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IN THIS ISSUE

POPULATION DYNAMICS OF VINE MEALYBUG AND ITS NATURAL ENEMIES IN THE COACHELLA AND SAN JOAQUIN VALLEYS, *Raksha Malakar-Kuenen, Kent Daane, Walter Bentley, Glenn Yokota, and Lee Martin, U. C. Kearney Agricultural Center; Kris Godfrey, and Joe Ball, California Department of Food and Agriculture.*

Serious infestations of the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), have been reported by table grape growers in the Coachella Valley (CV) since 1994 and the San Joaquin Valley (SJV) since 1998. This mealybug can feed on fruit, trunk, canes, leaves or roots. As vine mealybug (VMB) feeds, it excretes honeydew that promotes sooty mold growth. Any mealybug body part, honeydew or sooty mold in the grape bunch lowers crop quality (Geiger and Daane 2001).

VMB is similar in appearance to grape mealybug (the more common vineyard mealybug pest species). However, grape mealybug has longer caudal filaments (i.e., tails) and more cylindrical shaped waxy filaments around the edge of its body (Bentley 1999, Peacock et al. 2000). Also, the grape mealybug is found mostly on trunks and spurs (and bunches when present), whereas VMB is found all over the vine at some point in the season, including the roots (Malakar-Kuenen et al. 2001). There are only 2 generations of grape mealybug per year, in contrast to 5-6 generations per year of VMB. In 1998 and 1999, new findings of VMB were made in Kern and Fresno Counties, indicating VMB had moved north where it currently poses a serious threat to SJV vineyards. Previous studies conducted from 1995 – 1999 suggested that there might be differences in the biology and behavior of VMB between CV and SJV. Therefore, in 2000, studies were conducted in both valleys

in an attempt to further elucidate differences in the VMB between these two regions.

Materials and Methods

Coachella Valley. Three different sampling methods were applied concurrently to evaluate the impact of parasitoids on VMB and to elucidate changes in age structure oven space and time. Studies were conducted from February through November 2000 in a certified organic vineyard near Thermal (Riverside County). The imported parasitoids (*Leptomastidea abnormis* and *Anagyrus pseudococci*) previously had been released in this block (see González 1998).

Seasonal changes in VMB population age structure were investigated on different parts of the vine through the growing season. Each month, five vines were examined for the presence of VMB on the trunk, cordon and roots. Any VMB and/or mummies were collected, counted for each part of the vine, and held for parasitoid emergence and identification.

VMB parasitism levels were investigated using a "refuge site" method. To sample, each month a band of bubble wrap was placed around the trunk and one cordon on each of 20 vines. The wraps were removed after four weeks. The number of live VMB and mummies were recorded and held for parasitoid emergence and identification.

A group of 20 vines was used to determine the general pattern of activity of VMB and parasitoids. On each vine, a sticky tape trap was placed on the trunk and one cordon, and a yellow sticky card was hung in the canopy. The sticky traps and cards were replaced every 4 weeks. The numbers of each life stage of VMB and of adults of each parasitoid species were recorded.

San Joaquin Valley. VMB seasonal distribution was investigated in a 6 year old raisin vineyard near Sanger (Fresno County). Field counts were made weekly from May through October and once a month thereafter. To sample, each vine was divided into seven sections: ground (soil level to 1 ft. above the ground), trunk, armpit, old cane, new cane, leaves, and bunches (when present). Each section was searched for 3 minutes and all VMB (separated by development stage) and parasitoid "mummies" were recorded.

During harvest, economic damage was rated using a scale of 0–3 with 0 = clean, and 3 = totally damaged. Bunches touching wood were chosen for assessment because there is typically a higher rate of VMB infestation on these bunches (Geiger and Daane 2001). The damage was assessed on three bunches from just above the main trunk and three each from each side of the arm.

To determine the percent parasitism, destructive samples were taken from 2 raisin vineyards in Fresno County. In each vineyard, 50 leaves with VMB (about 100 individuals) were collected weekly, taken to the Kearney Agricultural Center insectary, and quarantined. The parasitoids that emerged were identified, and records of the species and sex of the parasitoid, as well as mealybug stage attacked, were kept.

Results and Discussion

Coachella Valley. The field count method revealed a peak in VMB density in April and May, with a second, smaller peak in August (Fig. 1). VMB was found only sporadically on root samples. In May and August, when substantial numbers of VMB were recovered on roots, individuals were found on one or two heavily infested vines and were not evenly distributed throughout the sample. No parasitoids were recovered from the VMB found on the roots.

Adult *A. pseudococci* and *L. abnormis* emerged from VMB and mummies collected with the refuge site method in March through June, and in November. *A. pseudococci* were recovered a few months prior to *L. abnormis*. It is not known if recovered *A. pseudococci* represent individuals that are progeny of the introduced or the native population.

The seasonal dynamics of VMB revealed using the trapping method were consistent with those revealed using the other sampling methods (Figs. 1 & 2). The peak male VMB density occurred in June, with a smaller, second peak in October and November. The second peak was most likely the result of the increase in density of VMB immatures in August. Adult *A. pseudococci* were captured in low numbers in March and April, with a peak in density in June. Density increased slightly in October and November. For the other parasitoid, *L. abnormis*, density began increasing in May with a peak occurring in July.

San Joaquin Valley. While there were some similarities in VMB seasonal abundance and distribution between CV and SJV, the differences might better explain VMB's lower pest status in SJV. In SJV vineyards, three distinct peaks in density of adult VMB were

observed during the summer and fall seasons (Fig. 3a). Sampling began in May, possibly missing one or more generations that occurred in the winter and early spring. What is surprising is that more adult peaks were found in the SJV than in the CV. Perhaps VMB have upper temperature thresholds, at which either development slows or VMB mortality is so high that individual generations cannot be distinguished during the summer months, as VMB density declines. This might explain the lack of multiple peaks in summer in CV.

Movement of VMB from trunk to leaves occurred in mid June, followed by movement to the bunches about a week later. Similar to CV, adult density in SJV in June was higher than that in August, September, and October (Fig. 3a). Adults were present in November and December, but in very low numbers, and most were located on the roots or on the trunk, deep under the bark clustering around feeding holes made by some lepidopteran larvae. Crawlers were visible during late spring, summer and early fall; they slowly disappeared after harvest (Fig. 3b).

In all SJV samples, there was a dramatic VMB reduction in August and September. This reduction came much later than that observed in CV, and was not accompanied by an increase in summer temperatures. In fact, all stages of VMB were found throughout the summer feeding on leaves and canes in exposed locations, suggesting that SJV temperatures had little effect on VMB densities. In contrast to CV, there was a later (e.g., beginning in August) and greater level of parasitoid activity that may be responsible for the decline in VMB density in the fall (Fig. 4).

Perhaps the most significant difference between CV and SJV was the level of resident parasitoid activity. The sample sites in the SJV have never had any releases of VMB parasitoids. Despite this, two parasitoid species were recovered. The dominant parasitoid was *A. pseudococci*, which attacks third instar (occasionally second) through adult VMB. The second parasitoid was an *Allotropa* sp. that was recovered from second and third instar VMB. *A. pseudococci* was observed for the first time during the first week of August. By mid August, percentage parasitism rose from 2 to 80%. Subsequently, parasitoid activity remained high on all VMB on leaves and canes (Fig. 5). No parasitoids emerged from mealybugs collected in December and January, from feeding holes of moth larvae or from roots.

Conclusion

Our most important contribution from the 2000 season was to define the differences between VMB seasonal abundance and parasitism between CV and SJV. In general, VMB in CV reached peak densities earlier in the growing season than those in SJV. The VMB densities in CV declined dramatically in mid summer. The VMB densities in SJV declined in early to mid fall, possibly as a result of increased parasitism by a native parasitoid. In addition, VMB could be found throughout vines at all times of the year in CV, whereas in SJV, the mealybugs tended to be lower on the trunk during the winter months. These differences in the seasonal abundance and parasitism must be considered when developing management plans for VMB. Clearly, individual plans must be developed for each grape growing region.

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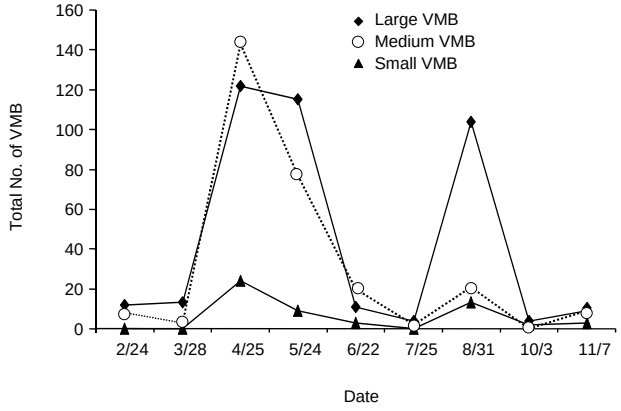


Figure 1. The total number of each life stage of vine mealybug (*Planococcus ficus)* found on the trunks of 5 vines in the field count study in the Coachella Valley in 2000.

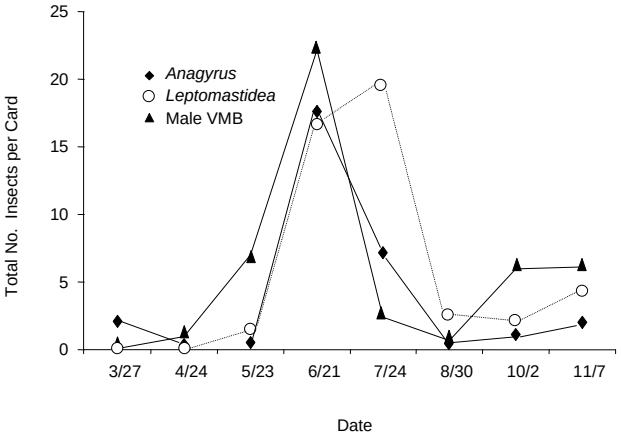


Figure 2. The total number of vine mealybug (*Planococcus ficus)* males and parasitoid adults found per yellow card placed in the grape canopy in the Coachella Valley in 2000.

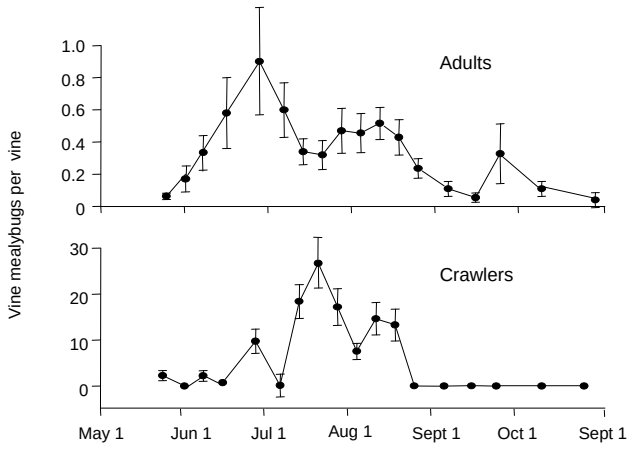


Figure 3. Vine mealybug (*Planococcus ficus)* (a) adult and (b) crawler density in San Joaquin Valley as measured from weekly, non-destructive samples.

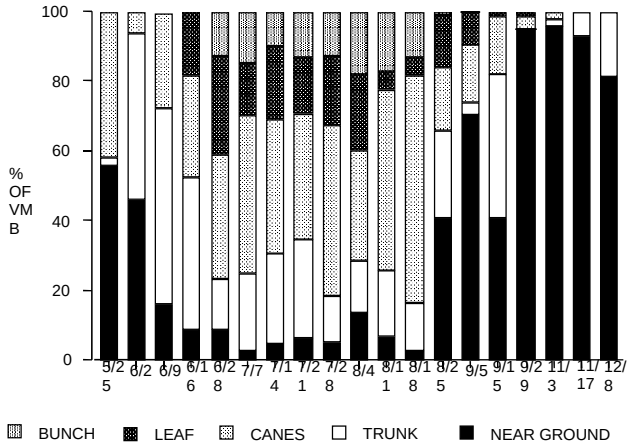


Figure 4. Vine mealybug (*Planococcus ficus)* population distribution (%) throughout the vine shows a continual presence of mealybugs on the trunk and on or under ground.

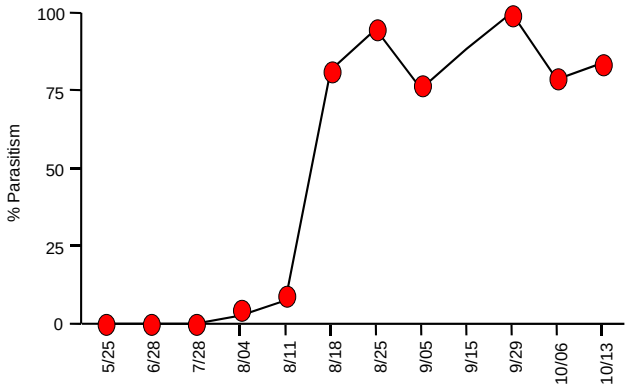


Figure 5. Percentage parasitism of vine mealybug (*Planococcus ficus)* shows high parasitoid activity after August. Over 95% of the parasitoids recovered were *Anagyrus pseudococci*.

GLASSY-WINGED SHARPSHOOTER IN SAN JOAQUIN VALLEY CITRUS, *Beth Grafton-Cardwell and Chris Reagan, U.C. Kearney Agricultural Center; Craig Kallsen and Marjie Bartels, UCCE Kern County.*

Glassy-winged sharpshooter, *Homalodiscus coagulata* (GWSS), has infested citrus in the Edison/Arvin area of Kern County for approximately 5 years. During the 1999-2000 field seasons, it became very numerous, especially along General Beale Road. GWSS feeds on the xylem of citrus, but it is generally not a serious pest of mature citrus. GWSS has many host plants, and citrus

is an important oviposition host during both egg-laying periods (April and July-September) in Kern County. While GWSS is not a serious pest of citrus, it is an effective vector of the *Xylella fastidiosa* bacterium that causes Pierce's Disease (PD), a devastating disease of grapes. During the 2000 field season, grape growers in the Edison/Arvin area became aware that citrus was generating high numbers of GWSS that were then flying into the vineyards and potentially spreading PD. The citrus growers were under great pressure to reduce GWSS numbers, especially if their orchards were located next to grapes. During 2000, a small number of grapevines with PD were found, thus emphasizing the need for GWSS management. The long-term objective of the GWSS suppression program is to reduce its numbers. However, until effective parasitoids are discovered and released, insecticides will remain the main method of control. The ultimate solution will not be insect control, but development of plant resistance to the disease.

During the 2000 growing season, we began several field trials to begin to understand which insecticides currently used in citrus might be helpful for reducing GWSS numbers. Figure 1 shows the results of the first field trial, testing the efficacy of 4 pints Lorsban (chlorpyrifos), 1 pint Danitol (fenpropathrin), 6.0 oz Success (spinosad), or 6.6 oz Nexter (pyridaben) in 200 gal water/acre. The treatments were applied with a commercial spray rig to five row by ten tree plots, replicated three times. The block was planted with mature 'Bonanza' navel oranges and interplanted with young 'Newhall' navel orange trees. In each plot, 20 young trees were examined for 3 minutes and all life stages of GWSS were counted. In mid-March, the adult GWSS numbered 0.3/tree examined. Between that date and the next sampling, the number of GWSS was greatly reduced due to high winds. Treatments had no effect on the number of fresh egg masses, which declined naturally over the 6-week sampling period due to hatching. The adults were suppressed most effectively by the broad-spectrum pesticides: the organophosphate Lorsban, the pyrethroid Danitol, and the insecticide/miticide Nexter. Their great mobility makes it difficult to accurately assess control of adult GWSS by insecticides. The nymphs, in contrast, cannot move between trees, and so give greater information about the efficacy of insecticides. Success showed no efficacy, Nexter showed moderate efficacy, and Lorsban and Danitol showed the most consistent and highest level of efficacy against nymphs. Even the most effective insecticides required several weeks for control, because the nymphs were gradually emerging from the egg masses over several weeks.

In the second trial, fresh glassy-winged sharpshooter egg masses were enclosed in cloth bags and treatments were applied to nine rows of a mature navel orange orchard in Kern County (adjacent to General Beale Road). There were 20 sets of caged egg masses for each treatment. The treatments consisted of 32 oz. of Admire (applied on April 10 through the irrigation system to three rows), 32 oz of Admire (applied on 24 May to an additional three rows), and three rows which were not sprayed. At weekly intervals, cloth bags were opened and the insides examined for live and dead GWSS. Figure 1 shows that the April 10 Admire treatment required 25 days to begin to significantly reduce GWSS nymphs emerging from the egg masses. The population continued to decline, and reached 86% total reduction compared to the controls. The May 25 treatment was too late to effect significant control of GWSS, compared to controls.

The third trial was conducted during the second generation of GWSS egg laying (July-September) in Kern County along General Beale Road. Each insecticide, Sevin (carbaryl), Baythroid (cyfluthrin), and Agri-Mek (abamectin) was sprayed in separate tree rows on 7 July 2000. Five mesh bags containing 5 adult GWSS each were placed on five trees in each of the treated rows on 10 August for one week and then a second set of bags was placed on the trees on 7 September for one week. We had hoped to study the effects of these insecticides on GWSS nymphal emergence. However, we were not able to do so, because parasitism of the egg masses by *Gonatocerus ashmeadi* was extremely high during this second generation of egg laying. Figure 2 shows that the 10-17 August exposure of adults resulted in 100% mortality on 5 week-old Sevin and Baythroid residues. GWSS mortality due to Agri-Mek was not much greater than the control mortality. On 7-14 September, the 9-week-old residues were no longer fully toxic to GWSS adults. These data suggest that the broad spectrum carbamate insecticide Sevin and the pyrethroid Baythroid residues are toxic to adult GWSS for at least 5 weeks.

Through field trials and observations of the response of GWSS to grower practices, it has become clear that soft pesticides (Veratran, Success, Agri-Mek, oil and Esteem) are not effective in controlling GWSS, and that broad spectrum pesticides in the organophosphate (Lorsban, Supracide, malathion, Cygon), carbamate (Sevin, Lannate), pyrethroid (Baythroid, Danitol), and neonicotinoid (Admire) groups are needed. Broad

spectrum foliar insecticides, such as organophosphates, carbamates, and pyrethroids were effective in reducing GWSS numbers for 4-6 weeks, approximately the length of one GWSS generation. Although Admire did not reduce GWSS numbers as much as the broad spectrum insecticides, its effect lasted much longer - approximately 6 months.

The current San Joaquin Valley citrus IPM program is shown in Figure 3. The success of this program is based on infrequent use or very low rates of certain broad spectrum pesticides, especially in the spring and fall when parasitoids are abundant. Thus, multiple applications of foliar insecticides for GWSS control needed during April-May and July-September are likely to severely disrupt the citrus IPM program. Spider mites and citrus thrips populations will increase where predacious mites are disrupted. California red scale and citrus cutworm populations will increase where parasitic wasps are disrupted. Cottony cushion scale outbreaks can be expected to occur following Admire use because of its toxicity to the predatory vedalia beetle. We will be observing the 10 Kern County IPM demonstration blocks during 2001 to see if GWSS treatments such as Admire and Lannate have reduced natural enemies and thrown the citrus IPM program out of balance.

While GWSS is not currently a major pest of citrus, and insecticide treatments are designed to reduce its disease vectoring potential for grapes, citrus growers do have cause for concern. GWSS is also a vector of the strain of *X. fastidiosa* that attacks citrus and causes the citrus variegated chlorosis disease. This disease has not yet been found in California, but it is present in South and Central America. Therefore, the potential for it to be moved to California via shipment of infected plants or by natural movement through transmission by sharpshooters, is high.

Table 1. Glassy-winged sharpshooter insecticide trial in Kern County. Treatments applied on 3/24/00.

Treatment	3/14/00	3/30/00	4/06/00	4/13/00	4/20/00	4/27/00
			Mean number of adult GWSS/tree			
Untreated	0.30a	0.07a	0.12a	0.17a	0.20ab	0.02a
Success 2SC	0.32a	0.03a	0.02a	0.02a	0.22a	0.00a
Nexter	0.30a	0.00a	0.02a	0.07a	0.00b	0.02a
Lorsban 4E	0.32a	0.00a	0.03a	0.05a	0.03ab	0.00a
Danitol 2.4EC	0.32a	0.00a	0.00a	0.07a	0.00b	0.02a
			Mean number of GWSS egg masses/tree			
Untreated	0	0.77a	0.67a	0.37a	0.05a	0.02a
Success 2SC	0	0.43a	0.58a	0.68a	0.13a	0.02a
Nexter	0	0.55a	0.52a	0.38a	0.12a	0.02a
Lorsban 4E	0	0.48a	0.32a	0.55a	0.10a	0.02a
Danitol 2.4EC	0	0.43a	0.32a	0.38a	0.02a	0.00a
			Mean number of GWSS nymphs/tree			
Untreated	0	0.00a	0.20bc	0.60a	0.28a	0.90a
Success 2SC	0	0.05a	0.78ab	1.15a	1.67a	0.53ab
Nexter	0	0.00a	0.90a	0.28a	0.45a	0.28bc
Lorsban 4E	0	0.05a	0.15c	0.02a	0.27a	0.00c
Danitol 2.4EC	0	0.03a	0.20bc	0.02a	0.00a	0.00c

Means for each life stage in each column followed by the same letter are not significantly different (LSD, P> 0.05)

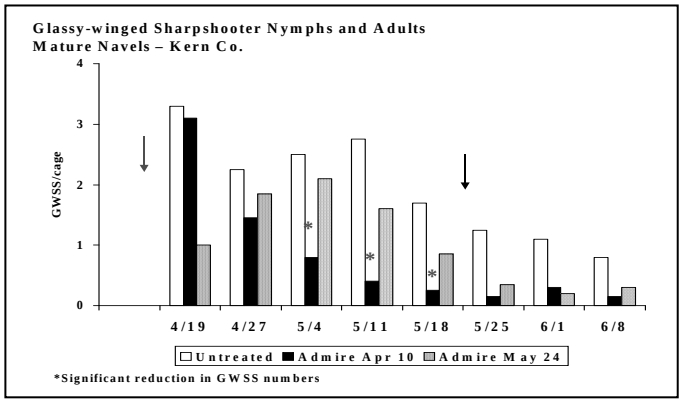


Figure 1.

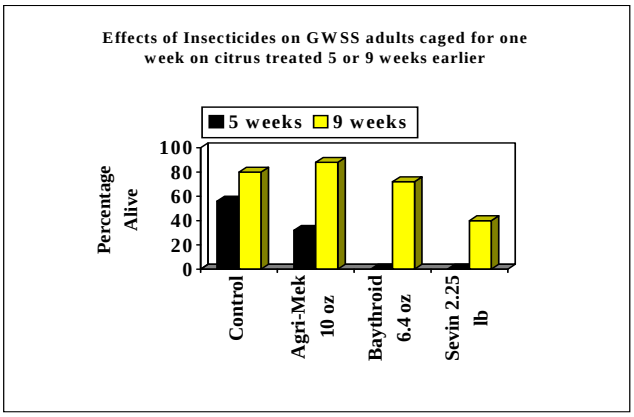
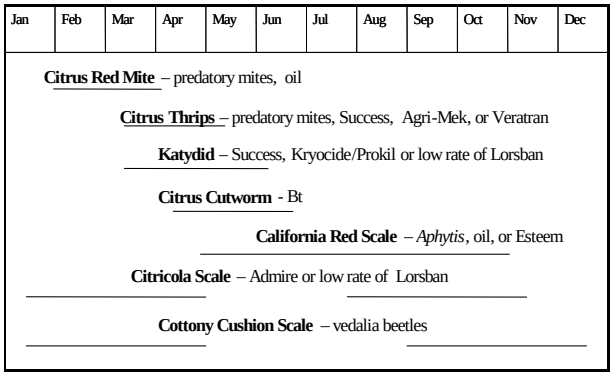


Figure 2

Table 2. Total number of glassy-winged sharpshooter nymphs and adults collected by time search (Apr-Oct) or beating sheet sampling (Nov-Jan 2001) in 20 trees per citrus orchard on a weekly (Apr-Sep) or monthly (Oct-Jan 2001) basis.

Orchard	Apr-May			Jun-Jul		Aug-Sep		Oct-Nov		Dec-Jan
	Thrips/katydid/mites Treatments	Red scale or GWSS Treatments	GWSS	Treatments	GWSS	Treatments	GWSS	Treatments	GWSS	GWSS
1	Veratran	Admire	8		7	Esteem + oil	5		9	21
2	Success+oil		0		161		240		21	41
3	Success or Agri-Mek + oil		7		154		137		15	19
4	Success	Admire	1		14		3		1	3
5	Success + oil		0		18		17		16	31
6	Agri-Mek + oil Veratran		0		1		3		29	12
7	Success + oil	Admire	9		7		0		10	12
8	Agri-Mek + oil	Admire	4		3	Lorsban	2	Lannate	10	1
9	Baythroid + oil		0	Esteem + oil	0		2	Lannate	1	1
10	Carzol+Lorsban	Esteem	0		12		99		11	21

San Joaquin Valley Citrus IPM Program



PENETRATION, DEVELOPMENT AND REPRODUCTION OF *MELOIDOGYNE ARENARIA* ON RESISTANT AND SUSCEPTIBLE *VITIS* SPP.

Safdar A. Anwar and M. V. McKenry, U. C. Kearney Agricultural Center.

Introduction

Host plant resistance restricts or prevents nematode reproduction by activating resistance mechanisms in response to nematode infection. By contrast, susceptible plants lack resistance, tolerance or both, making them good hosts for pathogen reproduction (Trudgill, 1991). Resistance that deters root-knot nematode can involve pre- or post-infection mechanisms (Huang, 1985). Pre-infection resistance may occur at the root surface or within the rhizosphere, thereby influencing nematode penetration. Plant-produced root exudates can also attract or repel root-knot nematodes. Post-infection resistance mechanisms can involve physiological processes within the roots which: 1) deter nematode feeding, 2) deter the establishment of feeding sites, 3) delay or prevent nematode development, or 4) inhibit reproduction (Trudgill, 1991).

Grape rootstocks 10-23B, *Vitis doanianna* Munson and RS-3 (*V. candicans* Engelmann × *V. rupestris* Scheele) × (*V. riparia* Michaux × *V. rupestris*) were chosen for study from among 520 *Vitis* selections by Drs. David Ramming and M. V. McKenry. These grape rootstocks are not only resistant to aggressive pathotypes of *Meloidogyne* spp. but possess "broad nematode resistance" against *Xiphinema index* Thorne & Allen, *Pratylenchus vulnus* Allen & Jensen, *Tylenchulus semipenetrans* Cobb plus some protection against *Criconemella xenoplax* (Raski) Luc & Raski and *X. americanum* sensu stricto Cobb.

The objectives of this study were to evaluate root-knot nematode penetration, development, and reproduction in susceptible and resistant rootstocks. The nematode studied is a pathotype capable of breaking resistance in the most popular nematode-resistant grape rootstocks. A more complete description of this research has been published (Anwar and McKenry, 2000).

Materials And Methods

The pathotype *M. arenaria* (Neal) Chitwood pt. Harmony was identified from a vineyard located near Livingston, California. Second stage juveniles (J2) were recovered from galled 'Harmony' roots using Baermann funnels placed in a mist chamber for 5 days. Suspensions of J2 were prepared in tap water to enable the desired inoculum density to be added in 10 cm^3 of water per plant. Two grape rootstocks, RS-3 and 10-23B, which possess resistance to root-knot nematode pathotypes, were compared to the susceptible 'Cabernet Sauvignon' (*V. vinifera*) control. Plants of each rootstock were

inoculated with 500 J2 of *M. arenaria* pt. Harmony by injecting at 4-inch depth on two sides of the plant.

At 4, 13, 21, 27 and 35 days after inoculation (DAI), five inoculated plants of each rootstock were harvested. The roots were washed free of soil, blotted with paper, damp-dried, and weighed. The whole root system of each plant at each harvest was stained with acid fuschin (Byrd et al., 1983). Each root system was spread in a film of glycerin between two glass plates (7.5 x 15 cm). Numbers of nematodes and stage of development within the roots were determined under a dissecting microscope. The numbers of nematodes in the stained root system recorded at each sampling were classified into four developmental stages (Anwar and McKenry, 2000) Vermiform, non-swollen J2; swollen, sausage shaped J2; globose, sub-spherical juveniles exhibiting spiked tail [J3 & J4]; adult fully differentiated female with or without eggs.

Infection sites were microscopically scored for host cell necrosis and appearance of juveniles. Necrosis was recorded when several deformed and brown cells appeared close to the nematode head. The appearance of a clear area in the intestine indicated starvation and severe reduction in body diameter and coiling indicated shriveling and death of nematodes (Van Gundy et al., 1967). Data were expressed as the percentage of juveniles showing necrosis in relation to total number of nematodes observed within root.

Root systems of plants harvested 21, 27 and 35 DAI were stained with Phloxine B (Holbrook et al., 1983) before staining with acid fuschin to assess the presence of egg masses. The root systems were rated for galling and egg mass presence on a 0 to 5 scale (Taylor and Sasser, 1978), where 0 = no gall or egg mass, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = > 100 galls or egg masses per root. At 27 and 35 DAI an additional 5 plants of each cultivar were harvested with five receiving stains and the other five incubated in a mist chamber for 5 days to hatch the eggs. Number of eggs per female was determined after dissolving the matrix of handpicked egg masses in 2% NaOCl (Hussey and Barker, 1973).

The data were subjected to analysis of variance using SAS software. Significant differences in means of nematode development were separated using Duncan's multiple range test.

Results

Penetration, development and reproduction of *M. arenaria* pt. Harmony differed among the three grape cultivars. At 4 DAI, a greater (P = 0.01) number of J2 penetrated susceptible Cabernet roots compared to the resistant 10-23B roots. Nematodes were not present in resistant RS-3 roots at 4 DAI. The infection sites of J2 in susceptible Cabernet were swollen, whereas there were no swollen infection sites in resistant 10-23B. The J2 were found clustered within the root apex of resistant 10-23B but a few had also migrated upward into the developing vascular cylinder.

At 13 DAI, more (P = 0.01) nematodes had reached the swollen stage of development in susceptible Cabernet roots than either of the resistant rootstocks. Roots of resistant RS-3 were penetrated by J2, but only 23% had advanced to a swollen stage of development, compared to 37% and 94% on resistant 10-23B and susceptible Cabernet, respectively. The number of vermiform and swollen J2 was equal in the roots of both resistant RS-3 and 10-23B rootstocks. Galls were visible on the root system of susceptible Cabernet and resistant 10-23B, with a mean gall index of 3.0 and 2.0, respectively. There were no galls on the roots of RS-3. However, infection sites of J2 in roots of both resistant rootstocks were found to be swollen. The root tissues of resistant RS-3 exhibited a hypersensitive reaction in response to 46% of the penetrated juveniles (Fig. 1). The entombed nematodes were starved, shrunken or dead.

By 21 DAI, a greater (P = 0.01) number of juveniles advanced to globose and adult female stages on the roots of susceptible Cabernet, compared to either resistant rootstock (Fig. 2, 21-DAI). At this time, the gall index on roots of susceptible Cabernet and resistant 10-23B had increased to 4.0. Resistant RS-3 roots showed a galling index of 3.0. Galls were largest on Cabernet roots, intermediate on roots of resistant 10-23B, and very small on roots of resistant RS-3. Egg masses were visible on roots of susceptible Cabernet, which had a 5.0 egg mass index, whereas egg masses were absent on the roots of both resistant rootstocks. The root tissues of resistant rootstocks exhibited vascular hypersensitive reaction associated with 73% and 50% of the total nematodes present within 10-23B and RS-3, respectively. The observed necrotic tissues always surrounded these nematodes during their vermiform J2 stage, thus arresting their development to J3 (Fig. 1).

By 27 DAI inoculation, significantly (P = 0.01) more juveniles had progressed to adult female stage in roots of

susceptible Cabernet compared to those in resistant rootstocks. The swollen development stage was more persistent in resistant 10-23B roots with 77% of the nematodes still present in this stage compared with 0% and 6% in susceptible Cabernet and resistant RS-3, respectively (Fig. 2, 27-DAI). The gall and egg mass indices were 5.0 on roots of susceptible Cabernet and 3.0 on resistant RS-3 roots, whereas on the resistant 10-23B the gall index reduced to 2.0. Most of the galls on the roots of 10-23B were either empty or contained fragments of nematodes or under-developed juveniles.

Numbers of J2 hatched per root system and females per gram of root were greater (P = 0.01) for susceptible Cabernet roots than for resistant 10-23B or RS-3 roots (Table 1). The hypersensitive reaction persisted and was 66% and 6% on roots of resistant 10-23B and RS-3, respectively (Fig 1).

At the 35-day sampling, more (P = 0.01) juveniles (45.4%) had developed to adult females on roots of susceptible Cabernet compared to roots of resistant rootstocks RS-3 (1%) and 10-23B (2%). All the juveniles had advanced to the adult stage, with or without egg masses, in all three grape rootstocks (Fig 2, 35-DAI). There was no change in gall and egg mass indices at this stage. However, the galls and adult females within Cabernet roots were largest in size, intermediate in size on roots of resistant 10-23B, and very small on the roots of resistant RS-3. The number of J2 hatched per root system and females per gram of root on susceptible Cabernet were also higher at the 35-day sampling, but the fecundity (eggs per egg mass) was higher (P = 0.05) in roots of resistant RS-3 than in susceptible Cabernet (Table 1). No egg masses were found on roots of 10-23B. Empty galls containing fragments of nematodes or under-developed juveniles were found in 10-23B rootstock 35 DAI. At 35 days there were only two under-developed juveniles remaining in RS-3, both having an associated hypersensitive reaction, whereas all under-developed juveniles on the roots of 10-23B had died.

Discussion

The observed host-parasite interactions resulted in important changes for both organisms. Compared to the susceptible rootstock where the nematodes developed, reproduced and induced gall formation, the resistant rootstocks RS-3 and 10-23B defended themselves against nematode infection by epidermal, cortical and vascular necrosis leading to reduced penetration, followed by limited development and reproduction.

The resistance response to *M. arenaria* pt. Harmony in the resistant grape rootstocks was expressed at the epidermal level by delayed J2 penetration and differential penetration compared with the susceptible grape rootstock. Failure of J2 to penetrate roots of resistant RS-3 indicates the existence of physical or chemical root barriers.

Juvenile infection sites occurring in susceptible Cabernet roots resulted in root tip swelling by 4 DAI, whereas the swelling of infection sites in roots of resistant rootstocks was delayed. This might be related to the behavior of J2 in roots. In Cabernet, the J2 were observed to migrate to the developing vascular cylinder, insert their heads within vascular tissues and orient parallel to the long axis of the root. In resistant cultivars the J2 remained clustered at the root apex.

The higher number of vermiform J2 at 13 days present in roots of resistant rootstocks compared to susceptible Cabernet was due to faster development of J2 in susceptible roots, compared to roots of resistant rootstocks. This delayed nematode development indicates the involvement of post-infection biochemical defense mechanisms (hypersensitive response). By contrast, roots of susceptible Cabernet did not exhibit hypersensitive reaction, nematode development was normal, and there appeared to be no biochemical defense mechanisms present.

The smaller number of adult females in RS-3 and 10-23B roots compared to susceptible Cabernet roots might be related to the number of juveniles per feeding site and intensity of hypersensitive reaction. We observed very strong necrosis of feeding sites parasitized by few nematodes, compared to little necrosis when there were multiple juvenile infections per feeding site. Once root-knot nematodes have reached the globose stage they can develop to adult females in these grape varieties. We observed no hypersensitive reaction associated with feeding sites of globose or adult females; still, the females were undersized in both resistant rootstocks and no reproduction occurred in roots of 10-23B. This suggests the occurrence of an additional mechanism leading to undersized females and limited reproduction.

The resistance response in 10-23B rootstock was more effective than that in roots of RS-3. Significantly fewer nematodes developed beyond the swollen stage, the gall index was lower and no egg masses developed on this rootstock. This suggests that 10-23B rootstock possesses very active defense mechanisms including hypersensitive reactions which limit nematode

development as well as reproduction. We observed very strong vascular hypersensitive reaction in roots of 10-23B. The presence of empty galls containing under-developed juveniles or fragments of nematodes indicates that nematodes were able to initiate the development of giant cells but further development of giant cells, was inhibited.

The resistant plants responded to nematode infection by producing necrosis around the nematode or its feeding sites, preventing further development. We observed epidermal hypersensitive reaction in response to epidermal penetration around the head of juveniles. These nematodes starved soon after penetration. There was also a cortical hypersensitive reaction entombing, shrinking and killing the nematodes, or delaying migration to vascular tissues to establish feeding sites during the first week after inoculation. Hypersensitive reactions also were common in response to vermiform J2 present along the developing vascular system. The presence of greater numbers of globose stage females in resistant 10-23 and RS-3 roots, compared to the susceptible Cabernet roots, appears to be associated with arrested development.

Although roots of resistant RS-3 supported less nematode development than susceptible Cabernet, the fecundity rate (eggs per egg mass) was high enough to cause concern about the durability of such resistance mechanisms. In the original screening for this rootstock, it was noted that some root-knot nematodes infected the periphery of the root system, but did not survive on older roots. However, that observation was beyond the scope of this study

This research demonstrated that resistant grape rootstocks express their genetic resistance at penetration, development and reproduction during the root-knot nematode infection process. The development of a hypersensitive reaction associated with slower rates of female development resulted in undersized adult females. There was also a mechanism during reproduction that limited formation of egg mass, thereby limiting fecundity. Our results provide an indication of diverse mechanisms of resistance at work in the root tips of these two promising grape rootstocks. Both rootstocks offer improvements over commercially available rootstocks, but the durability of their resistance remains unclear.

Acknowledgements

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Table 1. Number of *M. arenaria* pt. Harmony females, eggs and hatched juveniles on roots of susceptible Cabernet, and resistant 10-23B and RS-3 grape rootstocks.

Days after inoculation	Females per gram of root			Hatched J2 per gram of root			Eggs per female		
	Cabernet	10-23B	RS-3	Cabernet	10-23B	RS-3	Cabernet	10-23B	RS-3
27	91 a	14 b	9 b	101 a	0.71 b	14 b	-	-	-
35	108 a	1 b	3 b	810 a	1.40 b	81 b	107 b	0 c	157 a

Data are means of 5 replicates for females and hatched J_2 whereas eggs per female are means of 20 egg masses.
Within a row, means for female and hatched J_2 not followed by same letter differ significantly (P= 0.01), whereas means for eggs per female differ significantly (P = 0.05) according to Duncan's multiple range test.

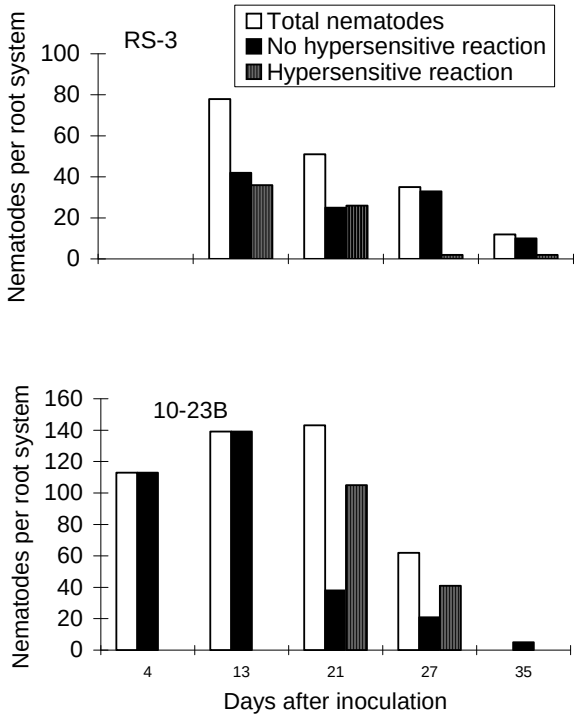

Fig. 1. Total number of *Meloidogyne arenaria* pt. Harmony in roots of resistant grape rootstocks over a period of 35 days and number of nematode-induced hypersensitive reactions.

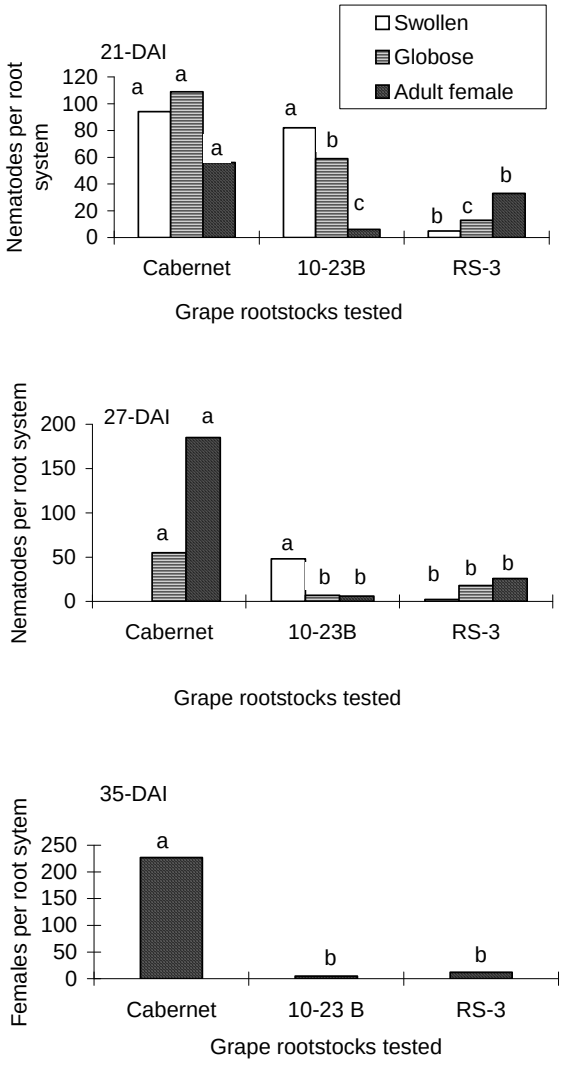

Fig. 2. Penetration and development of *Meloidogyne arenaria* pt. Harmony in roots of susceptible Cabernet and resistant 10-23B and RS-3 at 21, 27 DAI and 35 days after inoculation (DAI). Bars are means of five replicates. Bars of same pattern with different letters differ significantly (P = 0.05).