

## Chapter 2

### Literature Review of plum curculio and *Wolbachia*

**Life history.** The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is endemic to eastern North America and is a pest of stone and pome crops. Chapman (1938), Bobb (1952), and Racette et al. (1992) reviewed the life history of the plum curculio. The plum curculio attacks many fruit crops including apple, apricot, blueberry, cherry, nectarine, peach, pear, plum, and quince. Adults overwinter in woodlots, hedgerows, and abandoned trees (Butkewich and Prokopy 1993, Prokopy et al. 1999) and enter the orchard at bloom, with males entering a day or two before females (Chapman 1938).

Until immature fruits are available, the plum curculios feed on leaves, twigs and blossoms. Cryptic coloration, scotophasic periods of activity, and thanatosis (feigning death) make scouting for plum curculio difficult. When immature fruits are available, the weevils feed on the fruits (Figure 1) and the females will begin to oviposit in them. Often the presence of plum curculio in an orchard goes unnoticed until damage scars from early feeding or oviposition on the fruits become visible (Prokopy et al. 1980, Butkewich and Prokopy 1993). Heavy infestations in an orchard worsen early fruit abscission (June drop) (Bobb 1952) and can cause the loss of most of the crop.



Figure 1. Adult plum curculio feeding on apple



Figure 2. Oviposition damage on apple

A female plum curculio can lay up to 400 eggs in a single summer. The female cuts a small hole in the skin of the fruit, lays an egg under the skin, and then cuts a crescent shaped slit under the egg (Figure 2). The crescent relieves the larvae of pressure from the growing fruit. This strategy is ineffective in pear and apple early in the season,

because larvae are crushed in fruits that remain on the trees. In contrast, larvae develop successfully in infested fallen fruits (Chapman 1938). Larvae from eggs deposited late in the growing season can complete development in fruit remaining on the trees. After 6-7 days, the larvae eclose from the eggs, spend 16 days feeding and pass through four larval stages inside the fruit. Larvae then leave the fruit, burrow 2.5 to 5 cm into the soil and spend about one month in the soil as pupae. Adults emerge from the soil, enter the canopy and begin feeding. Males mate shortly after emergence and may mate up to three times in a day. Multivoltine females may begin laying eggs after a period of days; however, the univoltine females do not lay viable eggs until after winter diapause. Adult plum curculios generally survive in the field for one year. In the lab, however, some adults can live as long as 20 months (Dr. Leskey, USDA-ARS, Kerneysville, WV, personal communication).

**Significance as a fruit pest.** Virginia ranks 6<sup>th</sup> in the United States in apple production. With 7,530 ha of apples and 568 ha of peaches, total revenue of these two crops was \$36.2 million in 2001 (National Agriculture Statistics Service 2001).

Plum curculio is a major pest of pome and stone fruits and is currently effectively controlled by organophosphate (OPs) insecticides. However, with the passage of the Food Quality Protection Act (FQPA) by Congress in 1996 and subsequent reviewing of these compounds by the Environmental Protection Agency (EPA), many uses of OPs are being severely restricted, i.e. longer restricted entry intervals, lower application rates, and longer preharvest intervals. Several compounds, e.g. methyl parathion and malathion, are no longer registered for use on apples and peaches. Lack of chemicals or alternative control methods will leave growers vulnerable to severe infestations and crop losses due to plum curculio damage.

For some orchard fruit pests, like the apple maggot, *Rhagoletis pomonella* (Walsh), monitoring techniques have been developed that allow for precise timing of application of control techniques. Management systems for codling moth, *Cydia pomonella* (Linn.), and oriental fruit moth, *Grapholita molesta* (Busck), rely on degree-day models, effective trapping systems (Hogmire 1995), and alternative chemical controls such as insect growth regulators. For plum curculio no effective trapping or

monitoring system exists and there are few practical alternative chemicals to provide control of these pests.

Eller and Bartelt (1996) isolated and described a male-produced aggregation pheromone, (+)-(1*R*, 2*S*)-1-methyl-2-(1-methylethenyl) cyclobutaneacetic acid (Figure 3). Due to the pheromone's similarity to the boll weevil, *Anthonomus grandis grandis* (Boheman), pheromone, (+)-grandisol (Figure 3), the plum curculio pheromone was given the trivial name grandisoic acid. Landolt (1997) noted that host plant volatiles are often synergistic with pheromones produced by males. Butkewich and Prokopy (1993) reported that adult weevils located host fruits at close range using fruit odors, and that the adult weevils arrived at screen cages containing host apple branchlets more often than at screen cages containing non-host maple branchlets (Butkewich and Prokopy 1997). Prokopy et al. (1995) developed a still air olfactometer assay to test host fruit odors in the laboratory and showed that neither the sex of the weevil nor the direction the weevils had to move to locate the host odor had a significant effect on the response to a host odor. These findings suggested that both sexes should respond equally well to traps baited with plant volatile lures, regardless of the orientation of the trap.

Leskey and Prokopy (2000) reported that in still air olfactometer tests plum curculio adults were more attracted to hexane extracts of immature plum and McIntosh fruits than to hexane extracts of honeysuckle fruit. Prokopy et al. (2000) identified 30 components of the odor of unripe apple and plum fruits and evaluated them in field tests. Eight components showed good evidence of attractiveness: benzaldehyde, benzyl alcohol, decanal, ethyl isovalerate, geranyl propionate, hexyl acetate, limonene and trans-2-hexenal. The degree of attractiveness of these compounds varied in accordance with rates of release. Leskey et al. (2001) showed that linalool, 2-hexanone and 3-hydroxy-2-butanone also showed significant attractiveness in laboratory tests, but they reported that under field conditions only ethyl isovalerate and limonene were attractive.

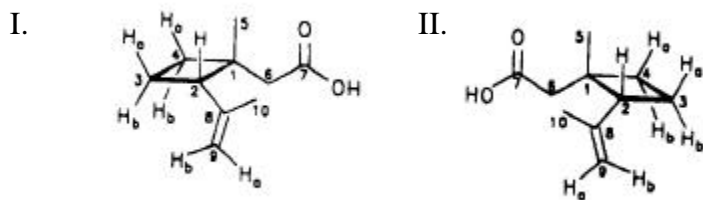


Figure 3. I. is the major component of the male specific pheromone in plum curculio and is the carboxylic analog of II. (+)-grandisol, the major component of the boll weevil pheromone.

Two strains of plum curculio exist: a univoltine strain and a multivoltine strain (Chapman 1938, Bobb 1952, Racette et al. 1992). Morphologically, the two strains appear identical. The univoltine strain is commonly referred to as the northern strain, as it is most often found in the northern U.S. and Canada. This strain undergoes an obligatory diapause (Bobb 1952). The multivoltine strain is commonly referred to as the southern strain, is found primarily in the southern U.S, and undergoes a facultative diapause (Bobb 1952). However, a multivoltine strain has been identified in New Jersey (Dr. Peter Shearer, Rutgers, personal communication). The multivoltine strain was first recorded near apple-packing facilities in New Jersey in 1999, and has since spread to other parts of the state. The implication is that the multivoltine strain arrived at the packing facilities as larvae in apples. The adults then established in adjacent orchards and have dispersed to other orchards and established in other areas of the state (Dr. Peter Shearer, Rutgers). Because the multivoltine strain may be present in the fruit at harvest, it is an export concern. Presently, plum curculio exists in its native range east of the Rocky Mountains in the eastern U.S. and Canada (Chapman 1938, Bobb 1952, Racette et al. 1992). States (e.g. California) and foreign countries (e.g. Brazil) that do not have plum curculio have imposed trade barriers against the importation of fruit from areas where the multivoltine strain of plum curculio exists. Although most of the fruit grown in Virginia is for processing or local fresh sales, fruit grown for export is at risk of being affected by such trade barriers.



Figure 4. Delineation of ranges of plum curculio as determined by Chapman (1938). The blue lines indicate the northern-most boundary (Maine) of plum curculio's range in the United States, and the southern-most boundary (tip of Florida). The black line is the western-most boundary of the plum curculio's range. The red line is the line set by Chapman (1938) delineating the ranges of the univoltine strain (above the line) and the multivoltine strain (below the line).

In 1938, Chapman determined the line delineating the ranges of the univoltine and multivoltine strains runs through the western part of Virginia (Figure 4). This is the region where most of the tree fruits in the state are produced. Due to the trade barriers, there is need for an accurate method to characterize the range of the two strains. A Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR) (Williams et al. 1990) assay has the potential to differentiate the strains genetically, and to test plum curculio from fruit growing regions in Virginia. If the multivoltine strain is present, its geographical distribution can be mapped and changes can be monitored. If the multivoltine strain is not present, the trade barriers imposed upon Virginia fruit can be lifted.

**RAPD-PCR.** RAPD-PCR uses single short (10 nucleotides) non-targeted primers that amplify arbitrary fragments of a genome. These small primers bind to homologous sites within the genome. If the primers bind at opposing sites about 3000 base pairs apart with the 3' ends oriented toward each other, amplification occurs (Loxdale and Lushai 1998). This results in a banding pattern when these fragments are electrophoresed in an

agarose or polyacrylamide gel. If there are mutations in the binding sites between individuals or between strains of a species, the primers are less likely to bind and no amplification will occur, resulting in the lack of a band. This allows dominant genes to be profiled (Loxdale and Lushai 1998) and individuals from sampled populations to be differentiated.

RAPD-PCR has been successfully used to differentiate populations of several extant insects: alfalfa weevil, *Hypera postica* (Gyllenhal) (Erney et al. 1996); a weevil pest of sugar beet, *Aubeonymus mariaefrancisciae* Roudier (Taberner et al. 1997); whiteflies (Gawell and Bartlett 1993); and the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius) (Lu and Rank. 1996).

**Reproductive incompatibility between plum curculio strains and the potential role of *Wolbachia*.** Stevenson and Smith (1961) reported that crossing a northern (univoltine) female with a southern (multivoltine) strain male resulted in significantly fewer eggs laid and fewer larvae eclosing from the eggs. Padula and Smith (1971) reported reproductive incompatibility from crosses of a southern female and a northern male. Similar reproductive incompatibility in invertebrates has been associated with infection of reproductive tissues by *Wolbachia* (Werren 1998, Stouthamer et al. 1999, Bandi et al. 2001).

Originally described in 1924 by Hertig and Wolbach (Werren 1997) from the ovaries of *Culex pipens* Linnaeus mosquitoes, several strains of this rickettsia-like (? - Proteobacteria) intracellular symbiont has been found in a large number of insects, mites and nematodes. Many strains of *Wolbachia* have been categorized into supergroups (A and B for insect infections), and some hosts are associated with more than one strain. The particular strain(s) of *Wolbachia* carried by a host organism may be associated with one of several types of reproductive alterations in the host: feminization of genotypic male offspring, female-biased sex ratios, parthenogenesis (Rousset et al. 1992, Bandi et al. 2001), thelytoky (Stouthamer et al. 1990, Vavre et al. 1999), increased fecundity (Vavre et al. 1999), reduced fecundity, and cytoplasmic incompatibility.

In diploid hosts, cytoplasmic incompatibility (CI) can decrease offspring viability or bias the sex ratio toward males. These anomalies occur when an infected male mates

with an uninfected female or a female that is infected with an incompatible strain of *Wolbachia*. A theoretical explanation for these phenomena is the sperm recovery theory (Werren 1997). The *Wolbachia* strain carried by the male leaves a molecular imprint on the sperm. Only if the female is infected with the same or a compatible strain of *Wolbachia* is the egg able to “recognize” the imprint and save the paternal genetic material. Otherwise, during embryonic meiosis the paternal genetic material is lost, and the resulting embryo is haploid. In diploid species the embryo dies, reducing the number of viable offspring produced from a mating. In haplodiploid species the embryo develops as a male, biasing the sex ratio among offspring toward males (Stouthamer et al. 1999).

Strong unidirectional cytoplasmic incompatibility has been shown in *Ephesia cautella* (Walker) (Lepidoptera), *H. postica* (Coleoptera) and *Laodelphax striatellus* (Fallen) (Homoptera) (O’Neill 1997). Laven (1959, 1967) developed the idea that bi-directional cytoplasmic incompatibility could lead to speciation. Shoemaker et al. (1999) reported *Wolbachia* could be an agent of speciation if the cytoplasmic incompatibility was unidirectional *and* coupled with another pre-zygotic or post-zygotic barrier as in *Drosophila recens* Wheeler and *D. subquinaria*.

Research by Szalanski et al. (1999) on western corn rootworm (WCR) and Mexican corn rootworm (MCR), *Diabrotica virgifera virgifera* LeConte and *Diabrotica virgifera zea* Krysan and Smith respectively, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the first internal transcribed spacer (ITS1) region and 75% of the mitochondrial genome detected only one significant polymorphism in the mitochondrial genome. Although the lack of genetic variation in the mitochondrial DNA suggests a recent divergence, the genetic boundary between these two *Diabrotica* species has remained discrete. Laboratory crosses between WCR and MCR showed reduced fecundity when female MCRs and male WCRs were crossed. *Wolbachia* was revealed in the male WCR by electron microscopy, but was not found in MCR. Because the cytoplasmic incompatibility was eliminated when the WCR were treated with tetracycline, the authors theorized *Wolbachia* was responsible for maintaining the genetic boundary between these *Diabrotica* species.

Bordenstein et al. (2001) reported that *Wolbachia* induced incompatibility has preceded other pre-zygotic and post-zygotic barriers in *Nasonia giraulti* (Darling) and

*Nasonia longicornis* (Darling). The cytoplasmic incompatibility between the two species of *Nasonia* was bi-directional and occurred at high levels since each species of wasp is infected with two strains of *Wolbachia*. The wasps are typically infected with a strain of *Wolbachia* from supergroup A and supergroup B. When the wasps were treated with antibiotics, crosses between the species resulted in viable offspring (F<sub>1</sub> generation), which were in turn capable of producing viable offspring (F<sub>2</sub> generation).

Originally *Wolbachia* was classified into clades according to the 16S rDNA gene sequence. Based on the 16S rDNA sequence analysis, Stouthamer et al. (1993) divided the genus *Wolbachia* into two clades: supergroup I and supergroup II. However the 16S rDNA gene is highly conserved across all eubacteria, making definitive phylogenetic analyses of species difficult. Werren et al. (1995) used the cell-cycle (*fts Z*) gene to classify the *Wolbachia* strains into two clades, supergroups A and B, correlating with the supergroups defined by Stouthamer et al. (1993). The *fts Z* gene evolves faster than the 16S rDNA gene (Werren et al. 1995). Faster evolution of a gene allows for a more definitive phylogenetic analysis within the clades when gene sequences are analyzed. Zhou et al. (1998) used the *wsp* (*Wolbachia* surface protein) gene sequence to classify strains of *Wolbachia*. The *wsp* gene evolves more rapidly than either the 16S rDNA or the *fts Z* genes previously used. Based on the *wsp* gene sequences, the genus *Wolbachia* is divided into two supergroups (A and B) for insects, one supergroup for mites (C) and one supergroup for nematodes (D). Within the clades the *wsp* gene sequence analysis allows for classification of *Wolbachia* by reference group and strain.



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