GENETIC VARIATION WITHIN AND BETWEEN RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* (KURDJUMOV), BIOTYPES FOUND IN THE UNITED STATES

By

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NOMENCLATURE

3-me- nC_X	methyl group on the third carbon of a straight chain alkane
bp	nucleotide base pair
C°	degrees Celsius
cm	centimeter
COI	cytochrome oxidase subunit I gene
*Dn*4	single dominant gene for Russian wheat aphid resistance in wheat
dNTP's	deoxynucleotide triphosphates
EDTA	ethylene diamine tetra acetic acid
FID	flame ionization detector
g	unit equal to the acceleration of gravity at the earth's surface
GC	gas chromatographer
h	hour
HCl	hydrogen chloride
HP	Hewlett Packard
IRDye 700	infrared pentamethane carbocyanine dye that fluoresces at 685nm
KCl	potassium chloride
L: D	refers to hours of light: hours of dark ratio
µl	microliter
M	a solution that contains one mole of solute per liter of solution
Mg	magnesium

$MgCl_2$	magnesium chloride
min.	minute
ml	milliliter
mm	millimeter
mM	millimolar
mtDNA	mitochondrial DNA
NaCl	sodium chloride
nC_X	normal straight chain alkane of X number carbon length
ng	nanogram
OD_{260}	optical density at a wavelength of 260 nanometers
pH	a measure of the acidity or alkalinity of a solution
pmol	picomole
RAPD-PCR	random amplified polymorphic DNA-polymerase chain reaction
RWA	Russian wheat aphid biotype
SDS	sodium dodecyl sulfate, used as a detergent
sec.	second
SSR	simple sequence repeat (synonymous with Microsatellites)
Taq	DNA polymerase from *Thermus aquaticus*
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
TE	Tris EDTA
Tris	tromethamine, white crystalline powder used in buffers
V	volts

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

History

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) was first described in the early 1900's when a large outbreak occurred in Moldavia and the Ukraine (Poprawski and Wraight 1992). The aphid damages wheat, *Triticum aestivum* (L.) and barley, *Hordeum vulgare* (L.), and is native to Central Asia between the Caucasus and Tian Shan Mountains. In 1978, the Russian wheat aphid was introduced into South Africa (Halbert and Stoetzel 1998). In 1980, it was first confirmed in Mexico, although it may have been present there for several years (Puterka *et al*. 1993). In 1986, an infestation of Russian wheat aphid occurred in Bailey County, Texas (Bush *et al*. 1989). By year's end, the Russian wheat aphid had spread to Eastern Colorado, Western Kansas, the Nebraska panhandle, New Mexico, Western Oklahoma, Western Texas, and Eastern Wyoming. The Russian wheat aphid reinfests these areas year after year, but can be found anywhere west of the 100º West longitude demarcation. Currently, the Russian wheat aphid occurs in all major cereal production areas on every continent, except Australia (Halbert and Stoetzel 1998).

Ecology

The Russian wheat aphid lacks developed cornicles, has a tubular body shape, and resides in a pseudo-leaf gall formed during leaf rolling (Burd *et al*. 1998). The tubular structure formed by the leaf serves as a niche for the Russian wheat aphid and is required for survival. The Russian wheat aphid not only feeds on phloem of young tillers and/or seedlings, but also feeds in heads.

The Russian wheat aphid is monoecious on graminae and oversummers on wild grasses and volunteer crops (Shufran *et al*. 1997). The most important non-cultivated

host crops for Russian wheat aphid are volunteer wheat and barley, crested wheatgrass, *Agropyron desertorum* (Fisch. ex Link) Schultes, and Canada wild rye, *Elymus canadensis* (L.) (Burd *et al*. 1998). In greenbug, *Schizaphis graminum* (Rondani) non-cultivated grasses can act as reservoirs for biotypic and genetic diversity (Anstead *et al.* 2003), which could also be true for the Russian wheat aphid.

Reproduction

Aphids reproduce asexually by apomictic parthenogenesis whereby viviparous females give birth to live female young (anholocycly). These offspring are genetically identical and are often referred to as clones. Anholocyclic reproduction allows for rapid reproductive rates and establishment of aphid genotypes (Dixon 1988). Aphids can also exhibit a sexual cycle (holocycly) for overwintering (Dixon 1988). In the United States, the Russian wheat aphid reproduces anholocyclically. Oviparae (egg laying females) have been found in the Northwestern United States in low numbers, but not males (Kiriac *et al*. 1990). In Southeastern Colorado, oversummering and overwintering viviparae both occur frequently (Elliott *et al*. 1998).

In Russia and other regions of Europe, holocycly or a sexual cycle occurs to produce eggs for overwintering (Kiriac *et al*. 1990) in response to shortening day-length in mid-summer. In sexual reproduction, recombination occurs during meiosis and forms new combinations of genes naturally, by crossing over, or by independent assortment. Genetic recombination during sexual reproduction may be a primary mechanism to produce new biotypes which are better adapted for survival on resistant crops (Puterka *et al*. 1993).

Damage

Damage from the Russian wheat aphid includes overall stunting of the plant, leaf streaking or folding, prostrate growth, and leaf rolling which leads to trapped leaves and heads (Morrison and Peairs 1998). Salivary enzymes injected into the plant breakdown the leaf chloroplasts, causing yellow, white, or purple longitudinal streaks (Pike 1991) that can accompany rolled leaves (Puterka *et al*. 1992). Prostrate growth consists of a flattened plant with many tillers, which leads to reduced height and yield. Leaf rolling consists of two types; the first is the inability of the young leaf to unroll, and the second is folding of expanded leaves (Burd *et al*. 1998), the prior being the most common with Russian wheat aphid damage. Trapped heads are due to rolled leaves that trap the flag leaf, preventing the head from emerging properly. Trapped heads mature slower and are common on stunted plants. Damage from the Russian wheat aphid causes decreased photosynthetic capacity and cold hardiness in infested wheat (Burd *et al*. 1998). Ultimately, the Russian wheat aphid can cause plant death or as much as 80% reduction in yield (Hein 1992).

Biotypes

Here in, a biotype is defined as a population of insects which is able to feed on and damage previously resistant sources (Claridge and Hollander 1983, Porter *et al*. 1997). However, biotypes can be determined by food or host preference, behavior, genetic variation, physiological responses to the environment and physical changes, reproductive examinations, seasonal patterns, migration and dispersal, and the ability to vector diseases (Diehl and Bush 1984, Saxena and Barrion 1987). In 1931, the first agriculturally important biotypes were documented in the Hessian fly, *Mayetiola*

destructor (Say), by the discovery of genetic polymorphisms (Saxena and Barrion 1987). Biotypes in arthropods can be found in 36 crop pest species, in 17 families, belonging to six orders (Saxena and Barrion 1987). Aphids account for half of the pest species showing biotypic variation, including: bird cherry-oat aphid, *Rhopalosiphum padi* (L.); corn leaf aphid, *R. maidis* (Fitch); pea aphid, *Acyrthosiphon pisum* (Harris); cotton/melon aphid, *Aphis gossypii* (Glover); and the green peach aphid, *Myzus persicae* (Sulzer) (Saxena and Barrion 1987). Biotypes have also been confirmed in greenbug (Porter *et al*. 1997) populations in the United States (Black *et al*. 1992). Currently, five Russian wheat aphid biotypes are present in the Western United States, according to plant response studies (Burd *et al.* 2006, Haley *et al.* 2004).

Current Pest Status

The Russian wheat aphid continues to be an economically important pest on small grains, specifically wheat and barley. From 1987-1993, the Russian wheat aphid directly and indirectly caused an estimated $800 million loss in the Western Unites States (Haley *et al*. 2004). The most cost effective control for the Russian wheat aphid is planting resistant varieties (Du Toit 1989). Resistant varieties are the preferred management tactic because it is less expensive and not detrimental to the environment or natural enemies (Mornhinweg *et al*. 2006). After the introduction of the Russian wheat aphid in 1986, no resistant varieties of wheat or barley were available. Between 1986 and 1994, the only means of control was using insecticides, the effectiveness of which was complicated by the refuge of Russian wheat aphids in rolled leaves (Voothuluru *et al*. 2006). It was not until 1994 that resistant wheat was available to producers.

From roughly 100 resistance sources, ten *Dn* genes from wheat and one from rye, *Secale cereale* (L.), were identified and exhibited resistance to the Russian wheat aphid (Voothuluru *et al*. 2006). In 1994, the *Dn*4 gene (in the wheat variety 'Halt') was used on a small scale for Russian wheat aphid resistance until it was registered for public use in 1996 (Quick *et al*. 1996). The *Dn*4 gene was also bred into other varieties of wheat including: 'Prairie Red', 'Yumar', 'Prowers', and 'Prowers 99' (Voothuluru *et al*. 2006). However, in 2003, Prairie Red showed symptoms of Russian wheat aphid damage in Walsh, Colorado (Haley *et al*. 2004). The Russian wheat aphid biotype damaging *Dn*4 wheat was named RWA-2, while the original, extant population was designated RWA-1 (Porter *et al*. 2005). At the time RWA-2 first occurred, 25% of the total wheat acreage planted in Colorado was a *Dn*4 resistant variety (Haley *et al*. 2004). Currently, there are no RWA-2 resistant varieties of wheat deployed in the field. The damaging RWA-2 biotype is also virulent to other wheat cultivars containing these genes: *Dn*1; *Dn*2; *dn*3; *Dn*5; *Dn*6; *Dn*8; and *Dn*9 (Table I) (Haley *et al*. 2004). Only the *Dn*7 gene provides resistance to the RWA-2 biotype (Table I). Another source of RWA-2 resistance controlled by two genes was recently identified in the wheat cereal introduction (CItr) 2401, along with 39 other accessions (Voothuluru *et al.* 2006). The RWA-3 biotype is virulent to all resistant wheat sources, while RWA-4 is virulent to all but the *Dn*4 and *Dn*6 genes (Table I). The RWA-5 biotype damages wheat with genes *Dn*1*, Dn*2*, dn*3*, Dn*8, and *Dn*9 (Table I) (Burd *et al*. 2006). In addition, RWA-5 is avirulent on susceptible wheat variety 'Yuma' (Burd *et al*. 2006) (Table I). One RWA-2 resistant barley variety, 'Burton' which contains the STARS 9301B germplasm is available for use in the field (Bregitzer *et al*. 2005).

Previous aphid studies

RAPD-PCR is a quick, easy and reproducible approach to assay interspecific variation. RAPD-PCR can yield bands of varying intensity and discrete DNA fragments that range from 100-3000 bp (Black *et al*. 1992). The primers used in RAPD-PCR are 10-mer (10 bp) in length which anneal to specific sequences on the DNA. The primer design is random and the area of the genome to which they anneal is unknown.

Genetic polymorphisms were detected between greenbug biotypes by using RAPD-PCR (Black *et al*. 1992). More recently, two genetically distinct bird cherry-oat aphid lineages were resolved in New Zealand, but low overall variability was detected between these two genotypes using RAPD-PCR analysis (Bulman *et al*. 2005). RAPD-PCR showed variation between worldwide populations of the Russian wheat aphid and little variation in United States populations (Puterka *et al*. 1993, Robinson *et al*. 1993, Shufran *et al.* 1997). In addition, United States populations of the Russian wheat aphid resembled those of Mexico, South Africa, Turkey, and France (Puterka *et al.* 1993).

mtDNA sequences were used to detect genetic diversity when studying variation between closely related insect populations (Anstead *et al*. 2002, Downie *et al*. 2001, Kourti 2006, Langor and Sperling 1997, Mun *et al*. 1999, Shufran *et al*. 2000, Viscarret *et al*. 2003). mtDNA is maternally inherited, circular, and does not recombine. Thus it acquires mutations independently of the nuclear genome and no sequence variation exists among members of the same aphid maternal lineage (i.e. clones), barring mutations. The COI gene of the mtDNA showed significant sequence variation between (Shufran *et al*. 2000) and within (Anstead *et al*. 2002) greenbug biotypes and is thus a good candidate for testing Russian wheat aphid biotypes. Sequencing of mtDNA is a useful technique

for phylogenetic studies, biotype occurrence and evolution, and population studies (Barrette *et al*. 1994, Frohlich *et al*. 1999, Shufran *et al*. 2000). In the pea aphid, the length of the mtDNA molecules was very diverse among aphid samples (Barrette *et al*. 1994). The COI gene codes for the enzyme cytochrome oxidase, which is involved in the electron transport chain.

Amplifying nuclear DNA, such as microsatellites, is a traditional PCR method used for DNA fingerprinting. Microsatellites markers (SSR's) are locus specific, highly polymorphic, co-dominant, and have been useful in aphid population genetics studies (Dolatti *et al*. 2005, Loxdale & Lushai 1998, Wilson *et al*. 2003). Microsatellite DNA consists of multiple, repeated 2-5 bp segments, located in the nuclear chromosome. Currently, microsatellites are the best resource to identify clones and clonal lineages (Wilson *et al*. 2004). Regional differences were detected among Iranian populations of the Russian wheat aphid (Dolatti *et al.* 2005). In bird cherry-oat aphid, microsatellites determined that asexual genotypes differ by fewer alleles than sexual genotypes, sexual populations are more genetically diverse than asexual populations, and the asexual populations are more heterozygous (Delmotte *et al*. 2002).

Whereas RAPD-PCR, mtDNA sequencing of the COI gene, and microsatellites are genotypic markers, cuticular hydrocarbon analysis is a phenotypic marker. Hydrocarbons in insects are produced by oenocytes associated with peripheral tissues (Howard and Blomquist 2005). Cuticular hydrocarbons have several functions essential to insects including chemical communication, waterproofing of the cuticle preventing desiccation, and also serving as a barrier to microorganisms (Lockey 1976, Howard and Blomquist 2005). In addition, summer beetles have shown a greater amount of

hydrocarbons, especially those with longer carbon chains, than winter beetles (Nation 2002). Cuticle lipids vary in mixture and composition, but as much as 75% is composed of hydrocarbons (Lockey 1976). Hydrocarbons can vary in length from 14-43 carbons long and are composed of many molecules including n-alkanes, unsaturated hydrocarbons, terminally branched monomethylalkanes, and internally branched monomethyl-, dimethyl-, and trimethyl alkanes (Lockey 1980). Cuticular hydrocarbon analysis is a species specific phenotypic marker (Howard and Blomquist 2005) and cuticular hydrocarbon profiles have been suggested for use as a taxonomic marker (Lockey 1976). Cuticular hydrocarbon analysis, corroborated with mtDNA sequencing, has been used intra-specifically, distinguishing populations in the subterranean termite, *Reticulitermes* (Copren *et al*. 2005). With greenbug, cuticular hydrocarbon profiles have differentiated seven biotypes found in the United States. (Dillwith *et al.* 1990).

Research Objectives

Biotypic variation information is extremely imperative when searching for plant resistance genes and developing resistant varieties (Puterka *et al*. 1992). Developing resistant varieities is time consuming, specifically in wheat, which can take at least 10 years (Sleper and Poehlman 2006.) Resistant lines must be laboratory and field tested and superior selections must be screened (Sleper and Poehlman 2006), in this case with the Russian wheat aphid. In 1992, prior to the occurrence of RWA-2, Puterka detected seven different virulence patterns from eight Russian wheat aphid isolates, including one isolate from the United States. Only one isolate from the Soviet Union was virulent to PI 372129, which is the donor of the *Dn*4 resistance gene in Halt and other varieties of

wheat. In 2003, Haley detected only one germplasm line, 94M370 (*Dn*7 gene), which provides resistance to RWA-2. Therefore, detection of variation within and between Russian wheat aphid biotypes is vital to researchers, plant breeders, pest management companies, consumers, and producers. The goals of the research are to identify and quantify variation between and within Russian wheat aphid biotypes and make inferences about biotype evolution or occurrence. Phenotypic variation has been shown within RWA-2 biotypes (Shufran *et al*. 2007b) present in the United States, but no current research has been conducted to measure genetic variation between or within Russian wheat aphid biotypes. To test for genetic diversity among and within Russian wheat aphid biotypes, 12 clones of RWA-1, 12 clones of RWA-2, and also a single clone of RWA-3, RWA-4, and RWA-5 (Burd *et al.* 2006) were assayed using three types of molecular markers: RAPD-PCR, mtDNA sequencing, and microsatellites. Cuticular hydrocarbon analysis was also used as a phenotypic marker.

CHAPTER II

METHODOLOGY

Insect Material

For the following experiments, I used Russian wheat aphid clones utilized in previous studies which are now published collected field samples (Table II). Russian wheat aphids were originally collected feeding on wheat, barley, rye, or other grasses in the Western United States (Table II). Biotypic status of Russian wheat aphid clones was determined according to plant response (Burd *et al*. 2006, Puterka *et al*. 2006, Shufran *et al*. 2007b). RWA1, RWA-2, RWA-3, RWA-4, and RWA5 biotypes were determined according to plant response to nine *Dn* genes (Burd *et al.* 2006). Russian wheat aphid clones were maintained on susceptible 'Schuyler' barley planted in conetainers with vented plastic covers to prevent contamination, using Fuller's earth fritted clay (Balcones Minerals Corp., Flatonia, Texas) as a planting medium and maintained in a growth chamber at 22:18ºC with a 14:10 h L:D photoperiod or a greenhouse. I evaluated apterous adult aphids belonging to 12 clones of each RWA-1 and RWA-2 biotype, and also from a single clone of RWA-3, RWA-4, and RWA-5 (Table II). The Western wheat aphid, *Diuraphis tritici* (Gillette), was used as an outgroup for interspecific comparison in mtDNA sequencing. Western wheat aphid samples were collected by Robert Hammon, June 25, 1966, near Meeker, Colorado on Mountain Brome, *Bromus marginatus* Nees ex Steud (Colorado Agricultural Experiment Station, Fruita Research Center, 1910 L Road, Fruita, Colorado 81521). The Western wheat aphid population was increased on wheat under greenhouse conditions, collected, and stored at -80°C prior to use.

DNA Extraction

For RAPD-PCR, DNA was extracted from five apterous adult aphids, belonging to 6 clones of RWA-1 biotype, 6 clones of RWA-2 biotype (Table II), and also from a single clone of RWA-3, RWA-4, and RWA-5 (Table II). Extraction procedures were adapted from Kambhampati & Smith (1995). The aphids were homogenized in 50 µl buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl pH 8.0, 0.5 M EDTA, 0.5% SDS) in a 1.5 ml centrifuge tube using a Teflon pestle. An additional 50 µl homogenizing buffer was used to wash the pestle. Each sample was centrifuged briefly then incubated in a 65°C water bath for 30 minutes. Following incubation, 30 µl of 8 M potassium acetate was added to each tube and placed on ice for 30 minutes. After centrifugation at 14,000 g for 15 minutes, the supernatant was transferred to a clean 1.5 ml tube. Two volumes (260 µl) of 100% ethanol was added to the tubes and inverted briefly. The tubes were incubated at -20ºC overnight or -80ºC for two hours and centrifuged again for 15 minutes at 14,000 g. The supernatant was carefully poured off and the DNA pellet was washed in 1 ml 70% ethanol. The tubes were centrifuged for an additional 5 minutes at 14,000 g and the supernatant discarded. Dried DNA pellets were suspended in 50 µl TE pH 8.0. For RAPD-PCR a Beckman DU 2400 spectrophotometer was used to calculate DNA concentrations at OD_{260} using 1:10 dilutions of each Russian wheat aphid sample.

For mtDNA sequencing, DNA was extracted using a single apterous adult aphid from 12 clones of RWA-1 biotype, 12 clones of RWA-2 biotype (Table II), and from a single clone of RWA-3, RWA-4, and RWA-5 (Table II). For microsatellites, DNA was extracted using a single apterous adult aphid from 11 clones of RWA-1 biotype, 11 clones of RWA-2 biotype (Table II), and from a single clone of RWA-3, RWA-4, and

RWA-5 (Table II). DNA extraction methods were adapted from Frohlich *et al.* (1999). Individual aphids were homogenized using 25 µl buffer (5 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.5% Nonidet P-40, 1 mg/ml proteinase K) in spot plates using a Teflon pestle. The homogenate was transferred to a 0.5 ml centrifuge tube and incubated at 65°C following the protocol of Frohlich *et al.* (1999). For microsatellite analysis, a NanoDrop ND-1000 was used to calculate DNA concentrations at OD_{260}.

RAPD-PCR

RAPD-PCR methods were adapted from Puterka *et al*. (1993) and Shufran *et al*. (1997). Variation in Russian wheat aphid biotypes was assayed using 10-mer primers C-01, C-04, C-06, C-07, and C-11 (designed by Operon Biotechnologies, Huntsville, Alabama), which showed variation between worldwide populations (Puterka *et al.* 1993), plus an additional 53 primers (Table III). PCR reactions were completed in 50 µl volumes using: 1X Mg free buffer containing: 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton® X-100 (Promega Corporation, Madison, Wisconsin); 2.25 mM $MgCl_2$ (Promega Corporation, Madison Wisconsin); 0.4 mM dNTP's; 50 pmol primer; 100 ng DNA template; 2.0 U *Taq* DNA polymerase. An MJ PTC-100 thermocycler was used with the following program: 1.) 96ºC for 5 min.; 2.) 80ºC for 1 min.; 3.) 34ºC for 1 min.; 4.) 72ºC for 2 min.; 5.) 94ºC for 1 min.; 6.) 34ºC for 1 min.; 7.) 72ºC for 2 min.; 8.) cycle to step 5, 34 times; 9.) 72ºC for 5 min.; 10.) and 4ºC hold. *Taq* DNA polymerase was added at step 2. For each PCR reaction, 5.0 µl of 6X tracking dye was added to 50 µl PCR product. PCR products were separated using agarose gel electrophoresis by loading 15 µl of the PCR product onto 1.2% 1X TAE high melting point agarose gels. The agarose

gels were run at a constant 70 V in Bio-Rad 11 x 14 cm submarine units until the bromophenol blue tracking dye migrated ¾ the length of the gel. DNA was visualized by staining with ethidium bromide. (Sambrook *et al*. 1989). Each RAPD-PCR reaction was replicated 3 times.

DNA Sequencing

mtDNA sequencing of the COI gene was conducted using methods adapted from Shufran *et al*. (2000). The Primers: C1-J-1718 (5'-TTTTTCTTTACACTTAGCA-3') and C1-N-2191 (5'-CATCCTGTTCCTGTTCCATT-3') were used to amplify a 525 bp section of the COI gene, according to Simon *et al*. (1994). Two PCR reactions were completed for each Russian wheat aphid sample in 50 µl volumes each using: 1X Mg free buffer containing: 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton® X-100 (Promega Corporation, Madison, Wisconsin); 2.5 mM $MgCl_2$ (Promega Corporation, Madison, Wisconsin); 0.2 mM dNTP's; 20 pmol of each forward and reverse primers; 2.0 µl DNA template solution; 2.0 U *Taq* DNA polymerase. PCR was completed using the following program: 1.) 96ºC for 3 min.; 2.) 94°C for 30 sec.; 3.) 50ºC for 30 sec.; 4.) 72ºC for 1 min.; 5.) cycle to step 2, 29 times; 6.) 72ºC for 5 min.; 7.) 4ºC hold. *Taq* DNA polymerase was added at step 3. For each Russian wheat aphid sample, 3.0 µl of product was diluted with 1.0 µl nuclease free water and 1.0 µl 6X tracking dye, loading 5.0 µls total. The size of the PCR products were checked by using 1.2% 1X TAE high melting point agarose gels against a 100 bp ladder (Promega Corporation, Madison, Wisconsin), and visualized by staining with ethidium bromide (Sambrook *et al*. 1989). PCR products for each sample were combined and purified using the Promega Wizard DNA

purification system, by centrifugation or vacuum. Concentration of purified DNA was estimated using a low DNA mass ladder (2000 bp-100 bp blunt-ended fragments, Invitrogen Life Technologies) using 2% high melting point 1X TAE mini agarose gels and stained with ethidium bromide (Sambrook *et al*. 1989). Purified DNA products were sequenced using BigDye™ (Applied Biosystems) terminated reactions and analyzed using an ABI Model 3700 DNA analyzer by the Oklahoma State University Recombinant DNA/Protein Resource facility. DNA sequences were aligned, truncated, and compared using the Lasergene 7 software package (DNASTAR, Inc., Madison, Wisconsin). Western wheat aphid was used as an outgroup.

Microsatellite DNA

Methods used for microsatellite DNA analysis were adapted from Simon *et al*. (1999) and Wilson *et al*. (2004). PCR products were visualized in Real-time using a LI-COR DNA analyzer (LI-COR Biosciences, Lincoln, Nebraska). Eight primer pairs were used to amplify microsatellite DNA at specific loci: Sm10, Sm11, Sm12, S16b, S17b, S23, S49, and Sa4.Σ (Simon *et al*. 1998, Wilson *et al*. 2004) (Table IV). These primers were developed from other species of aphid, specifically the English grain aphid, *Sitobion avenae* and *Sitobion miscanthi* (Takahashi). However, they were shown to cross amplify in other aphids, including the Russian wheat aphid. PCR reactions for primers Sm10, Sm12, S16b, S17b, S49, and Sa4.Σ were completed for each Russian wheat aphid sample in 10 µl volumes using: 1X Green or Colorless GoTaq™ Flexi Buffer (Promega Corporation, Madison, Wisconsin); 2.0 mM $MgCl_2$; 0.2 mM dNTP's; 4.0 pmol of each forward (containing the M13 forward/IRDye 700 primer) and reverse primer; 1.0 ng DNA template; 0.2 pmol M13 reverse/IRDye 700 primer; 1.25 U GoTaq™ DNA

polymerase (Promega Corporation, Madison, Wisconsin). The M13 /IR Dye 700 primer pair serves as a label, which allows visualization of DNA fragments without staining. A solid-state diode laser excites the dye on the DNA fragments as they migrate past a detector window at the bottom of the gel plate. A focusing florescence microscope containing a solid-state silicon avalanche photodiode scans back and forth across the width of the gel collecting data in real time. Primers Sm11 and S23 were obtained from LI-COR Biosciences (Lincoln, Nebraska) and already contained the IRDye 700. PCR reactions for primers Sm11 and S23 were completed for each Russian wheat aphid sample in 10 µl volumes using: 1X Green or Colorless GoTaq™ Flexi Buffer (Promega Corporation, Madison, Wisconsin); 2.0 mM $MgCl_2$; 0.2 mM dNTP's; 4.0 pmol of each forward and reverse primers; 1.0 ng DNA template; 1.25 U GoTaq™ DNA polymerase (Promega Corporation, Madison, WI). PCR reactions were completed using the following program: 1) 96ºC for 3 min.; 2.) 94°C for 30 sec.; 3.) 55ºC for 30 sec.; 4.) 72ºC for 30 sec.; 5.) cycle to step 2, 29 times; 6.) 72ºC for 2 min.; 7.) 4ºC hold. Three different thermocycler programs were used because of differences in primer annealing temperatures. The annealing temperature for primers Sm10 and S16b was 45ºC, and primer Sm11 was 53°C. The annealing temperature for Sm12, S17b, S23, S49, and Sa4.Σ was 55ºC. For each PCR reaction, 1:40 dilutions were made and 2.5 µl LI-COR loading buffer was added to each 10 µl product. To visualize results, PCR products were run in a LI-COR model 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, Nebraska), in 1X TBE buffer, using a 96-well, 0.25 mm 6.5% polyacrylamide gel. Each well was loaded with 0.8 µl total PCR product using an 8-channel 0.2 mm Hamilton (Reno, Nevada) syringe. PCR reactions were run against a 350 bp ladder (LI-COR Biosciences,

Lincoln, Nebraska), at 1500 V, for approximately 1.5 hours. Microsatellite PCR reactions and electrophoresis were replicated 3 times for accuracy. Gel images were captured and bands were sized using Saga^{GT} (LI-COR Biosciences, Lincoln, Nebraska) software.

Cuticular Hydrocarbon Analysis

Insect material used for cuticular hydrocarbon analysis consisted of 3 replications of a single RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5 biotype clone (Puterka *et al*. 2006) and consisted of 200 apterous adult aphids each. All Russian wheat aphid samples were collected and stored at -80°C prior to use.

Methods used for cuticular hydrocarbon analysis were adapted from Bergman *et al*. (1990). Cuticular lipids were extracted from 200 aphids belonging to each Russian wheat aphid clone using 6.0 ml aliquots of hexane for 5 minutes each. Cuticle extracts were evaporated under nitrogen at 60ºC then redissolved in 200 µl of hexane. Dissolved extracts were applied to a Pasteur pipette column containing activated silica gel (Sigma-Aldrich Inc.). Hydrocarbons were then eluted in 6.0 ml of hexane, and again evaporated at 60ºC under nitrogen. The cuticular extract was then redissolved in 50 µl hexane. The hydrocarbon components of Russian wheat aphids were differentiated using a DB-1, 100% methylsilicone capillary column with 15 m, 0.25 mm i.d., 0.1µm film thickness. (J. and W. Scientific Folsom, California) in a HP-5890 GC with a splitless injector and FID. Helium was the carrier gas with the temperature programs as follows: 1.) 50ºC (1.75 min. with purge off); 2.) 40°C/ min. to 180ºC, 8ºC/ min. to 320ºC; 3.) hold at 320ºC for 3 min. Elution times for standard n-alkanes were used to determine equivalent chain lengths.

Raw data on the percentage of primary cuticular hydrocarbons were analyzed by principal component analysis in SAS statistical software (Cary, North Carolina) (Appendix Figure A2). JMP software (SAS Software Company, Cary, North Carolina) was used for cluster analysis by the Ward method (Ward 1963), canonical discriminate analysis, and plotting of the hierarchical dendrogram and the 2-D canonical plot.

Table I: Russian wheat aphid biotype distinction based on phenotypic response to *Dn* resistance genes and wheat varieties (Burd *et al.* 2006).

	Biotype				
Gene/Variety	**RWA-1**	**RWA-2**	**RWA-3**	**RWA-4**	**RWA-5**
Dn1	+	+	+	+	+
Dn2	-	+	+	+	+
dn3	-	+	+	+	+
Dn4	-	+	+	-	-
Dn5	-	+	+	+	-
Dn6	-	+	+	-	-
Dn7	-	-	+	+	-
Dn8	+	+	+	+	+
Dn9	+	+	+	+	+
'Yuma'	+	+	+	+	-
'Custer'	+	+	+	+	+
'TAM 105'	+	+	+	+	+

+ = Virulent to Resistance source
- = Avirulent to Resistance source

Table II: A summary of Russian wheat aphid biotypes used in this research (Burd *et al.* 2006, Puterka *et al.* 2006, Shufran *et al.* 2007b).

Location	Collection Date (mm/ yyyy)	Host	Collector	Biotype
Stillwater, OK[2]	Nov-94	Barley	D. Mornhinweg	RWA-1
Fort Collins, CO[2]	May-03	Barley	F. Peairs	RWA-1
Walsh, CO[2]	May-03	Wheat	T. Walker	RWA-1
Walsh, CO[2]	May-06	Wheat	T. Walker	RWA-1
Bushland, TX[1,2]	May-03	Wheat	J. Michels	RWA-1
Scottsbluff, NE[1,2]	May-03	Wheat	J. Thomas and G. Hein	RWA-1
Akron, CO[1,2]	Jun-03	Wheat	D. Mornhinweg	RWA-1
Banner County, NE[1,2]	Jun-03	Wheat	J. Thomas and G. Hein	RWA-1
Walsh, CO[2]	May-03	Wheat	T. Walker	RWA-1
Hays, KS[1,2]	Nov-03	Wheat	T. Harvey	RWA-1
Prosser, WA	Nov-04	Wheat	G. Puterka	RWA-1
Walsh, CO[1,2]	May-03	Wheat	T. Walker	RWA-2
Walsh, CO[1,2]	May-03	Wheat	T. Walker	RWA-2
Walsh, CO[1,2]	May-03	Wheat	T. Walker	RWA-2
Lubbock, TX[1,2]	May-03	Rye	S. Armstrong	RWA-2
Fort Collins, CO[1,2]	May-03	Wheat	T. Walker	RWA-2
Quinlan, OK	May-06	Wheat	G. Puterka	RWA-2
Shallow Water, TX	May-05	Wheat	G. Puterka	RWA-2
Kimball, NE	Apr-05	*Agropyron desertorum*[+]	G. Puterka	RWA-2
Worland, WY	Jul-05	Barley	G. Puterka	RWA-2
Park Co., WY	Jul-05	Barley	G. Puterka	RWA-2
Fort Collins, CO*	May-03	Wheat	G. Puterka	RWA-2
Bailey County, TX[1]	Jun-02	Wheat	Burd *et al.* 2006	RWA-1
Walsh, CO[1]	Jun-02	Wheat	G. Puterka	RWA-2
Floydada, TX[1]	May-02	Wheat	Burd *et al.* 2006	RWA-3
Idalou, TX[1]	May-02	Wheat	Burd *et al.* 2006	RWA-4
Powell, WY[1]	Aug-03	Barley	Burd *et al.* 2006	RWA-5

[1]=Biotypes used in RAPD-PCR
[2]=Biotype clones from Shufran *et al.* 2007b
*=Only used in mtDNA sequencing of the COI gene
[+]=Non-native, Invasive Species

Table III: Primer sequence information used in RAPD-PCR analysis of Russian wheat aphid biotypes.

Name	Sequence	Name	Sequence
A-01	5'-CAG GCC CTT C-3'	B-10	5'-CTG CTG GGA C-3'
A-02	5'-TGC CGA GCT G-3'	B-11	5'-GTA GAC CCG T-3'
A-03	5'-AGT CAG CCA C-3'	B-12	5'-CCT TGA CGC A-3'
A-04	5'-AAT CGG GCT G-3'	B-13	5'-TTC CCC CGC T-3'
A-05	5'-AGG GGT CTT G-3'	B-14	5'-TCC GCT CTG G-3'
A-06	5'-GGT CCC TGA C-3'	B-15	5'-GGA GGG TGT T-3'
A-07	5'-GAA ACG GGT G-3'	B-16	5'-TTT GCC CGG A-3'
A-08	5'-GTG ACG TAG G-3'	B-17	5'-AGG GAA CGA G-3'
A-09	5'-GGG TAA CGC C-3'	B-18	5'-CCA CAG CAG T-3'
A-10	5'-GTG ATC GCA G-3'	B-19	5'-ACC CCC GAA G-3'
A-11	5'-CAA TCG CCG T-3'	B-20	5'-GGA CCC TTA C-3'
A-12	5'-TCG GCG ATA G-3'	C-01	5'-TTC GAG CCA G-3'
A-13	5'-CAG CAC CCA C-3'	C-04	5'-CCG CAT CTA C-3'
A-14	5'-TCT GTG CTG G-3'	C-06	5'-GAA CGG ACT A-3'
A-15	5'-TTC CGA ACC C-3'	C-07	5'-GTC CCG ACG A-3'
A-16	5'-AGC CAG CGA A-3'	C-11	5'-AAA GCT GCG G-3'
A-17	5'-GAC CGC TTG T-3'	D-01	5'-ACC GCG AAG G-3'
A-18	5'-AGG TGA CCG T-3'	D-02	5'-GGA CCC AAC C-3'
A-19	5'-CAA ACG TCG G-3'	D-03	5'-GTC GCC GTC A-3'
A-20	5'-GTT GCG ATC C-3'	D-04	5'-TCT GGT GAG G-3'
B-01	5'-GTT TCG CTC C-3'	D-05	5'-TGA GCG GAC A-3'
B-02	5'-TGA TCC CTG G-3'	D-06	5'-ACC TGA ACG G-3'
B-03	5'-CAT CCC CCT G-3'	D-07	5'-TTG GCA CGG G-3'
B-04	5'-GGA CTG GAG T-3'	D-08	5'-GTG TGC CCC A-3'
B-05	5'-TGC GCC CTT C-3'	D-09	5'-CTC TGG AGA C-3'
B-06	5'-TGC TCT GCC C-3'	D-10	5'-GGT CTA CAC C-3'
B-07	5'-GGT GAC GCA G-3'	D-11	5'-AGC GCC ATT G-3'
B-08	5'-GTC CAC ACG G-3'	D-12	5'-CAC CGT ATC C-3'
B-09	5'-TGG GGG ACT C-3'	D-13	5'-GGG GTG ACG A-3'

Table IV: Primers used to amplify microsatellite loci of Russian wheat aphids (Simon *et al*. 1999, Wilson *et al*. 2004).

Name	Sequence
Sm10-F	5'-TCT TCT CTA TAC ACC TAT AAA C-3'
R	5'-TTA TGC TAA TCT CAC AAT AC-3'
Sm11-F	5'-AAC CCT ACG GGT AAC GCC-3'
R	5'-GGT ACC CCT ATG TTA TTA CGC G-3'
Sm12-F	5'-TTC GGT ATA ATA GTG CGT G-3'
R	5'-GGC GAT GCG ACT AAA C-3'
S16b-F	5'-ATA AAA CAA AGA GCA ATT CC-3'
R	5'-GTA AAA GTA AAG GTT CCA CG-3'
S17b-F	5'-TTC TGG CTT CAT TCC CGG TCG-3'
R	5'-CGT CGC GTT AGT GAA CCG TG-3'
S23-F	5'-GGT CCG AGA GCA TTC ATT AGG-3'
R	5'-CGT CGT TGT CAT TGT CGT CG-3'
S49-F	5'-CGC ATT TAG GAG GTT TCG AC-3'
R	5'-CAT GTG CAG TGG ACG AGG AA-3'
Sa4.Σ-F	5'-GTG ACG TAT ACG CGA TGC G-3'
R	5'-GAC GTC GAT ATT AGC CTA GCC-3'

CHAPTER III

RESULTS

RAPD-PCR

Using any of the 58 primers, only two detected polymorphic bands: primer B-04 and primer B-07 (Figures 2 and 4). Primer B-04 detected variation in RWA-5 and also clone 7 (RWA-2) (Figure 2). Both RWA-5 and clone 7 were missing the band in the 1,200 bp region, which was present in all other biotypes and clones (Figure 2). Primer B-07 was able to detect variation between biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5 (Figure 4). A single polymorphic band of approximately 800 bp was found in biotype RWA-5, but was absent in all other biotypes (Figure 4). The single polymorphic bands were repeated in three different PCR reactions. No other variation was detected between or within Russian wheat aphid clones or biotypes (Figures 1, 3, 5, 6, and 7).

DNA Sequencing

No variation was found in mtDNA sequences of the COI gene between or within biotypes RWA-1 and/or RWA-2 (Appendix, Figure A1). Also, no sequence variation was found between RWA-3, RWA-4, and RWA-5 (Appendix, Figure A1). All DNA sequences were submitted to GenBank with accession numbers 21093-21120. However, there was interspecific variation between Russian wheat aphid and the Western wheat aphid (Appendix, Figure A1). Between these two species, there were 22 residue substitutions including: 15 cytosine-thymine; 5 adenine-thymine; 1 adenine-cytosine; and 1 adenine-guanine. The bp frequencies between the species differed slightly; 75.92% of the bp present in the Russian wheat aphid consisted of adenine (A) and thymine (T); whereas the Western wheat aphid consisted of 75.69% A and T (Table V). The majority (93%) of transitions were cytosine-thymine, while the majority of transversions (83%)

were adenine-thymine. In addition, the purines adenine and guanine (75%) were the dominating nucleotides in the fragment (Table V). In greenbug, 84% of the third codon position consisted of adenine and guanine (Shufran *et al*. 2000). In the corn stalk borer (*Sesamia nonagrioides*), adenine and guanine composed 70% of the nucleotides in the COI gene (Kourti 2006).

Microsatellite DNA

Primer Sm12 failed to amplify any alleles. In the remaining seven loci, no variation was found between or within biotypes RWA-1 or RWA-2, or between RWA-3, RWA-4, and RWA-5. The primers detected a maximum of 2 bands at each locus, ranging from 101 bp -201 bp (Table VI, Figures 8-12). Dolatti *et al*. (2004) detected 1-4 alleles per locus in the Russian wheat aphid, ranging from 90 bp-187 bp, using the same primers I evaluated. Wilson *et al*. (2004) detected 1-12 alleles per locus in *S. miscanthi*, ranging from 86 bp-227 bp, using primers Sm10, Sm12, S16b, S17b, S23, and S49. In addition, Simon *et al*. (1999) found 4-16 alleles per locus in the English grain aphid, which ranged from 139 bp-240 bp, using primers Sm10, Sm11, Sm12, and Sa4.Σ. All microsatellite loci I tested were homozygous, except for S17b and Sa4.Σ, which were heterozygous (Table VI, Figures 8 and 9). According to Dolatti *et al*. (2004), primer S17b was homozygous, although a deficit in heterozygosity was also detected using these primers.

Cuticular Hydrocarbon Analysis

The majority of Russian wheat aphid cuticular hydrocarbons consisted of n-alkanes and 3-methyl-branched hydrocarbons ranging from 23-33 carbons in length. The cuticular hydrocarbons analyzed included: nC_{23}; nC_{25}; 3-me-C_{25}; nC_{27}; 3-me-C_{27}; nC_{29}; 3-me-C_{29}; nC_{31}; and nC_{33}. The Ward method (Ward 1963) was used to construct a hierarchical tree, separating biotypes. I chose this method because it is a way of analyzing inter-cluster distance based on the sum of squares between each individual biotype principal component cluster, summed over all variables (Ward 1963). In the hierarchical dendrogram constructed using SAS JMP software (Figure 13), a darker red color means a higher percentage of a particular hydrocarbon in the cuticle, where a dark blue color distinguishes a lower percentage of the hydrocarbon in the cuticle. The hierarchical dendrogram clusters each repetition of RWA-1 and RWA-2 biotypes, according to the relative percentages of each hydrocarbon mixture. For example, RWA-1 and RWA-2 are widely separated by color: RWA-1 has a higher percentage of the hydrocarbons nC_{23}, nC_{25}, 3-me-C_{25}, nC_{27}, and 3-me-C_{27} (red), while RWA-2 has a higher percentage of longer carbon chain hydrocarbons such as 3-me-C_{29}, nC_{31}, and nC_{33} (blue). RWA-3, RWA-4, and RWA-5 were not clustered by each repetition (Figure 13). In contrast, the canonical plots made using the SAS JMP software showed hydrocarbon profiles characteristic for each five Russian wheat aphid biotype examined. In the 2-D canonical cluster assembled using the SAS JMP software (Figure 14), the three repetitions of biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5 were clearly separated. However, in the canonical plot RWA-1 and RWA-2 are the most widely separated biotypes based on hydrocarbon composition.

Figure 1: RAPD-PCR gel showing monomorphic bands in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Primer A-18

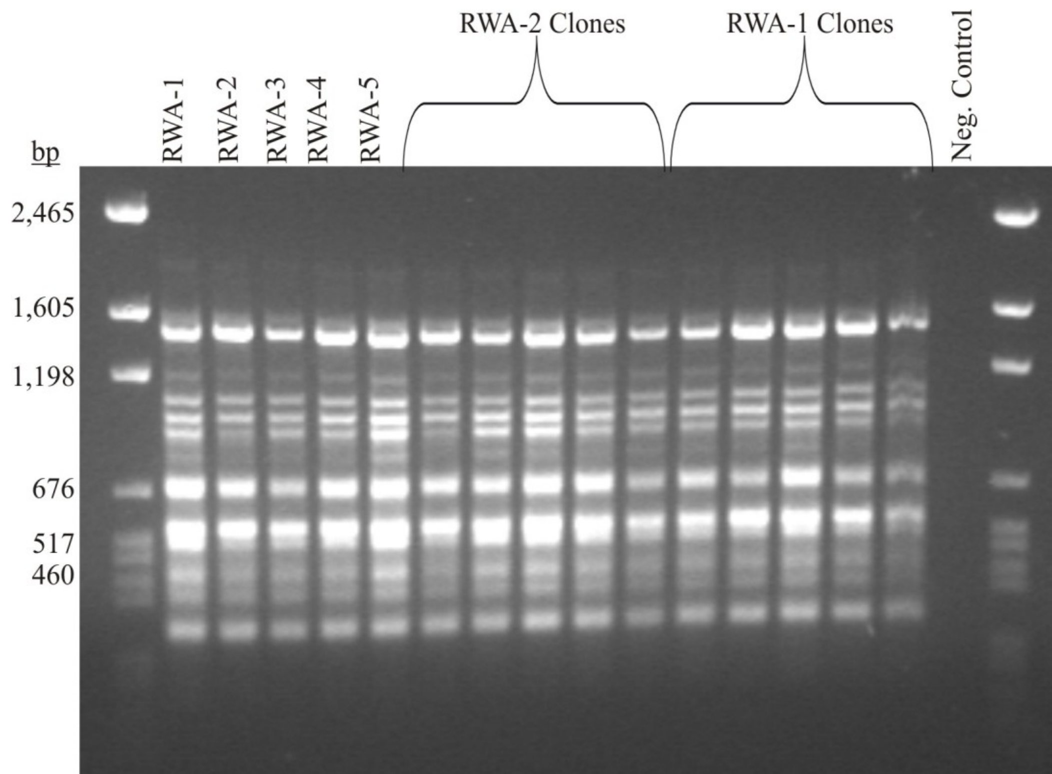

Figure 2: RAPD-PCR gel showing polymorphic bands in RWA-5 and clone 7 (RWA-2) at the 1,200 bp region. All Russian wheat aphid biotypes are monomorphic. Primer B-04.

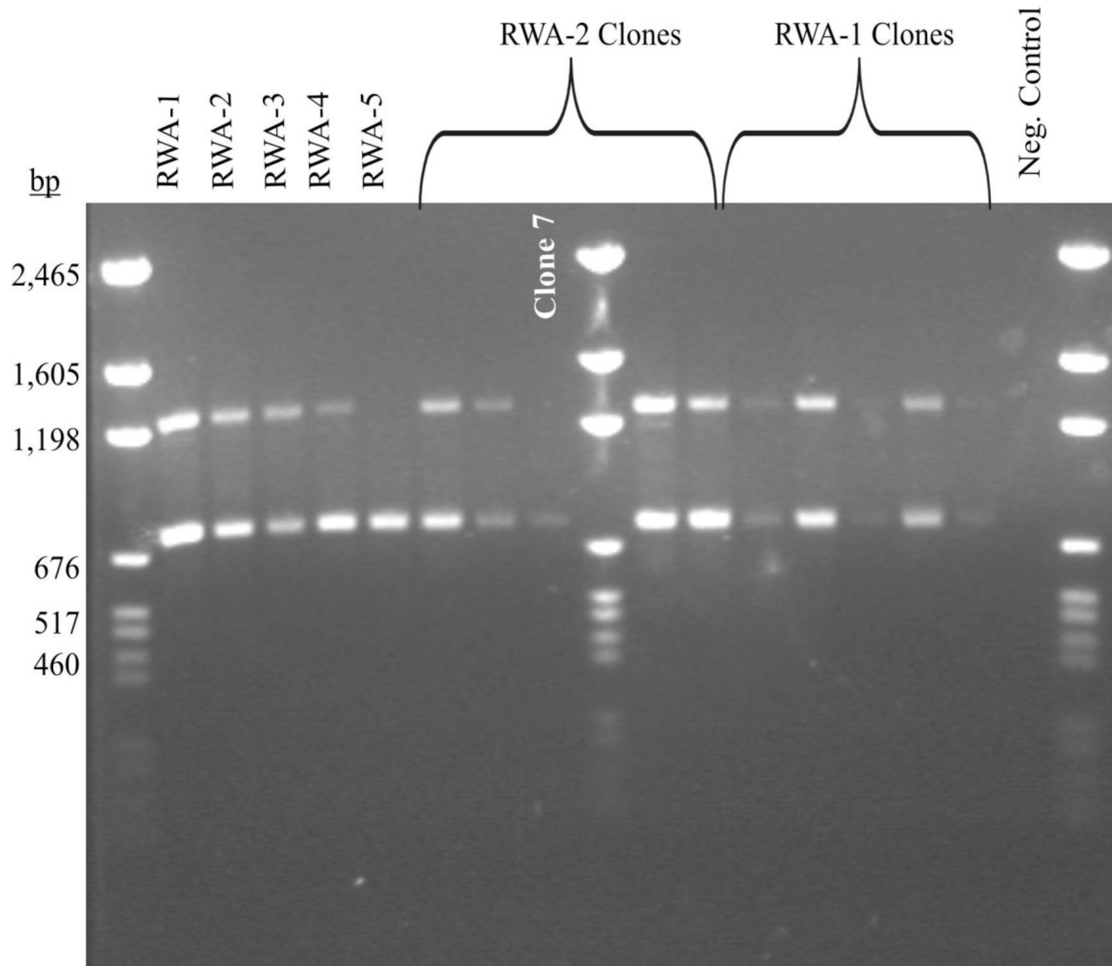

Figure 3: RAPD-PCR gel showing monomorphic bands in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Primer B-06.

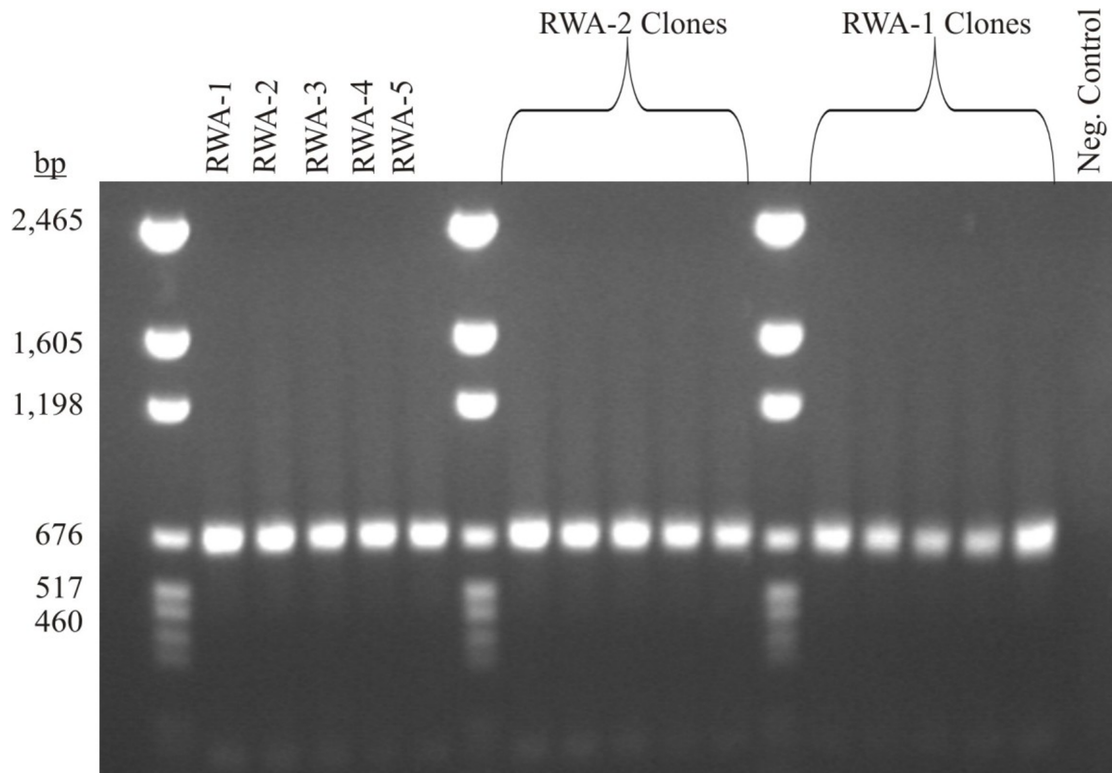

Figure 4: RAPD-PCR gel showing a single polymorphic band in RWA-5 at 800 bp. All other Russian wheat aphid biotypes are monomorphic. Primer B-07

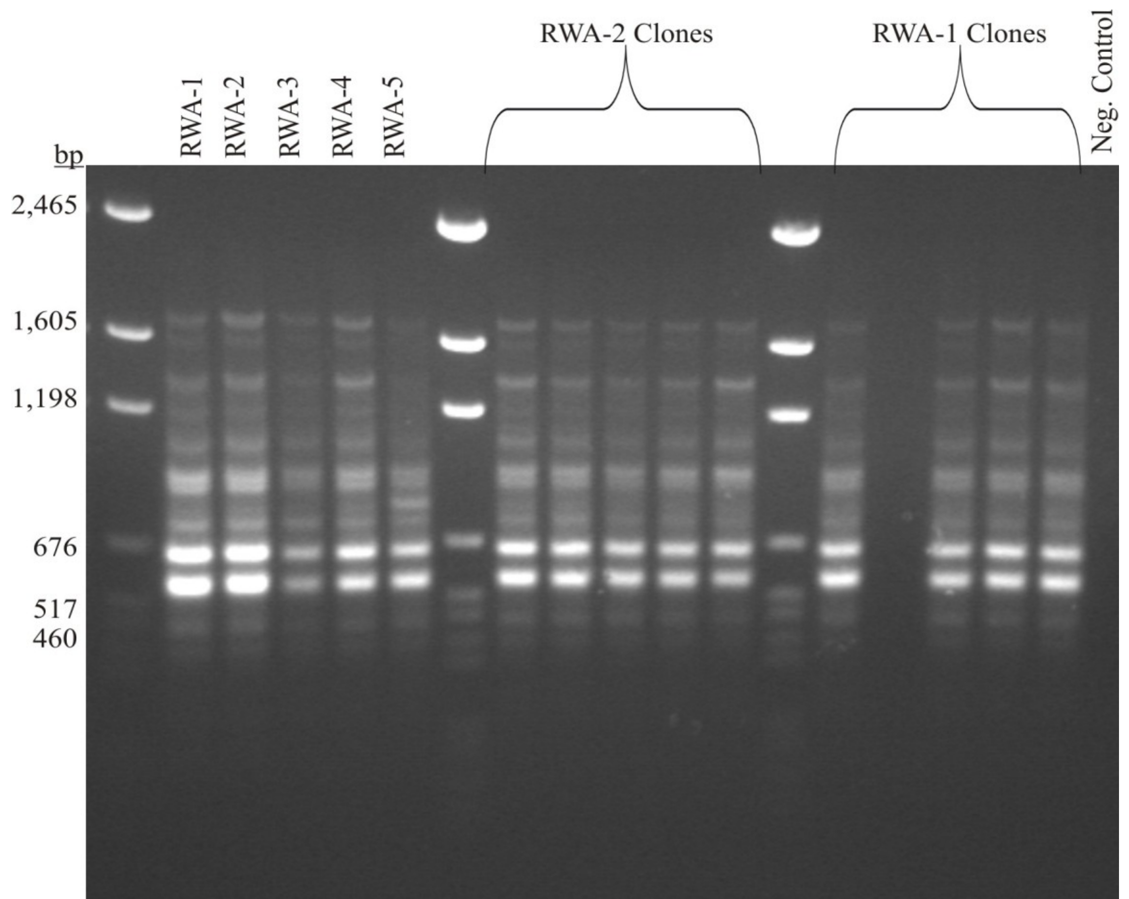

Figure 5: RAPD-PCR gel showing monomorphic bands in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Primer B-18

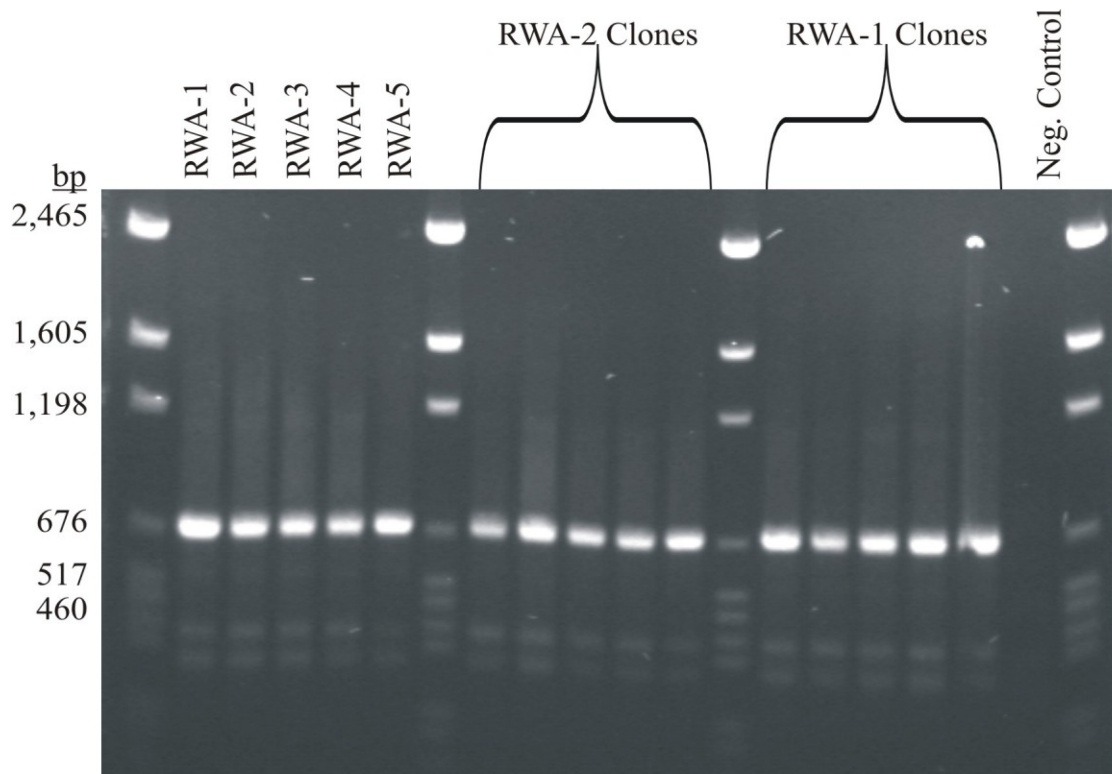

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Figure 6: RAPD-PCR gel showing monomorphic bands in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Primer C-01.

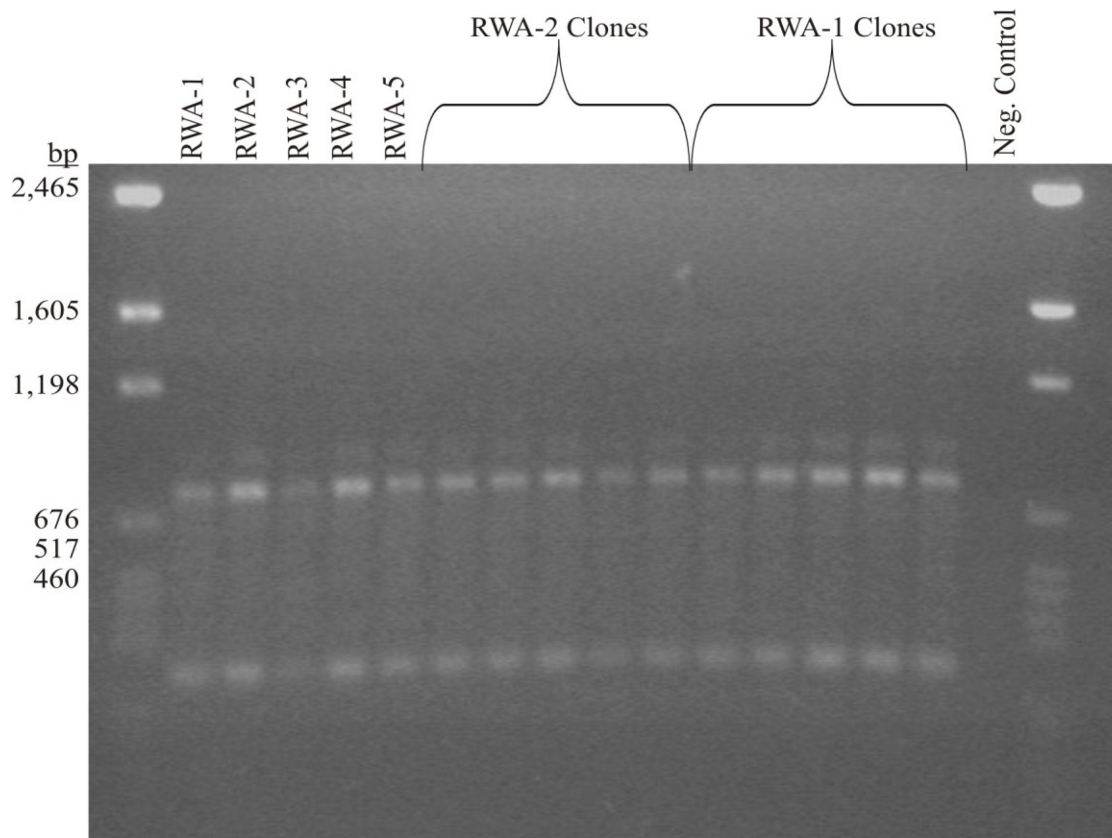


Figure 7: RAPD-PCR gel showing monomorphic bands in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Primer D-13.

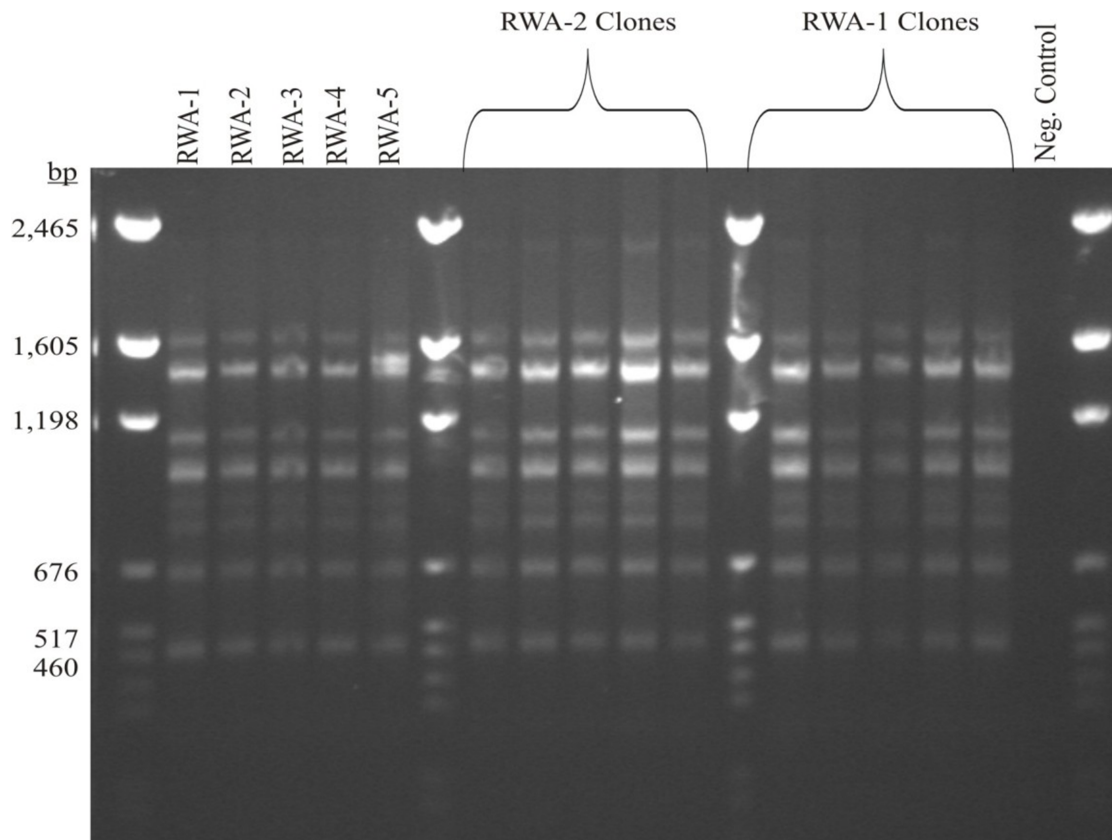


Table V: Differences between Russian wheat aphid and Western wheat aphid mtDNA COI nucleotide percentages. Data collected from 12 clones of each Russian wheat aphid biotype RWA-1 and RWA-2, a single clone of RWA-3, RWA-4, and RWA-5, and a single clone of the Western wheat aphid.

Nucleotide	Russian wheat aphid %	Western wheat aphid %
A	35.09	34.86
T	9.4	9.63
G	40.6	41.06
C	14.91	14.45

Table VI: Number and size of alleles amplified in microsatellite loci of Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

Loci	No. Alleles	Size (bp) in 25 individuals
Sm10	1	185
Sm11	1	153
S16b	1	201
S17b	2	173,185
S23	1	102
S49	1	101
Sa4.Σ	2	178,180

Figure 8: Microsatellite loci Sa4.Σ showing monomorphic heterozygous alleles of 178 bp and 180 bp in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

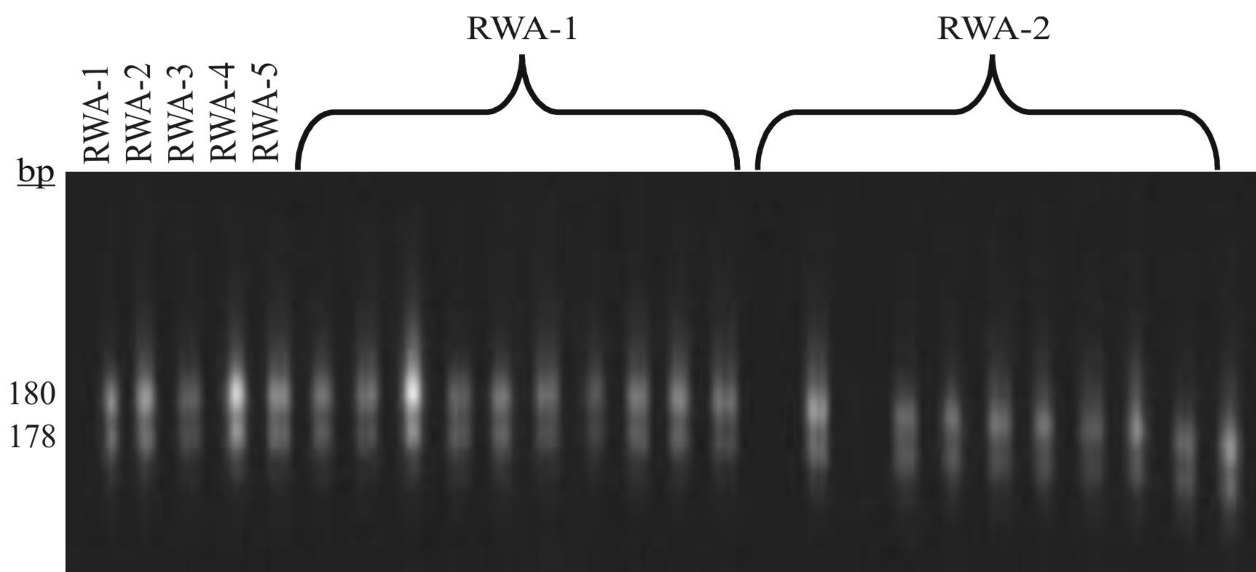


Figure 9: Microsatellite loci S17b showing monomorphic heterozygous alleles of 173 bp and 185 bp in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

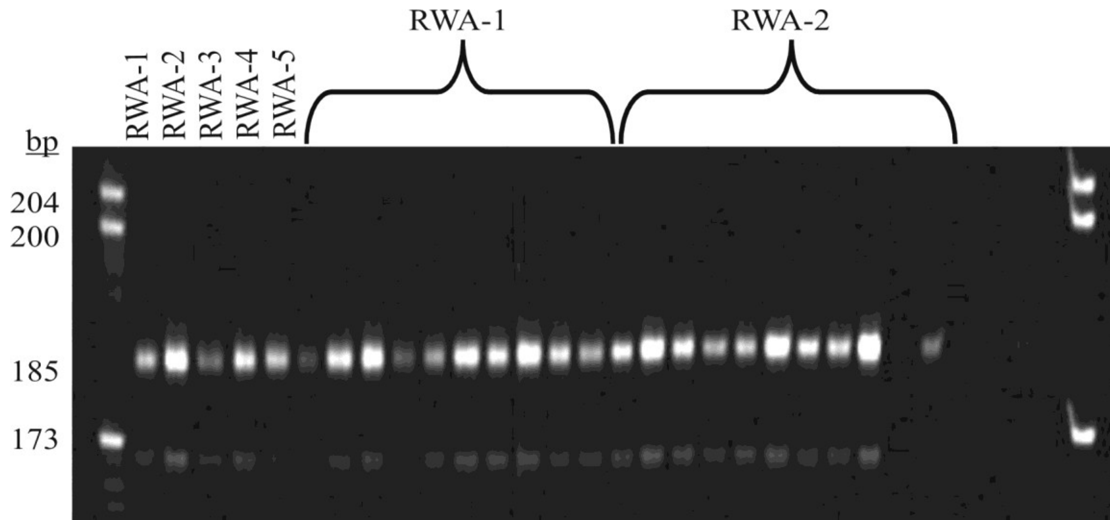


Figure 10: Microsatellite marker Sm10 showing monomorphic homozygous alleles of 185 bp in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

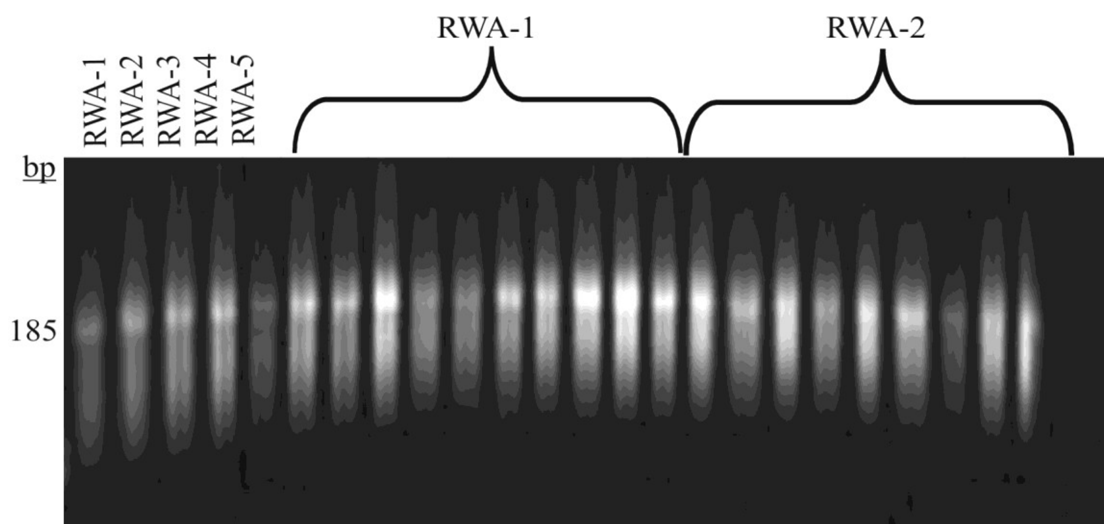


Figure 11: Microsatellite primer S16b showing monomorphic homozygous alleles of 201 bp in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

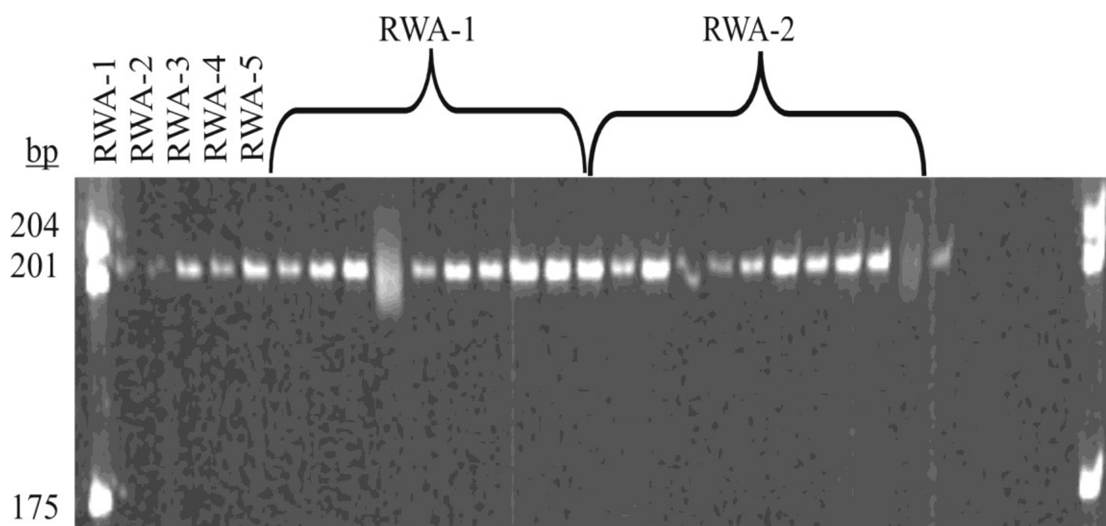


Figure 12: Microsatellite primer S49 showing monomorphic homozygous alleles of 101 bp in Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

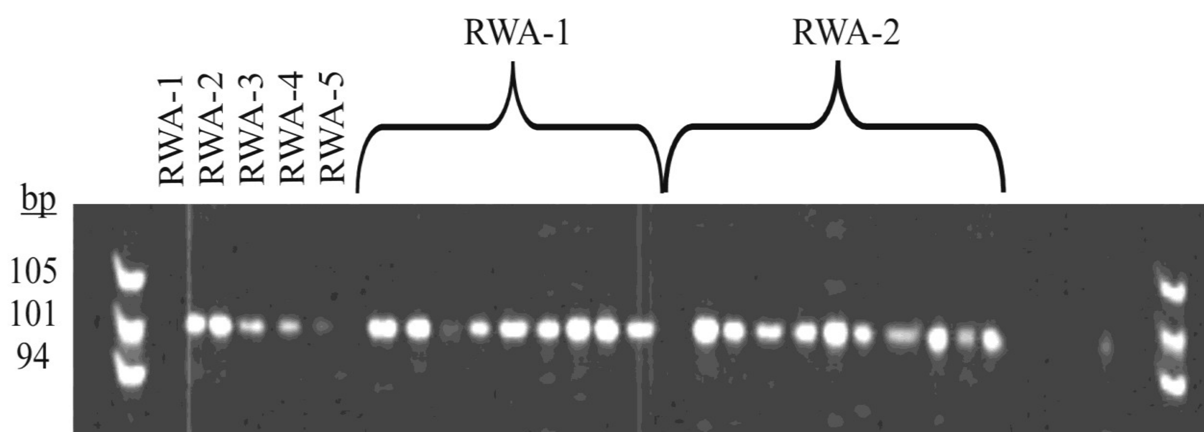


Figure 13: Hierarchical cluster dendrogram constructed using the Ward method (Ward 1963) based on principal component analysis of cuticular hydrocarbons of each Russian wheat aphid biotype RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Red = higher percentage, whereas blue = a lower percentage of a hydrocarbon in the cuticle.

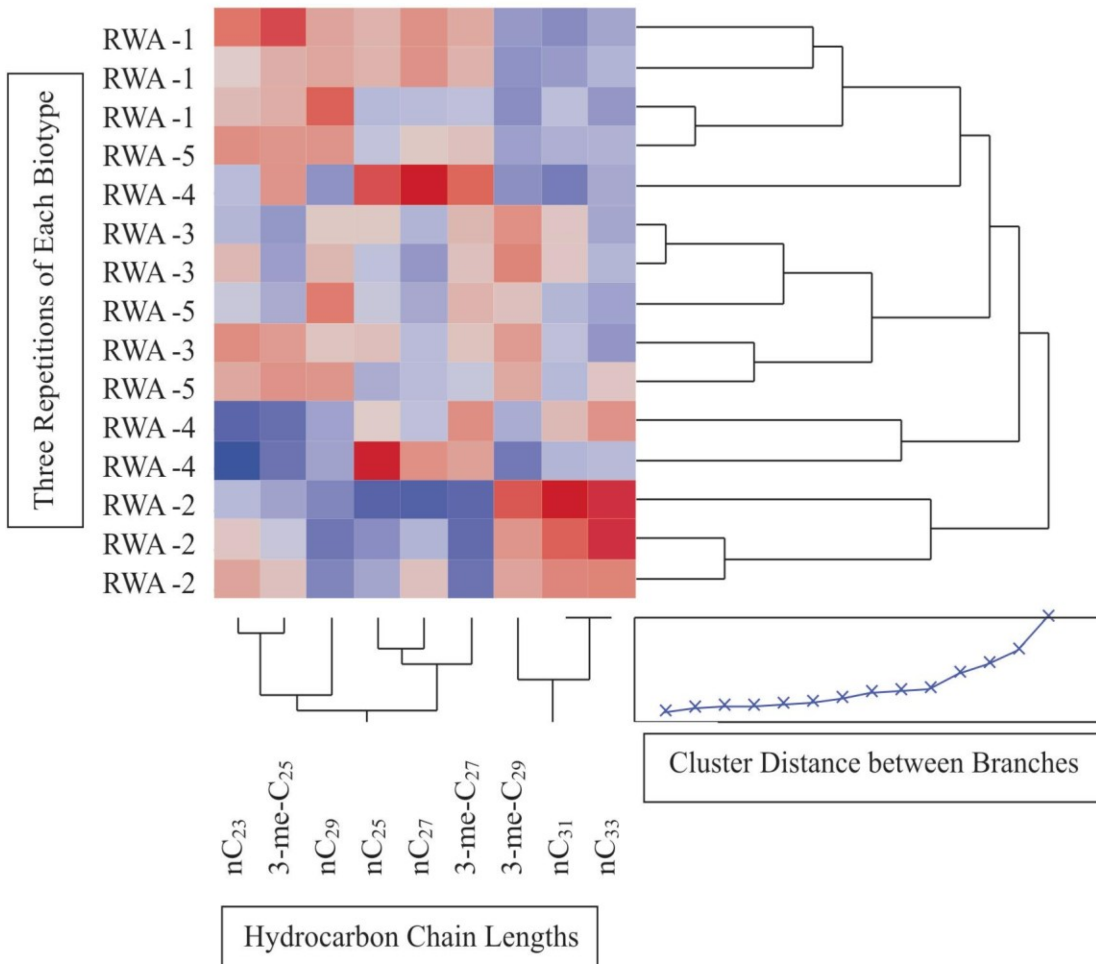
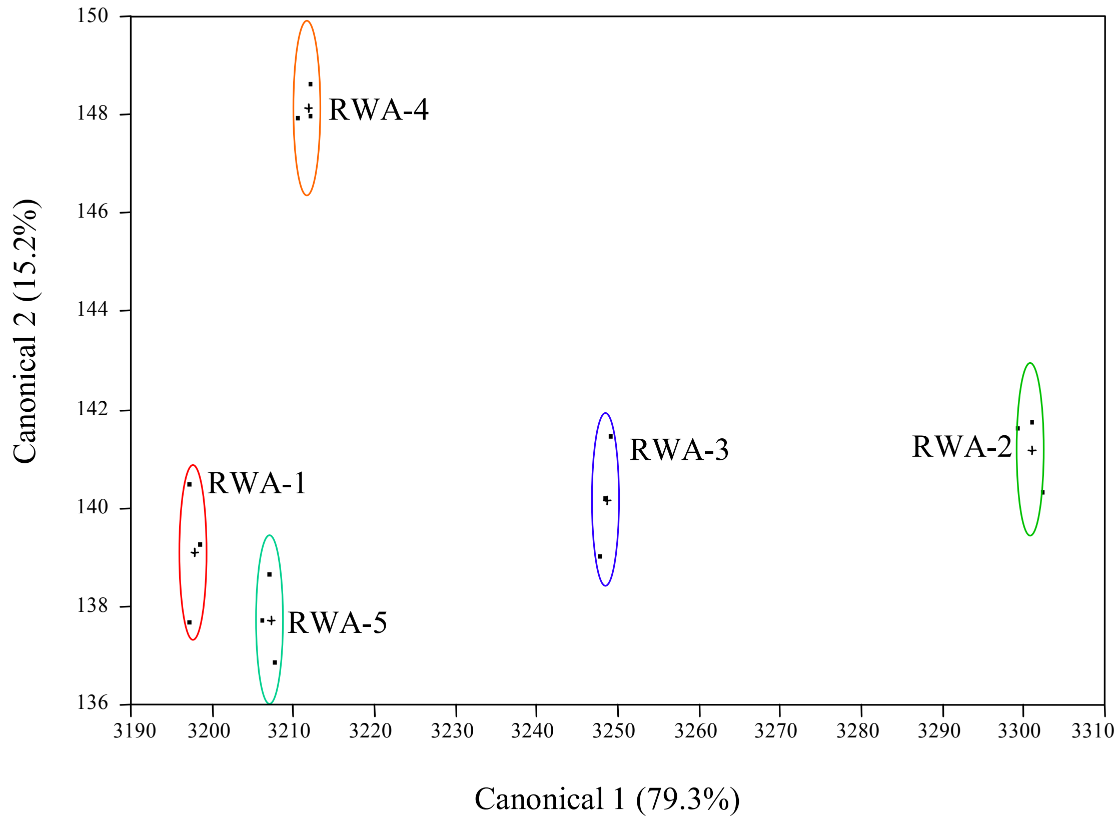


Figure 14: 2-D canonical plot determined by discriminate analysis separating biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. Based on principal component of Russian wheat aphid cuticular hydrocarbons.



CHAPTER IV

DISCUSSION AND CONCLUSIONS

Puterka *et al.* (1993) concluded there was a considerable amount of genetic variation using RAPD-PCR among Russian wheat aphid populations present in North America, using samples from Kansas, California, Montana, Oregon, New Mexico, Nebraska, Colorado, Idaho, and Canada. Although the populations differed slightly from one another, the North American populations were the most biotypically similar to the Russian wheat aphids from France, but genetically similar to populations from Mexico, South Africa, and Turkey. The most genetically diverse Russian wheat aphids included populations from Kirghiz, Jordan, Syria, and Moldavia. This finding suggests the initial population present in the United States involved a single introduction from the population which originated in Turkey and was introduced into South Africa and Mexico. In addition, Shufran *et al.* (1997) showed no biotypic differences in Russian wheat aphid populations in the United States according to plant response. Currently, Russian wheat aphid biotypes RWA-1 and RWA-2 vary according to plant response with wheat containing the *Dn* genes (Burd *et al*. 2006), specifically *Dn*4 (Haley *et al*. 2004). It is theorized the occurrence of RWA-2 in 2003 could have resulted from a second introduction of the Russian wheat aphid (Haley *et al*. 2004). Using RAPD-PCR, three single polymorphic bands were detected: two in RWA-5; and one in clone 7 (RWA-2) (Figures 2 and 4) and was absent in all other biotypes and individuals (Figures 1, 3, 5, 6, and 7). Compared to Puterka *et al*. (1993), Robinson *et al.* (1993), and Shufran *et al.* (1997) this RAPD-PCR experiment shows the Russian wheat aphid populations in the United States have become slightly more varied. The lack of significant polymorphic bands infer the biotypes present in the United States, especially RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5 are derived from a common source or population. If a greater

amount of polymorphic bands were detected between Russian wheat aphid biotypes, the presence of corresponding polymorphic bands could be similar to those detected in Russian wheat aphids from outside North America, such as Black *et al*. (1992) detected among South African populations and Puterka *et al.* (1993) found among populations in Moldavia and Syria. Furthermore, multiple clones of RWA-2 vary in the degree of plant response, based on chlorosis, shoot dry weight, and plant height (Shufran *et al*. 2007b). This finding suggests that the Russian wheat aphid biotypes in the United States have increased in phenotypic diversity, but decreased genotypically.

Shufran *et al*. (2000) was able to differentiate among greenbug biotypes using mtDNA sequence divergence of the COI gene. It was also concluded greenbug biotypes consisted of host-adapted races, not unique biotypes. A host race can be formed by the exploitation of a new host by a pest (Marchetti *et al*. 2007). Anstead *et al*. (2002) showed a greater amount of diversity among greenbug clones collected from non-cultivated hosts and suggested virulence genes could be exchanged among the biotypes due to a sexual cycle in the greenbug. However, I detected almost no variation within or between Russian wheat aphid biotypes RWA-1 and RWA-2, or between single clones of RWA-3, RWA-4, and RWA-5 (Appendix, Figure A1). Interspecific variation was present between the Russian wheat aphid and the Western wheat aphid. This shows there is variation in mtDNA between the two species and verifies sequencing was completed correctly. The mtDNA in the Russian wheat aphid and Western wheat aphid is very similar, except for 22 variable nucleotides (Appendix, Figure A1). The lack of variation in Russian wheat aphid mtDNA illustrates that the biotypes are very closely related, possibly arising from the same maternal lineage. Russian wheat aphid samples taken in

the United States from 1986-2006 also lack variation, showing the homogeneity in this area of the COI gene in the Russian wheat aphid population since it was introduced (Shufran *et al*. 2007a). Furthermore, it can be concluded the Russian wheat aphid biotypes could consist of unique clones. Currently, the only way to identify biotypes is according to phenotypic plant response, and plant response can also be variable within a biotype (Shufran *et al*. 2007b).

Simon *et al.* (1999) and Wilson *et al.* (2004) showed that microsatellite loci on the recombinant nuclear DNA can cross amplify between species, specifically from *S. miscanthi* and *S. avenae*, to the Russian wheat aphid. In my experiments, no variation was detected among Russian wheat aphid samples in all seven loci tested (Figures 8, 9, 10, 11, and 12). The majority of the microsatellite loci tested consisted of homozygous alleles (Figures 10, 11, and 12). However, with primers S17b and Sa4.Σ, two alleles were amplified, meaning the loci were heterozygous (Figures 8 and 9). This shows that highly variable, microsatellite DNA is uniform between and within Russian wheat aphid clones of RWA-1 and RWA-2, and also between a single clone of RWA-3, RWA-4, and RWA-5 biotypes. Since no variation was found in Russian wheat aphid clones or biotypes, it can be inferred no crossing over or mutations have occurred in the loci tested that could account for the differences in plant response of the biotypes. Dolatti *et al*. (2004) suggested since the Iranian populations consisted of a high level of unique genotypes and genotypic diversity, sexual reproduction in Iranian populations of the Russian wheat aphid is likely. The Russian wheat aphid is indigenous to Iran and also exhibits obligate parthenogenesis in this region, but it has only recently become a pest in this area (Dolatti *et al.* 2004). Similar to the United States biotypes and clones tested, the majority of the

loci tested in Russian wheat aphids from Iran were also homozygous (Dolatti *et al*. 2004). However, S17b only amplified one allele at this locus in the Iranian populations, but two alleles were amplified in the Russian wheat aphids I tested (Figure 9). This finding could mean the Russian wheat aphid biotypes in the United States possibly did not originate from Iranian populations. In addition to genotypic studies, Dolatti *et al*. (2004) also observed different damage ratings on wheat and barley for Iranian populations of the Russian wheat aphid, which is similar to the observations of Shufran *et al*. (2007b) within RWA-2 clones. Overall, it can be concluded that Russian wheat aphid populations have greater variability closer to their origin (i.e. Iran) than areas of more recent introduction (i.e. North America). This could be due to a bottlenecking of populations, or a founder effect, where a very small population has given rise to a large, uniform population.

Dillwith *et al.* (1990) showed that cuticular hydrocarbon analysis could distinctly separate greenbug biotypes, especially biotypes B and F. Greenbug biotypes B and F were separated in a 3-D canonical diagram based on principal component analysis (Dillwith *et al*. 1990). Biotypes B, E, and C were more closely clustered, while biotypes F, G, and H were separated. The cuticular hydrocarbons extracted from the Russian wheat aphid also showed distinct differences between RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5 (Figure 13). In the dendrogram, RWA-1 and RWA-2 are the most widely separated and biotypes RWA-3, RWA-4, and RWA-5 follow no distinct pattern (Figure 13). It appears that RWA-1 and RWA-2 have the most clearly diverse cuticular hydrocarbon mixture, and are easily identifiable from the plot. This correlates with Puterka *et al*. (2007), which determined RWA-2 and to a lesser extent RWA-1 to be the most abundant biotypes present in the United States. In addition, RWA-2 has a higher

percentage of longer chain hydrocarbons than RWA-1, specifically nC_{31} and nC_{33} (Figure 13). This could correlate to longer chain hydrocarbons being present in summer and/or desert dwelling insects, especially since RWA-2 arose during a large drought. In contrast, the 2-D canonical plot indicates five distinct Russian wheat aphid groupings consistent with the designated Russian wheat aphid biotypes tested. RWA-1 and RWA-5 appear to be the most closely related, and it is interesting that the two are also avirulent to the resistance genes *Dn*4, *Dn*5, *Dn*6, and *Dn*7 (Burd *et al*. 2006). Also, RWA-2 and RWA-3 are the farthest apart from the other biotypes, and they also happen to be the only biotypes that are virulent to *Dn*4 and *Dn*6, while RWA-1, RWA-4, and RWA-5 are avirulent (Burd *et al*. 2006). RWA-3 appears to be located in the middle of the other biotypes, which is important in noting that it is the most virulent, damaging all resistance genes (Figure 14, Table I). From the canonical plot, biotypes RWA-1 and RWA-2 are the most distinctive according to cuticular hydrocarbon composition, since they are located the farthest apart (Figure 14). From this experiment, RWA-1 and RWA-2 are the most distinctive biotypes, but all Russian wheat aphid biotypes tested were separated by their specific hydrocarbon mixture (Figure 14). This experiment on Russian wheat aphid biotypes was conducted only once, therefore more replications, including multiple clones of each biotype, should be performed to support these findings.

It can be concluded that Russian wheat aphid populations present in the United States are very uniform, possibly resulting from a single introduction. In addition, from DNA sequencing, the current biotypes could have arisen from the extant population (RWA-1). It could also be inferred that Russian wheat aphid populations in the United States may not have undergone a sexual cycle to produce new genotypes. From cuticular

hydrocarbon analysis, RWA-1 and RWA-2 are the most distinctive biotypes currently present in the Western United States. For the Russian wheat aphid biotypes in the United States to become more genetically similar and less phenotypically similar, a change in the environment could have occurred. This could be due to a founder effect, or the result of several bottlenecks. More Russian wheat aphid samples should be evaluated, specifically using cuticular hydrocarbon analysis and possibly RAPD-PCR, to further evaluate the status of the populations in the United States.

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APPENDIX

Figure A1: Alignment of mtDNA COI sequences from Russian wheat aphid biotypes: 12 clones of RWA-1; 12 clones of RWA-2; one clone of RWA-3, RWA-4, RWA-5; and the Western wheat aphid.

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                          ----------------+-------------------+-------------------+-------------------+-------------------+---
                                         10                  20                  30                  40                  50
                          ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)  C A T A T C T T T T C C A C G A T T A A A T A A T A T T A G A T T C T G A T T A T T A C C T C C T T   71
RWA-1 Stillwater, OK      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   71
RWA-1 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   83
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   61
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   64
RWA-1 Bushland, TX        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   53
RWA-1 Scottsbluff, NE     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   59
RWA-1 Akron, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   67
RWA-1 Scottsbluff, NE     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   61
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   65
RWA-1 Hays, KS            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   71
RWA-1 Prosser, WA         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   68
RWA-2 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   70
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   60
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   50
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   61
RWA-2 Lubbock, TX         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   62
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   72
RWA-2 Quinlan, OK         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   69
RWA-2 Shallow Water, TX   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   77
RWA-2 Kimball, NE         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   65
RWA-2 Worland, WY         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   67
RWA-2 Park Co., WY        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   68
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   72
RWA-3 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   64
RWA-4 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   67
RWA-5 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   70
D. tritici                T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . G . . . C . T . . . . . . .   59
```

Figure A1 : Continued

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                          ----------------+-------------------+-------------------+-------------------+-------------------+---
                                         60                  70                  80                  90                 100
                          ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)C A T T A A T A A T A A T A A T T T G T A G A T T C A T A A T T A A T A A T G G A A C A G G A A C A   121
RWA-1 Stillwater, OK    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   121
RWA-1 Fort Collins, CO  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   133
RWA-1 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   111
RWA-1 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   114
RWA-1 Bushland, TX      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   103
RWA-1 Scottsbluff, NE   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   109
RWA-1 Akron, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   117
RWA-1 Banner Co., NE    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   111
RWA-1 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   115
RWA-1 Hays, KS          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   121
RWA-1 Prosser, WA       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   118
RWA-2 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   120
RWA-2 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   110
RWA-2 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   100
RWA-2 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   111
RWA-2 Lubbock, TX       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   112
RWA-2 Fort Collins, CO  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   122
RWA-2 Quinlan, OK       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   119
RWA-2 Shallow Water, TX . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   127
RWA-2 Kimball, NE       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   115
RWA-2 Worland, WY       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   117
RWA-2 Park Co., WY      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   118
RWA-2 Fort Collins, CO  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   122
RWA-3 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   114
RWA-4 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   117
RWA-5 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   120
D. tritici              . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . .   109
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Figure A1 : Continued

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                                 ----------------+-------------------+-------------------+-------------------+-------------------+---
                                                110                 120                 130                 140                 150
                                 ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)G G A T G A A C T A T T T A T C C A C C A T T A T C A A A T A A T A T T G C T C A T A A T A A T A T           171
RWA-1 Stillwater, OK    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           171
RWA-1 Fort Collins, CO  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           183
RWA-1 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           161
RWA-1 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           164
RWA-1 Bushland, TX      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           153
RWA-1 Scottsbluff, NE   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           159
RWA-1 Akron, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           167
RWA-1 Banner Co., NE    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           161
RWA-1 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           165
RWA-1 Hays, KS          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           171
RWA-1 Prosser, WA       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           168
RWA-2 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           170
RWA-2 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           160
RWA-2 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           150
RWA-2 Walsh, CO         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           161
RWA-2 Lubbock, TX       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           162
RWA-2 Fort Collins, CO  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           172
RWA-2 Quinlan, OK       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           169
RWA-2 Shallow Water, TX . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           177
RWA-2 Kimball, NE       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           165
RWA-2 Worland, WY       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           167
RWA-2 Park Co., WY      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           168
RWA-2 Fort Collins, CO  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           172
RWA-3 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           164
RWA-4 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           167
RWA-5 (Burd et al. 2006). . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .           170
D. tritici              . . . . . . . . A . . . . . C . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . .           159
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Figure A1 : Continued

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                            ----------------+-------------------+-------------------+-------------------+-------------------+---
                                           160                 170                 180                 190                 200
                            ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)    T T C A G T T G A T T T A A C T A T T T T T T C T T T A C A C T T A G C A G G A A T T T C A T C A A    221
RWA-1 Stillwater, OK        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    221
RWA-1 Fort Collins, CO      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    233
RWA-1 Walsh, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    211
RWA-1 Walsh, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    214
RWA-1 Bushland, TX          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    203
RWA-1 Scottsbluff, NE       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    209
RWA-1 Akron, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    217
RWA-1 Banner Co., NE        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    211
RWA-1 Walsh, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    215
RWA-1 Hays, KS              . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    221
RWA-1 Prosser, WA           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    218
RWA-2 (Burd et al. 2006)    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    220
RWA-2 Walsh, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    210
RWA-2 Walsh, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    200
RWA-2 Walsh, CO             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    211
RWA-2 Lubbock, TX           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    212
RWA-2 Fort Collins, CO      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    222
RWA-2 Quinlan, OK           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    219
RWA-2 Shallow Water, TX     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    227
RWA-2 Kimball, NE           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    215
RWA-2 Worland, WY           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    217
RWA-2 Park Co., WY          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    218
RWA-2 Fort Collins, CO      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    222
RWA-3 (Burd et al. 2006)    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    214
RWA-4 (Burd et al. 2006)    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    217
RWA-5 (Burd et al. 2006)    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    220
D. tritici                  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . .    209
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Figure A1 : Continued

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                          ----------------+-------------------+-------------------+-------------------+-------------------+---
                                         210                 220                 230                 240                 250
                          ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)  T T T T A G G A G C A A T T A A T T T T A T T T G T A C A A T T T T A A A T A T A A T A C C T A A T    271
RWA-1 Stillwater, OK      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    271
RWA-1 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    283
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    261
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    264
RWA-1 Bushland, TX        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    253
RWA-1 Scottsbluff, NE     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    259
RWA-1 Akron, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    267
RWA-1 Banner Co., NE      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    261
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    265
RWA-1 Hays, KS            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    271
RWA-1 Prosser, WA         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    268
RWA-2 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    270
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    260
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    250
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    261
RWA-2 Lubbock, TX         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    262
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    272
RWA-2 Quinlan, OK         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    269
RWA-2 Shallow Water, TX   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    277
RWA-2 Kimball, NE         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    265
RWA-2 Worland, WY         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    267
RWA-2 Park Co., WY        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    268
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    272
RWA-3 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    264
RWA-4 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    267
RWA-5 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    270
D. tritici                . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . .    259
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Figure A1 : Continued

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                          ----------------+-------------------+-------------------+-------------------+-------------------+---
                                         260                 270                 280                 290                 300
                          ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)  A A T T T A A A A T T A A A T C A A A T T C C T C T T T T T C C A T G A T C A A T T C T A A T T A C    321
RWA-1 Stillwater, OK      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    321
RWA-1 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    333
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    311
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    314
RWA-1 Bushland, TX        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    303
RWA-1 Scottsbluff, NE     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    309
RWA-1 Akron, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    317
RWA-1 Banner Co., NE      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    311
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    315
RWA-1 Hays, KS            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    321
RWA-1 Prosser, WA         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    318
RWA-2 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    320
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    310
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    300
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    311
RWA-2 Lubbock, TX         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    312
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    322
RWA-2 Quinlan, OK         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    319
RWA-2 Shallow Water, TX   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    327
RWA-2 Kimball, NE         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    315
RWA-2 Worland, WY         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    317
RWA-2 Park Co., WY        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    318
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    322
RWA-3 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    314
RWA-4 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    317
RWA-5 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    320
D. tritici                . . . A . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . A . . . . . . .    309
```

Figure A1 : Continued

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                           ----------------+-------------------+-------------------+-------------------+-------------------+---
                                          310                 320                 330                 340                 350
                           ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)   A G C T A T T C T A T T A A T C T T A T C T T T A C C A G T T T T A G C T G G A G C T A T T A C A A   371
RWA-1 Stillwater, OK       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   371
RWA-1 Fort Collins, CO     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   383
RWA-1 Walsh, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   361
RWA-1 Walsh, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   364
RWA-1 Bushland, TX         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   353
RWA-1 Scottsbluff, NE      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   359
RWA-1 Akron, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   367
RWA-1 Banner Co., NE       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   361
RWA-1 Walsh, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   365
RWA-1 Hays, KS             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   371
RWA-1 Prosser, WA          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   368
RWA-2 (Burd et al. 2006)   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   370
RWA-2 Walsh, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   360
RWA-2 Walsh, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   350
RWA-2 Walsh, CO            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   361
RWA-2 Lubbock, TX          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   362
RWA-2 Fort Collins, CO     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   372
RWA-2 Quinlan, OK          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   369
RWA-2 Shallow Water, TX    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   377
RWA-2 Kimball, NE          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   365
RWA-2 Worland, WY          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   367
RWA-2 Park Co., WY         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   368
RWA-2 Fort Collins, CO     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   372
RWA-3 (Burd et al. 2006)   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   364
RWA-4 (Burd et al. 2006)   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   367
RWA-5 (Burd et al. 2006)   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   370
D. tritici                 . . . . . . . T . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . .   359
```

Figure A1 : Continued

```
                              ----------------+-------------------+-------------------+-------------------+-------------------+---
                                             360                 370                 380                 390                 400
                              ----------------+-------------------+-------------------+-------------------+-------------------+---
RWA-1 (Burd et al. 2006)      T A T T A T T A A C T G A T C G A A A C T T A A A T A C T T C A T T T T T T G A T C C A G C A G G A    421
RWA-1 Stillwater, OK          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    421
RWA-1 Fort Collins, CO        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    433
RWA-1 Walsh, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    411
RWA-1 Walsh, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    414
RWA-1 Bushland, TX            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    403
RWA-1 Scottsbluff, NE         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    409
RWA-1 Akron, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    417
RWA-1 Banner Co., NE          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    411
RWA-1 Walsh, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    415
RWA-1 Hays, KS                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    421
RWA-1 Prosser, WA             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    418
RWA-2 (Burd et al. 2006)      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    420
RWA-2 Walsh, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    410
RWA-2 Walsh, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    400
RWA-2 Walsh, CO               . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    411
RWA-2 Lubbock, TX             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    412
RWA-2 Fort Collins, CO        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    422
RWA-2 Quinlan, OK             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    419
RWA-2 Shallow Water, TX       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    427
RWA-2 Kimball, NE             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    415
RWA-2 Worland, WY             . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    417
RWA-2 Park Co., WY            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    418
RWA-2 Fort Collins, CO        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    422
RWA-3 (Burd et al. 2006)      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    414
RWA-4 (Burd et al. 2006)      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    417
RWA-5 (Burd et al. 2006)      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    420
D. tritici                    . . C . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .    409
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Figure A1 : Continued

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                           ----------------+-------------------+-------------------+---------------
                                          410                 420                 430
                           ----------------+-------------------+-------------------+---------------
RWA-1 (Burd et al. 2006)  G G A G G A G A T C C T A T T T T A T A T C A A C A T T T A T T C T G A       457
RWA-1 Stillwater, OK      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       457
RWA-1 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       469
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       447
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       450
RWA-1 Bushland, TX        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       439
RWA-1 Scottsbluff, NE     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       445
RWA-1 Akron, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       453
RWA-1 Banner Co., NE      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       447
RWA-1 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       451
RWA-1 Hays, KS            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       457
RWA-1 Prosser, WA         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       454
RWA-2 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       456
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       446
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       436
RWA-2 Walsh, CO           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       447
RWA-2 Lubbock, TX         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       448
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       458
RWA-2 Quinlan, OK         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       455
RWA-2 Shallow Water, TX   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       463
RWA-2 Kimball, NE         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       451
RWA-2 Worland, WY         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       453
RWA-2 Park Co., WY        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       454
RWA-2 Fort Collins, CO    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       458
RWA-3 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       450
RWA-4 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       453
RWA-5 (Burd et al. 2006)  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .       456
D. tritici                . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . T . . .       445
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Figure A2: SAS program commands used in cuticular hydrocarbon analysis of Russian wheat aphid biotypes RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5.

```
proc import out=RWA
        datafile="H:\My Documents\Consulting Clients\AG\Payton\Payton - RWA data.xls"
                        dbms=excel2000 replace;
getnames=yes;
proc print;
data RWA;
set RWA;
drop CL25_7 CL27_7 CL29_7;
*Tree Diagram;
proc princomp data=RWA covariance out=RWA;
var CL23--CL33;
proc means data=RWA noprint;
by Biotype;
var Prin1-Prin7;
output out=RWAMean mean(Prin1-Prin7)=Prin1-Prin7;
proc standard data=RWAMean out=RWAMean mean=0 std=1;
var Prin1-Prin7;
proc cluster data=RWAMean outtree=RWATree method=centroid;
var Prin1-Prin3;
id Biotype;
proc tree data=RWATree horizontal;
id Biotype;
*3D Plot;
proc candisc data=RWA out=RWA;
class Biotype;
var CL23--CL33;
run;
data RWA;
set RWA;
if Biotype=1 then do;
        shape='balloon';
        color='blue';end;
if Biotype=2 then do;
        shape='heart';
        color='red';end;
if Biotype=3 then do;
        shape='club';
        color='green';end;
if Biotype=4 then do;
        shape='diamond';
        color='orange';end;
if Biotype=5 then do;
        shape='cross';
        color='purple';end;
proc g3d data=RWA;
scatter Can1*Can2=Can3 / color=color shape=shape;
proc gplot data=RWA;
plot Can2*Can1=Biotype;
*/run;
```

VITA

Tracey Lee Payton

Candidate for the Degree of

Master of Science

Thesis: GENETIC VARIATION WITHIN AND BETWEEN RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* (KURDJUMOV), BIOTYPES FOUND IN THE UNITED STATES

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Major Field: Entomology

Scope and Method of Study: The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), is a pest on wheat and barley in the Western United States. Management is achieved primarily by resistant varieties. In 2003, the Russian wheat aphid caused damage to wheat with the *Dn4* resistance gene in Southeastern Colorado. The damaging biotype was designated RWA-2 and the extant population RWA-1. Plant response experiments showed variation within RWA-2 clones based on chlorosis, plant height, and shoot dry weight to wheat with the *Dn4* resistance gene. Phenotypic variation found within RWA-2 argues against the hypothesis that RWA-2 populations consist of a single, genetically identical clone.

Findings and Conclusions: I conducted studies to observe variation between and within Russian wheat aphid biotypes using 12 clones of RWA-1 and RWA-2 biotype, and a single clone of: RWA-3; RWA-4; and RWA-5 biotypes, determined according to plant response to *Dn* genes. Using RAPD-PCR, three single band polymorphisms were detected distinguishing RWA-5 and clone 7 (RWA-2) after screening all Russian wheat aphids with 58 primers. I found no sequence variation within or between biotypes in a 436 bp fragment of the COI gene in the mtDNA. Seven microsatellite DNA markers were used to evaluate clonal diversity, but no variation was found. In addition, cuticular hydrocarbon analysis was performed on multiple aphids belonging to a single clone of RWA-1, RWA-2, RWA-3, RWA-4, and RWA-5. This method showed RWA-1 and RWA-2 had unique hydrocarbon mixtures, based on principal component analysis. Ultimately all five biotypes could be separated according to canonical variables. Using genotypic markers, Russian wheat aphid biotypes are homogenous, and consist of a single introduction into the United States. Using phenotypic markers, RWA-1 and RWA-2 are more distinctive of the five Russian wheat aphid biotypes. Lack of genotypic variation could have resulted from a founder effect upon introduction into North America, followed by bottlenecking of populations in the United States.

ADVISOR'S APPROVAL: Dr. Kevin Shufran