PROJECT NO.: ARS (Final Report)

TITLE: Development of Genetic Markers for Differentiation of Intercepted Immature Lepidoptera Pests in Deciduous Fruits

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REPORTING PERIOD: 2000-2002

ACCOMPLISHMENTS:

1. Extracted DNA from 6 species of Lepidoptera, codling moth, lesser apple worm, oblique-banded leafroller, omnivorous leafroller, Lacanobia fruitworm, oriental fruit moth.
2. Tested, using polymerase chain reactions, 13 primer sets designed to amplify mitochondrial DNA (7 for COI and 6 for COII) and 2 primer sets to amplify genomic DNA (ITS region).
3. Differences in banding patterns were noted for 4 of the COI primer sets, all 6 of the COII primer sets, and both of the ITS primer sets.
4. Sequencing of the bands obtained from C and I primer sets of COI were performed..
5. Comparisons of the banding patterns of 5 populations of codling moth using primer sets N and O of the ITS region were made. These are in the process of sequencing.

RESULTS:
We have perfected a DNA extraction method for Lepidopteran larvae, and have obtained good quality DNA from the extraction method. The DNA was used in amplification reactions using primers designed to amplify specific regions of mitochondrial DNA (primer sets A-J for the COI region and primer sets D-M for the COII regions). Sequence analysis of bands obtained from primer sets C and I of the COI region indicate 2 to 3 areas of polymorphism (differences) which may be useful in species identification. Additional bands obtained from primer sets G, F, and K are currently being sequenced.

Primer set N of the ITS region was used to amplify DNA from 12 to 13 individual codling moths obtained from 5 different populations in Washington State. A number of differences in the banding pattern among individuals were noted, as well as differences between populations. The 1200 bp band was the most common amplification product. Population #5 yielded the most different sizes of amplified bands among individuals. It appears that the number and size differences in bans obtained from primer set N may not be as useful in population identification as other primer sets. Primer set O is currently half-way through this process, and appears to have a more consistent banding pattern among individuals in populations. Once the sequences are complete for the above mentioned primer sets, DNA analysis will be performed on various populations of these species. Also, searches will be made on GenBank to compare our sequences against published sequences for other insect species. Once a unique sequence is identified for a specific species, a primer set will be made specifically to that region, and then tested against various populations of that species and against other species.

Figure 1. 1.8% Agarose gel electrophoretic pattern of moth mitochondrial DNA amplified with SR-J-14233 and T1-N-24 mitochondrial primers. LAW-Lesser Apple Worm, OLR-Omnivorous Leafroller; OBLR-Oblique-banded Leafroller; OFM-Oriental Fruit moth; CM-Codling moth; LS-Lacanobia Fruitworm (from 1st report).

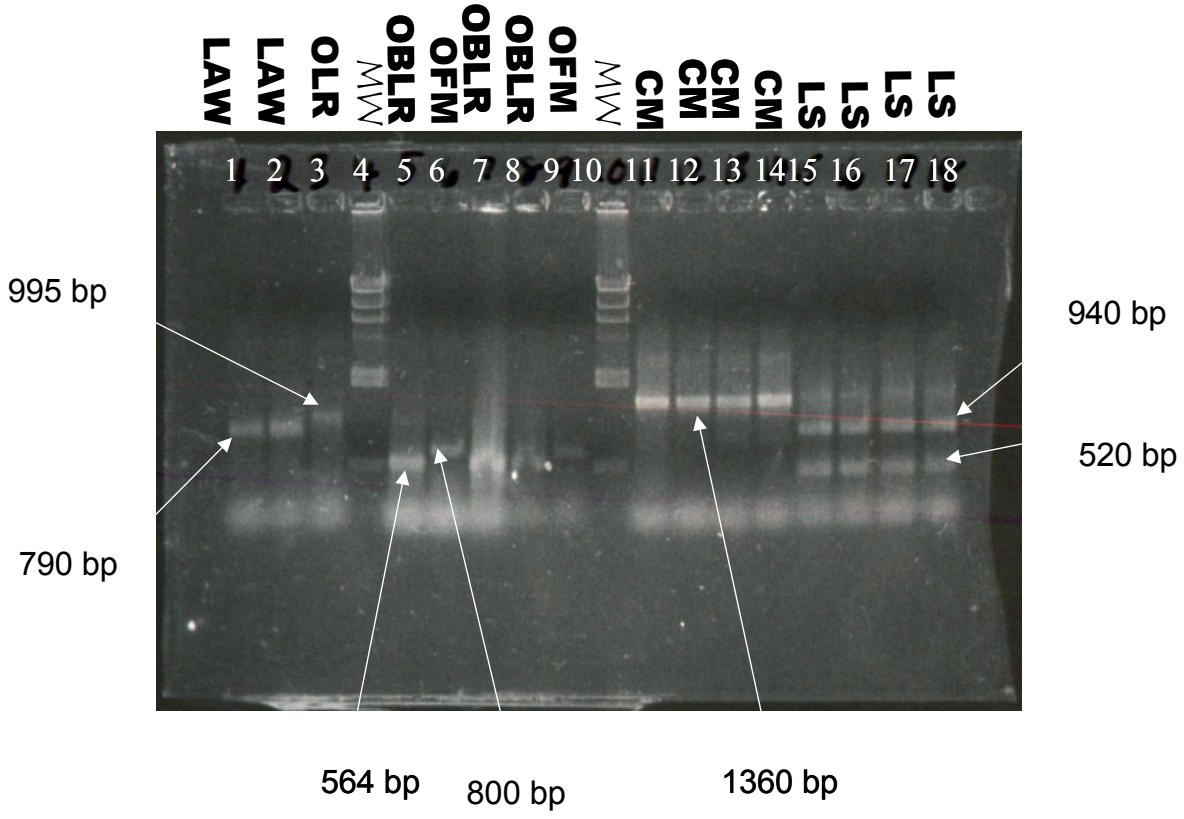


Table 1. Description of samples and band molecular weights resulting from DNA amplification using SR-J-14233 and T1-N-24 mitochondrial primers. LAW-Lesser Apple Worm, OLR-Omnivorous Leafroller; OBLR-Oblique-banded Leafroller; OFM-Oriental Fruit moth; CM-Codling moth; LS-Lacanobia Fruitworm (from 1st report).

LANE	INSECT	LENGTH
1	LAW	790 bp
2	LAW	790 bp
3	OLR	995 bp
4	MW marker	Marker
5	OBLR	564 bp
6	OFM	800 bp
7	OBLR	564 bp
8	OBLR	564 bp
9	OFM	800 bp
10	MW marker	Marker
11	CM	1360 bp
12	CM	1360 bp
13	CM	1360 bp
14	CM	1360 bp
15	LS	940 bp + 520 bp
16	LS	940 bp + 520 bp
17	LS	940 bp + 520 bp
18	LS	940 bp + 520 bp

Table 2. Description of primer sets used for DNA amplification of mitochondrial and genomic sequences. Primer sequences are given in Simon et al. (1994)*.

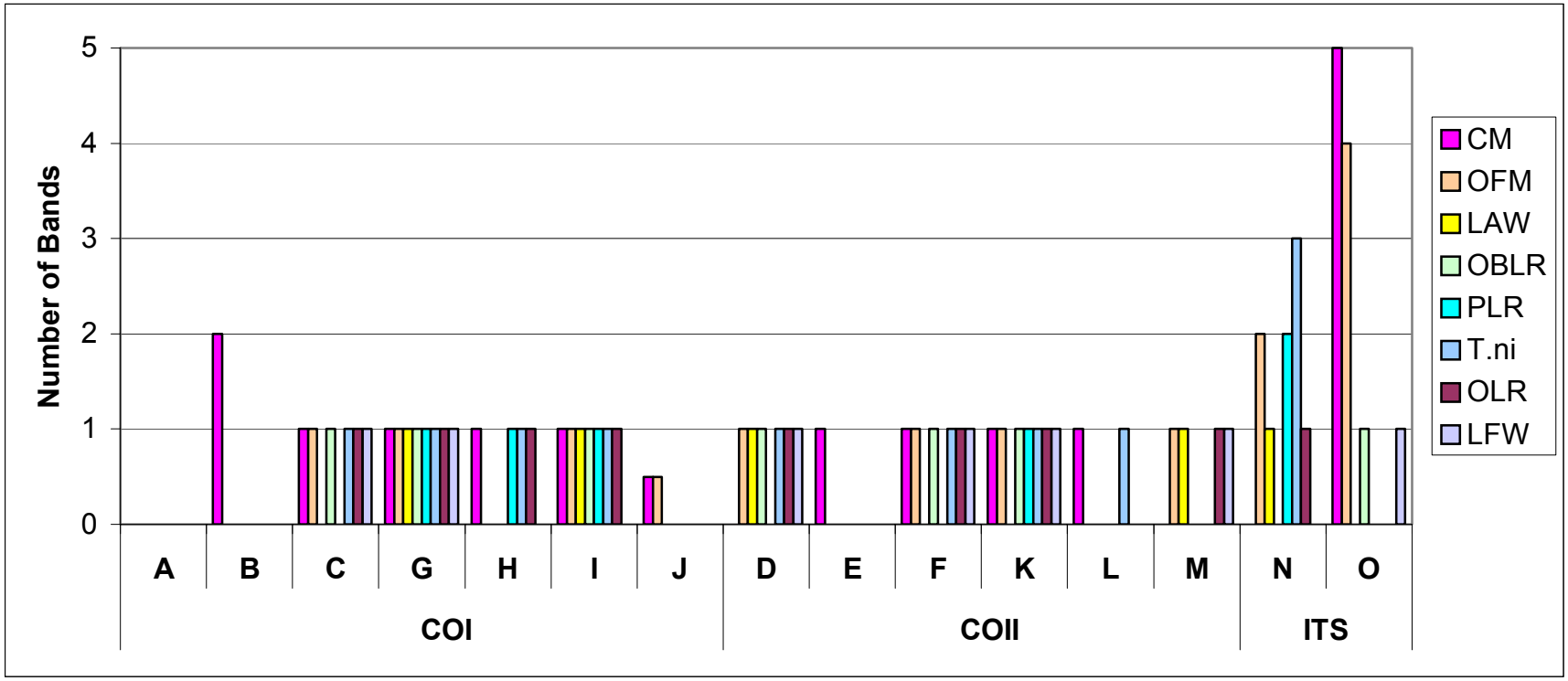
Region Amplified	Primer Set	Primer 1	Primer 2
Cytochrome Oxidase I	A	C1-J-1751	C1-N-2191
	B	C1-J-1752	TL2-3014
	C	C1-J-2183	TL2-3014
Cytochrome Oxidase II	D	C2-J-3400	C2-N-3661
	E	C2-J-3279	C2-N-3389
	F	C2-J-3400	TK-N-3785
Cytochrome Oxidase I	G	C1-J-1859	C1-N-2191
	H	C1-J-1859	L2-N-3014
	I	C1-J-2195	C1-N-2329
	J	C1-J-2195	L2-N-3014
Cytochrome Oxidase II	K	C2-J-3138	C2-N-3389
	L	C2-J-3571	C2-N-3661
	M	C2-J-3571	TK-N-3785
ITS	N	ITS 4	ITS 5
ITS	O	ITS 2-5'	ITS 2-3'

**Simons, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a complication of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87(6): 651-701.*

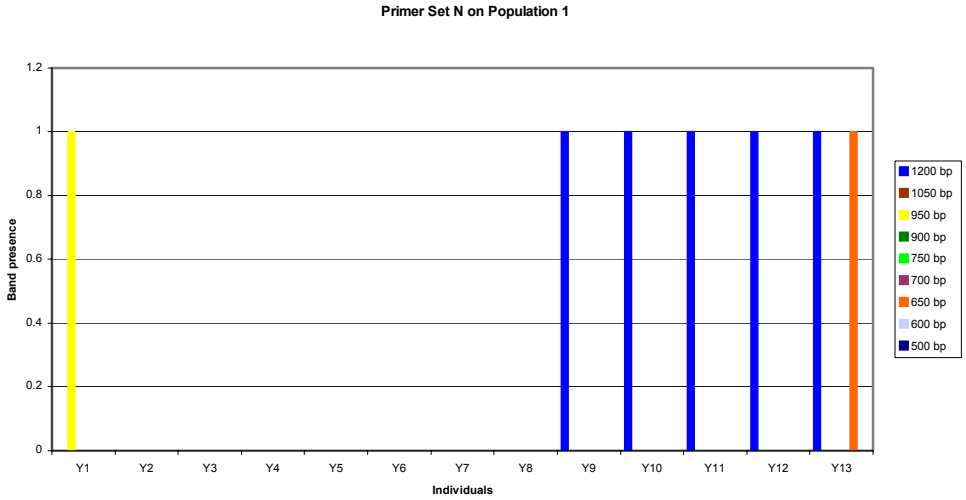
Table 3. List of the number and size of amplified bands obtained from the DNA of codling moth (CM), Oriental Fruit moth (OFM), Lesser Apple Worm (LAW), Oblique banded Leafroller (OBLR), Pandemis Leafroller (PLR), Cabbage looper (T.ni), Omnivorous leafroller (OLR), and Lacanobia Fruitworm (LFW), using various primer sets for COI, COII, and ITS (as listed in Table 2). Green indicated bands which have been sequenced and yellow are bands in the process of being sequenced.

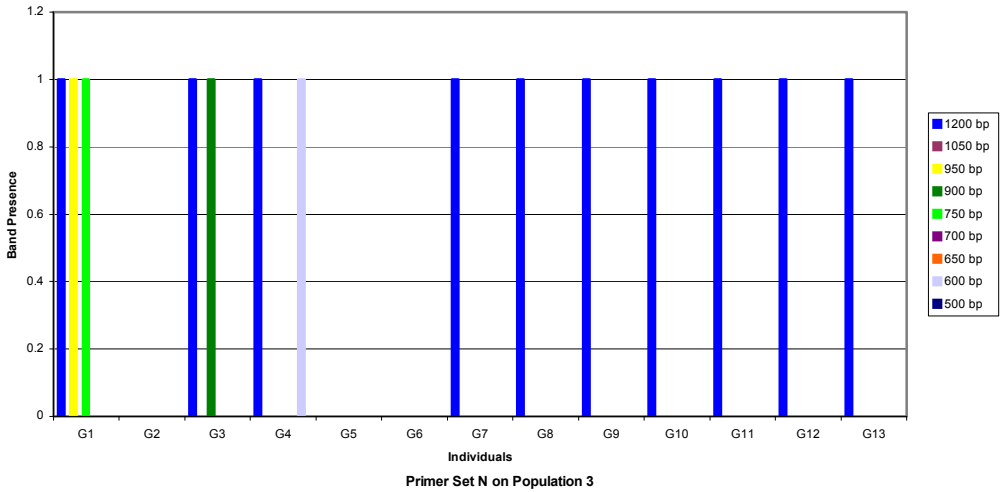
Primers			CM	OFM	LAW	OBLR	PLR	T.ni	OLR	LFW	Size
	COI	**A**	0			0					None have been found to work yet
		B	2			0					One is ~800 bp and the other is ~700 bp (Found using 500 ng of DNA)
		C	1	1	0	1	0	1	1	1	All are ~900 bp except **LFW** >~100 bp
		G	1	1	1	1	1	1	1	1	All are ~350 bp
		H	1	0	0	0	1	1	1	0	All are ~ 1100 bp
		I	1	1	1	1	1	1	1	0	All are ~200 bp
		J	0.5	0.5	0	0	0	0	0	0	**CM** smear from ~1000 bp to ~100 bp **OFM** Smear from top to a band at ~1000 bp
	COII	**D**	0	1	1	1	0	1	1	1	All are ~350 bp
		E	1			0					Faint band at ~200 bp (Found using 500 ng of DNA)
		F	1	1	0	1	0	1	1	1	All are ~450 bp
		K	1	1	0	1	1	1	1	1	All are ~ 200 bp, worked best at 51deg for annealing
		L	1	0	0	0	0	1	0	0	**CM** ~650 bp, **T.ni.** ~250 bp
		M	0	1	1	0	0	0	1	1	All are ~200 bp
	ITS	**N**	0	2	1	0	2	3	1	0	**OFM** ~1200, ~1100 **LAW** ~1300 **PLR** ~1400, ~1300 **T.ni** ~1400, ~1300, ~1050 **OLR** ~1050 bp
		O	5	4	0	1	0	0	0	1	**CM** ~1600, ~1400, ~1300, ~1050, ~450 **OFM** ~1400, ~1050, ~800, ~550 **OBLR** ~450 **LFW** ~700 bp

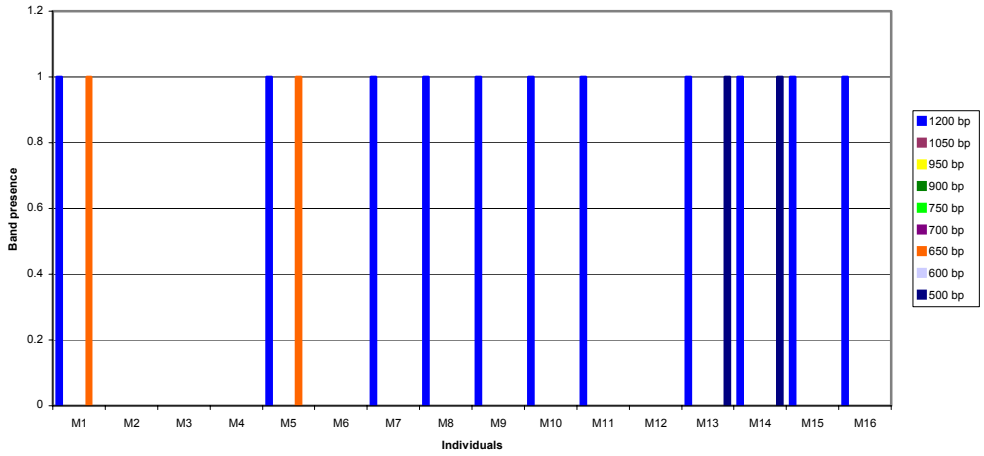
Figure 2. Number of bands from amplification of DNA from codling moth (CM), Oriental Fruit moth (OFM), Lesser Apple Worm (LAW), Oblique banded Leafroller (OBLR), Pandemis Leafroller (PLR), Cabbage looper (T.ni), Omnivorous leafroller (OLR), and Lacanobia Fruitworm (LFW), using various primer sets for COI, COII, and ITS.

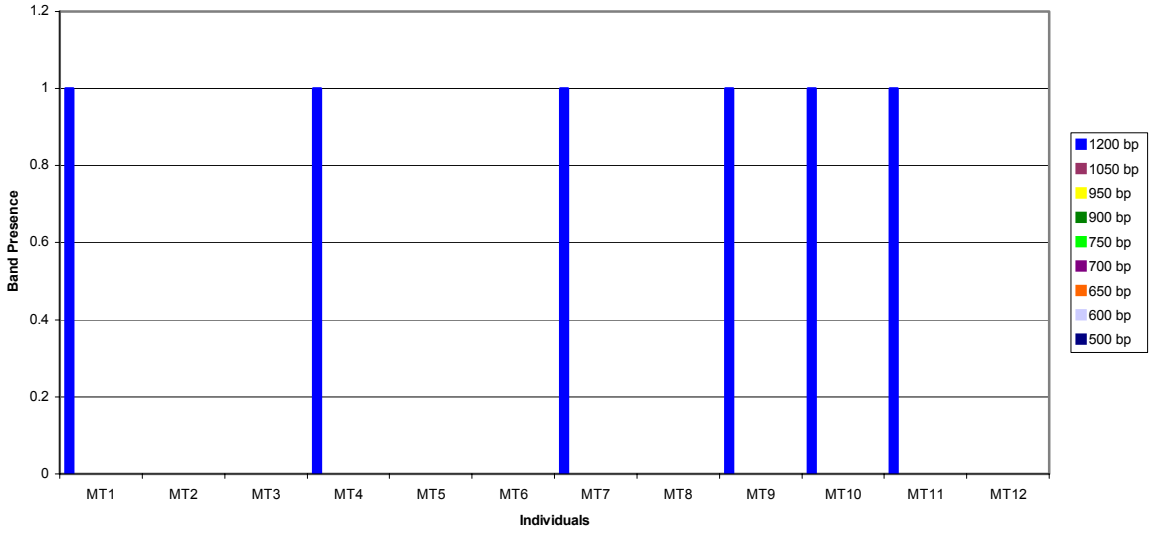


populations in Washington State.









Primer Set N on Population 5