Mann-Whitney U rank test (Kruskal Wallis two-sample test) and showed no significant differences in any of the activities when grouped by date (early summer or late summer season) or when the data was grouped by morning or afternoon observations. However when comparing observations across sex, in general behavioral observations were similar between males and females, but feeding that lead to ingesteion was significantly different ($p < 0.05$), females spent more time eating than males (Figure 15).

DISCUSSION

The results suggest that factors other than food source in the plant community may be important to ZBWG life history and fitness. Gangwere (1961) was among the first to point out that a number of factors determine the food eaten by grasshoppers. Ehrlich and Raven (1964) also discuss factors determining food choice of phytophagous insects. They point to ecological, mechanical and chemical factors as well as relationships with predators or parasites. Bernays and Chapman (1994) impart a great deal of information on those factors and more, including accounts of behavioral factors and the general evolution of phytophagous insects host range, in their book about host-plant selection. Factors that have been identified as affecting microhabitat choice include sun exposure and social groupings (Main 1987), predator avoidance (Stevenson 1985) and habitat structure and substrate choice (Joern and Lawlor 1980).

The fact that the ZBWG is found in areas of less total species cover (Figure 6) suggests that the Zayante band-winged grasshopper actively selects open microhabitats when possible. The differences seen in grasshopper chosen quadrats versus random quadrats shows that the species avoids microhabitats dominated by nonnatives (Figures 11-14) as well. The ZBWG is likely to seek open, microhabitats with native plant species that it may have co-evolved with, and as such it may be important to maintain these habitats along with its host plants. ZBWG may be using these open areas for thermoregultion, to find mates or to avoid predators. Restriction of host use as a result of restriction on habitat use has been noted for a number of butterflies that are restricted to particular habitats of open grasslands, to forests, or even areas of light in a forest

(Bernays and Chapman 1994) so that plant structure, arrangement and distribution in nature can also affect food selection by herbivores.

B. diandrus in the Sand Hills was observed qualitatively to form clusters of plants in semi-disturbed areas, bordering chaparral areas. *Hypochaeris glabra*, a non-native from the family Asterace was another plant that Zayante band-winged grasshoppers tended to stay away from (from cover and abundance data), these plants also were seen to have formed tight groups of many individual plants along and within chaparral areas. Microhabitat areas with *B. diandrus* and *Hypochaeris glabra* may form too dense of plant cover for ZBWG to maintain thermoregulatory activities, or to suitably avoid predators or find mates. Native *Calyptritdium umbellatum* was positively associated with the grasshopper. *C. umbellatum* is an annual herb, that was found in the habitat along many trails (which tend to be open and sandy). ZBWG appears to be preferentially choosing areas that are typical of a healthy sand parkland community, those which would have more open, sandy areas, and fewer non-natives or encroaching chaparral species.

ZBWG appears to be a disjunct oligophage, the feeding pattern seen when a very small number of plants from different families are eaten (Bernays and Chapman 1994). Results suggest that ZBWG prefers native over non-native host plants, a result that may indicate the importance of the fact that *Lupinus* species contain quinolizidine alkaloids. Adult ZBWGs may have made metabolic adjustments (evolutionarily) to this secondary substance, since typically alkaloids are a repellent to most insects (Ehrlich and Raven 1964). Other native insects, including butterfly species endemic to California, have formed special utilization with native *Lupinus* species to help in predator avoidance. The chemical composition of the plant can also change with age, sun exposure or other environmental factors such as rainfall. Therefore, it may be important that qualitatively observed data in this study showed when *Lupinus* leaves were ingested that they appeared to be from new leaves.

Interestingly, ZBWG microhabitat preference (open areas and non-native avoidance) did not correspond directly with host plant choice (*L. Albifrons* and *H. sessiflora*). This result may indicate that other factors such as predator avoidance, reproductive behaviors, or plant structure and resultant shading and angles to the sun may also determine microhabitat choice rather than host plant.

Lupinus albifrons (Fabaceae) and *Heterotheca sessiliflora* (Asteraceae), the plants that appeared to be the most preferred host for the ZBWG, are some of the few plants that stay green (Appendix B) through the hot (30-35 °C) and dry summer of the arid Sand Hills habitat (Appendix E) and are native plants that may have coevolved with the ZBWG. Moisture content of the leaves may play a part in food selection (Bernays and Chapman, 1970). Differences in plant species compared between sites showed that more non-native grasses were used at the less pristine North Ridge site than at the South Ridge site, indicating possible facultative use of non-native plants by ZBWG when habitat is less than optimal. Similarly the slight use of the other species in Asteraceae may indicate opportunistic use of those species in the ZBWG diet, when the plant species are more abundant in the plant community.

It is important to note that the ZBWG population at the more degraded site (North Ridge) is smaller, with 63% of all sitings of ZBWGs in 1999 occurring in one transect at the more pristine site with fewer nonnatives (South Ridge) (Arnold 1999). Therefore, it is possible that the North Ridge population is declining as a result of nonnative plant invasion into open areas and displacement of native host plants. This potential merits further study. Increased plant cover may also be responsible for the decreases seen in the North Ridge ZBWG population. ZBWGs may need sites like those of South Ridge, with less overall cover of vegetation, (leaving more open sandy areas) but with more total species diversity present. North Ridge also had three cases where non-native plants (*Hypochaeris glabra, Bromus diandrus* and *Vulpia myuros*) occurred significantly more often (in cover and abundance) in tests of significant differences. This may be a contributing factor as to why North Ridge is less suitable habitat than South Ridge, as indicated by the fact that fewer ZBWGs are found there.

Loss of the natural disturbance regime in the Sand Hills may also be affecting the ZBWGs status. With less fire, more plant cover (encroaching chapparal) has developed in the Sand Hills habitat, leaving less open areas of habitat that the ZBWG prefer. *Lupinus albifrons*, one of ZBWGs preferred host plants, is frequently observed as an early successional species in disturbed areas (Williams 1974), which again may indicate the importance of maintaining the natural disturbance regime.

Observations in general support data on host plants found from frass analysis. 55% of the observations that included information on feeding that led to ingestion showed that the grasshopper was eating *Lupinus albifrons*, while 18% of those same observations showed that a grasshopper was found eating *Heterotheca sessiliflora*. The remaining 27% of those observations showed other dead and unidentifiable plant material being eaten. Observations also indicated that grasshoppers spent nearly half of the over thirty hours of observation time idle, which could indicate the importance of thermoregulation in the ZBWG lifestyle. The other large portion of ZBWG time was spent moving about the habitat in locomotive activities. Comparatively little time was spent in the rest of the categories of reproduction (mating, courtship, males posturing and ovipositing) and feeding (palpitating, biting and ingestion). Females spent significantly more time in the activities of feeding than did males, presumably this feature of ZBWG life history is related to typical behavior where females need to have more energy available to reproduce and lay eggs which are a high energy sink. More future observations could reveal further differences in behavior between the sexes and could also provide valuable information on oviposition sites.

Limitations

The fact that only adults of the Zayante band-winged grasshopper were studied is an important limitation to the host plant information obtained in this study. Further taxonomic information on the nymphs is needed before proper juvenile host plant identification can be made. Oviposition was also not studied here, another associated component in the study of host plants. It may be important as insects often lay their eggs near an appropriate food source. Less than forty hours of observation data was taken, certainly more hours could reveal more behavioral trends of ZBWGs. Finally only two site locations were sampled during the season, there are a number of other smaller locations (populations) of ZBWGs that could be studied (Figure 1), but were not here due to time constraints.

CONCLUSION

Recommendations

The Sand Hills habitat is a complex system with at least 5 endangered species and a whole suite of coevolved rare flora and fauna. Natural disturbance regimes will likely prove to be an important factor to the health of sand parkland, as with many other habitats in California, since the natural fire cycles and influences of human activity have changed many landscapes dramatically in the last 100 years.

Microhabitat maintenance and the use of the identified host plants in revegetation efforts should help the recovery of the ZBWG. Maintaince of the preferred ZBWG habitat (open and sandy, yet diverse in native plant species), in conjunction with thorough study and protection of the remaining Sand Hills ecosystem is necessary to find the appropriate habitat conditions for optimal ZBWG fitness.

With host plants discovered and the restoration efforts that are currently underway (in areas where harsh mining practices have left the landscape altered with steep slopes and compacted soils that tend not to hold plants) we may now begin to ensure that the appropriate resources are present in the ZBWG habitat. Clearly however, finding the host plants and learning about the microhabitat of plant species diversity is only one important part of understanding ZBWG's life history. Further studies to investigate reproduction (oviposition sites), juvenile life history, and intra-specific interactions (competition, predation or parasitism) are all merited.

Monitoring and continued improved life history and ecosytem information will add to the necessary knowledge base needed to update the recovery plan, so that it doesn't become out-dated and so that there may be a better understanding of the ecosystem as a whole. Caution should be used as an ecosystem plan is developed for the region, so that individual species are not lost in the shuffle or jeopardized by lack of funding and effort when looking at the overall habitat. The continued use of a variety of participants in research and recovery plan development, who are studying all aspects of the Sand Hills biodiversity, along with education for all involved will help support and protect the Zayante Sand Hills and the species that live in it.

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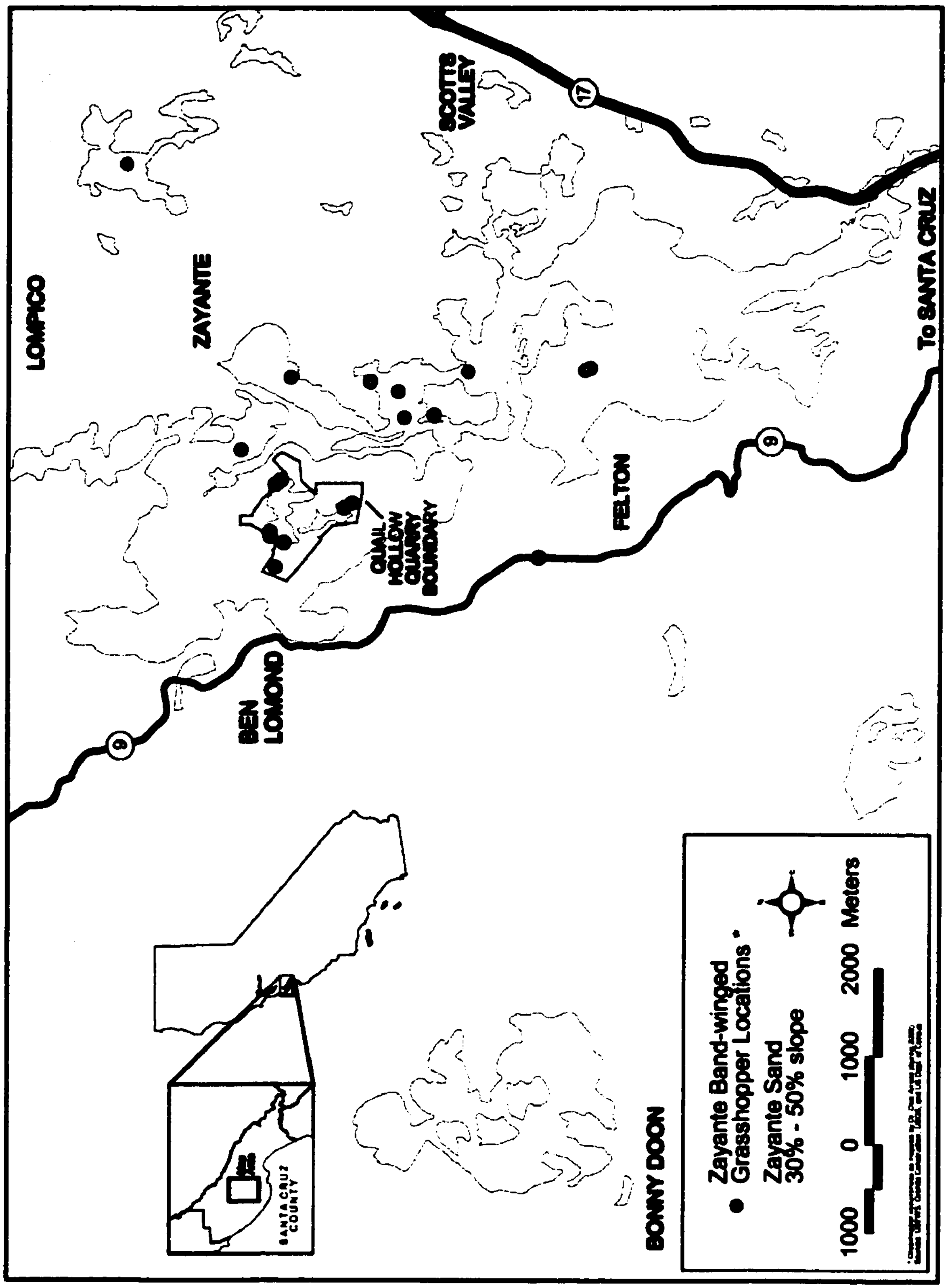
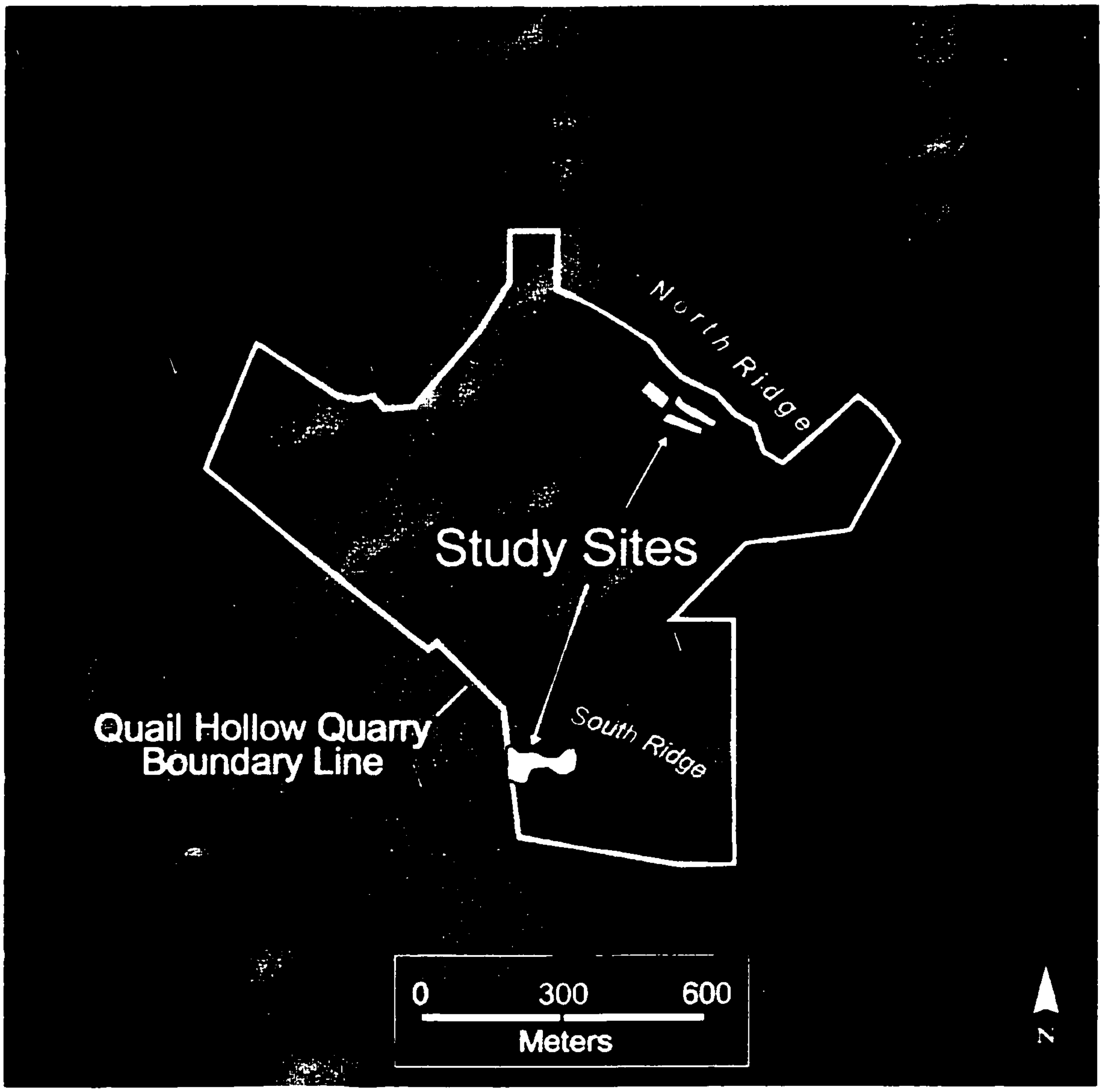
Figures

Figure 1- Zayante Band-winged Grasshopper Occurences, Santa Cruz County, California



Map by Tom Robinson

Figure 2 - Locations of Zayante Band-winged Grasshopper Host Plant Study Sites



Map by Tom Robinson

Figure 3 - Aerial View of Landscape Around Quail Hollow Quarry

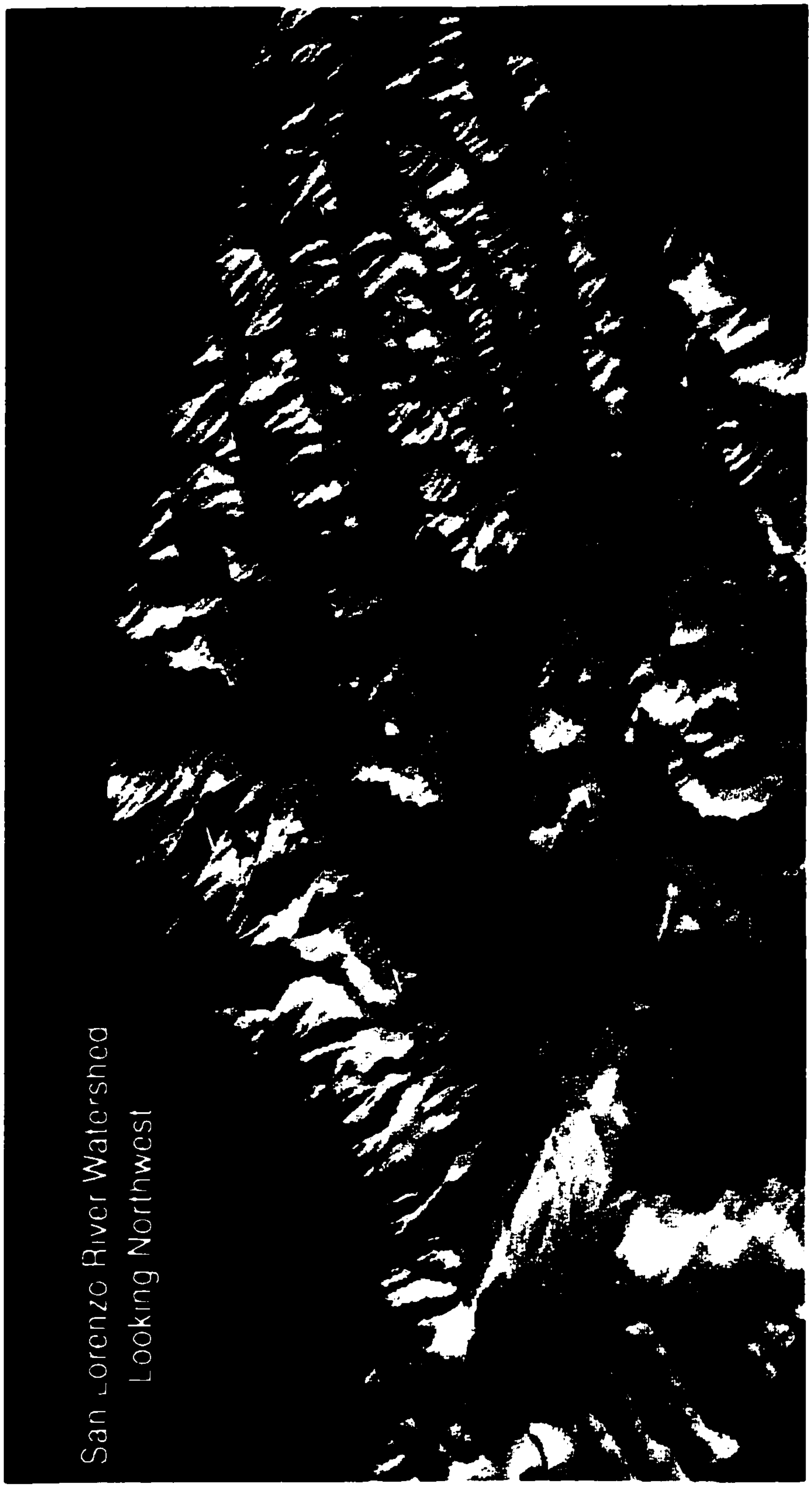


Image by Tom Robinson

Figure 4 - North Ridge and South Ridge
Vegetation Sampling and 1m2Cicular Sampling Quadrat

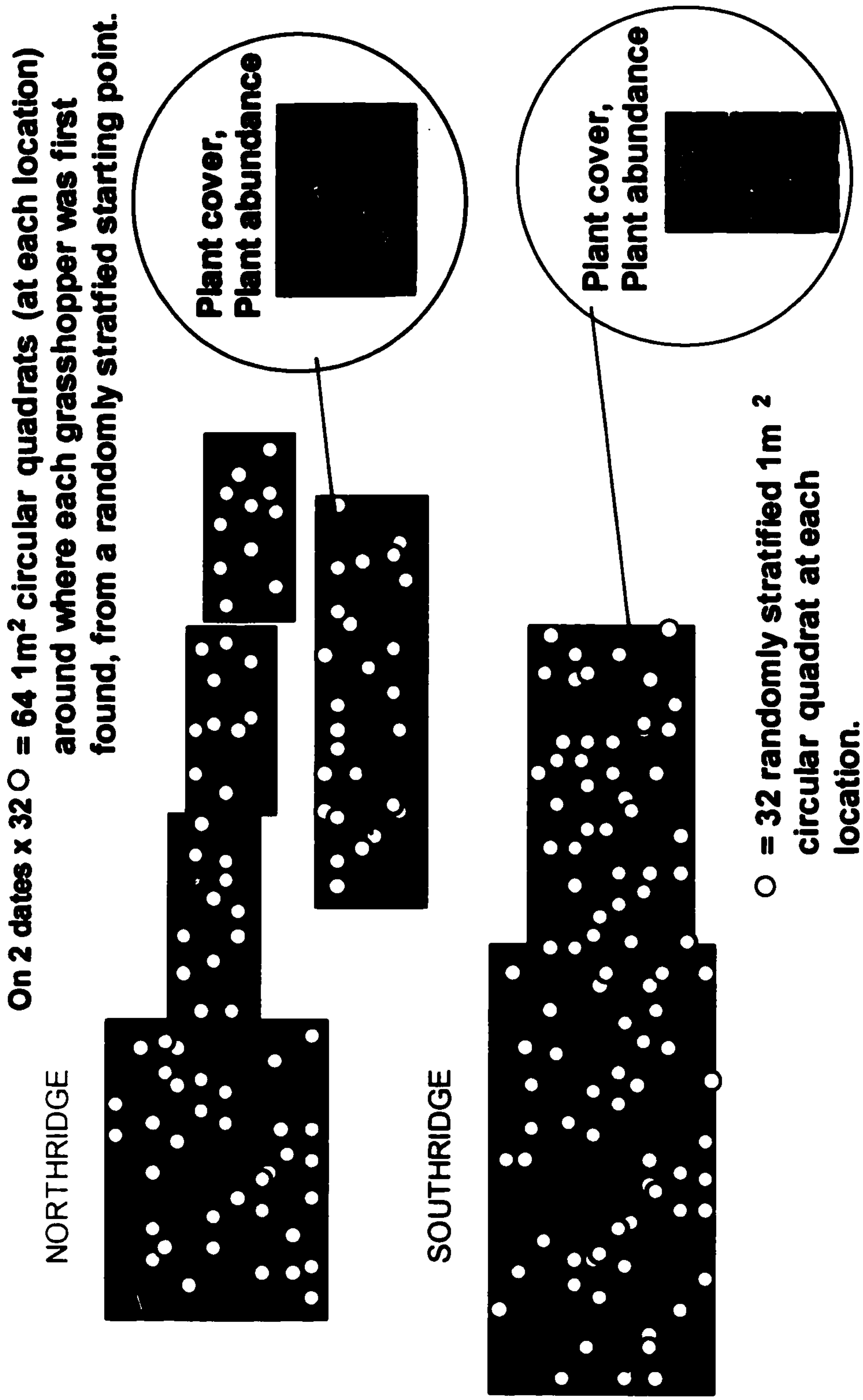


Figure 5 - Sample Photo Records of Plant Species

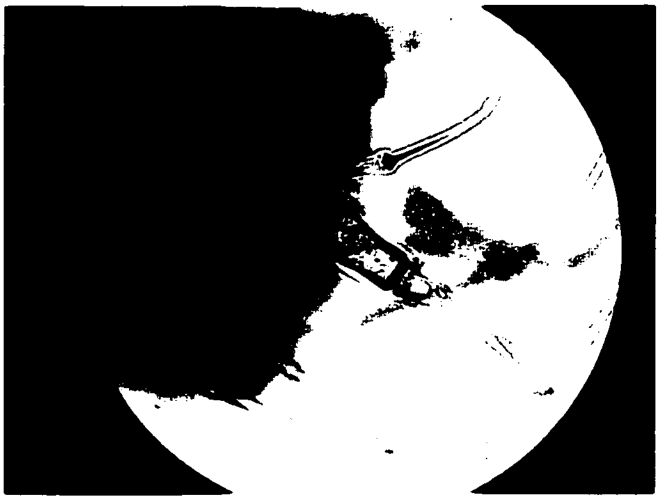


Photo by Jennifer Chu

Heterotheca sessiliflora prepared in glycerin. (40x objective)

Photo by Jennifer Chu

Filago californica prepared in glycerin. (40x objective)

Photo by Jennifer Chu

Lupinus albifrons prepared in glycerin. (40x objective)

Figure 6 - Plant Cover in Grasshopper Quadrats
vs. Random Quadrats Across All Sites

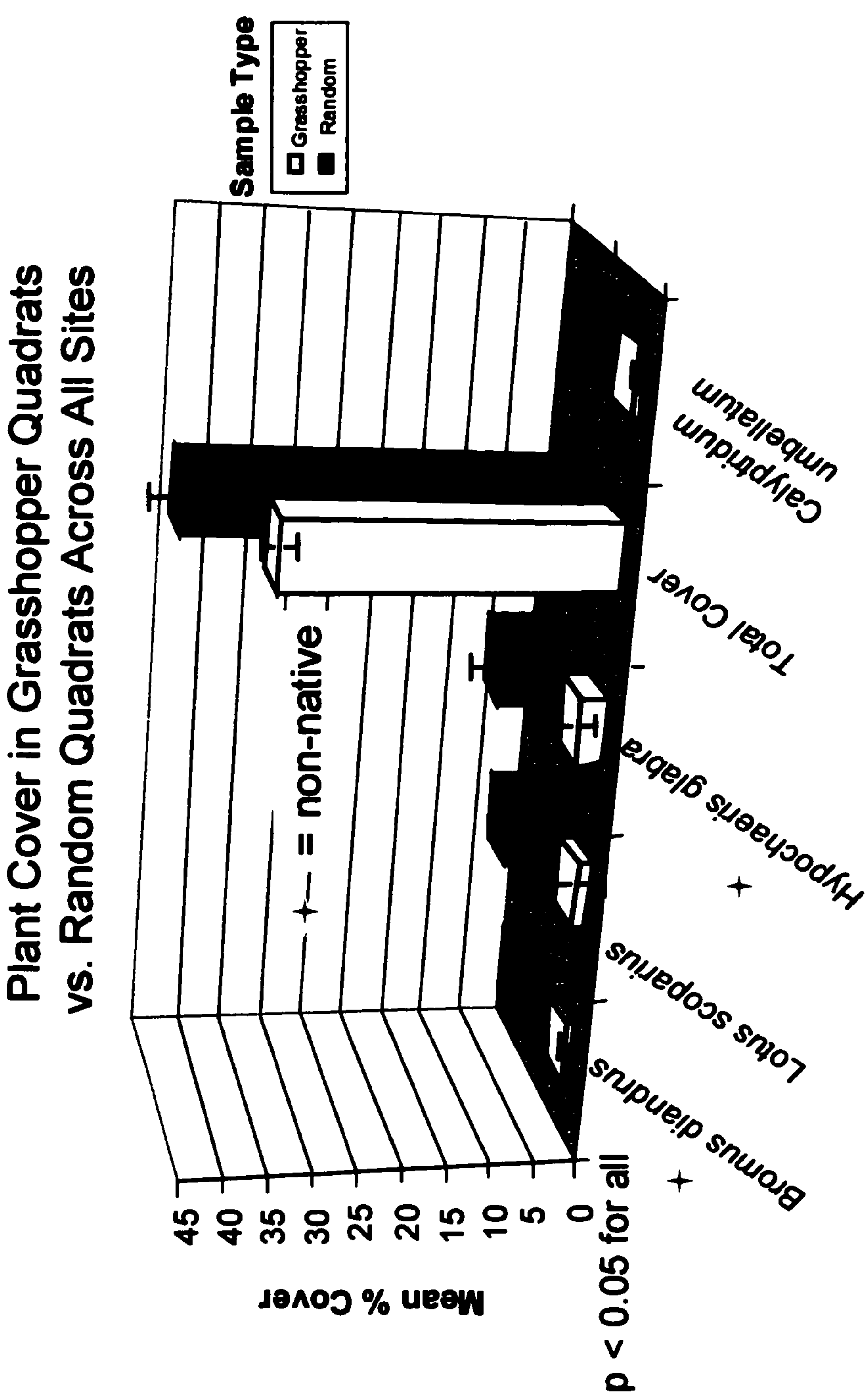


Figure 7 - Average Number of Plant Individuals for Grasshopper Selected vs Random Quadrats

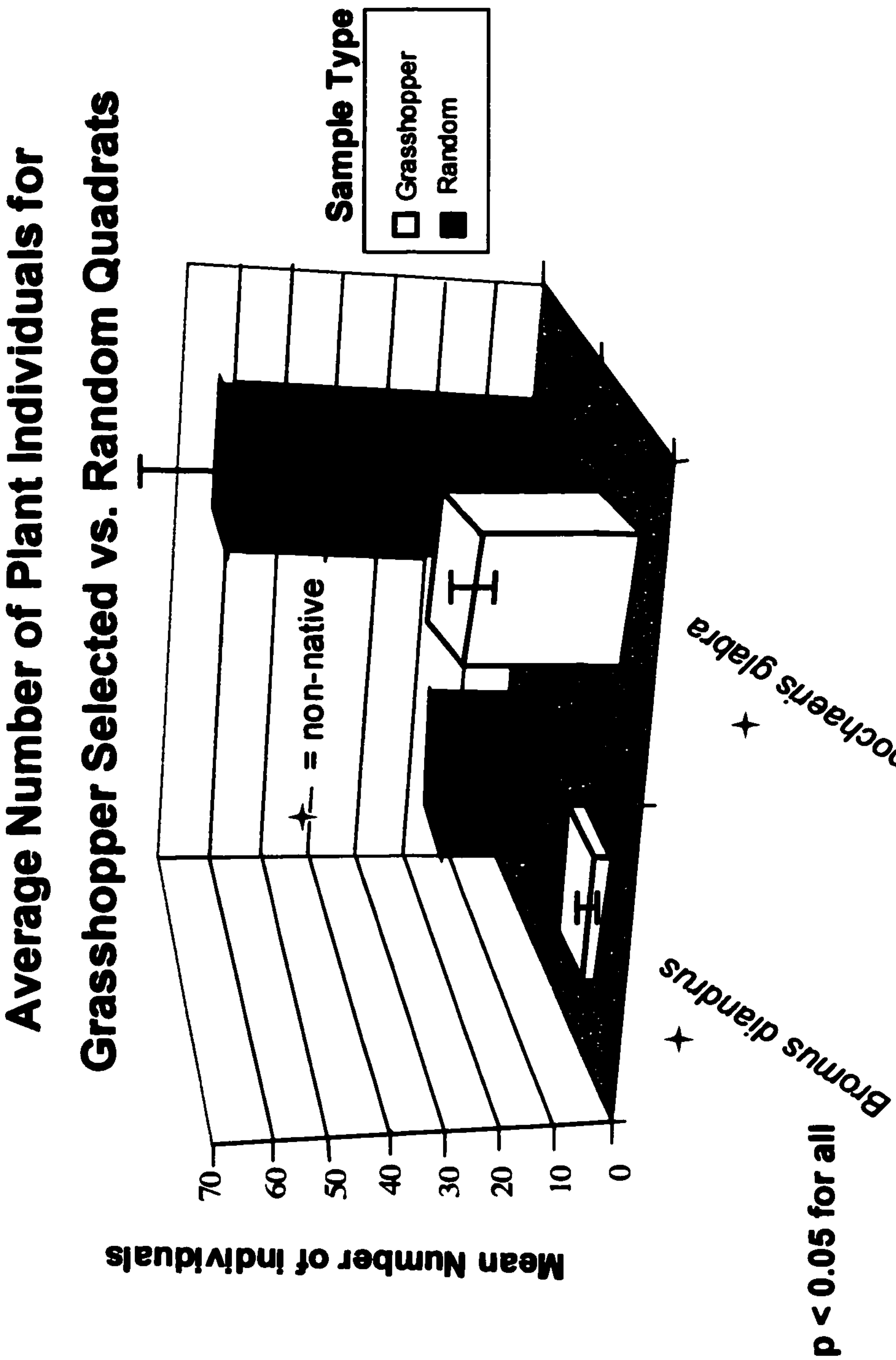


Figure 8 - Total Plant Cover
North Ridge vs. South Ridge

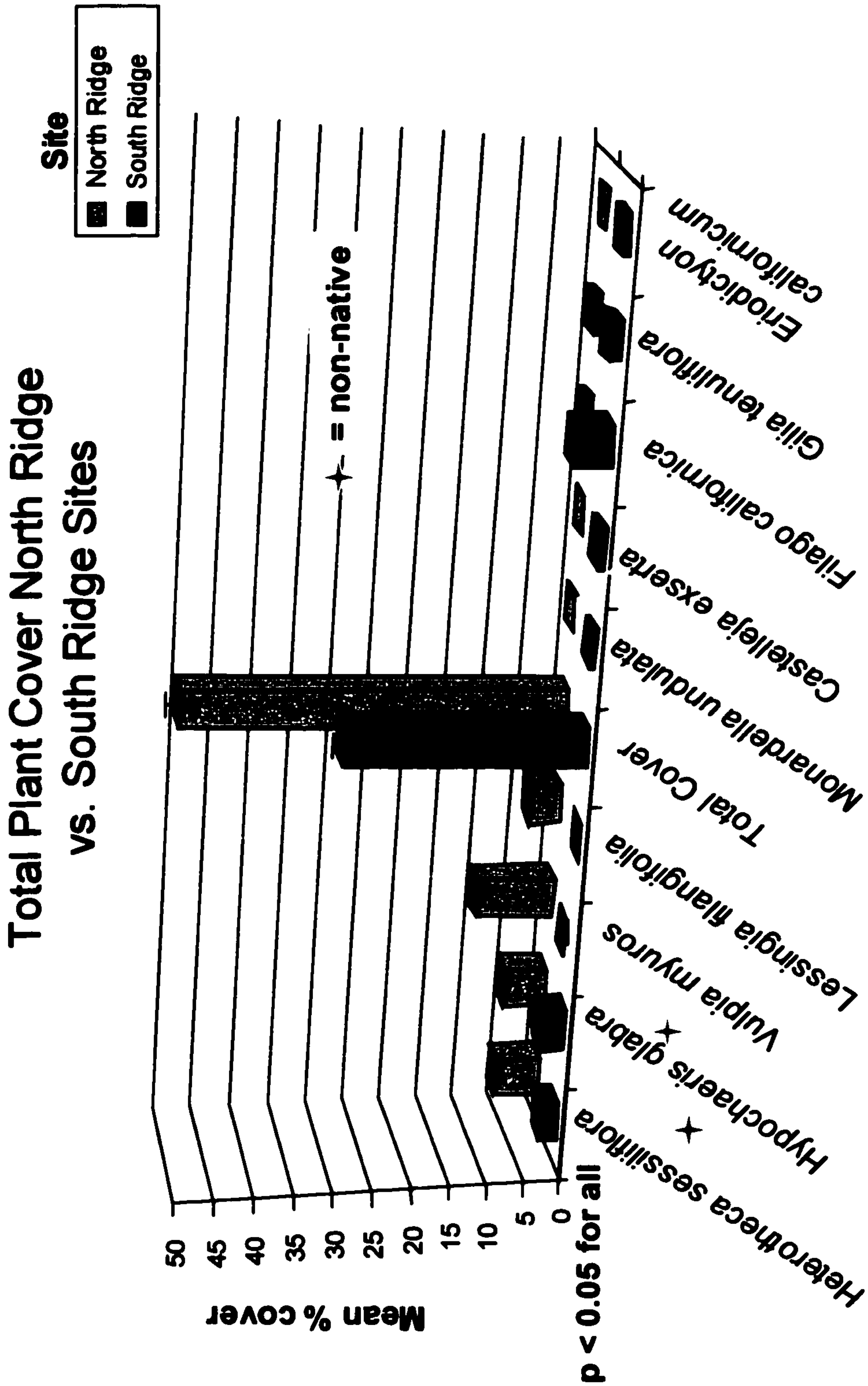


Figure 9 - Average Number of Species / 1m^{2}Quadrat

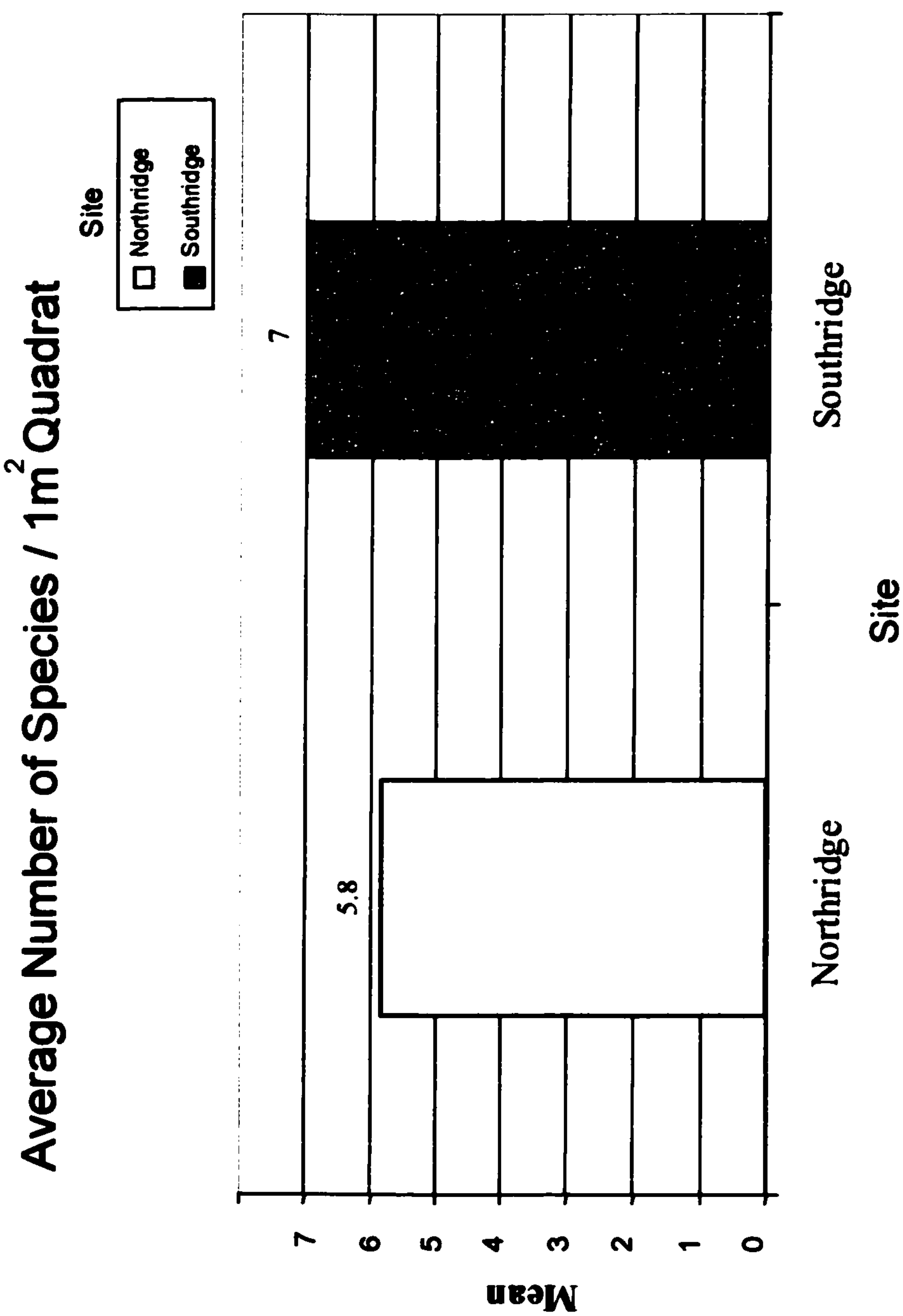


Figure 10 - Total Number of Individuals
North Ridge vs. South Ridge

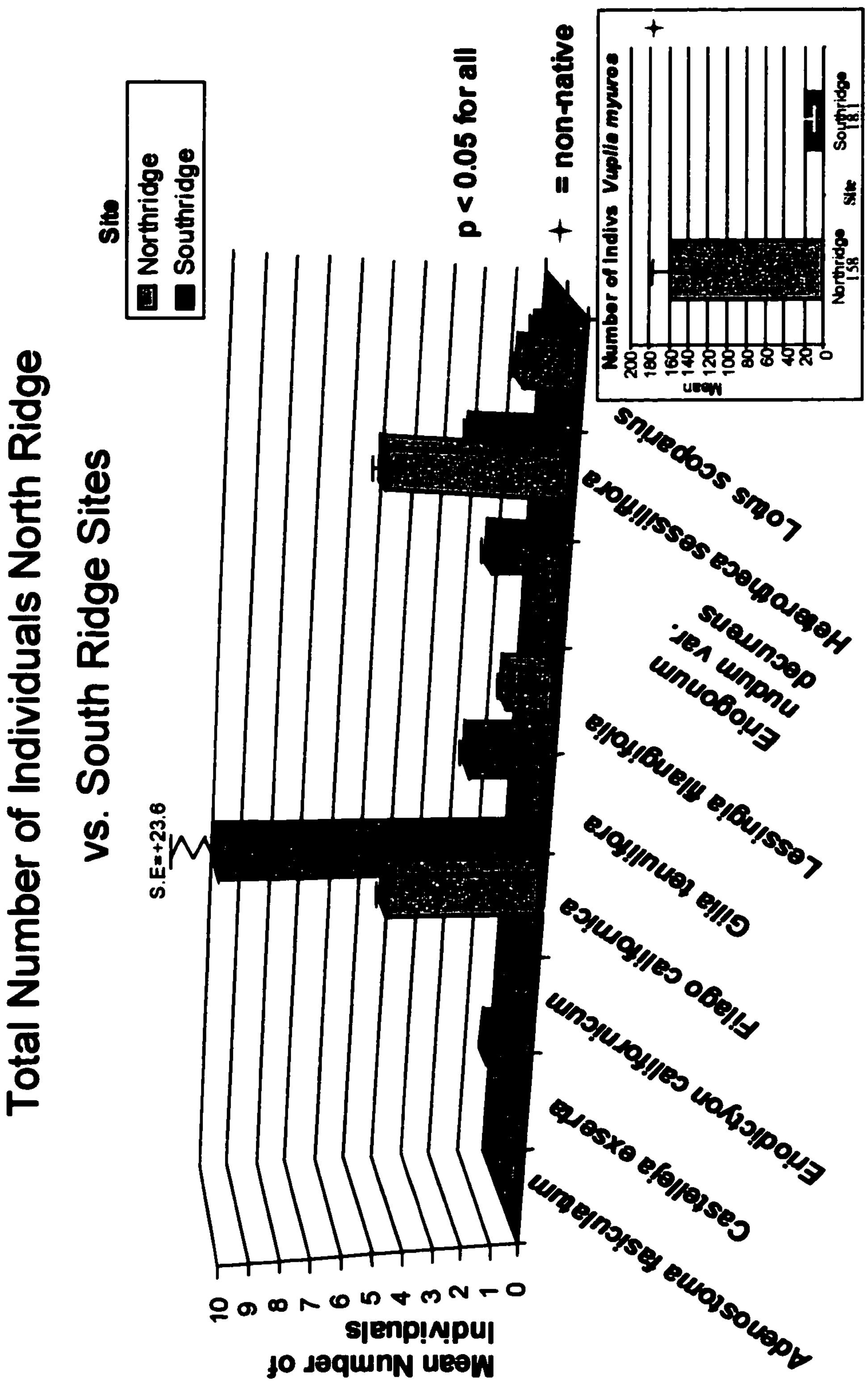


Figure 11 - Percent Cover for North Ridge Grasshopper vs. Random Quadrats and Percent Cover for South Ridge vs Random Quadrats

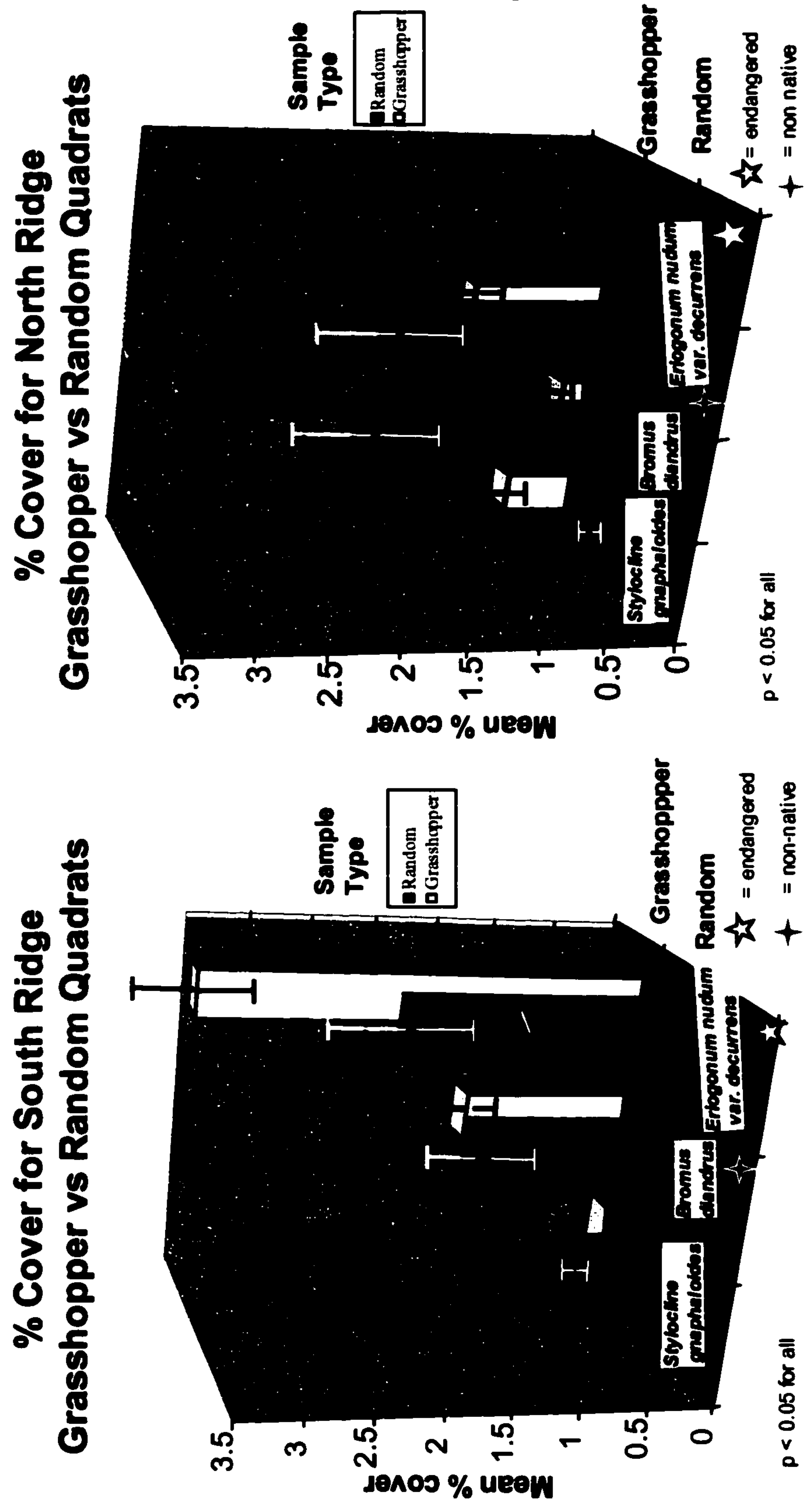


Figure 12- Total Overall Species Found in Frass

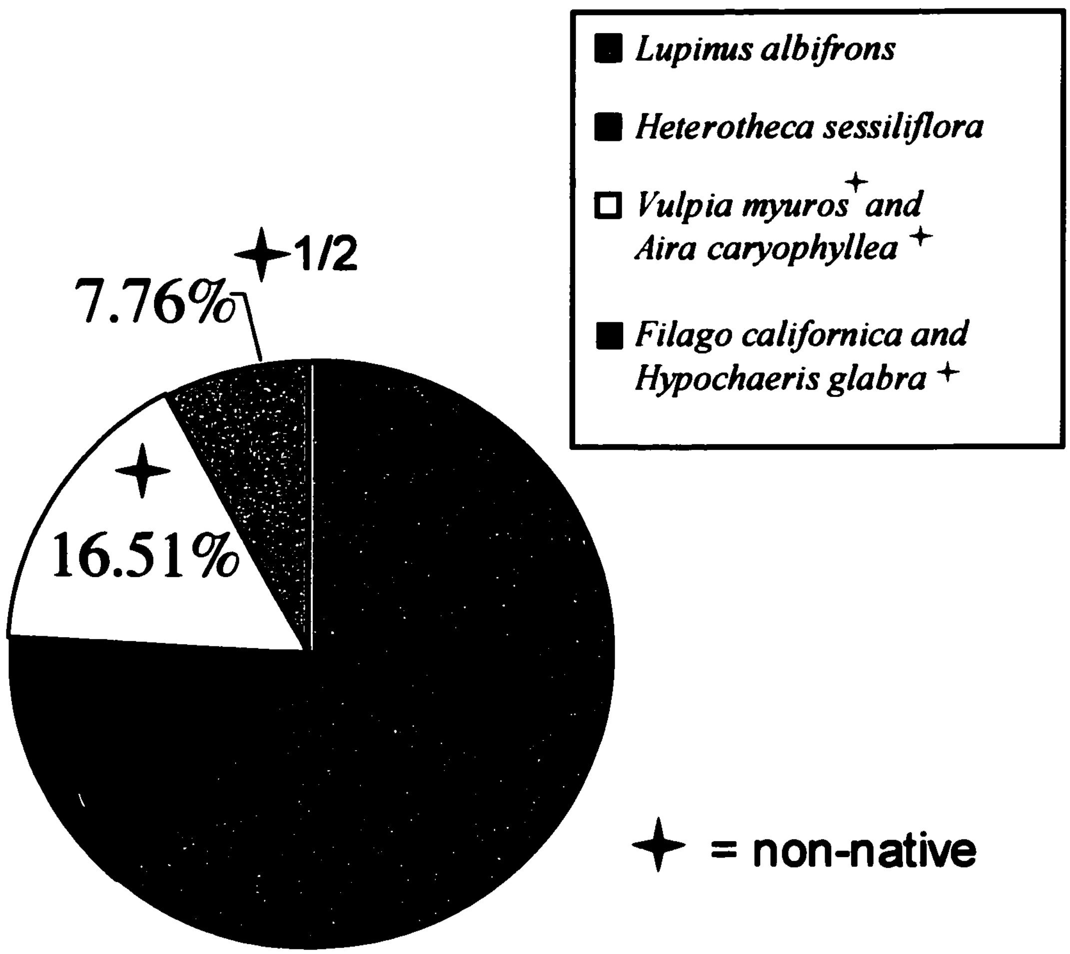


Figure 13 - South Ridge plant species found in frass and North Ridge Plant species found in frass

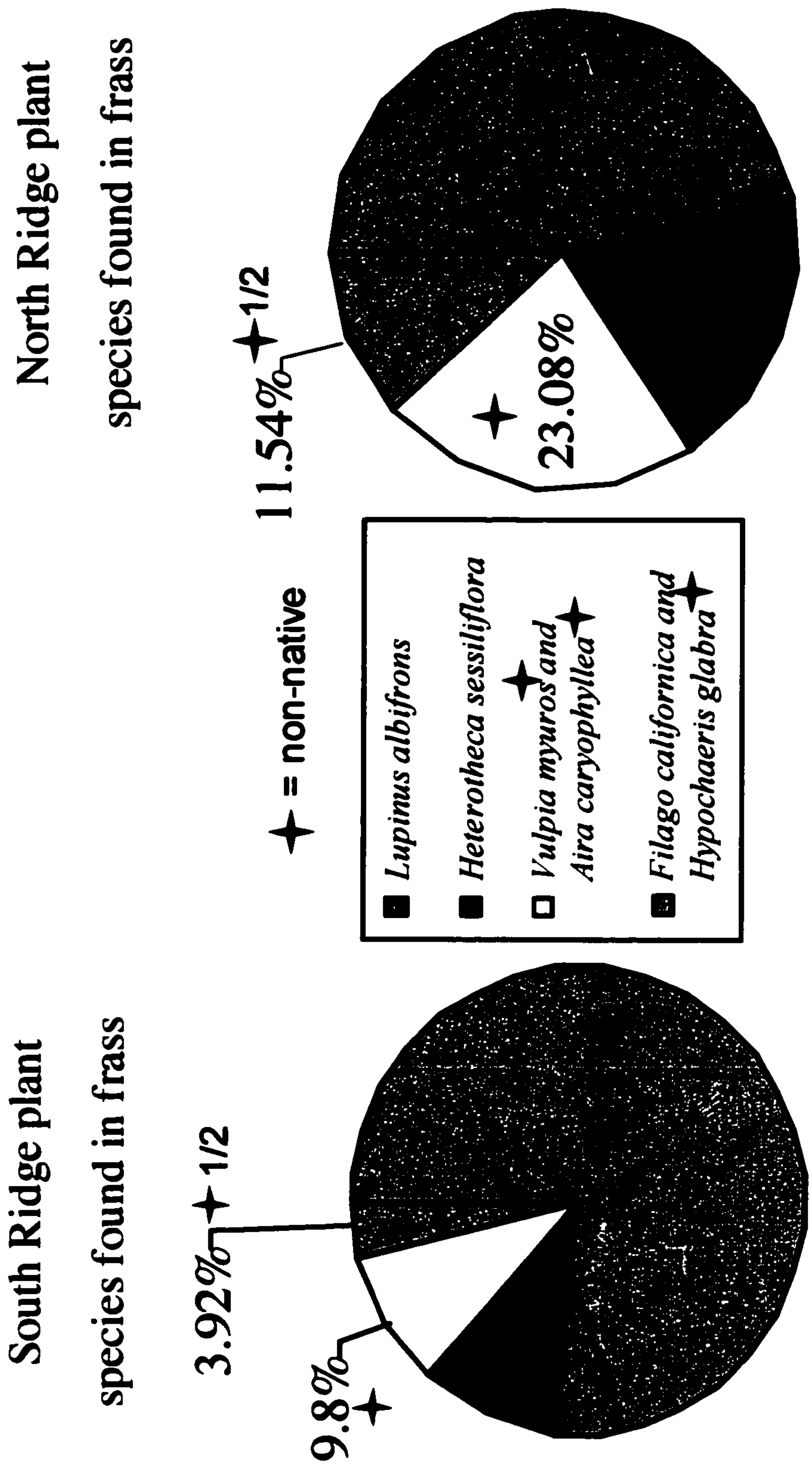
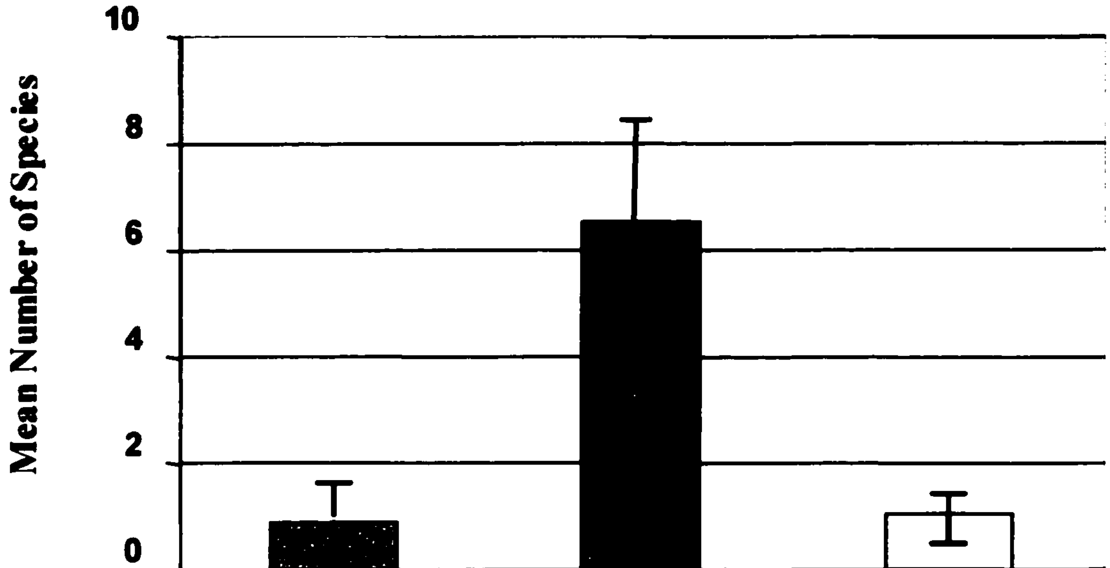


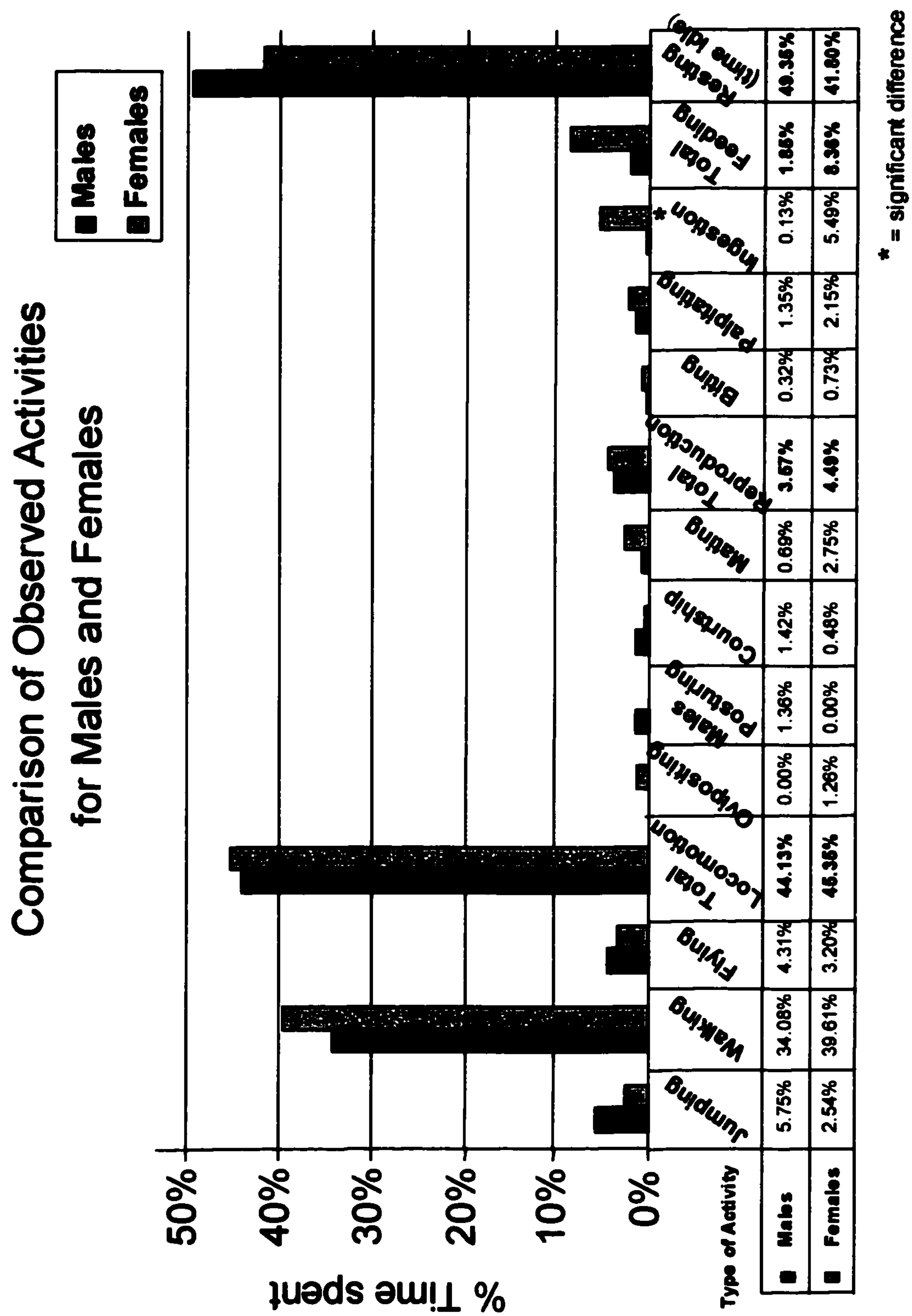
Figure 14 - Average Number of Species Found in Each Sample Type



Data type	Frass	Grasshopper microsite	Observation *
	Mean =0.92, S.E. = ± 0.61	Mean = 6.55, S.E. = ± 1.93	Mean = 1.0, S.E. = ± 0.5

* = Total # of species observed to be eaten

Figure 15 - Comparison of Observed Activities for Males and Females



Appendix A – Complete List of Sandhills Plants

Genus and Species	Family	Common Name (when known)
Toxicodendron diversilobum	Anacardiaceae	Poison Oak
Ahillea millefolium	Asteraceae	Yarrow Milfoil
Artemisia pycnocephala	Asteraceae	Coastal sagewart
Baccharis pilularis	Asteraceae	Dwarf Chaparral Broom/Fuzzy Wuzzy
Cynara sp.	Asteraceae	Artichoke Thistle
Ericameria ericoides	Asteraceae	Goldenbush
Eriophyllum confertifolium	Asteraceae	Golden Yarrow
Filago californica	Asteraceae	California Filago
Gnaphalium californicum	Asteraceae	California Cudweed
Gnaphalium canescens ssp. Beneolens	Asteraceae	Cudweed
Heterotheca grandiflora	Asteraceae	Telegraph weed
Heterotheca sessiliflora ssp. echioides	Asteraceae	Goldenaster
Hypochaeris glabra	Asteraceae	Smooth Cat's Ear
Hypochaeris radicata	Asteraceae	Rough Cat's Ear
Lasthenia californica	Asteraceae	Goldfields
Layia platyglossa	Asteraceae	Layia
Lessingia filaginifolia var. filaginifolia	Asteraceae	California Aster
Madia madioides	Asteraceae	
Malacothrix clevelandii	Asteraceae	
Malacothrix floccifera	Asteraceae	
Senecio sylvaticus	Asteraceae	
Stephanomeria virgata	Asteraceae	
Stylocline gnaphaloides	Asteraceae	Nest Straw
Tragopogon sp.	Asteraceae	
Cryptantha clevelandii	Boranginaceae	
Cryptantha micromeres	Boranginaceae	
Cryptantha muricata	Boranginaceae	
Pectocarya penicillata	Boranginaceae	
Plagiobothrys tenellus	Boranginaceae	
Erysimum teretifolium	Brassicaceae	Ben Lomand Wallflower
Thysanocarpus curvipes	Brassicaceae	Common Fringe-Pod, Fringe Pod
Campanula angustiflora	Campanulaceae	Eastwood's harebell
Cardionema ramosissimum	Caryophyllaceae	Sandmat, Sandcarpet
Loeflingia squarrosa	Caryophyllaceae	Spreading Loeflingia
Minuartia californica	Caryophyllaceae	California sandwort
Minuartia douglasii	Caryophyllaceae	Douglas' stitchwort, Douglas' sandwort
Silene verecunda ssp. platyota	Caryophyllaceae	San Francisco campion
Helianthemum scoparium	cistaceae	Common Sun-Rose
Crassula connata	Crassulaceae	Pygmy-Weed, Sand Pygmyweed

Appendix A - cont.		
Dudleya palmeri	Crassulaceae	Palmer's Dudleya
Cupressus abramsiana	Cupressaceae	Santa Cruz Cypress
Carex globosa	Cyperaceae	Round-Fruit Sedge
Pteridium aquilinum var. pubenscens	Dennstaeditiaceae	Bracken Fern
Arctostaphylos nummularia	Ericaceae	Glossy Leaf Manzanita
Arctostaphylos silvicola	Ericaceae	Bonny Doon Manzanita
Arctostaphylos tormentosa ssp crinita	Ericaceae	
Vaccinum ovatum	Ericaceae	
Genista monspessulana	Fabaceae	French Broom
Lotus scoparius	Fabaceae	Common Lotus
Lotus strigosus	Fabaceae	Stirgose Lotus
Lupinus albifrons	Fabaceae	(Smaller) Silver Bush Lupine
Lupinus arboreus	Fabaceae	(Larger) Coastal Bush Lupine)
Lupinus bicolor	Fabaceae	Miniature Lupine
Chrysolepis chrysophylla	Fagaceae	
Lithocarpus densiflora	Fagaceae	
Quercus agrifolia	Fagaceae	Coast Live Oak
Quercus chrysolepis	Fagaceae	
Quercus wislizeni	Fagaceae	
Erodium cicutarium	Gerianiaceae	Redstem Filaree
Ribes divaricatum	Grossulariaceae	Spreading Gooseberry
Eriodictyon californicum	Hydrophyllaceae	Yerba Santa
Nemophila pedunculata	Hydrophyllaceae	Meadow Nemophila
Phacelia distans	Hydrophyllaceae	Common Phacelia
Phacelia douglasii	Hydrophyllaceae	Douglas' Phacelia
Phacelia nemoralis	Hydrophyllaceae	Woods Phacelia
Phacelia ramosissima	Hydrophyllaceae	Branching Phacelia
Luzula comosa	Juncaceae	Hairy Wood Rush
Monardella undulata	Lamiaceae	Curly-Leaved Monardella
Monardella villosa	Lamiaceae	Coyote Mint
Salvia columbariae	Lamiaceae	Chia Sage
Salvia mellifera	Lamiaceae	Black Sage
Scutellaria tuberosa	Lamiaceae	Common Skullcap
Calochortus venustus	Liliaceae	Butterfly Mariposa
Chologalum pomeridianium	Liliaceae	
Dichelostemma capitatum	Liliaceae	Blue Dicks
Muilla maritima	Liliaceae	Common Muilla
Camissonia contorta	Onagraceae	Contorted Suncup (Evening Primrose)
Camissonia micrantha	Onagraceae	Small-Flowered Evening-Primrose.
Clarkia purpurea	Onagraceae	Purple Clarkia
Clarkia rubicunda	Onagraceae	Reddebed Clarkia

Appendix A - cont.		
Clarkia unguiculata	Onagraceae	Woodland Clarkia
Epilobium minutum	Onagraceae	Little Willowherb, Slender Annual Fireweed
Dendromecon rigida	Papaveraceae	Bush Poppy
Eschscholzia californica	Papaveraceae	California Poppy
Meconella linearis	Papaveraceae	Narrow-Leaved Meconella
Pinus attenuata	Pinaceae	Knobcone Pine
Pinus ponderosa	Pinaceae	Ponderosa Pine
Pinus sabiniana	Pinaceae	Foothill Pine, California
Pseudotsuga menziesii var. menziesii	Pinaceae	Douglas-Fir
Plantago erecta	Plantaginaceae	California Plantain
Armeria maritima ssp. californica	Plimbaginaceae	California Seapink
Aira caryophyllea	Poaceae	Silver Hairgrass
Briza maxima	Poaceae	Big Quaking Grass
Bromus diandus	Poaceae	
Bromus laevipes ssp rubens	Poaceae	
Bromus tectorum	Poaceae	Cheatgrass, Downy Brome
Cynosurus echinatus	Poaceae	Hedgehog Dogtail-Grass, Annual Dogtail
Koeleria macrantha	Poaceae	Junegrass
Poa secunda ssp.secunda	Poaceae	One-Sided Blue Grass
Vulpia microstachys. var. ciliata	Poaceae	Eastwood Fescue
Vulpia microstachys. var. confusa	Poaceae	Confusing Fescue
Vulpia microstachysvar. pauciflora	Poaceae	Pacific Fescue
Vulpia myuros	Poaceae	Rattail Fescue
Vulpia octoflora var. hirtell	Poaceae	Hairy Six-Weeks Fescue
Gilia tenuiflora	Polemoniaceae	Slender-Flowered Gilia
Linanthus parviflorus	Polemoniaceae	Common Linanthus
Navarettia atractyloides	Polemoniaceae	
Chorizanthe diffusa	Polygonaceae	Diffuse Spineflower
Chorizanthe pungens var hatwegiana	Polygonaceae	Ben Lomand Spineflower
Eriogonum nudum var decurrens	Polygonaceae	Ben Lomand Buckwheat
Eriogonum vimineum	Polygonaceae	Wicker Buckwheat
Rumex acetosella	Polygonaceae	Common Sheep Sorrel
Polypodium californicum	Polypodiaceae	California Polypody
Calyptridium umbellatum	Portulaceae	Pussy Paws
Monia fontana	Portulaceae	
Pellaea mucronata	Pteridaceae	California Cliff-Brake
Pentagramma triangularis	Pteridaceae	Gold-Back Fern
Delphinium parryi	Ranunculaceace	An Bernardino Larkspur
Ceanothus cuneatus var. cuneatus	Rhamnaceae	Buck Brush
Ceanothus papillosus	Rhamnaceae	Wartleaf Ceanothus

Appendix A - cont.

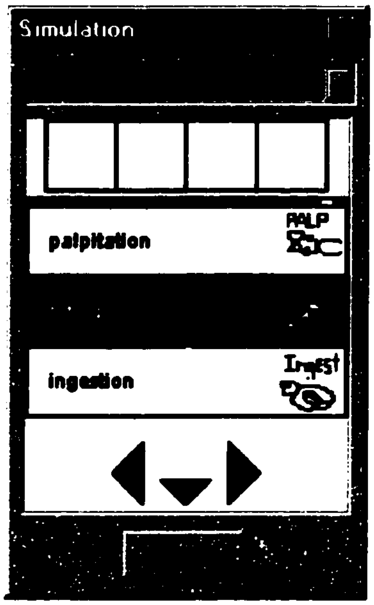
Rhamnus californica	Rhamnaceae	California Coffeeberry
Adenostoma fasciculatum	Rosaceae	Chamise
Heteromeles arbuifolia	Rosaceae	
Horkelia cuneata ssp. cuneata	Rosaceae	Wedge-Leaf Horkelia Coast
Horkelia cuneata ssp. Sericea	Rosaceae	Kellogg's Horkelia.
Galium spp.	Rubiaceae	Bedstraw
Saxifraga californica	Saxifragaceae	California Saxifrage
Antirrhinum multiflorum	Scrophulariaceae	Chaparral Snapdragon
Castilleja affinis	Scrophulariaceae	Indian Paintbrush
Collinsia bartsiifolia	Scrophulariaceae	White Collinsia
Linaria canadensis var. texana	Scrophulariaceae	Rough-Seeded Blue Toad-Flax
Mimulus androsaceus	Scrophulariaceae	Androsace Monkeyflower
Mimulus aurantiacus	Scrophulariaceae	Sticky Monkeyflower
Orthocarpus purpurescens	Scrophulariaceae	
Sequoia sempervirens	Taxiodiaceae	Coast Redwood

Appendix B – Sandhills Plant Species found at Study Sites

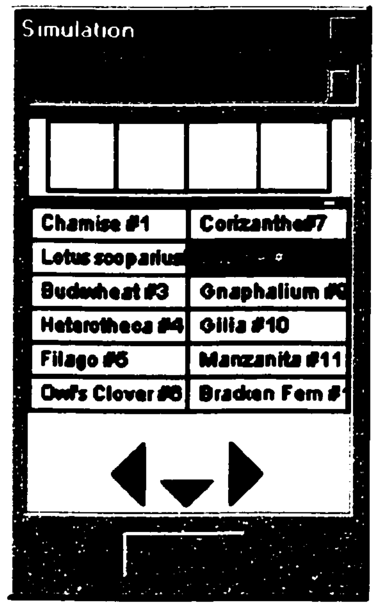
Genus and Species	Family	Green or Senesced in Summer
Lupinus albifrons	Leguminosae(Fabaceae)	Green
Heterotheca sessiliflora	Asteraceae	Green
Bromus diandrus	Poaceae	Senesced
Bromus tectorum	Poaceae	Senesced
Eriogonum nudum	Polygonaceae	Green
Lotus scoparius	Leguminosae(Fabaceae)	Green
Lotus strigosus	Leguminosae(Fabaceae)	Senesced
Monerdella undullata	Lamiaceae	Senesced
Chorizanthe pungens var. hartwegiana	Polygonaceae	Senesced
Hypochaeris glabra	Asteraceae	Senesced
Stylocline gnaphaloides	Asteraceae	Senesced
Filago californica	Asteraceae	Senesced
Erysimum teretifolium	Brassicaceae	Senesced
Adenostoma fasciculatum	Rosaceae	Green
Gnaphalium californicum	Asteraceae	Senesced
Calyptridium umbellatum	Portulacaceae	Green
Castelleja exserta	Scrophulariacee	Senesced
Arctostaphylos silvicola	Ericaceae	Green
Eriodictyon californicum	Hydropyllaceae	Green
Pteridium aquilinum var. pubescens	Pteridaceae	Senesced
Gilia tenuiflora	Polemoniaceae	Senesced
Vulpia myuros	Poaceae	Senesced
Aira caryophyllea	Poaceae	Senesced
Avena fatua	Poaceae	Senesced
Silene verecunda ssp. platyota	Caryophyllaceae	Senesced
Senecio sylvaticus	Asteraceae	Senesced
Lessingia filaginigolia var.filaginifolia	Asteraceae	Green
Pinus Ponderosa	Pinaceae	Green
Mimulus auranticus	Scrophulariaceae	Senesced
Quercus agrifolia	Fagaceae	Green
Camissonia contorta	Onagraceae	Senesced

Appendix C- Screen Sequences from Cybertracker™

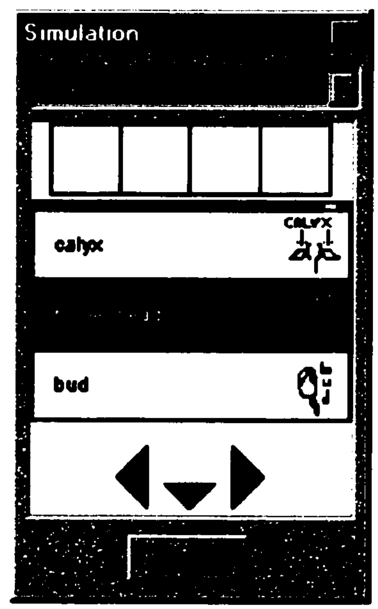
Sample screen sequences for feeding activities:



Type of Feeding



Plant Type

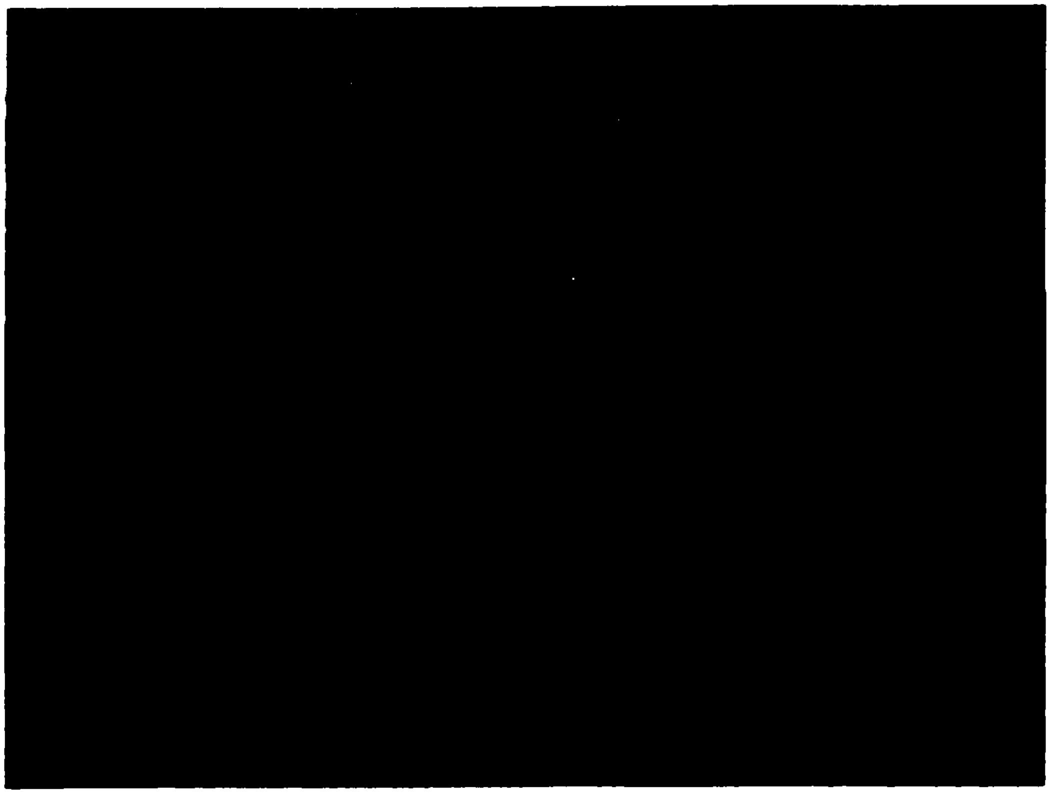


Plant Part

Similar screen sequences were used for reproductive and locomotive activities.

Appendix D- Photos of the Zayante Band-winged Grasshopper (*Trimerotropis infantilis*)

Appendix E- Sand Hills habitat as seen on South Ridge, Quail Hollow Quarry.



Lupinus albifrons, host plant to the Zayante Band-winged Grasshopper