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Seasonal Phenology of *Amphorophora agathonica* (Hemiptera: Aphididae) and Spread of Viruses in Red Raspberry in Washington

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ABSTRACT *Amphorophora agathonica* (Hottes) is the primary vector of aphid-transmitted viruses in red raspberry in the Pacific Northwest region of the United States. To better understand the biology of the aphid, we estimated the lower developmental threshold and studied the seasonal activity of *A. agathonica* in commercial fields in northern Washington state. In addition, we monitored the spread of raspberry viruses (raspberry latent virus and raspberry leaf mottle virus, RLMV) to determine how rapidly fields became infected and whether there was a relationship between aphid presence and infection. The lower developmental threshold of *A. agathonica* was estimated to be 2.7°C. In the field, apterous and alate aphid populations began rapidly increasing at ≈800 growing degree-days and peaked at 1,050 growing degree-days. RLMV spread rapidly, with 30–60% of plants in four different commercial fields testing positive after three growing seasons. There was no discernible relationship between the presence or abundance of aphids based on 10 leaves sampled per plant location, and the odds of that plant becoming infected with RLMV.

RESUMEN *Amphorophora agathonica* (Hottes) es el principal vector de virus transmitidos por áfidos en cultivos de frambuesa en la región Noroeste del Pacífico de los Estados Unidos. Para obtener un mejor entendimiento sobre la biología de A. agathonica, SE estimó el umbral inferior de desarrollo y su actividad estacional en cultivos comerciales en el norte del Estado de Washington. Adicionalmente, SE monitorearon índices de diseminación del Virus latente de la frambuesa (por sus siglas en inglés) y Virus del moteado de la hoja de la frambuesa (RLMV) a nivel de campo, así como la existencia de una relación entre presencia de áfidos y niveles de infección de los virus. El umbral inferior de desarrollo de A. agathonica fue estimado en 2.7°C. En campo, poblaciones de áfidos ápteros y alados empezaron rápidamente a aumentar a aproximadamente 800 grados día de desarrollo llegando a un máximo de 1,050 grados día de desarrollo. RLMV SE diseminó rápidamente, evidenciado por un 30 a 60% de plantas positivas para el virus en cuatro diferentes campos comerciales después de tres temporadas de cultivo. No existió relación discernible entre la presencia o abundancia de áfidos, basado en 10 hojas muestreadas por planta por localidad, y la posibilidad de que dicha planta sea infectada por RLMV.

KEY WORDS closteroviridae, reoviridae, RpLV, RLMV, raspberry aphid

Amphorophora agathonica (Hottes), sometimes referred to as large raspberry aphid, is a common pest found in commercial red and black raspberries (*Rubus idaeus* L. and *Rubus occidentalis* L.) across the northern United States and Canada. Feeding damage resulting from *A. agathonica* is limited; however, it is a crop contaminant and important vector of economically damaging viruses (Kieffer et al. 1983, Isaacs and Trefor Woodford 2007). In black raspberries, *A. aga-*thonica is the main vector of black raspberry necrosis virus (family *Secoviridae*, genus unassigned *Secoviridae* species), which is responsible for loss of plant vigor and field decline (Halgren et al. 2007). In red raspberries, *A. agathonica* is responsible for transmission of raspberry leaf mottle virus (RLMV; family *Closteroviridae*, genus *Closterovirus*) and raspberry latent virus (RpLV; family *Reoviridae*, genus unassigned *Reoviridae* species). These viruses, when found in combination with raspberry bushy dwarf virus (RBDV; family unassigned, genus *Idaeovirus*), cause crumbly fruit disease, which decreases fruit quality and marketability (Martin et al. 2013).

A. agathonica is monoecious, a nonhost alternating aphid that uses *Rubus* spp. as its only host. Reported hosts include commercially planted black raspberry and red raspberry, as well as a suite of wild native

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Rubus (*Rubus parviflorus* Nuttall, *Rubus ursinus* Chamisso & Schlechtendal, *Rubus odoratus* L., *Rubus armeniacus* Focke and *Rubus phoenicolasius* Maximovich; Blackman and Eastop 2000). There are scattered reports of *Fragaria* × *ananassa* Duschesne as also being an accepted host, although the degree to which *Fragaria* are used is unknown (Stultz 1968). *A. agathonica* may be found on *Rubus* from early spring until late fall, where it overwinters as an egg which is laid on the undersides of leaves and, rarely, on the cane itself (Winter 1929).

Aphids, with short generation times and rapid population growth, can be efficient transmitters of plant viruses. Thus, it is important to have a good knowledge of the seasonal phenology of a given aphid species to predict when populations will be greatest and implement management strategies that have the greatest impact (Poehling et al. 2007). The seasonal phenology of *A. agathonica* has been documented in the past in New York (Kennedy and Schaefers 1974), but the populations may have different trends in the Pacific Northwest, where the summer and winter climate is more mild. By determining the lower developmental threshold of the aphid, the seasonal development through use of degree-days can be calculated and compared between growing seasons to observe when management strategies may be most usefully applied.

The research objectives for this study were to determine the lower temperature threshold and monitor the seasonal phenology of *A. agathonica.* In addition, we monitored raspberry fields in northern Washington for infection with RLMV, RpLV, and RBDV to look for potential relationships between aphid populations and virus infection.

Materials and Methods

Determination of Temperature Thresholds. The raspberry cultivar used for all studies was 'Meeker' (*R. idaeus*) obtained as planting stock from Sakuma Brothers Inc. (Burlington, WA). Canes were planted in 10-cm pots and grown in a greenhouse set at 16°C night and 21°C day temperatures and a photoperiod of 16:8 (L:D) h.

The aphid colony was begun with adult *A. agathonica* collected from commercial raspberry fields in Whatcom County, WA, in June 2010. Ten aphid adults were used to begin the colony, so the colony was not clonal. Because aphids in the colony were observed to undergo genetic drift by exhibiting decreased acquisition rates of plant viruses (D.Q.-A., unpublished data), the colony was restarted with field-collected aphids every October and June. Aphids were reared on Meeker plants in a growth chamber under fluorescent growth lights at 22°C and a photoperiod of 16:8 (L:D) h. New plants were added weekly to maintain high plant quality.

To determine the lower developmental threshold of *A. agathonica*, aphid development was measured at five different temperatures in growth chambers (Percival Scientific Inc., Perry, IA): 10, 14, 18, 22, and 26°C. A HOBO datalogger (Onset Computer Corp., Bourne,

MA) recorded the temperature and humidity in each chamber. Three days before the study, actively growing Meeker plants with 15- to 30-cm-tall primocanes were placed into the growth chambers to acclimate. A cohort of aphid nymphs was obtained by isolating adult aphids in a petri dish with a leaf. After 12 h, aphid nymphs were removed and placed into the different temperature treatments. Nymphs were caged to a terminal leaflet of a young fully expanded leaf using clip cages made from 15-ml plastic tubes that were cut into 2-cm lengths. Clip cages were attached to the leaf with a rubber-coated washer and metal hair clip. The hair clip was affixed to a binder clip on a wooden stake to reduce the stress to the petiole of the leaf. Only one nymph was caged on each plant. When the leaf began to turn yellow and leaf quality declined, aphids were moved to a new leaflet. The experiment was replicated five times, with six aphids per treatment per replicate for a total of 30 aphids observed at each temperature.

Once nymphs were caged, they were checked every 24 h for molting into the next nymphal instar, as indicated by the presence of aphid exuviae inside the clip cage. The number of days to reach adulthood and the number of days from adulthood until the first nymph born (prereproductive period) was recorded. The development rate (y) of each insect was calculated as $y = 1/d$ where d was the number of days required for the insect to develop into the next life stage (Andrewartha and Birch 1954, Campbell et al. 1974), and regressed against the temperature. The degree-day model

$$y = a + bT$$

was fit over the linear portion of the regression, where T was the temperature at which the insect developed and a and b are regression constants. The lower developmental threshold was calculated as $-a/b$, and the number of degree-days required for development (K) was calculated as $1/b$ (Campbell et al. 1974). Values of y and K were calculated for birth to adulthood, the prereproductive period (time from adult to first nymph born), and from birth to laying of first nymph.

Field Monitoring. Aphid populations and virus infection levels were surveyed in four commercial Meeker red raspberry fields located in Whatcom Co., WA. Meeker is the most commonly grown cultivar in the region (Washington Red Raspberry Commission [WRRC] 2008). Fields were located within a 7-km radius and were managed conventionally with four to seven insecticide sprays each year. Raspberries were planted at 0.6-m spacing, with 3- to 3.5-m spacing between rows.

In September 2010, 108 plants were flagged across the four commercial fields. Field 1, planted in spring 2010, was 12.5 acres and had two plants flagged per row across 18 rows for a total of 36 plants. Flagged plants were ≈90 m apart within a row, 13 m apart between rows, and 10–40 m from the ends of the rows. Fields 2 and 3, both planted in spring 2010, were 18 acres and 58 acres, respectively. Each field had two plants flagged per row across 12 rows for a total of 24 plants

per field. Flagged plants were ≈50 m apart within a row, 10–15 m apart between rows, and 30–40 m from the ends of the rows. Field 4, planted in spring 2009, was 56 acres and also had two plants flagged per row across 12 rows for a total of 24 plants. Flagged plants in field 4 were ≈50 m apart within a row, 7 m apart between rows, and 30 m from the ends of the rows.

To monitor population dynamics of *A. agathonica*, leaves were collected weekly from March to October 2011 and April to October 2012. Fifty locations were selected for weekly sampling which were a subset of the 108 locations where plants were flagged and tested for viruses. At each sample location, 10 leaves were collected randomly at different heights. Only fully expanded leaves near the meristem were collected because these are preferential feeding locations for *A. agathonica* (Kennedy and Schaefers 1974). Leaves were frozen to stop aphid reproduction until processing in the laboratory. Under a dissecting microscope, both sides of the leaves were checked and all arthropod stages present were counted and recorded (e.g., aphids, insect eggs, and mites). Aphids found on the leaves were stored in 70% EtOH.

A. agathonica collected from the leaf samples were identified as a member of three different age classes: nymphal instars I-II, instars III-IV, and adults. Adults were easily distinguished by the presence of a protruding cauda and nymph eye spots. Nymphs were sorted into the two ages classes based on size. Because the size of aphids may vary depending on plant quality (Kennedy 1974), aphid size was compared within each trap date to account for variation in plant quality throughout the growing season. Aphids with wings, visible wing buds, males, and oviparae (egg-laying females, determined through dissection of adults) were also recorded.

Raspberry plants were sampled for viruses by collecting a single young fully expanded leaf from each of the 108 flagged plants, and stored at 4°C until testing. Plants were sampled in September 2010, May 2011, September 2011, and September 2012. In all, 66% of the plants originally flagged in 2010 were found during the three subsequent sampling periods; other plants that had died or were removed were replaced with a neighboring plant. Each sample was tested for RLMV, RpLV, and RBDV by reverse transcription-polymerase chain reaction (RT-PCR) using total RNA as initial template. RNA was extracted using a combination of the methodologies described by Halgren et al. (2007) and Rott and Jelkmann (2001). Briefly, 100 mg of leaf tissue was ground in extraction buffer and precipitated in isopropanol followed by resuspension in 500 μl of wash buffer and 25 μl of glass milk. The RNA was eluted in 150 μl of water and stored at −80°C until used.

RT reactions were performed using random primers as described in Halgren et al. (2007). In all, 2.5 μl of the RT product was used as template for the PCR in a final volume of 25 μl. The reaction was carried out according to the polymerase manufacturer's instructions (TaKaRa Bio Inc. Shiga, Japan). Primers developed by Tzanetakis et al. (2007a) and Quito-Avila et al. (2011) were used for detection of RLMV and RpLV, respectively. RBDV was detected by using the degenerate primers F:AAAGACKYSCAGAAATC-CGTTA and R:TGWAWARGAAGTTDGCCCATTT (K. Keller, unpublished). The PCR program for amplification of the targets consisted of initial denaturation for 4 min at 94°C followed by 40 cycles with denaturation for 40 s at 94°C, annealing for 25 s at 58°C (RLMV and RpLV) or 55°C (RBDV) and extension for 40 s at 72°C, with a final 7 min extension step at 72°C. To assess the RNA quality and effectiveness of the RT reaction and RNA quality, the highly conserved plant gene NADH dehydrogenase ND2 subunit (ndhB) was used as endogenous control to verify the RNA quality and RT reaction by amplification of a 721 bp transcript region (Thompson et al. 2003, Tzanetakis et al. 2007).

Statistical Analysis. The proportion of aphids in each size class (instar I–II, instar III–IV, and adult) was regressed against the accumulated growing degree-days (GDD) to determine whether population composition varied throughout the growing season. Temperature data were acquired from the AgWeatherNet (Washington Agricultural Network) weather station located in Lynden, WA.

Binomial logistic regressions were run to investigate the relationship between observed aphid counts at each sampling location on the probability of a plant becoming infected with RLMV. A plant was counted as infected if it tested negative for RLMV at the beginning of the growing season and positive at the end. Models were developed to explore 1) whether aphid population numbers at different times of the growing season and 2) whether peak aphid abundance influenced the probability of infection with RLMV. In the first model, the predictor variable was the number of aphids per location per week (representing time over the course of the growing season). The percentage of field infection at the beginning of the growing season was included as a covariate. A full model was fit with all sampled weeks, and nonsignificant weeks were removed in a stepwise process. Separate models were run using aphid counts in 2011 and 2012. In the second model, the predictor variable was the maximum sampled aphid count per location, with the percentage of plants infected at the beginning of the growing season included as a covariate. 2011 and 2012 data were combined to increase the number of infected plant observations. All analyses were carried out in SAS (Proc Glimmix, version 9.3.2, SAS Institute 2008). Similar models were not fit for RpLV because infection events with RpLV were rare.

Results

Aphid Monitoring. The number of days aphids spent in each development stage is shown in Table 1. The lower developmental threshold from birth through the prereproductive period was calculated as 2.7°C (Table 2; Fig. 1). The threshold remained fairly consistent throughout the stages of nymphal development, although was lower (1.2°C) for the prereproductive period (adult to first nymph born, Table 2).

Table 1. Mean number of days (±SD) to reach adulthood and lay first nymph at constant temperatures

Avg temp (°C)	Avg no. days of development ± SD (sample size)		
	Nymph to adult	Prereproductive	Birth to laying first nymph
9.28 ± 1.7	31.2 ± 6.4 (24)	6.85 ± 2.6 (21)	38.14 ± 8.5 (21)
13.75 ± 0.7	18.5 ± 2.4 (22)	3.57 ± 1.1 (21)	22.28 ± 2.8 (21)
17.55 ± 0.6	14.8 ± 3.1 (27)	2.30 ± 0.7 (27)	17.43 ± 3.7 (27)
21.49 ± 0.1	11.1 ± 1.4 (23)	2.00 ± 0.8 (23)	13.39 ± 1.7 (23)
25.22 ± 0.5	9.23 ± 1.4 (21)	2.12 ± 0.9 (17)	11.24 ± 1.4 (17)

Parentheses after the means represent the sample size of aphids at each temperature.

The development time, K, was ≈250 DD from birth until development into a reproductively mature adult.

In the field, the peak populations of aphids were observed ≈9 d earlier in 2012 (27 June) than in 2011 (5 July; Fig. 2A). To examine whether the lower developmental threshold could be incorporated into a model that could reliably anticipate aphid population increases and peaks under field conditions, GDD were calculated for each growing season using the calculated threshold of 2.7°C and a biofix of 1 January. Using the GDD model, the timing of aphid appearance and population growth was similar in 2011 and 2012 (Fig. 2B). Aphids were first detected as early as 350 GDD. However, aphid populations increased most rapidly beginning at ≈800 GDD and peaked between 1,000 and 1,100 GDD (Fig. 2B). Population numbers decreased sharply after this peak because of the application of insecticides for routine preharvest clean-up sprays common in raspberry production. After the initial peak, aphid populations increased and decreased marginally throughout the latter half of the growing season, but never attained the high populations seen early in the growing season.

Winged *A. agathonica* were collected at two main points in the growing season. The first flight period coincided with the period of largest population growth. Like with the general aphid populations, alate populations peaked ≈9 d earlier in 2012 than 2011, or around 1,000–1,100 GDD (Fig. 2D). A second smaller peak in winged morphs was observed near the end of the growing season (≈2,000 GDD). In all, 15–40% of aphids collected during the end of the growing season were winged males.

Throughout the growing season, adult aphids comprised 10% of the overall aphid population on average. Young nymphs (instar I–II) accounted for the majority of the aphids collected, averaging 62% of the aphids at each collection point, while older nymphs (instar

Table 2. Lower developmental threshold, generation time (K), and the regression equation for *A. agathonica* at each development stage

Development stage	Lower threshold (°C)	K	Regression equation	R^2
Nymph to adult	2.9	204	$y = -0.0145 + 0.0049T$	0.88
Prereproductive	1.2	38.9	$y = -0.032 + 0.0257T$	0.36
Birth to first nymph	2.7	250	$y = -0.0109 + 0.004T$	0.88
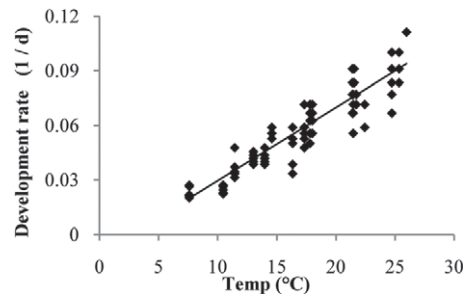
Fig. 1. Development rate of aphids from birth to first nymph deposited when reared at constant temperatures. Regression equation: $y = -0.0109 + 0.004T$; $R^2 = 0.88$.

III–IV) made up 26% (Fig. 3). There was no effect of time within the growing season or year on the age-structure of the populations (Table 3). The remaining 2% collected were sexual aphid morphs collected at the very end of the growing season.

Virus Monitoring. None of the three viruses was detected in any of the newly planted raspberry fields, indicating that the growers were using clean planting stock and that nurseries were doing a good job of virus control during the plant propagation cycles. The virus with the highest rate of spread was RLMV. One year after planting, fields had an infection rate of 0–20% (Fig. 4). By 3 yr after planting, 30–60% of the raspberry plants tested positive for RLMV. The infection rates for RpLV and RBDV were much lower. RpLV was not detected in any of the fields tested during the first 2 yr. Two fields had plants that tested positive for RpLV in yr 3, with only 3–4% of the plants infected, while a 4-yr-old field had 8% of plants infected. RBDV was not detected in any of the fields until yr 3. At yr 3, infection rates were ≈15%, and increased to 37% in the 4-yr-old field.

Relationship Between Aphid Presence and Virus Infection. The virus incidence in a given field in the prior year was not a significant predictor of the probability of infection in subsequent growing seasons (Table 4). In 2011, the aphid counts at 2 out of 12 wk were correlated with the probability of a given plant becoming infected with RLMV. The collection on 5 July 2011 (1,076 GDD) was negatively associated with RLMV infection (Table 4), with the odds of infection being 1.15 times lower with each additional aphid counted. This week corresponded to the highest numbers of aphids collected, as well as the peak flight of the alate adults. Unfortunately, the numbers of alate aphids was not recorded on a per-site basis in 2011, so the influence of alate vs. apterous aphids could not be examined further. In 2011, the collection on 14 August 2011 (1,660 GDD) was positively associated with RLMV infection (Table 4), with odds of infection increasing 1.28 times with each additional aphid counted. This collection corresponds with the second greatest peak in aphid counts in 2011. In 2012, none of the aphid counts (total, alate, or apterous) during the 12 wk was a significant predictor of the probability of a plant testing positive for RLMV. Finally, there was
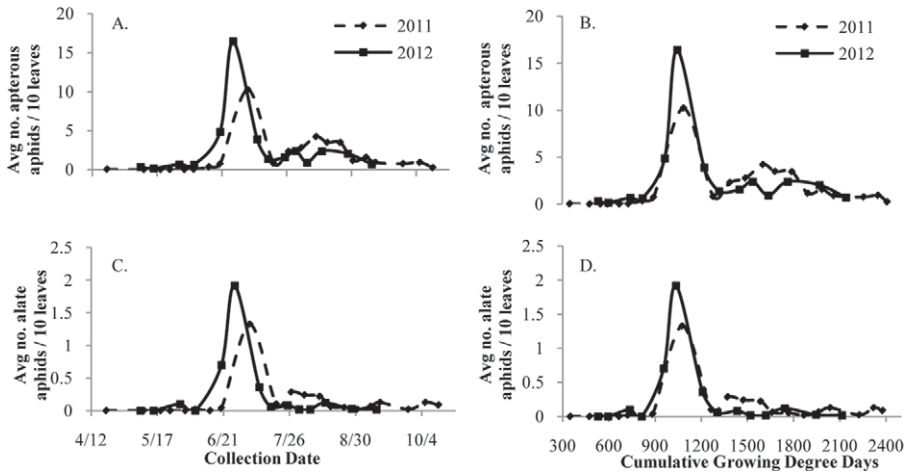
Fig. 2. (A and B) Average number of aphids of all ages counted per 10-leaf collection in 2011 (dotted line) and 2012 (solid line) as a function of calendar collection dates (A) and the GDD model (B). (C and D) Average number of aphids with wings or wingbuds counted per 10-leaf collection in 2011 and 2012 as a function of calendar collection dates (C) and the GDD model (D).

no correlation between the maximum number of aphids detected at a given site, and the probability of a plant in that area becoming infected during the 2-yr period (Table 4).

Discussion

A. agathonica is a pest of *Rubus* across North America, but this is the first study of *A. agathonica* biology in the Pacific Northwest region. Previous work has surveyed the seasonal phenology of this aphid, with the most detailed work occurring at the New York State Agricultural Experiment Station (Geneva, NY); Kennedy and Schaefers 1974). The major difference between the aphid phenology in New York and Washington were the periods of aphid flight. Anticipating and controlling alate aphids is important because these aphids may act as primary vectors into newly planted or previously uninfected fields. In New York, a large number of alate aphids were counted in June with subsequent survey dates turning up no detectable numbers of alate individuals (Kennedy 1974). However, in this study, we observed two periods

where alate aphids were frequently caught: at ≈1,000 GDD (approximately late June or early July) and a lesser numbers during a second period at ≈2,000 GDD (early September).

The levels of RLMV in the four 3-yr-old commercial fields surveyed averaged 50% infection at 3 yr of age. Five- to seven-year-old commercial fields surveyed throughout northern Washington in 2011 ranged from 60 to 100% infection (Quito-Avila 2011). When RLMV is found co-infecting plants with RBDV, RBDV titers increase 400 fold (Quito-Avila and Martin 2012) and therefore RLMV control may be the most important factor in limiting the spread of RBDV and the impact of crumbly fruit disease in red raspberry. Rates of RpLV were much lower in our surveyed fields, with infection levels in 3- to 4-yr-old fields remaining under 10%, although other surveys conducted in 5- to 7-yr-old fields showed rates of RpLV at up to 80% (Quito-Avila 2011). *A. agathonica* is an inefficient transmitter of RpLV (Quito-Avila et al. 2012); thus, spread of RpLV is likely dependent on high populations of *A. agathonica.*

Integrated management decisions for aphid control should ultimately be based on accurate timing and population threshold levels; however, establishment of treatment thresholds is difficult in systems where the insect pest transmits a virus. The relationship be-
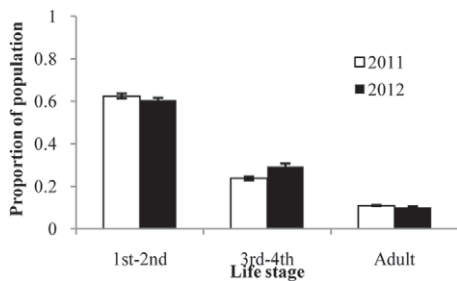

Fig. 3. Proportion of different age categories of the total aphid population collected throughout 2011 and 2012. There was no difference in the population structure between years.

Table 3. Effect of time (GDD) and year on the proportion of the population each stage comprises

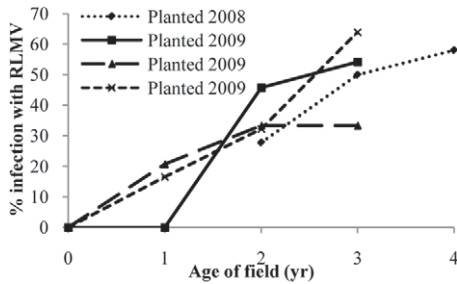| Stage	Factor	*F* value	*P* value
First–second instar	Degree day	2.66	0.116
	Year	0.83	0.371
Third–fourth instar	Degree day	0.28	0.599
	Year	1.17	0.291
Adult	Degree day	0.71	0.407
	Year	0.01	0.922

Fig. 4. Rate of infection with RLMV of four different commercial 'Meeker' raspberry fields in Whatcom Co., WA.

tween aphid population levels and odds of virus infection were not readily apparent from our data. In one instance, a plant tested positive for RLMV when there were eight aphids sampled in that location over the entirety of the growing season. For comparison, multiple sites within the same field had aphid counts as high as 65 aphids in one sampling period alone. A correlation between vector population levels and vector spread is often not apparent even in well-studied systems. No relationship between cereal aphid and barley yellow dwarf virus incidence levels in wheat and barley was found by Poehling et al. (2007), nor was there a link between *Graphocephala atropunctata* (Signoret) abundance and Pierce's disease (Redak et al. 2004). In the case of Pierce's disease, a partial explanation for this observation is that inoculations made later in the season did not become chronic infections, unlike plants inoculated with the bacterium earlier in the season (Redak et al. 2004).

Multiple factors are likely confounding detection of a direct relationship between aphid counts and virus infection in our models. First, *A. agathonica* transmission rates of RLMV are <100%; thus, only a subset of aphids exposed to an infected plant is able to successfully transmit the disease. Second, in fields with low infection rates, apterous aphids are less likely to ever come into contact with an infected plant because of their relatively limited movement, and as a result are not vectoring the disease. Fields planted with clean

rootstock are likely to remain uninfected until viruliferous alate aphids migrate into the field and begin the primary infection cycle. Long-range movement of *A. agathonica* is also restricted during most of the growing season, with only limited windows of flight occurring around 1,050 GDD and again at 2,000 GDD. Third, the raspberry cropping system itself presents additional variability in the data because the canes are perennial. As a result, the amount of initial inoculum present at the beginning of the growing season increases from year to year until the field is replanted. Lastly, aphid counts may not have been a significant factor in the constructed models because of the relatively small sample size at each of the 50 locations (10 leaves per wk). In a study on cereal aphids, population growth in individual plots was unable to be tracked when aphid densities were low, whereas data pooled over all plots were more accurate (Jarosik et al. 2002). Increasing the sample size in each location will give a better estimate of true aphid population densities and possibly shed more light on the relationships with virus spread.

Use of calendar dates alone to anticipate aphid population peaks were not consistent from year to year. There was approximately a 9-d difference between population peaks in 2011 and 2012. However, the GDD model developed showed consistent large aphid population counts around 1,050 GDD in both growing years, followed by a rapid population decline. This decline is expected from the preharvest "clean-up" insecticide spray that is routinely applied in raspberry production to remove contaminant pests such as leafhoppers, leafrollers, and spiders (DeFrancesco 2012). The latter half of the growing season was inconsistent and revealed no easily identifiable patterns in aphid population peaks or declines. Aphid populations may be more variable because of a number of nonindependent factors, such as continued insecticide applications throughout the harvest period, high levels of parasitism and fungal infection of aphids late in the growing season (D.M.L., unpublished data), raspberry plant nutritional quality, and proximity to other raspberry fields, which may influence the numbers of successful alate migrants.

The consistency observed between 2011 and 2012 allows for anticipation of when aphid populations will begin to increase and peak. Future work should examine the efficacy of applying insecticides earlier than the typical timing of the "preharvest spray" to prevent the large aphid population peak observed between 800 and 1,000 GDD while still providing control against crop contaminants. In addition, these studies should determine whether aphid control during this period decreases the infection rates of RLMV over the course of several growing seasons. Identification of ideal timing for insecticide applications has been shown to decrease yield loss owing to *Aphis glycines* Matsumura (Myers et al. 2005). Because aphid control is needed to limit the damage caused by RLMV and RpLV, further research is needed to better time insecticide applications to prevent the greatest number of infections.

Table 4. Model estimates from binomial logistic regressions on the probability of plants testing positive for RLMV (probability of infection)

Year	Factor	Estimate	df	F value	P value
2011[a]	% prior infection	0.025 ± 0.77	34	0.43	0.516
	Aphid count on 5 July 2011	−0.13 ± 0.07	34	4.23	0.047
	Aphid count on 14 Aug. 2011	0.27 ± 0.14	34	3.88	0.057
2012[b]	% prior infection	−0.10 ± 0.08	22	1.59	0.221
2011	% prior infection	0.027 ± 0.02	64	1.91	0.629
2012[c]	Max aphid no.	0.012 ± 0.03	64	0.24	0.172

[a] Probability of infection = % prior infection + July 5 Count + August 14 Count + Error.
[b] Probability of infection = % prior infection + Error.
[c] Probability of infection = % prior infection + Max weekly aphid count + Error.

The percentage of plants positive in the field at the beginning of the growing season (% prior infection) was included as a covariate in all models.

Acknowledgments

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