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### ADMIN: Reason(s) Not Eligible

## John Z. Duling Grant Application

## Please note: This application is available for viewing year-round, but may only be submitted July 1 - October 1.

If you have any questions, please email dhettinger@treefund.org or call 630-369-8300 x204.

# Applicant

## Principal Investigator

Prefix	Dr.
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Last name	Cranshaw
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CranshawWhitney	'
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Email address	whitney.cranshaw@colostate.edu
Phone number	970-491-6781
Degrees	<ul> <li>B.A. (1976) Hampshire College, Amherst, Massachusetts</li> <li>M.S. (1979) University of Minnesota (Entomology) Thesis title -</li> <li>"Contributions to potato pest management in the Red River Valley"</li> <li>Ph.D. (1981) University of Minnesota (Entomology) Dissertation title</li> <li>- "Integrated control of insect contaminants of processed peas"</li> <li>Minors - Plant Pathology, Horticulture</li> </ul>
Relevant citations authored	<ul> <li>Minors - Plant Pathology, Horticulture</li> <li>Books:</li> <li>Cranshaw, W. (2015). Garden Insects of North America. Princeton University Press. Princeton, NJ. 678 pp.</li> <li>Cranshaw, W., D. Leatherman, W. Jacobi and N. Tisserat. (2014).</li> <li>Insects and Diseases of Woody Plants of the Central Rockies.</li> <li>Cooperative Extension Bulletin 506A.</li> <li>Articles:</li> <li>Cranshaw, W. (2011). Recently recognized range extensions of the walnut twig beetle, Pityophthorus juglandis Blackman (Coleoptera: Curculionidae: Scolytinae), in the western United States. The Coleopterists Bulletin 65(1) 48-49. doi: http://dx.doi.org/10.1649/0010-065X-65.1.48</li> <li>Cranshaw, W. and M. Camper. (2007). Management of poplar twiggall fly on nursery-grown aspen. Journal of Environmental Horticulture 25(1): 33-35.</li> <li>Cranshaw, W. and N. Tisserat. (2010). Thousand Cankers Disease: A Recently Recognized Threat to North American Black Walnut.</li> <li>Arborist News 19 (1): 52-56.</li> <li>Sclar, D. C., and Cranshaw, W. S. (1996). Evaluation of new systemic insecticides for elm insect pest control. Journal of Environmental Horticulture, 14, 22-26.</li> <li>Sclar, D. C., Gerace, D., and Cranshaw, W. S. (1998). Observations of population increases and injury by spider mites (Acari: Tetranychidae) on ornamental plants treated with imidacloprid. Journal of Economic Entomology, 91(1), 250-255.</li> <li>Eckberg, T. B., and Cranshaw, W. S. (1994). Occurrence of the Oak Rough Bulletgall Wasp, Disholcaspis quercusmamma (Walsh) (Hymenoptera: Cynipidae), as a Street Tree Pest in Colorado. Journal of the Kansas Entomological Society, 290-293.</li> <li>Leslie, C. A., Seybold, S. J., Graves, A. D., Cranshaw, W., and Tisserat, N. (2009). Potential impacts of thousand cankers disease</li> </ul>
	on commercial walnut production and walnut germplasm conservation. In VI International Walnut Symposium 861 (pp. 431- 434). Tisserat, N., Cranshaw, W., Leatherman, D., Utley, C., and Alexander, K. 2009. Black walnut mortality in Colorado caused by the walnut twig beetle and thousand cankers disease. Online. Plant Health Progress 11 August, 2009. doi:10.1094/PHP-2009-0811-01-
Has this investigator previously received funding from the TREE Fund?	No

## CranshawWhitney

project?

Previous TREE Fund awards N/A

## Co-Principal Investigator (if applicable)

Prefix	Mrs.
First name	Rachael
Last name	Sitz
Status	Other
Title	Graduate Research Assistant (PhD student)
Organization	Colorado State University
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Phone number	402-990-2133
Degrees	B.S. (2011) University of Nebraska-Lincoln (Plant Biotechnology and Insect Science) undergraduate thesis title- Evaluation of tetraploid switchgrasses for resistance to the greenbug (Schizaphis graminum) M.S. (2013) Colorado State University (Entomology) thesis title- Research on the walnut twig beetle, Pityophthorus juglandis, including establishment of lower lethal temperature limits, measures to sanitize infested felled logs, and techniques to sterilize beetles of Geosmithia morbida
Relevant citations authored	Luna, E.K., Sitz, R.A., Cranshaw, W.S., and N.A. Tisserat. (2013). The effect of temperature on survival of Pityophthorus juglandis (Coleoptera: Curculionidae). Environ. Entomol. 42(5):1085-1091.
Has this investigator previously received funding from the TREE Fund?	No
If yes, was the funding for this project?	No
Previous TREE Fund awards	N/A

## Students/Interns (if applicable)

## CranshawWhitney

Name

Department or major

Status

#### Student/Intern 2

Name

Department or major

Status

### Student/Intern 3

Name

Department or major

Status

Project	
Project title	Improving Management Tools for Drippy Blight of Red Oaks
Research area	Plant health care Urban forestry
Project summary	Our project aims to provide the first thorough depiction of drippy blight disease affecting red oaks in the Colorado Front Range. Drippy blight is a striking disease where the unique association between a kermes scale insect and a bacterium contribute to severe tree decline. This research will increase knowledge of the bacterial associate by detecting means of disease dissemination, modes of infection, and determining if the bacteria is systemic. Other investigations will establish the insect host range, detail the life history and biology of the kermes scale insect, and explore the feeding damage to tree tissues. A method to mitigate the spread of drippy blight is also central to this research along with keeping pin oak and red oak as viable planting options.
Statement of problem	<ul> <li>Drippy blight is a recently recognized condition that seriously affects several kinds of red oak (e.g., Quercus rubra, Q. palustris).</li> <li>Approximately one thousand drippy blight affected oak trees were documented last year by municipal foresters in Boulder and Denver, Colorado. This condition appears to be caused by the combined activity of two organisms, a kermes scale insect (Allokermes rattani) and the bacterium Lonsdalea quercina. Symptoms on Northern red oak and pin oak include branch dieback, stunted growth, and production of witches' brooms; in severe cases it contributes to such substantial tree decline that removal is indicated. Furthermore, the massive oozing of bacteria from infected branches creates sticky film on leaves and surfaces on which it drops.</li> </ul>

Lonsdalea quercina has an extensive host range, as it is also present on California live oaks (Q. agrifolia and Q. wislizenii), Spanish holm and Pyrenean oaks (Q. ilex and Q. purenaica respectively), and hybrid poplar (Populus x euramericana). The bacterium has previously been classified as Brenneria quercina and Erwinia quercina.

Infection of oak trees with Lonsdalea quercina has been reported from Spain, California, and Colorado. However, in other sites the bacterium is associated with acorns, producing a condition known in California as "drippy nut". The extensive involvement of twigs and branches, and the apparent involvement of a kermes scale that has been observed in Colorado constitutes a very different disease and one that is much more damaging to host plants.

Drippy blight has spread steadily through red oak plantings in the greater Denver Metro area (including Boulder) and has caused extensive damage to many plantings, with tree removals associated with this problem increasing in recent years. Due to its increasing widespread occurrence and the serious damage being sustained, several communities are looking for management options. Unfortunately, progression of drippy blight symptoms have not been consistently well managed by applications of neonicotinoids or horticultural oils directed at the kermes scale. There are several possible reasons for such failures including poor treatment timing, coverage failures, use of insecticides that are ineffective against kermes scales, and complications from involvement of a bacterium along with an insect in producing disease symptoms. Identifying suitable control measures for drippy blight is central to this project. In addition, project objectives include studies that will allow foresters a better understanding of the causal agents and how they produce disease, which can have important implications in developing management strategies. Studies focused on the scale insect will establish its host range, document the life history and biology, and examine the feeding damage on oak branches. We will research the nature of the disease, the relative contributions and interactions of the two organisms (A. rattani, L. guercina), the means of its spread. To address these objectives, research is proposed that will help improve health of red oaks where this disease becomes established.

Drippy blight has emerged as the most important pest problem affecting red oaks grown in Colorado. Communities in the Front Range lack a variety of options for shade trees, and red oaks are a highly valued group used to diversify shade tree plantings. Many diseases and insect pests have already demonstrated the risks of single species or genera plantings in this region, including Dutch elm disease, thousand cankers disease of black walnut, and emerald ash borer.

Currently, we have observed kermes scales on Northern red and pin oaks, which are the only species in the red oak group widely planted in the region. It is essential to protect this group of trees. It is also critical to further the information available on the scale insect causing the damage. Not only does drippy blight endanger oaks in the Front Range, but it also poses a potential risk to red oaks elsewhere in the state and country. If drippy blight spreads, it could potentially colonize other species in the red oak group that are widely

Significance of your proposed project as it relates to the profession of arboriculture or urban forestry Description of what is currently known about proposed project area planted street trees throughout the United States and an important part of North American native forests. If left uncontrolled, drippy blight could compromise red oaks. It is important to increase our understanding of this disease before it spreads further, and the Front Range provides an opportunity.

Kermes scales are an understudied yet economically important group of insects (Turner et al 2005). Of the resources on the insect family Kermesidae, only MacGillivray (1921), Balachowsky (1950), and Bullington and Kosztarab (1985) mention Allokermes rattani. The information available is limited to a description of post-reproductive females (Bullington and Kosztarab 1985) and collection information. Allokermes rattani was collected in southern and central California, Santa Catalina and Santa Cruz California respectively. In Santa Catalina the scale was found on canyon live oak (Q. chrysolepis), and chiquapin (Chrysolepis chrysopylla and C. sempervirens), whereas in Santa Cruz it was found on canyon live oak. This is the first potential documentation of A. rattani feeding on trees in the red oak family, and would be the first record of Rattan's kermes in Colorado.

Our preliminary observations on the biology of A. rattani indicate that it has a one-year life cycle. First instars, or crawlers, are mobile after eclosion in September and move to protective crevices within the bark to overwinter (i.e. bud scars, yearly growth rings, wounds, furrowed areas). As soon as new growth begins, usually in April and May, female scales begin migrate and the majority appear to settle at the base of new season growth. Furthermore, histological techniques including fixation and embedding protocols have been established for oak branches containing settled adult kermes scales in order to explore their feeding damage.

In low infestations of kermes scales, control tactics include removing infected branches by hand and encouraging natural predation (Turner et al 2005). For heavy infestations, Turner and Buss (2005) recommend monitoring for nymphs then using horticultural oils or insecticides. Acephate was the most effective chemistry when compared to bifenthrin, imidacloprid, and horticultural oil, on a control trial with A. kingii (Turner and Buss 2005), but this insecticide cannot be used at all of our experimental sites. Azadiractin products have been effective on other hard scales (Juang-Horng et al 2015), and therefore we would like to set up experiments testing azadiractin.

To our knowledge, only one trial has been conducted to concurrently manage two organisms. Fettig et al (2013) used emamectin benzoate and propiconazole to treat mountain pine beetle infested trees. Both the beetle and blue stain fungus were targeted with their combination treatment. Management of drippy blight may similarly involve a novel use of concurrent controls for two organisms, in this case a scale insect and a bacterium. Our preliminary laboratory studies used the antibiotics oxytetracyline, streptomycin, and kasugamycin to evaluate in vitro bacterial sensitivity. Two hundred parts per million of active ingredient was placed onto a confluent L. quercina colony ( $6.5 \times 105$  colony forming units). Results showed the oxytetracyline as the most effective antibiotic (P<0.05 Students t) as it inhibited bacterial growth by 59 percent when compared to the

control. Due to the promising in vitro results, we would like to try field experiments with oxytetracycline as well.

Information is fairly limited on the bacterial causal agent of drippy blight. Researchers have documented drippy nut in California on live oak (Hildenbrand and Schroth 1996; Swiecki and Bernhardt 2006), drippy blight in Colorado on red oaks (Snelling et al 2011; Ibarra Caballero et al 2014), oak decline in Spain on oaks (Biosca et al 2003), and in both Hungary and China on poplar (Tóth et al 2013; Li et al 2014 respectively). The classification of the bacteria and phylogeny including relationships between the subspecies of Lonsdalea quercina is described (Brown et al 2000; Brady et al 2012), the genome has been sequenced (Ibarra Caballero et al 2014), Lonsdalea virulence factors were documented (Yang et al 2014), and bacteria detection methods were found (Poza-Carrión et al 2008; Shang et al 2014).

Although Lonsdalea quercina occurs in California, it affects oaks differently in Colorado. The disease is associated with oviposition wounds of a cynipid gall wasp and nut feeding weevils in California and symptoms include frothing or oozing from branches and acorns. Most of the tree damage is incurred on the acorns, and tree decline is minimal (Swiecki and Bernhardt 2006). In Colorado, kermes scales feed expansively on the entire tree. They are most commonly found on small branches in the canopy, but can also infest large diameter branches and the trunk. Symptoms are primarily seen in the form of extensive twig flagging, which can cause progressive decline of trees.

Numerous questions remain in regard to the insect and bacterial causal agents of drippy blight.

All references are available upon request.

Summary of project goals Scale insects are important pests of many ornamental, forest, and fruit and nut production trees. The current chemical regime is not working to suppress kermes scales in Colorado and there is not an available control for the bacterial causal agent, necessitating this research to find an effective solution. More accurate predication of scale life stage, knowledge of its feeding wounds, and better timing of pesticide applications would aid in management decisions. Our preliminary life history information is central to making sound management decisions, but it needs to be repeated to account for year-to-year variation. Currently, the tissues kermes scales feed on are unknown. Histological studies would shed light on feeding wounds made by kermes scales. Moreover, data is lacking on management for either of the two organisms that produce the drippy blight disease. Therefore, a primary goal of this project is to identify new uses of existing products in order to better manage drippy blight. Screening oaks (Quercus) to determine the host range of this insect is also of interest and will apprise the risk to other important trees in our region. We would like to expand on the current knowledge of this disease by exploring the modes of bacterial entry into the host plant, determine whether or not the bacteria can move systemically in the plant, and characterize the modes of bacterial dissemination. Answering these questions will aid in our ability to decrease

Description of measurable outcomes expected

Project plan including design, hypotheses, methodology and analyses It is essential to understand drippy blight disease in order to provide suitable controls, and currently green industry representatives in Colorado need clear recommendations for drippy blight treatments. Arborist Stakeholder groups with an interest in drippy blight include arborists, foresters, nursery growers, garden managers, chemical companies, tree care businesses, landscape and garden professionals.

Limited information is available on A. rattani life history; therefore this research can be used to target the most vulnerable life stage of the insect and guide best management practices. More accurate predication of scale location, insect feeding habits, and pesticide efficacy would lead to better predictions of pesticide application and timing.

The results of this research could be applied to similar disease dynamics or pests related to Allokermes rattani and Lonsdalea quercina. Information about A. rattani will also serve as a management guideline for other gall like scales. Furthermore, the antibiotic efficacy information can be applied to other Lonsdalea quercina bacterial infections.

A method to mitigate the spread of drippy blight is central to keeping pin oak and red oak as viable planting options. It would benefit oak nurseries and landscape suppliers to maintain or expand the market they currently have for oaks rather than see their market share lost to drippy blight.

Life History and Biology of Allokermes rattani:

An understanding of the life history of A. rattani is fundamental to the development of suitable management methods. Our initial life history study has documented the seasonal life history of A. rattani, the immature overwintering locations, and the placement of settled adult females on the oak branches. Further observations are needed to confirm and expand upon these basic outlines of life history and habit.

Methodology and Design: First instar overwintering locations and placement after a second movement in the spring will be documented on approximately 50 cm of infested red oak branches. From May through October weekly collections at two sites in Boulder, CO will document insect life history and disease progression. Cutting colonized branches will provide female specimens while male specimens will be obtained with an aspirator from the main trunk or reared in the lab from pupal cases. Analysis: Percentages will be calculated to determine the relative amount of immature scales in each overwintering location. An ANOVA will also be performed to determine if there are any differences between the number of scales in each overwintering location.

Chemical Control Trial:

To date, efforts by tree care companies and municipalities to chemically control progression of drippy blight have been unsuccessful. Part of the objective to determine the appropriate management of this disease complex is addressed with improvements in understanding the life history and seasonal distribution of the insect (aforementioned project). To more fully address this objective, we will evaluate systematic treatments for their efficacy against both the insect and bacteria. Methodology and Design: Several chemicals will be tested to determine their effectiveness controlling drippy blight. The experiment will include the following treatments: untreated control, an insecticide (acephate, dinotefuran, and/or azadirachtin), an antibiotic (oxytetracycline), and a combination of the insecticide with the antibiotic. Chemical treatments will be injected by an ISA certified applicator in late spring when the nymphs are beginning to move onto new growth.

At two time points throughout the 2016 growing season, four branch samples consisting of at least five years growth will be taken from each tree. Branch samples will be transported to a laboratory at Colorado State University where the number of scales will be counted, and their life stage and placement on the branch will be documented. Data will also be taken in early spring 2017 in order to quantify the number of overwintered immature scales and their placement (i.e. the growth year and anatomical part of the branch they settle on).

Analysis: An ANOVA will be used to detect differences in scale abundance on trees treated with the varying chemical treatments. Trends in the location of kermes scales throughout the branch will also be analyzed.

Insect Host Range:

Allokermes rattani has been documented on two genera within the Fagaceae family, Chrysolepis and Quercus. Within the genus Quercus, this scale is reported to feed on both red and white oaks, although in Colorado A. rattani and associated development of drippy blight has only been noted on Northern red and pin oak. This objective will evaluate the ability of A. rattani to colonize other Quercus species that may be regional candidates for use as alternative shade trees.

Methodology and Design: Branch sections containing venters full of developed eggs will be taken from infested oaks in September or October. These venters will be placed at the base of various Quercus spp. allowing crawlers to move onto and establish on the candidate host plants. Species of interest that are potentially important regionally will be the primary focus of this study (e.g., Q. gambelii, Q. macrocarpa, Q. muehlenbergii, Q. coccinea, Q. palustris, Q. rubra, and Q. schumardii).

Analysis: Host suitability will be measured by determining the relative number of scales able to complete development on the tested hosts, and they will be compared using an ANOVA model. Description of the Feeding Damage Associated with A. rattani: Kermes scales are known to damage trees in large numbers by penetrating plant twigs with their stylets, but their feeding is uncharacterized. Our goal is to visualize feeding tracks and cell changes in order to determine what plant tissues are damaged and the nature of the injury. Due to the extensive flagging and subsequent branch abscission on drippy blight inflicted trees, we hypothesize the feeding occurs in the xylem, and that stylet penetration is not superficial. This objective proposes use of histological tools to establish the nature of plant injury associated

with the feeding site of A. rattani.

Methodology and Design: Fixation and embedding protocols are developed for our samples. Approximately twenty twig samples containing a feeding adult A. rattani will be fixed in a series of weeklong ethanol soaks. Cut samples will be immediately submerged in 30% ethanol for one week, and then exposed to a week in each of the following ethanol grades: 50, 70, 90, 95, and 100 percent. Samples will be embedded in hydroxypropyl methylacrylate plastic then sliced in 5  $\mu$ m increments using a microtome. Sections will be stained with toluidine blue to visualize both plant and scale cells. 3-D reconstruction will be done in order to better visualize the amount of tissue affected.

Analysis: Healthy and diseased cells will be identified, and with 3-D reconstruction we can determine the average area of affected tissues.

Lonsdalea quercina Dissemination:

Although Allokermes rattani feeding sites frequently are points where bacterial ooze emerges from plants, the limited mobility and feeding method of this insect seem to make it a poor candidate for transmission of the bacteria within and between trees. However, the bacterial exudates of Lonsdalea guercina are attractive to many more mobile insects, including various hymenopterans (ants, bees, wasps, and hornets), beetles and flies. Also, several species of treehoppers are associated with red oaks and these are species that can produce twig wounds during oviposition. Preliminary data shows L. guercina is present on several insects associated with red oak and this objective will seek to determine which species are most important in spread of the pathogen between and within trees. Methodology and Design: Insects associated with at least two diseased oaks will be collected weekly. Trapping methods have been identified where a subset of the insects fall into Lindgren funnel traps and others are captured on or flying around the tree. DNA extractions will be performed on the sampled insects, then PCR and gel electrophoresis will be used to detect L. guercina. Primers developed by Shang et al 2014 work well to detect bacteria in insect samples collected from traps.

Analysis: The presence or absence of bacteria will be noted for all insects found on and around diseased oaks.

Entry and Host Infection by Lonsdalea quercina:

It is unknown how L. quercina enters hosts and establishes infections. Feeding wounds produced by A. rattani are one possible entry court, although sites of bacterial infection are not consistently associated with scale feeding sites. This objective will seek to determine what are ways that this pathogen can colonize oak. Methodology and Design: Sapling red oaks will be inoculated in several ways with a suspension of approximately 1 x 108 colony forming units of L. quercina. Leaves and branches will be either sprayed with inoculum or disks containing the bacteria will be placed on the tissue. On the main trunk, the bacterial suspension will be injected into a wound or will be surface sprayed. All symptoms will be recorded and cankers will be measured. Water checks will be used as a control.

Analysis: Cankers will be photographed, and canker areas obtained

with an image processing software program. Differences in canker size will be compared using a linear model analysis of variance (ANOVA).

Determination of Distribution and Systemic Movement of L. quercina in Red Oak:

In another bacterial disease infecting trees, fire blight, Erwinia amylovora is able to move through xylem tissues and spread more quickly throughout the plant. It is unknown whether or not L. quercina is able to move systemically throughout the host plant. It is an objective of this study to determine the distribution of L. quercina within trees and to determine if it can develop systemically or is limited to production of localized cankers.

Methodology and Design: Various tissues including the periderm, xylem, phloem, and heartwood will be obtained from oaks showing symptoms of drippy blight will be tested for the bacteria. Tissues at the site of a canker will be sampled, and then tissues from increasing distances away from the canker center will be excised. Additionally, samples from several larger sized branches and the main trunk will be taken. All samples will be placed in individual tubes containing 2 mL of broth (Luria-Bertani or LB) for 48 hours at 25°C. The DNA from organisms that grow in the broth will be extracted, and then L. quercina detection will be based on PCR and gel electrophoresis. We have detected L. quercina in host plant tissue using these techniques.

Analysis: The presence or absence of bacteria will be noted for all of the tissues tested.

The information developed from this project will provide the fundamental resources needed to best manage this emergent disease. In order to supply information, we will present at a professional meeting(s) to raise awareness about the occurrence of drippy blight. The presentation will inform green industry members of our research progress and summarize our data. We will create management guidelines for city foresters and distribute the information at municipal forester meetings. Management guidelines will also be sent out via e-mail on a Front Range green industry list serve, Pestserv-I. Our lab also has strong collaborations with several Extension Agents and the State Forest Service Entomologist who can help disseminate the information to other parts of the state. We expect multiple educational materials and scholarly publications to materialize from this research. Our information will be used to update the Colorado State University Extension website by adding a fact sheet entitled "Drippy Blight of Red Oak". My PhD dissertation will include this research, and it will be available through Colorado State University's digital repository. Finally, we project this research to stimulate at least two peer-reviewed journal articles. Data collected to describe the life history, chemical control options, host range, and feeding wounds of A. rattani will contribute to additional journal articles. Furthermore, the information gathered on the disease dissemination and colonization will render further publications. Our manuscripts will be geared toward journals such as Arboriculture and Urban Forestry, Arborist News, Journal Environmental Horticulture, Journal of Economic Entomology, or Forest Pathology.

Description of plan for disseminating the results of this project

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Geographic range of project USA & Canada

# Budget

## Compensation/Stipend

Project completion date

Proposed project budget	\$7,776
Requesting from TREE Fund	\$7,776
Funding from other sources	0
Value of in-kind support from other	0
sources	

#### **Employee Benefits**

Proposed project budget	\$560
Requesting from TREE Fund	\$560
Funding from other sources	0
Value of in-kind support from other	0
0001000	

## Travel (> 50 miles)

Proposed project budget	0
Requesting from TREE Fund	0
Funding from other sources	0
Value of in-kind support from other	0
sources	

#### Local Transportation (< 50 miles)

Proposed project budget	\$1,100
Requesting from TREE Fund	\$1,100
Funding from other sources	0
Value of in-kind support from other sources	0

## Equipment (vehicles, growth chambers, etc.)

#### **CranshawWhitney**

Proposed project budget	0
Requesting from TREE Fund	0
Funding from other sources	0
Value of in-kind support from other sources	0

#### Supplies (paper, ink, toner, etc.)

Proposed project budget	0
Requesting from TREE Fund	0
Funding from other sources	0
Value of in-kind support from other	0
sources	

#### Contract Labor (contractor, speaker, etc.)

Proposed project budget	0
Requesting from TREE Fund	0
Funding from other sources	0
Value of in-kind support from other	0
sources	

#### Other/Misc.

Proposed project budget	\$564
Requesting from TREE Fund	\$564
Funding from other sources	0
Value of in-kind support from other sources	0
Description of other/misc. expenses	Samp (\$10 (

Sampling and field supplies: An order of 30 bare-root sapling trees (\$10 each), 1 pack of histology slides (\$50), 1 pack of microcentrifuge tubes (\$14), and 2 DNA Extraction Kits (\$100 each). These supplies are needed to preform host specificity tests (bare-root saplings), visualize the insect's feeding tracks (histology slides), and detect bacteria in host plant tissues and on associated insects (DNA extraction kits and microcentrifuge tubes). The total budget for materials is \$564.

Notes on the budget:

The University will leverage the following resources to support the project: PI (Cranshaw) time as needed will be covered as part of his University appointment. The PI's department will provide lab supplies

and cover additional travel and printing costs as needed. In addition, the University will cover the indirect costs applicable to this project. In addition, other resources such as salary, travels costs, additional supplies and printing costs will be contributed but will not be quantified or tracked as match. Similarly, in-kind support is being contributed, but not being tracked as match.

#### Total

Proposed project budget	10000
Requesting from TREE Fund	10000
Funding from other sources	0
Value of in-kind support from other sources	0
Funds already received from other sources	0
Funds pending from other sources	\$25,302
Value of in-kind support already received from other sources	0
Value of in-kind support pending from other sources	0

How did you hear about this	TREE Fund website
grant?	

Applications will be scored on the following scale:

- Applicant is qualified (0-10)
- Applicant has experience (0-5)
- Project has potential to result in transformative research ideas or approaches (0-5)
- Project directly meets one or all TREE Fund priorities (0-10)
- Project has clearly stated need (0-10)
- Project is clearly linked to arboriculture and/or urban forestry (0-5)
- Research has practical application (0-10)
- Project design is scientifically sound, methods are clear and analysis is appropriate (0-15)
- Project is likely to result in peer review publication (0-10)
- Objectives are achievable within proposed time frame (0-5)
- Objectives are achievable within proposed budget (0-5)
- Requested funds have potential to leverage future support from other funding sources (0-10)
- NOTE: proposals with documented cash or in-kind match will be awarded an additional 1-5 points

#### Your application will not be available for editing after it has been submitted. Please review your application for completion before submission.