**Problem:** Crapemyrtle bark scale (CMBS), *Eriococcus lagerstroemiae* or *Acanthococcus lagerstroemiae* (Kuwana) (Eriococcidae), is an emerging new pest threatening the production and landscape use of crapemyrtles (Lagerstroemia spp.), a \$66 M/yr wholesale value crop (USDA NASS 2015). CMBS was recognized among the top 9 pests reported in the past two years by the Greenhouse Grower magazine and was listed as one of "key management arthropods."

Use of Biological Control. Biological controls and beneficial insects. Biological controls may provide a more sustainable method, compared to chemical treatments, to reduce populations of Crapemyrtle Bark Scale in nurseries and landscapes. In the most recent Pest Management Strategic Plan for Container and Field-Produced Nursery Crops in FL, GA, KY, NC, SC, TN, and VA: Revision 2015 [(PMSP) coordinated by Southern Nursery Integrated Pest Management Working Group], it was noted that "no biological control of the crapemyrtle bark scale is known". Being a native in Asia and only first reported in the U.S. in 2004 in Richardson, TX (Merchant et al., 2014), CMBS has no specialized natural enemies here, and a classical importation biological control approach represents a historically successful method for achieving sustainable, economical, and environmentally safe control of this exotic pest.

The Classic Biological Control to Be Performed ---Heinz/Gu/Woolley. Following the importation protocol outlined by Van Driesche and Bellows (1996), the goals of the proposed work are to: i) obtain and introduce into quarantine candidate natural enemies (with an emphasis on parasitoids), ii) process shipped material to destroy undesirable organisms, and iii) conduct research as necessary in quarantine on natural enemies to define their host associations and biology. The work proposed here will be performed by an entomology doctoral student in the Heinz lab.

Obtain and introduce into quarantine candidate natural enemies and eliminate undesirable organisms. Professor Juan Shi of Beijing Forestry University (see letter of support) will help collect natural enemies from crapemyrtles in three locales with climates similar to the mid-south U.S. Collections will be made during the first two years of the project and on an "as needed" basis thereafter from i) at least three different cultivars, ii) at low, moderate, and high densities of CMBS, and iii) during each the fall, winter, spring, summer. Material will be received into the Texas A&M University Department of Entomology Quarantine facility (see letter of support) and opened by the quarantine officer. This will allow for inspection, separation of contents, and containment of any under-desirable organisms. We have applied for an APHIS-PPQ 526 permit (#P526-170222-017) to move materials collected by Professor Shi to the quarantine facility. Potentially beneficial organisms will be separated by species, collection locality, cultivar, and season. Dr. Gu will provide CMBS-infested crapemyrtles with taxonomic identity like the collection cultivars, and we will initiate rearing of the parasitoids following the methods outlined by Guzmán-Larralde et al. (2013) to prevent parasitoids inadvertently adapting to the rearing environment. Mr. Peter Krauter, quarantine officer for the facility with over 25 years of experience in classical biological control and arthropod mass-rearing, will supervise the quarantine rearing efforts.

Examples of successful classical biological control targeting scale insects on woody ornamental plants (Flanders, 1952; Dreistadt et. al. 2016) leads us to focus our importation efforts on CMBS parasitoids. A sample from each generation of parasitoid rearing will be provided to Dr. James B. Woolley, Professor of Entomology at Texas A&M University and an expert in parasitic Hymenoptera systematics, for taxonomic identification and vouchering. Authoritative taxonomic determinations of parasitic Hymenoptera will also identify any potential hyperparasitoids present. Specimens used for taxonomic identification will be vouchered at the Insect Museum, Chinese Academy of Forestry, Beijing, China; the

U.S. National Museum of Natural History, Washington, DC; and the Texas A&M University Insect Collection, College Station, TX. A sample from each parasitoid generation (of both live and dead insects) will be examined for the presence of unwanted biotic contaminants following the methods outlined by Goettel and Inglis (2006) in order to identify and eliminate contaminants that are potential threats to the parasitoids themselves or the ecosystem. Only healthy appearing parasitoids will be used to start subsequent generations within quarantine. Any diseased organisms will be sent to insect pathologists for identification and hyperparasitoids will be eliminated from cultures.

Conduct research in quarantine on natural enemies to define their host associations and biology. For each parasitoid population, life tables will be constructed following the approach described by Pilkington and Hoddle (2007). Data will be collected from 10-20 parasitoid cohorts maintained in temperature cabinets set to low (15°C), moderate (22.5°C) and high (30°C) average monthly temperatures associated with Richardson, TX (the location of the first CMBS discovery). The goals of these studies are: 1) to assess whether the parasitoids can survive in the target release climate, 2) to compare the reproductive and development biology of the parasitoids, and 3) to assess the impact on CMBS in terms of parasitism and host feeding rates. Results from these studies will be used to prioritize the order of parasitoids for further study and to eliminate those that are poorly adapted to the conditions of their targeted release location.

Estimating host ranges of candidate parasitoids will focus on scales with similar life histories and geographic distributions as CMBS, rather than taxonomic relatedness. The systematics of Eriococcidae is not completely resolved as recent research using ribosomal DNA has shown that the family is not a single monophyletic group but is an aggregation of several different groups (Cook et al., 2002). The Eriococcidae contain taxa exhibiting several different life history strategies. For example, several clades induce plant galls (Cook and Gullan, 2004) that will require that parasitoids of these clades to have adaptations not found in the CMBS parasitoids.

CMBS has the same incomplete metamorphosis as other species in the superfamily Coccoidea (Kondo et al., 2008). The female is paedomorphic, meaning that its form resembles that of a nymph (Gu et al., 2014; Wang et al, 2015). The male turns into an alate without mouthparts after the prepupal and the pupal stage (Wang et al., 2015). We can use this to our advantage as parasitoids adapted to this life history are less likely to attack grossly different groups, such as the Eriococcidae taxa that induce plant galls (Cook and Gullan, 2004). Parasitoids of these clades are likely to have adaptations that make CMBS and similar species unacceptable hosts. Further, our temperature tolerance data will demarcate the possible geographic ranges of candidate releases species. Scale insects outside the physiological ranges of candidate species are not likely to be unintended hosts. For example, some North American species, like CMBS, are tree pests that include European elm scale (Gossyparia spuria (Modeer), Hemiptera: Eriococcidae) which infests American elm and other native elms primarily in the cooler Midwest; azalea bark scale (Eriococcus azaleas Comstock) which attacks four species of azalea, rhododendron, "flowering cherry," and huckleberry in the cooler East and upper Midwest of the United States; and oak eriococcin (Eriococcus quercus (Comstock) Hemiptera: Eriococcidae) which is primarily located in the cooler eastern U.S.

The ability of a parasitoid to locate a host requires several levels of behavioral integration, including responding positively to olfactory cues emitted by the host (Vinson, 1976). Van Driesche and Murray (2004) have suggested testing for responses to olfactory cues could be useful for assisting to define host

range. We will use the "Nomina Insecta Nearctica; a check list of the insects of North America" (Poole, 1997) and Miller and Miller's (1992) treatise on the systematics of Acanthococcus in the Western United states to identify potential non-target scales of similar life form and geographical distribution as CMBS. We will obtain fresh samples of these insects and use them in both choice (non-target vs. blank and non-target vs. CMBS) and no-choice (blank vs. blank and non-target vs. same non-target) two-arm olfactometer studies. Dependent upon the amount of available insect material, our goal is to test 30 individual parasitoids for each comparison, and differences will be detected using paired comparison statistics.

## Potential for Additional Studies (Just a few of many possible examples):

A current master's student working on the factors influencing the abundance and distribution of CMBS and its natural enemies has started to catalogue a rich community of predators and parasitoids. How might these communities provide opportunities and limitations for releases of natural enemies used in future classical biological control programs?

How does the structure of the existing natural enemy community affect population numbers of CMBS? The pattern analysis conducted by the MS student will hopefully provide some insights, but will have completed only one manipulative study at the most.

Using information collected from empirical studies, construct and evaluate one or more mathematical models to assess the risks of imported natural enemies to move onto non-target hosts when released into the field.