

Chapter 6

Summary and Future Research

Through RAPD-PCR and *Wolbachia* strain analysis, plum curculios of unknown strains (univoltine vs. multivoltine) can quickly be categorized in the laboratory. This will allow testing of the plum curculio taken in transects across Virginia. If no multivoltine populations are found, then the export barriers currently being sanctioned against Virginia fruit are unfounded and could be lifted. Periodic testing of the populations to determine the arrival or natural and human-mediated spread of multivoltine populations should be done.

Testing plum curculio in Virginia using the RAPD assay and a *Wolbachia* assay will allow a better understanding of the genetics of plum curculio in this state. Other states can adapt these assays to test their populations of plum curculio. Multivoltine population ranges can then be mapped and a ‘big picture’ approach to dealing with this strain can be developed.

Because there is no genetic information in GenBank on plum curculio, future work arising from the RAPDs should include the characterization of some of the amplifiers that characterize one population from the other. Building a body of genetic information will allow researchers to look for more differences between the two strains of plum curculio on a molecular level. This could eventually payoff in greater understanding of the differences of behaviour between the two strains. A better understanding of the behaviours will allow for better management of this pest.

The discovery of *Wolbachia* infections in plum curculio indicates that *Wolbachia* infection may impact strain variation and voltinism. The fitness costs of *Wolbachia* infections are unknown. The histological changes seen by Padula and Smith (1971) may be caused by the *Wolbachia* infection, or may be a separate prezygotic barrier to interbreeding strains of plum curculio. Based on Shoemaker et al. (1999) and Bordenstein et al. (2001) this may be the beginning of speciation between the univoltine strains and the multivoltine strains of plum curculio. Or it may only establish a *status quo* between the two populations in areas where both strains of plum curculio are present.

The effect of the host on the *Wolbachia* is also unknown. Specific strains of *Wolbachia* are closely linked to certain mitochondrial haplotypes in *Drosophila* spp. (O'Neill 1997). Perhaps the mitochondria of the univoltine and multivoltine strains have evolved so that the infections of *Wolbachia* in plum curculio are linked also. Then the mitochondria within a population of plum curculio would control the infection of *Wolbachia* present in that population, thus creating even more definitive strains of plum curculio. This along with the seasonal differences in behaviour and reduced fecundity may be evidence enough to separate the strains by giving them a tri-nomial.

The trapping studies suggest that no effective trapping systems are available for plum curculio in Virginia orchards. These results; however, contradict the literature. Perhaps some geographical influence is responsible for these differences.

There is much more research to be done with plum curculio. For the growers of fruit in Virginia and elsewhere, effective trapping systems must be found. This is a basic requirement for IPM programs. Efforts are ongoing in Virginia and West Virginia to unravel the visual and olfactory cues that are attractive to plum curculio. Another area of research is the cues that are repellent to the plum curculio adults. This work has been started by Dr. T. Leskey at USDA-ARS in West Virginia.

References

- Bordenstein, S. R., F. P. O'Hara, and J. H. Werren. 2001.** *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature* 409: 707-710.
- O'Neill, S. L., A. Hoffmann, and J. H. Werren. 1997.** Influential Passengers: Inherited microorganisms and arthropod reproduction. Oxford University Press, Oxford.
- Padula, A., and E. H. Smith. 1971.** Reproductive incompatibility between univoltine males and multivoltine females of the plum curculio. *Annals of the Entomological Society of America* 64: 665-668
- Shoemaker, D. D., V. Katju, and J. Jaenike. 1999.** *Wolbachia* and the evolution of reproductive isolation between *Drosophila recens* and *Drosophila subquadrata*. *Evolution* 53: 1157-1164.
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Appendix A
Nucleotide and Predicted Protein Sequence Similarities for 29 *Wolbachia* *wsp* gene sequences from different hosts.

Above the line is nucleotide similarity, below the line of asterisks is predicted protein similarity.

	wDro	Atab-1	Wwhi	wNov	wNaw	wDes	FLA	Aus	wAscD	GA
<i>Trichopria drosophilae</i>	*	99.6	94.85	91.68	88.71	90.29	89.9	89.7	89.5	89.1
<i>Asobara tabida</i>	99.33	*	94.85	91.68	88.71	90.29	89.9	89.7	89.5	89.1
<i>Lutzomyia shannoni</i>	90	90	*	91.29	87.52	89.5	89.11	88.91	88.71	88.71
<i>Aedes novoniveus</i>	85.33	85.33	87.33	*	88.32	89.7	89.5	89.11	89.31	88.92
<i>Telenomus nawai</i>	78.67	78.67	78	78	*	86.93	86.53	86.34	86.14	86.14
<i>Dacus destillatoria</i>	85.33	85.33	86	85.33	81.33	*	99.6	99.01	98.81	98.81
<i>Florida Curculios</i>	85.33	85.33	86	85.33	81.33	100	*	98.61	98.41	98.41
<i>Callosobruchus chinensis</i>	83.33	83.33	84	83.33	79.33	98	98	*	97.82	97.82
<i>Bactrocera</i> sp.	84.67	84.67	86.67	87.33	78.67	94.67	94.67	92.67	*	97.62
<i>Georgia Curculios</i>	72.67	72.67	72.67	72	67.33	80.67	80.67	79.33	78.67	*
<i>Muscidifurax uniraptor</i>	74.67	74.67	76	78	71.33	87.33	87.33	86.67	82.67	68
<i>Pachycrepoideus dubius</i>	76	76	77.33	78	71.33	87.33	87.33	86.67	82.67	68
<i>Armigeres subalbatus</i>	74	74	75.33	74.67	67.33	83.33	83.33	83.33	80	66
<i>Phlebotomus papatasi</i>	80	80	81.33	81.33	75.33	91.33	91.33	90	86.67	72
<i>Phlebotomus perniciosus</i>	72	72	72	71.33	74	72	72	70.67	72	62
<i>Bemisia tabaci</i> 5	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	61.33
<i>Bemisia tabaci</i> 2	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	61.33
<i>Bemisia tabaci</i> 1	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	61.33
Dryinid wasp	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	61.33
<i>Coquillettidia crassipes</i>	72	72	72	71.33	74.67	72.67	72.67	71.33	72.67	60.67
<i>Aleurotrachelus</i> sp.	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	61.33
<i>Culex sitiens</i>	72	72	72	72	75.33	73.33	40	72	73.33	61.33
<i>Torymus bedeguaris</i>	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	61.33
<i>Tribolium confusum</i>	72.67	72.67	72.67	72	75.33	73.33	73.33	72	73.33	62
<i>Bactrocera pyrifoliae</i>	72.67	72.67	72.67	71.33	75.33	74	74	72.67	72.67	61.33
<i>Bactrocera cucurbitae</i>	72.67	72.67	72.67	71.33	75.33	74	74	72.67	72	61.33
<i>Acraea encedon</i>	74	74	74	72.67	77.33	74.67	74.67	73.33	74	62.67
<i>Massachusetts Curculios</i>	73.33	73.33	73.33	70.67	73.33	74	74	72.67	72	64
<i>Perithemis tenera</i>	72.67	72.67	72.67	71.33	74	72.67	72.67	71.33	72.67	62.67

Trichopria drosophilae = wDro

Lutzomyia shannoni = wWhi

Telenomus nawai = wNaw

Florida Curculios = FLA

Bactrocera sp.= wAscD

Muscidifurax uniraptor =wUni

Armigeres subalbatus = wSub

Phlebotomus perniciosus = wPrn

Dryinid wasp = wDry

Aleurotrachelus sp. = wAlesp

Torymus bedeguaris = wBed

Bactrocera pyrifoliae = wPyr

Acraea encedon = wEnc

Perithemis tenera = wTen

Asobara tabida = Atab-1

Aedes novoniveus = wNov

Dacus destillatoria = wDes

Callosobruchus chinensis = Aus

Georgia Curculios= GA

Pachycrepoideus dubius = Pdub

Phlebotomus papatasi = wPap

Bemisia tabaci (5, 2, 1) =Btab 5, 2, 1

Coquillettidia crassipes = wCra

Culex sitiens = wSit

Tribolium confusum = wCon

Bactrocera cucurbitae = wCur

Massachusetts Curculios = MA

	wUni	Pdub1	wSub	wPap	wPrn	wBtab5	wBtab2	wBtab1	wDry	wCra
<i>Trichopria drosophilae</i>	83.36	83.96	82.38	80.99	84.15	84.36	84.36	84.36	84.36	84.16
<i>Asobara tabida</i>	83.76	84.36	82.38	80.99	84.15	84.36	84.36	84.36	84.39	84.16
<i>Lutzomyia shannoni</i>	82.77	83.37	82.38	81.58	81.98	81.98	81.98	81.98	81.98	81.78
<i>Aedes novoniveus</i>	82.97	83.17	80.99	80.59	80.79	81.39	81.39	81.39	81.39	81.19
<i>Telenomus nawai</i>	79.6	79.8	77.03	77.03	80.99	80.79	80.79	80.79	80.79	80.59
<i>Dacus destillatoria</i>	89.11	89.31	85.35	84.95	80.99	81.39	81.39	81.39	81.39	81.19
Florida Curculios	88.71	88.91	84.95	84.55	80.59	80.99	80.99	80.99	80.99	80.79
<i>Callosobruchus chinensis</i>	88.51	88.71	85.15	84.55	80.59	80.99	80.99	80.99	80.99	80.79
<i>Bactrocera</i> sp.	87.92	88.12	84.16	83.76	80.99	80.79	80.79	80.79	80.79	80.59
Georgia Curculios	87.92	88.12	84.16	83.96	79.8	80.2	80.2	80.2	80.2	80
<i>Muscidifurax uniraptor</i>	*	99.4	92.47	84.36	77.03	77.42	77.42	77.42	77.42	77.23
<i>Pachycrepoideus dubius</i>	98.67	*	93.07	84.95	77.23	77.62	77.62	77.62	77.62	77.42
<i>Armigeres subalbatus</i>	90.67	92	*	82.57	77.62	77.62	77.62	77.62	77.62	77.42
<i>Phlebotomus papatasi</i>	90	91.33	85.33	*	76.43	77.23	77.23	77.23	77.23	77.03
<i>Phlebotomus perniciosus</i>	68.67	68.67	66.67	71.33	*	96.24	96.24	96.24	96.24	96.04
<i>Bemisia tabaci</i> 5	70	70	67.33	72.67	94	*	100	100	100	99.8
<i>Bemisia tabaci</i> 2	70	70	67.33	72.67	94	100	*	100	100	99.8
<i>Bemisia tabaci</i> 1	70	70	67.33	72.67	94	100	100	*	100	99.8
Dryinid wasp	70	70	67.33	72.67	94	100	100	100	*	99.8
<i>Coquillettidia crassipes</i>	69.33	69.33	66.67	72	93.33	99.33	99.33	99.33	99.33	*
<i>Aleurotrachelus</i> sp.	70	70	67.33	72.67	94	100	100	100	100	100
<i>Culex sitiens</i>	70	70	66.67	72.67	93.33	99.33	99.33	99.33	99.33	98.67
<i>Torymus bedeguaris</i>	70	70	67.33	72.67	93.33	98.67	98.67	98.67	98.67	98
<i>Tribolium confusum</i>	70	70	67.33	72.67	92.67	99.33	99.33	99.33	99.33	98.67
<i>Bactrocera pyrifoliae</i>	70.67	70.67	67.33	73.33	92.67	98	98	98	98	97.33
<i>Bactrocera cucurbitae</i>	70.67	70.67	67.33	73.33	92.67	97.33	97.33	97.33	97.33	96.67
<i>Acraea encedon</i>	70.67	70.67	68	73.33	91.33	96	96	96	96	95.33
Massachusetts Curculios	68.67	70	68	74	90	85.33	85.33	85.33	85.33	84.67
<i>Perithemis tenera</i>	68	68	66	72	92.67	86.67	86.67	86.67	86.67	86

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Lutzomyia shannoni = wWhi
Telenomus nawai = wNaw
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Muscidifurax uniraptor =wUni
Armigeres subalbatus = wSub
Phlebotomus perniciosus = wPrn
 Dryinid wasp = wDry
Aleurotrachelus sp. = wAlesp
Torymus bedeguaris = wBed
Bactrocera pyrifoliae = wPyr
Acraea encedon = wEnc
Perithemis tenera = wTen

Asobara tabida = Atab-1
Aedes novoniveus = wNov
Dacus destillatoria = wDes
Callosobruchus chinensis = Aus
 Georgia Curculios= GA
Pachycrepoideus dubius = Pdub
Phlebotomus papatasi = wPap
Bemisia tabaci (5, 2, 1) =Btab 5, 2, 1
Coquillettidia crassipes = wCra
Culex sitiens = wSit
Tribolium confusum = wCon
Bactrocera cucurbitae = wCur
 Massachusetts Curculios = MA

	wAlesp	wSit	wBed	wCon	wPyr	wCuc	wEnc	MA	wTen
<i>Trichopria drosophilae</i>	84.36	83.96	84.55	84.55	83.76	83.56	85.54	81.58	82.77
<i>Asobara tabida</i>	84.36	83.96	84.55	84.55	83.76	83.56	85.54	81.58	82.77
<i>Lutzomyia shannoni</i>	81.98	81.58	82.18	82.18	81.58	81.39	82.38	79.41	81.39
<i>Aedes novoniveus</i>	81.39	80.99	81.19	81.19	80.2	80.2	81.39	77.23	79.6
<i>Telenomus nawai</i>	80.79	80.39	80.59	80.59	79.6	79.6	81.19	78.22	79.8
<i>Dacus destillatoria</i>	81.39	80.99	81.39	81.58	80.39	80.39	81.39	77.42	81.39
Florida Curculios	80.99	80.59	80.79	81.19	80	80	80.99	77.03	80.99
<i>Callosobruchus chinensis</i>	80.99	80.59	80.79	81.19	80	80	80.99	77.03	80.99
<i>Bactrocera</i> sp.	80.79	80.39	80.59	80.99	79.8	79.8	80.79	77.23	81.19
Georgia Curculios		80.2	79.8	80	80.39	79.21	79.21	80.2	76.24
<i>Muscidifurax uniraptor</i>	77.42	77.03	77.23	77.62	76.24	76.24	77.42	71.48	75.64
<i>Pachycrepoideus dubius</i>	77.62	77.23	77.42	77.82	76.43	76.43	77.62	72.08	75.84
<i>Armigeres subalbatus</i>	77.62	77.23	77.82	78.22	76.83	76.83	77.62	72.47	76.04
<i>Phlebotomus papatasii</i>	77.23	76.83	77.03	77.23	76.04	76.04	76.83	71.68	74.85
<i>Phlebotomus perniciosus</i>	96.24	95.84	96.04	96.63	95.44	95.44	94.85	89.5	91.09
<i>Bemisia tabaci</i> 5	100	99.6	99.4	98.41	98.41	98.22	98.22	88.71	90.1
<i>Bemisia tabaci</i> 2	100	99.6	99.4	98.41	98.41	98.22	98.22	88.71	90.1
<i>Bemisia tabaci</i> 1	100	99.6	99.4	98.41	98.41	98.22	98.22	88.71	90.1
Dryinid wasp	100	99.6	99.4	98.41	98.41	98.22	98.22	88.71	90.1
<i>Coquillettidia crassipes</i>	99.8	100	99.4	99.6	98.22	98.22	98.02	88.51	89.9
<i>Aleurotrachelus</i> sp.	*	99.6	99.4	98.41	98.41	98.22	98.22	88.71	90.1
<i>Culex sitiens</i>	99.33	*	99.01	98.02	98.02	97.82	97.82	88.31	89.7
<i>Torymus bedeguaris</i>	98.67	98	*	98.22	98.22	98.02	98.02	88.12	89.5
<i>Tribolium confusum</i>	99.33	98.67	98	*	98.02	97.82	97.42	88.12	89.9
<i>Bactrocera pyrifoliae</i>	98	97.33	96.67	97.33	*	99.8	97.82	87.52	88.71
<i>Bactrocera cucurbitae</i>	97.33	96.67	96	96.67	99.33	*	97.62	87.52	88.71
<i>Acraea encedon</i>	96	95.33	94.67	95.33	95.33	94.67	*	87.33	88.51
Massachusetts Curculios	85.33	85.67	84	84.67	84.67	84.67	84	*	91.48
<i>Perithemis tenera</i>	86.67	86	86	86	86	86	85.33	97.33	*

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