North Dakota Industrial Commission
Outdoor Heritage Fund Grant Application

Name of Organization * North Dakota State University

Federal Tax ID# * 45-6002439

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MAJOR Directive:

Choose only one response

O **Directive A**. Provide access to private and public lands for sportsmen, including projects that create fish and wildlife habitat and provide access for sportsmen;

√ **Directive B. Improve, maintain, and restore water quality, soil conditions, plant diversity, animal systems and to support other practices of stewardship to enhance farming and ranching;**

O **Directive C**. Develop, enhance, conserve, and restore wildlife and fish habitat on private and public lands; and

O **Directive D**. Conserve natural areas for recreation through the establishment and development of parks and other recreation areas.

Additional Directive:

Choose all that apply

O **Directive A**. Provide access to private and public lands for sportsmen, including projects that create fish and wildlife habitat and provide access for sportsmen;

O **Directive B**. Improve, maintain, and restore water quality, soil conditions, plant diversity, animal systems and to support other practices of stewardship to enhance farming and ranching;

√ **Directive C. Develop, enhance, conserve, and restore wildlife and fish habitat on private and public lands; and**

O **Directive D**. Conserve natural areas for recreation through the establishment and development of parks and other recreation areas.

Type of organization:

√ **State Agency**

O Political Subdivision

O Tribal Entity

O Tax-exempt, nonprofit corporation, as described in United States Internal Revenue Code (26 U.S.C. § 501 (c)

Project Name* Behavioral Attractants for Monitoring Important Insect Pollinators of North Dakota

Abstract/Executive Summary (493 words)
The grasslands of North Dakota are among the most productive communities in the world. Grasslands rely on connections between species, examples being beneficial relationships between plants and insect pollinators. By pollinating a diversity of plants, insects make contributions by increasing plant genetic diversity, reproduction and population growth. Plant diversity is essential to rangeland managers, increasing forage production for livestock and wildlife, contributing to better soil structure, and promoting resilience to disturbance. The argument can be made that grasslands will only maintain their diversity if a diversity of insect pollinators is also maintained. Unfortunately insect pollinators have suffered significant declines in recent years. Efforts to help insect pollinators have been hampered by the difficulty of monitoring populations. Pollinator species are highly mobile and change flight patterns from day to day as different floral resources become available across the landscape. We propose a research initiative that will create tools for monitoring and conserving insect pollinators, the tools being natural chemicals that serve as attractants. Unlike humans, insects rely on volatile chemicals to achieve two fundamental aspects of reproduction, finding mates (e.g. pheromones) and finding plants that provide nectar and serve as hosts for offspring (e.g. kairomones). Future uses of chemical attractants are: 1) for a yearly census of pollinator species, and 2) as a means of assessing interventions to increase pollinator populations. Examples of interventions to maintain a diversity of pollinators are supplementing nectar sources and larval host plants. The five hawkmoth species that we will investigate are important pollinators of the rare western prairie fringed orchid, *Platanthera praeclara*. We have studied the orchid and it's pollinators every summer since 2002, study sites being in southeastern North Dakota, the location of one of three remaining orchid metapopulations in North America. We have recently published results of our research on orchid-pollinator interactions in the journal *Biological Conservation* (Fox et al. 2013). Key results were: 1) the orchid cannot produce seeds without the aid of pollinators, 2) five hawkmoth species act as orchid pollinator, and 3) only one species, the leafy spurge hawkmoth, is abundant and present each year. Clearly there is a need to increase the diversity of orchid pollinators. The objectives of our proposed research are: 1) to identify the sex pheromone of the five hawkmoth pollinators, 2) to identify plant volatiles, including orchid volatiles, that attract the five pollinators, 3) to develop a live-trapping system that allows non-destructive sampling of hawkmoths for future monitoring and experiments. In our budget we have requested $197,220 to support the stipend of a PhD student, two hourly workers, equipment and supplies for chemical identifications, travel to the field and scientific meetings, and supplies and greenhouse fees for insect and plant colonies. Participants are Dr Stephen Foster, a natural products chemist who has been identifying insect attractants for over 25 years and Dr Marion Harris, who has expertise in insect behavior and insect-plant interactions and 12 years of experience studying the orchid and its hawkmoth pollinators in North Dakota.

Amount of Grant request: $197,220

Total Project Costs: $363,248

Amount of Matching Funds: $166,028 ($80,879 Cash and $85,149 Indirect)

Source of Matching Funds: North Dakota Agricultural Experiment Station and North Dakota State University

Certifications

√ I certify that this application has been made with the support of the governing body and chief executive of my organization.

√ I certify that if awarded grant funding none of the funding will be used for any of the exemptions noted on Page 1 of this application.

Project Narrative

Organization Information

North Dakota State University first opened as a public land grant institution in Fargo, North Dakota, in 1890, shortly after North Dakota officially became a state in November 1889. Initially known as the North Dakota Agricultural College, the college's name was changed to North Dakota State University in 1960. The North Dakota Agricultural Experiment Station and NDSU Extension Service are integral parts of the University. NDSU accepted its first graduate students in 1895.

NDSU has enjoyed steady growth, with enrollment now exceeding 14,500 students and over 700 faculty members. NDSU offers over 100 undergraduate and approximately 100 graduate programs in a wide variety of fields, with degrees awarded at the doctoral, master's, professional, and baccalaureate levels. In addition to their academic studies, students have opportunities to participate in approximately 300 student organizations, leadership development, civic engagement activities, fine arts, athletics, and study abroad.

NDSU is part of the North Dakota University System (NDUS) which includes 11 campuses across the state. The State Board of Higher Education (SBHE) is the policy-setting and governing body for the NDUS. The SBHE is made up of seven citizen members appointed to four-year terms by the governor, one student appointed by the governor to serve a one-year term, a non-voting faculty advisor and a non-voting staff advisor. NDSU is headed by a President, with a Provost who provides administrative leadership for all academic activities, including eight academic colleges and the graduate school.

NDSU's mission statement: "With energy and momentum, North Dakota State University addresses the needs and aspirations of people in a changing world by building on our land-grant foundation." With its land-grant mission to provide quality education, leading-edge research and excellent service, NDSU is acknowledged as a national leader among its peers.

Purpose of Grant

Proposed Research: We propose research to create tools for monitoring insect pollinators that play an important role in the grasslands of North Dakota. The North Dakota pollinators that are the focus of our research are the night-flying hawkmoths that pollinate many grassland plants, including the rare western prairie fringed orchid. The orchid cannot reproduce through seed production without the aid of pollinators. The tools that we will create are natural insect and plant chemicals that will serve as attractants for hawkmoth pollinators. Two future uses of these attractants are for 1) a yearly census of hawkmoth species and 2) to evaluate interventions to support a greater diversity of pollinators. The aim of our research fits the Heritage Fund's directive to "Improve, maintain, and restore water quality, soil conditions, plant diversity, animal systems and to support other practices of stewardship to enhance farming and ranching." If hawkmoths can be viewed as 'wildlife', the research also fits the directive to "Develop, enhance, conserve, and restore wildlife and fish habitat on private and public lands."

Research Objectives:
1) To identify the sex pheromones of five North Dakota hawkmoth pollinators,
2) To identify plant volatiles that attract the five pollinators, and
3) To develop a trapping system for non-destructive sampling of pollinators.

Introduction to Insects Pollinators: The grasslands of North Dakota are among the world's most productive communities (Samson and Knopf 1996, Grygiel et al. 2009). Grasslands rely on a particular physical environment, e.g. soil and water, as well as countless connections between species (Travers et al. 2011). Beneficial relationships between plants and insect pollinators are examples of such connections (Faegri and van der Pijl 1979, Barth 1981). Insects visit plants to obtain food, i.e., nectar and pollen, and plants benefit by increasing their genetic diversity, reproduction, and population growth (Menz et al. 2011). Plant diversity is essential to rangeland managers, increasing forage production for livestock and wildlife, contributing to better soil structure, and promoting resilience to disturbance (Jones and Cushman 2004). The argument has been made that grasslands will only maintain these features if a diversity of insect pollinators is also maintained (Travers et al. 2011).

Unfortunately for the plants of the world and for agriculture in particular, insect pollinators have suffered significant declines in recent years (National Research Council 2007, Potts et al. 2010). The realization that this was happening first came from apiarists, who move honey bee colonies around the United States to pollinate fruit crops, vegetables, seed crops and nuts such as almonds. Investigations of other pollinators, such as bumblebees (Westphal et al. 2008), showed similar population declines and possible disappearances of some species, such as *Bombus franklini*. Declines are related to increased parasite load (e.g. *Varroa* mite), new diseases (viruses, fungi, protozoans), fragmentation of habitat (Pauw 2007), and new insecticides (e.g. neonicotinoids). While all of these things are harmful to bee pollinators, the Dakotas (North and South) are essential to the health of these pollinators, being the

place where bees come to recover (but not rest!) for several months every summer from their efforts in U.S. agriculture. The grasslands of the Dakotas are a paradise for pollinators. As a result, North Dakota is the number one U.S. honey producer, South Dakota being second.

While the focus has been on managed insect pollinators because of their critical role for production agriculture, there are real concerns about non-managed pollinators that rely on wild plants for nectar and pollen, and produce offspring on wild plants (Kearns et al. 1998, Larson et al. 2001, Corbet 2006, National Research Council 2007, Menz et al. 2011, Winfree et al. 2011). These pollinators, including bees, moths, butterflies, flies, and beetles, contribute the lion's share of pollination services in the United States (Travers et al. 2011). Moths, being highly mobile and active at night (when many flowers bloom), are seen as particularly important for maintaining grassland diversity, but are rarely studied in their native environments. Efforts to determine the health of these wild insect pollinators have been hampered by the difficulty of monitoring populations (Neff et al. 1993, Vazquez et al. 2005). Wild pollinators, being highly mobile and surprisingly intelligent, change flight patterns from day to day as different floral resources become available across the landscape. Pollinators that are active at night are even harder to monitor, especially when visits to nectar plants are on-the-wing, as is the case with hawkmoths that forage like hummingbirds (von Arx et al. 2013).

Orchids are well-known for their reliance on insect pollinators, having elaborate features to attract and sometimes deceive insect pollinators (Darwin 1877, Barth 1981). Sexual reproduction is essential for maintaining extant populations and creating new populations. Therefore understanding orchid pollination is essential for conservation and recovery (Tremblay et al. 2005, Bernhardt and Edens-Meier 2010).

History of the Study of Chemical Ecology: The chemical identification of natural attractants began in the 1950s (Wyatt 2014) when chemists gained the analytical tools (e.g. gas chromatography) to begin investigations of the well-known ability of female moths to attract male moths over long distances (over hundreds of feet). The volatile chemicals that were identified were called sex pheromones and are remarkable for triggering robotic behaviors in male moths, including upwind flight over long distances, courtship, and attempts to mate (Tumlinson et al. 1989). The power of these sex pheromone attractants has led to their use in agriculture, forestry, and public health (Foster and Harris 1997), where today they are used to: 1) monitor pest populations to make management decisions, 2) disrupt pest reproduction by inundating habitat with sex pheromones, and 3) mass trap to reduce pest populations. Subsequent to the identification of insect sex pheromones was the discovery insects also have strong behavioral responses to chemicals produced by plants (Miller et al. 1984, Harris et al, 1987), including plants that provide nectar and pollen and plants that are needed by adult female insects (Raguso et al. 1996), which play a critical role in reproduction by finding host plants for larval offspring. While not as powerful as sex pheromones, plant attractants have also been used successfully in agriculture and forestry (Foster and Harris 1997). The identification and use of the behaviorally active chemicals of insects led to discoveries of similar chemicals in many other animals, including reptiles, birds,

and mammals, and gave rise to a new interdisciplinary field in science called Chemical Ecology (with its own scientific journal called *Journal of Chemical Ecology*) that has combined the expertise of biologists, with their knowledge of natural history and behavior, with the expertise of chemists, with their ever-expanding analytical technology (Wyatt 2014).

Hawkmoth Pollinators of the North Dakota Grasslands. Hawkmoths are important pollinators of many plants throughout the United States (Tuttle 2007). Nevertheless little is know about them except they are exceptional flyers, capable of migrating hundreds or even thousands of miles (e.g. Bartholomew and Casey 1978, Keaster et al. 1996), and have elaborate sensory systems, especially antennae and compound eyes, for finding mates, nectar plants, and host plants for larval offspring (Schiestl and Schluter 2009). All hawkmoth species use powerful sex pheromones for mate location (Tumlinson et al. 1989, Tuttle 2007). Sex pheromones have been identified and synthesized for a number of hawkmoth species, a prime example being *Manduca sexta* (Tumlinson et al. 1989), which is the 'lab rat' of the insect world, being a model system for developmental biology, neurophysiology, and behavior (e.g. Krenn 2010, Schiestl and Schluter 2009). Plant volatiles are also very attractive to hawkmoths (Raguso et al. 1996, Plepys et al. 2002).

Aside from *Manduca sexta* and a small number of hawkmoth species (one of which is a pollinator of the orchid, *Hyles lineata*), little is known about biology and natural history of most hawkmoths (Tuttle 2007). The only reason this is not true for the hawkmoths of southeastern North Dakota is their association with the western prairie fringed orchid, *Platanthera praeclara*, a federally listed threatened plant in the United States and Canada (USFWS 1989, Environment Canada 2006). The orchid only has only three remaining metapopulations, one each in North Dakota, Minnesota, and Manitoba (Borkowsky 2006). A management plan for orchids in the United States was published in 1996 (USFWS 1996). The western fringed prairie orchid has a sibling species (Sheviak and Bowles 1986), the eastern prairie fringed orchid, *Platanthera leucophaea*, which also is a federally listed threatened species and occurs mostly east of the Mississippi, with some overlap between the two sibling species in Iowa. Its populations have declined so much that plants near Chicago are hand-pollinated by humans (http://archive.audubonmagazine.org/features0705/habitat.html).

In 2002, at a meeting between North Dakota State University and Dakota Prairie Grasslands (USDA Forest Service), we were introduced to the orchid and asked by scientists in the US Forest Service to address two questions: How important are pollinators for orchid reproduction? And are there enough pollinators to support North Dakota's orchid metapopulation? This research was seen as complimenting research on orchid reproduction (Sieg and Bjugstad 1992, Sieg 1995, Wolken et al. 2001, Alexander 2006). A summary of our 10-year research on orchid-pollinator interactions was recently published in the journal *Biological Conservation* (Fox et al. 2013).

Key results of our 10-year study were: 1) the orchid cannot produce seeds without the aid of pollinators, 2) the orchid has floral features (i.e., a pollination syndrome), including

an unusually long nectar spur (5 cm long), that match the features of the head of hawkmoth pollinators, 3) there are five hawkmoth pollinator species in southeastern North Dakota, and 4) only one of the five pollinator species, the leafy spurge hawkmoth (Jordan et al. 2006), is abundant and is probably doing the lion's share of orchid pollination. Given that the host plant of this keystone pollinator species is leafy spurge, a noxious weed that we have hopes of eradicating from southeastern North Dakota (Setter and Lym 2013), it seems unwise to rely so heavily on this one pollinator species, which in fact was introduced to the United States from Europe as a biological control agent for leafy spurge (Tuttle 2007), in addition to several other biological control agents, including beetles, moths, and gall midges (Setter and Lym 2013). The orchid's four other hawkmoth pollinators are native resident species of North Dakota: *Eumorpha achemon*, *Sphinx drupiferarum*, *Lintneria eremitus*, and *Hyles lineata*. A sixth hawkmoth species, *Hyles gallii*, has been found carrying orchid pollen in Canada (Westwood and Borkowsky 2004) and is captured in southeastern North Dakota (Fox et al. 2013) but not carrying orchid pollen.

Management implications of our study are that we need to do more to support a diversity of pollinators for the orchid and other grassland species (Fox et al. 2013). We proposed two ways of doing this: 1) create a greater diversity of nectar plants to support pollinator survival and reproduction before and after the orchid flowers in July, and 2) create places (refuges) where the host plants of hawkmoth larvae are abundant. Since hawkmoths fly long distances, it seems likely that the locations of these interventions could be at some distance from the orchids, for example in residential areas or wetlands. Having the tools provided by attractants would make possible testing for effective distances between orchids and the other plants that hawkmoth pollinators need to survive and reproduce in southeastern North Dakota. We are confident that this can be done alongside the stewards of the land where the orchid and its pollinators live, i.e. ranchers, farmers, Dakota Prairie Grasslands, and the Nature Conservancy.

Why We Need Funding For Research on Hawkmoth Pollinators: We are the only scientists studying pollinators of the orchid in North Dakota and also are the primary research group studying orchid pollinators in the United States. Our research was launched by generous funding from Dakota Prairie Grasslands, which provided funds from 2002 to 2009, supporting travel to field sites (near Lisbon) and a talented graduate student (Kristina Fox) who spent four summers working in conjunction with a pollination biologist (Dr Pati Vitt, Chicago Botanic Garden). We have continued research since 2009, monitoring hawkmoth and orchid populations, filming orchid-moth interactions (http://www.ndsu.edu/entomology/research/videos/) and expanding studies to nectar larceny (from long-tongued hawkmoths and bees) and population genetics. The latter research, done in collaboration with Dr Steve Travers (NDSU Biological Sciences), has resulted in the creation of genetic molecular markers (Ross et al. 2013) that are now being used to study inbreeding depression. As has been studied in the eastern prairie fringed orchid (Bowles et al. 2002, Wallace 2003), we also have studied the reproductive consequences of self- versus outcross-pollination, showing that orchid seed capsules produced by selfing (i.e. hawkmoths moving pollen between flowers within the

inflorescence) have less than one-third as many viable seeds as capsules produced by hawkmoths moving pollen between distant plants (Travers et al. in preparation).

As has been the case for many studies of threatened plant species, funding in recent years has been harder to come by. In our last four years of research in southeastern North Dakota we have received a total of $3000 in outside funding from Jeff Towner of USFWS (in 2012). Given that it costs about $3500 just for gas mileage each summer, most of our research in the past four years has been self-funded, a situation that is not sustainable. This past summer (2013) DNR orchid researchers studying the orchid metapopulation east of Crookston, Minnesota, suggested that they might be able to provide funding (state, USFWS) if we moved our pollinator studies to Minnesota. We would like to stay in North Dakota.

Methods: Identification and identification of both sex pheromone and plant volatiles will be carried out by standard chemical ecology methods. Briefly, volatiles (either from female insects or plants) will be collected by passing charcoal-filtered air over the enclosed material and collecting the effluent on Tenax GC adsorbent. After solvent elution from the Tenax, extracts will be analyzed by coupled gas chromatography/electroantennogram detection (GC/EAD; Myrick and Baker, 2012) using the antenna of a male moth antenna (for both pheromone and plant volatiles). Essentially, the technique allows isolation of individual compounds that cause depolarization (stimulation) of antennal neurons. Such compounds are likely to be responsible for the attraction of male moths to females or of both sexes to the orchids for feeding/pollination. After isolating the GC/EAD-active chemcials, we will identify them by gas chromatography/mass spectrometry, microchemical reactions, and use of authentic standards (purchased from Pherobank, Wageningen, The Netherlands, or Sigma-Adrich, St Louis, MO). Following isolation of the compounds, we will test and optimize their ability to attract moths by formulating various mixtures and testing these with moths in a wind tunnel (in the laboratory) and in the field using sticky traps baited with a slow release formulation of the compounds.

Management of Project: A timetable for our research is given in Table 1. Drs. Stephen Foster will devote 10% of his time during each of the three years of this project. Dr Foster has over 25 years experience as a natural products chemist and has published 80 peer-reviewed scientific papers. He will be responsible for identifying the chemical attractants using coupled gas-chromatography/ electroantennogram detection. One of his current research projects is identifying volatile chemical attractants for the blue orchard bee, *Osmia lignaria*, is a commercially- developed alternative pollinator for the honey bee, *Apis mellifera*. Dr Foster is an Associate Editor for the *Journal of Chemical Ecology* (for the past seven years) and is president-elect of the International Society of Chemical Ecology. A number of his relevant publications in chemical ecology are listed in the 'Literature' section at the end of the proposal (see papers with S.P. Foster as author). Dr Marion Harris will devote 8% of her time during each of the three years of this project. She has over 25 years experience researching insect-plant interactions, in particular insect mate- and host- finding behavior, and has published 83 peer-reviewed scientific papers. She recently (2013) served as Panel Manager for the USDA's NIFA-

AFRI Foundational Research Program for Plant-Associated Insects and Nematodes, Organismal Program. This is the primary competitive research fund available to researchers in the United States who study non-molecular insect-plant interactions, including interactions with pollinators. Dr Harris will be responsible for trapping moths, rearing moths, and developing behavioral assays to test the attractiveness of plant and insect extracts in the laboratory and field. A number of her relevant publications in chemical ecology are listed in the 'Literature' section at the end of the proposal. In their respective roles, Dr Foster and Dr Harris started collaborating on chemical ecology research in 1988, results being the identification of number of insect attractants, including sex pheromones and chemical attractants originating from host plants. A sampling of their joint publications is in the 'Literature' section at the end of the proposal.

Table 1. Tentative timeline for our 3-year research project.

Date	Activity
Summer 2014	1) Collect hawkmoths from SE North Dakota using established protocols 2) Establish rearing methods for captured hawkmoth species 3) Make pheromone collections 4) Make collections of orchid volatiles 5) Conduct GC/EADs to isolate potential attractants
Fall/Winter/Spring 2014/2015	1) Chemically identify candidate chemicals 2) If insects are available, use antennae to continue to explore extracts for candidates and conduct wind tunnel studies testing attraction
Summer 2015	1) Develop methods to deploy attractants in traps and complete wind tunnel trials. 2) Collect hawkmoths from SE North Dakota using established protocols 3) Establish rearing methods for new hawkmoth species 4) Make pheromone collections for new moth species 5) Make collections of orchid volatiles 6) Conduct GC/EADs to isolate potential attractants
Fall/Winter/Spring 2015/2016	1) Chemically identify candidate chemicals 2) If insects are available, use antennae to continue to explore extracts for candidates and conduct wind tunnel studies testing attraction
Summer 2016	1) Develop methods to deploy attractants in traps and complete wind tunnel trials. 2) Chemistry to identify candidate chemical 3) Collect volatiles from host plants
Fall/Winter/Spring 2016/2017	1) Write publications

Evaluation – Describe your plan to document progress and results. *
Each attractant chemical that is identified and synthesized will be a measure of success. Given that we have the aim of finding attractants for five hawkmoth species (attractants being sex pheromones and/or plant volatiles), finding five attractant chemicals or attractant blends of chemicals will be our specific measure of success. Each discovery of an attractant and associated behavioral responses will be published in a peer-reviewed scientific journal. An additional measure of success will be the development of a trapping system that will deploy attractants in a manner that allows non-destructive sampling of hawkmoth pollinators. Yearly census of hawkmoth populations will also be published as will experimental studies to test the efficacy of interventions to enhance pollinator populations.

Financial Information (Detailed Budget Narrative follows)

Project Expense	OHF Request	Applicant's Match Share (Cash)	Applicant's Match Share (In-Kind)	Applicant's Match Share (Indirect)	Other Project Sponsor's Share
Salaries	$101,628	$62,214			
Fringe Benefits	$3,048	$18,665			
Equipment = > $5,000	$8,000				
Travel	$25,549				
Supplies	$50,675				
Fees	$4,320				
Publications	$4,000				
Subawards					
Other Direct Costs					
Unrecovered Indirect Costs				$85,149	
Total Project Costs	$197,220	$80,879	0	$85,149	0

√ I certify that a project budget will be sent to the Commission*

Sustainability *

The goal of our research is to identify attractants for hawkmoth pollinators that can be conveniently deployed in traps to provide a yearly census of pollinator populations or to design and evaluate interventions to conserve and recover pollinator populations. We have every expectation to achieve this goal within the three years allotted to the project. Once the attractants are available and a trapping system developed, we expect to partner with land stewards (ranchers, farmers, Dakota Prairie Grasslands, and the Nature Conservancy) so that the work is done locally. Since using the tools we develop will be relatively simple, a nice approach would for local, state, or federal funds to be used to hire local high school or college students in the summer. This would be an inexpensive way to monitor the health of pollinators, which would occur in parallel with federally mandated 'orchid counts' conducted by Dakota Prairie Grasslands during peak orchid flowering in July of each summer.

Partial Funding *

Identification of behaviorally-active chemicals is not an easy task, requiring both intensive chemistry in the lab and extensive behavioral testing in the lab and field and will not be accomplished in less than three years. This means there is little 'wiggle room' in the budget. The graduate student is needed to do the research under the supervision of Drs Foster and Harris. The hourly workers are needed to rear insects and host plants and the greenhouse fees are necessary for growing plants and housing insects. The electroantennogram equipment is essential for identifying the chemicals. The chemical supplies are essential for making extracts, and the pumps required for collecting volatile chemicals from orchids in the field. Travel between Fargo and field sites is essential. If necessary, funds budgeted for travel to scientific meetings could be reduced from $15,658, for 3 years, to $5,000 for one year, the last year of the project when we will have lots of results to report. The $4,000 budgeted for publications could be reduced to $3,000.

Detailed Budget Narrative

Total Funds Requested = $197,220

1. Salaries (Total = $101,628 for three years)

A. **$61,818 for one PhD student at $20,000/year at 3% inflation/3 yrs**
The student will be trained in natural products chemistry and responsible for making extracts for chemical analysis, behavioral and electrophysiological assays of extracts, chemical analysis via Gas-Chromatography/Mass Spectrometry, and synthesis of behaviorally-active chemicals. He/she will also be responsible for overseeing collecting and rearing of insects and host plants,

B. **$39,810 for two hourly workers**
The student hourly workers will help collect and rear insects and host plants in the laboratory and greenhouse. They will be paid $11.50/hour for 560 hours/year at 3% yearly inflation over 3 yrs.

2. Fringe Benefits (Total = $3,048 for three years)

A. $1,855 for benefits (3%) for one PhD student over 3 years

B. $1,194 for benefits (3%) for two hourly student workers over 3 years

3. Equipment (Total = $8,000 for one year)

A. Electroantennogram equipment ($8,000) purchased in first year
The insect antennae is well-known for its perfectly tuned ability to sense odors that are important for finding mates (sex pheromone) or finding host plants for larvae to feed on (kairomones). This means that the insect antennae can also be used by researchers to identify behaviorally-active chemicals by running a

chemical extract through gas chromatography to separate out the chemical components and then use electrical output from the insect antennae to identify the chemical in the extract that is detected by the antennae and controlling attraction. We will purchase electroantennogram equipment for coupled gas chromatography-electoantennogram detection analyses and isolation of behaviorally active volatile chemicals from Syntech Inc (Hilversum, Netherlands). The equipment includes pre-amplified probes, an A:D converter and amplifier, and the volatile chemical delivery system.

4. Travel (Total = $25,549 for three years)

A. **$10,094 Travel to North Dakota field sites for three years**
The western fringed prairie orchid is found in only one location in North Dakota, i.e., the southeastern part of the state, ca. 10 miles east of Lisbon.
Driving back and forth from Fargo, plus driving around the Sheyenne Grasslands to various sites is a 200 to 250-mile round trip. Moths start flying as early as May and continue flying through September. This would allow about 25 trips over the five-month period, at five trips a month (5000 miles per year). We have assigned to us a State Fleet SUV, which has a cost of $0.64/mile.

B. **$15,455 Travel by Researchers**
Budgeting $5000 (plus 3% inflation) per year would allow 3-4 people to attend a state or regional meeting and two people to attend a national meeting (for example: North American Congress on Conservation Biology, American Orchid Society).

5. Supplies (Total = $50,675 for three years)

A. **$37,091 for 3 years of chemistry supplies ($12,000 year plus inflation)**
Chemical analysis of natural compounds is an expensive process. The requested funds would covers the costs of chemical analysis including chromatographic gases, columns and inlet liners, adsorbents, air filters and vessels for collecting volatiles, glassware, including connectors, vials, collection jars, syringes, and solvents.

B. **$3,000 for two portable pumps purchased in first year**
Some of the plants that produce volatiles that attract our pollinators are rare or threatened species. This means that we will need to collect volatiles from plants growing in the field, an example being the western prairie fringed orchid. To collect volatiles we will need to purchase two portable stainless steel pumps and four needle valve air-flow controllers ($1500/pump, Grainger Industrial Supply).

C. **$2,500 for 10 light traps purchased in first year at $250/trap**
Light traps are used to collect live moth pollinators, which fly at night and are attracted to black lights. Traps will be purchased from BioQuip Products (Rancho Dominquez, California), which includes black light, bucket trap, photoelectric switch, DC battery, Plexiglas panels and metal top.

D. $1,902 for 30 net traps purchased in first year at $63.40/trap

Net traps are used to collect live moth pollinators by placing them above attractive plants, for example the western prairie fringed orchid. Traps will also be used to field test the attractive chemicals that we identify, using a randomized complete block design at three sites, testing two treatments, the putative attractive chemical versus a control solvent. Traps will be purchased from BioQuip Products (Rancho Dominquez, California).

E. $6,182 for rearing supplies for insects for three years

Materials would include: cages for insects, pots for plants, soil, fertilizer, and purchase of perennial and annual host plants, insect diet, and materials to build field traps.

6. Fees (Total = $4,320 for three years)

Over the three years, a greenhouse room in the new NDSU Greenhouse facility will be needed for rearing plants and insects. Rental for a 200 square foot room is $120/ month x 36 months = $4,320.

7. Publications (Total = $4,000 for three years)

We expect to publish four scientific papers over the course of the three years. Since the research is on attractants for insects, good places to publish are journals of the Entomological Society of America. Page charges for an article of average length (10-15 pages) is $1000.

8. Subawards- None
9. Other Direct Costs- None
10. Unrecovered Indirect Costs- $85,149

Non-federal Matching Funds

Source of Matching Funds: North Dakota Agricultural Experiment Station and North Dakota State University

1. Salaries and Fringe Benefits Matching

Drs. Stephen Foster will devote 10% of his time during each of the three years of this project. Dr Marion Harris will devote 8% of her time. Their salaries and fringe benefits total $80,879. Dr Foster has over 25 years experience as a natural products chemist and will be responsible for identifying the chemical attractants using coupled gas-chromatography/ electroantennogram detection. One of his current research projects is identifying volatile chemical attractants for the blue orchard bee, *Osmia lignaria*, is a commercially- developed alternative pollinator for the honey bee, *Apis mellifera*. Dr Harris has over 25 years experience researching insect-plant interactions, in particular insect mate- and host- finding behavior. She will be responsible for developing behavioral assays to test the attractiveness of plant and insect extracts in the laboratory and field. In these respective roles, Dr Foster and Dr Harris have collaborated many times in the past, the result being the identification of number of insect attractants, i.e., sex pheromones originating from insects and chemical attractants originating from host plants.

Non-federal Matching Funds (continued)

2. Indirect Costs Matching
NDSU's federally approved indirect cost rate is 45% of modified total direct costs, thus: $197,220 minus equipment ($8,000) = $189,220 * 45% = $85,149.

Awarding of Grants

All decisions on requests will be reported to applicants no later than 30 days after Industrial Commission consideration. Applicants whose proposals have been approved will receive a contract outlining the terms and conditions of the grant. Please note the appropriate sample contract for your organization on the website at http://www.nd.gov/ndic/outdoor-infopage.htm that set forth the general provisions that will be included in any contract issued by the North Dakota Industrial Commission. Please indicate if you can meet all the provisions of the sample contract. If there are provisions in that contract that your organization is unable to meet, please indicate below what those provisions would be.

Should this project be selected for funding and a contract is issued to NDSU, we request the sample language in article 11 and 20 be deleted and the following replacement language be inserted in articles 11 and 20 of the sample contract for a state entity.

11. Ownership of Work Product, Equipment and Materials
Title to all inventions and discoveries made solely by Contractor inventors resulting from the Agreement shall reside in Contractor; title to all inventions and discoveries made solely by Commission inventors resulting from the Agreement shall reside in Commission; title to all inventions and discoveries made jointly by Contractor and Commission inventors resulting from the Agreement shall reside jointly in Contractor and Commission. Inventorship shall be determined in accordance with U.S. Patent Law.

20. Compliance with Public Records Law
Contractor understands that, except for disclosures prohibited in this Agreement, the Commission must disclose to the public upon request any records it receives from the Contractor. Contractor further understands that any records that are obtained or generated by the Contractor under this Agreement, except for records that are confidential under this Agreement, may, under certain circumstances, be open to the public upon request under the North Dakota open records law. Contractor agrees to contact the Commission immediately upon receiving a request for information under the open records law and to comply with the Commission's instructions on how to respond to the request.

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NDSU NORTH DAKOTA STATE UNIVERSITY

GRANT APPLICATION TRANSMITTAL

This page indicates university endorsement of the referenced proposal and is intended to be submitted to the sponsor organization.

Sponsor Organization:	**ND Industrial Commission**
Project Title:	Behavioral Attractants for Monitoring Important Insect Pollinators of North Dakota
Principal Investigator/	**Stephen Foster**
Department:	Entomology
Project Budget:	
Direct Costs $197,220	
F&A N/A	
Total Project	$197,220
Authorized University Representative:	Amy B. Scott
Title:	Assistant Director, Sponsored Programs Administration
Address:	North Dakota State University 1735 Research Park Drive Fargo, ND 58105-5756
Phone:	(701) 231-8045
Signature:	Amy B Scott
Date:	11-25-13

Any future notifications regarding this proposal, including award notices, should be directed to the authorized university representative at the address listed above.
Thank you.

NDSU NORTH DAKOTA STATE UNIVERSITY

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21 November 2013

North Dakota Industrial Commission
ATTN: Outdoor Heritage Fund Program
State Capitol- Fourteenth Floor
600 East Boulevard Ave, Dept 405
Bismarck, North Dakota 58505

Dear North Dakota Industrial Commission,

Enclosed is our application for the Outdoor Heritage Fund Program titled "Behavioral Attractants for Monitoring Important Insect Pollinators of North Dakota". Our proposal addresses Directive B "Improve, maintain, and restore water quality, soil conditions, plant diversity, animal systems and to support other practices of stewardship to enhance farming and ranching."

We have also enclosed a reprint of our recently published article on the hawkmoth pollinators that will be studied if we receive research funds through the Outdoor Heritage Fund Program.

We have not enclosed paperwork from Research Administration at North Dakota State University because this paperwork is still being processed. Once we receive this paperwork, we will send via email a PDF of it, along with an electronic Word file of the application, and a PDF of the research article.

Please contact me by email (Stephen.Foster@ndsu.edu) if you have questions or additional requests for information.

Sincerely,

Stephen P. Foster
Professor, Department of Entomology

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