

**BEHAVIORAL AND DEVELOPMENTAL RESPONSE OF GRAPE ROOT BORER LARVAE
TO ROOTS OF NATIVE AND COMMERCIAL GRAPE**

Proposed initiation date: July 1, 2007

Proposed project duration: 1 year

Objectives:

1. Compare the suitability of native and commercial grape rootstocks as hosts for newly-hatched GRB larvae
2. Measure preferences of GRB larvae for root sections from native and commercial grape rootstocks

Justification:

The grape root borer (GRB) poses a potentially significant risk to vineyards located near woodlands containing its native hosts, and many vineyards in Virginia are currently infested. Management options for GRB are limited in number and are often of limited effectiveness and/or operationally difficult to implement. There are fundamental gaps in our knowledge regarding the suitability of commercially important rootstocks in Virginia for the establishment and development of GRB larvae. There is no information on the performance of GRB larvae on the roots of native species of *Vitis* and other potential native hosts in the family Vitaceae. This proposal addresses basic questions about the behavioral response of grape root borer larvae to root tissue from different sources and about the survival and developmental rate of larvae on the same food sources. The information generated by this research is intended to improve our understanding of the risk factors associated with GRB infestation and potentially to lead toward new management tactics for it.

Background:

The grape root borer, *Vitacea polistiformis* Harris, can be a serious and destructive pest of commercial grapevines from Pennsylvania to Florida and west to Arkansas and Ohio, and has been a limiting factor in grape production in some southeastern states. Indigenous to the eastern US, larvae of this clearwing moth utilize the roots of native species of *Vitis* as hosts and are believed to be host-specific on members of the family Vitaceae. Bergh et al. (2005) conducted surveys of the abundance of GRB using sex pheromone traps deployed in 19 vineyards in the northern and central portions of Virginia and captured male GRB moths at all sites. Subsequently, Bergh (2006) deployed GRB pheromone traps in five commercial apple orchards that were adjacent to forest but not near commercial vineyards and in five commercial vineyards that were also next to woodlands but not near commercial apple orchards. During a trapping interval that spanned the peak flight period of GRB in Virginia (mid-July – mid-August), averages

of 113 moths/site and 126 moths/site were captured in apple orchards and vineyards, respectively (total captures were 564 and 630 male GRB at orchards and vineyards, respectively). Since GRB has a male:female ratio of 1:1, these trap captures approximate only half of the larval population that were emerging as adult moths in the vicinity of the traps. These data showed that GRB is ubiquitous and abundant in Virginia where wild hosts occur and that all vineyards located near native forest with wild grapes are vulnerable to attack and infestation. Female GRB deposit their eggs on vines, trellis posts and weeds within the vineyard. Larvae hatch from these eggs and burrow down through the soil to locate and establish on vine roots, where they feed for a period of two years. Larval feeding on roots can seriously impair the vigor and yield of vines, cause slow decline that is not readily distinguishable from other causes of vine decline, and can kill vines and entire plantings.

Management options for GRB are limited. Chemical control relies exclusively on soil drench applications of the organophosphate pesticide, chlorpyrifos (Lorsban). This highly toxic and broad-spectrum material is somewhat effective as a barrier to the movement of newly-hatched larvae through the soil, but is disruptive to the ecological balance within the vineyard rhizosphere. Lorsban is scheduled for re-registration review in 2009. Given the recent loss of some organophosphate pesticides (e.g. methylparathion and azinphosmethyl) and increasing restrictions on the use of others (e.g. phosmet) under the Food Quality Protection Act, the future availability of chlorpyrifos remains uncertain. Cultural practices for GRB control, such as berming or the use of plastic ground covers, are disruptive, labor intensive, costly and impermanent. Control using nematodes has been achieved on infested vines in pots, but has been less consistent under field conditions. Mating disruption continues to be explored, but does not account for the possibility of immigration of mated females from native hosts outside a vineyard. The potential for use of resistant rootstocks has also been explored, but there remain fundamental, unanswered questions about the suitability of commercially important rootstocks used in Virginia for the survival and development of GRB larvae. Furthermore, the suitability of roots of native grape and other possible host plants for the survival and development of GRB larvae has not been documented. In 2005, we found that GRB larvae fed and developed on roots of Virginia creeper, *Parthenocissus quinquefolia*, which is also a member of the Vitaceae family. Given that Virginia creeper and native species of *Vitis* are common and abundant components of Virginia forests, understanding their roles in producing and sustaining populations of GRB is an important criterion for risk assessment and vineyard site selection.

My research in 2006 on the attraction of GRB larvae to alcohol-based extracts of grape roots from various sources has revealed statistically significant differences in the behavioral response of larvae to extracts from different cultivars. In choice-test bioassays, larvae responded significantly more strongly to root extracts from 3309 and the native grape, *V. cordifolia*, than to extracts from Virginia creeper, while there was no difference in the response to 3306 vs *V. cordifolia*. However, when extracts of 3309 were paired with extracts of 420-A and *V. riparia*, there was a consistent and significantly greater response to both 420-A and *V. riparia*. Complete results of the extract bioassays are contained within the quarterly reports submitted in 2006. These results suggest biochemical differences among grape hosts that may be

useful in assessing differences in their susceptibility and/or resistance to GRB. Furthermore, identification, purification and/or synthesis of the behaviorally active compounds may ultimately lead to using them in a management tactic based on disrupting the ability of GRB larvae to orient to roots in the soil.

This proposal addresses basic aspects of the host-plant relations and behavior of newly hatched GRB larvae and is intended to provide baseline information that pertains to the potential development of novel management approaches as well as to risk assessment for commercial plantings based on rootstock susceptibility and proximity to native hosts.

Procedures:

Insects. For all of the following experiments, the majority of GRB larvae will be obtained from eggs provided by Dr. John Meyer, North Carolina State University, under an ongoing collaborative agreement. Supplemental sources of eggs and larvae will be generated by capturing mated female moths in commercial vineyards in Virginia and allowing them to deposit eggs in containers in the laboratory. Individual GRB females lay between 200 and 500 eggs. Eggs will be maintained under suitable environmental conditions in small Petri dishes and all experiments will be initiated with larvae that are ≤ 2 -hours-old.

Rootstocks. Based on their relative importance to wine grape production in Virginia, this research will focus primarily on the rootstocks 3309, 420-A and 101-14, but aspects of the work will include *V. aestivalis* ('Norton') and *V. riparia* 'gloire' as well. The native grape, *V. cordifolia* and Virginia creeper will also be used and roots of apple will be used as a non-host control. All roots will be collected from plantings at the Virginia Tech AHS-AREC in Winchester and/or from commercial plantings in northern Virginia. Roots will be used in experiments on the same day that they are collected and will be held in humid and dark conditions between collection and use.

Test arenas. Test arenas will consist of small, white plastic cups (size??) with white plastic lids and will be lined with filter paper discs moistened with distilled water. During testing, the cups with root sections and larvae will be held under dark and humid conditions within a controlled environment chamber at constant 25°C.

Objective 1. Compare the suitability of native and commercial grape rootstocks as hosts for newly-hatched GRB larvae

This test will measure the establishment, survival and growth of GRB larvae on grape roots under "no-choice" test conditions. Individual pieces (4 cm long) will be pruned from field-collected sections of small, fibrous roots and their diameters will be measured. Each piece will be placed individually in a cup and a single larva will be transferred to each. Twenty-five root pieces from each of the following rootstocks will be tested: 3309, 420-A, 101-14, *V. aestivalis*, *V. riparia* 'gloire', *V. cordifolia*, Virginia creeper and apple.

At 24-hr intervals over the first five days and then at 72-hr intervals thereafter (through day 20), the following information will be recorded for each larva: 1. the presence of fresh frass (i.e. larval excrement), 2. the location of the larva (mining root, on root exterior, off of root) and 3. the status of the larva (alive or dead). For larvae that die during the experiment, the head capsule width will be measured at the time they are recorded dead. This measurement will provide an indication of the amount of growth that has occurred. As well, the frass produced by each larva that dies during the experiment will be collected and held individually in small plastic cups to dry. After thorough drying, the frass produced by each larva will be weighed. The amount of frass produced will provide an indication of the amount of feeding that has occurred. The same measurements (i.e. head capsule measurements and dry weight of frass produced) will be recorded from all larvae recovered on day 20.

Statistical comparisons of survivorship (days), developmental rate (head capsule measurements) and feeding activity (frass dry weight) of larvae will be compared among the root sources using analysis of variance and Tukey's HSD tests.

Objective 2. Measure preferences of GRB larvae for root sections from native and commercial grape rootstocks

This test will employ a choice-test protocol to compare the establishment of GRB larvae on pairs of root sections (2 cm long) offered simultaneously within a test arena. The rootstocks for this test are selected based on the results of the root extract bioassays conducted in 2006. It is anticipated that the different behavioral responses shown by larvae to extracts from different roots under choice-test conditions will also be reflected in this experiment, in which they will be presented with pieces of "whole" root. Pair-wise comparisons will include: 3309 vs *V. cordifolia*, 3309 vs Virginia creeper, *V. cordifolia* vs Virginia creeper, 3309 vs 420-A, 3309 vs 101-14, 420-A vs 101-14 and, as a control that includes a non-Vitaceae host, 3309 vs apple. Each pair of root sections will be presented simultaneously in the same white, covered plastic dishes described above. Root sections will be placed in opposing quadrants within each cup and held in place by small pieces of clay. Five larvae (≤ 2 -hr-old) will be transferred to a small circular release area marked on the damp filter paper in the center of each cup and all cups will be covered and held in a controlled environment chamber under the conditions described above. At 24 h intervals for 5 days, the location of all five larvae within each cup will be recorded. Five replicates of each pair combination of rootstocks will be tested.

The paired-sample *t*-test will be used to compare the cumulative number of observations of larvae on each rootstock during the 5-day experiment.

Personnel and Facilities: Chris Bergh, Asst. Professor of Entomology, will act as project leader and will be directly involved in all aspects of this research, including training and supervision of activities of

summer wage employee. Facilities of the Winchester Agricultural Research and Extension Center are available for this research.

Other Entities: There are no other entities directly involved with the proposed research.

Source of Other Funds: No other sources of funds are being sought to support the Objectives proposed herein. A submission (1-12-2007) by C. Bergh, J. Meyer and A. Zhang to the Viticulture Consortium-East titled "Toward the Identification of Behaviorally Active Compounds from Grape Roots that Attract Grape Root Borer Larvae" has requested support to chemically fractionate grape roots extracts and to isolate and assay the compounds that attract GRB larvae. These proposals contain Objectives that are complimentary to one another but that do not overlap.

Budget:

	2007
Personnel Salaries and Fringe Benefits	
0.5% Summer wage employee (\$11.00/hr x 20 hrs/week x 8 weeks)	1,760
Fringe benefits @ 8.5%	150
Materials and Supplies	
Laboratory and field supplies for collecting and maintaining GRB and for conducting bioassays	250
Travel	
Gasoline for lease vehicle \$150.00 month x 2 months	300
Contractual Services	
Lease of VT Motor Pool vehicle for travel to field sites for collection of insects and plant material- \$338/month x 2 months	676
Total Cost	\$3,136

Literature Cited

Bergh, J.C., D.G. Pfeiffer and K.P. Love. 2005. Survey of grape root borer, *Vitacea polistiformis* (Harris), using pheromone traps in Virginia vineyards. J. Entomol. Sci. 40:337-342.

Bergh, J.C. 2006. Trapping grape root borer (Lepidoptera: Sesiidae) in vineyard and non-vineyard habitats in Virginia. J. Entomol. Sci. 41: 253-256.