Effect of Contact Pesticides on Vine Mealybug Parasitoids, *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae)

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Anagyrus species near _pseudococci_ (Girault) and _Coccidoxenoides perminutus_ (Timberlake) (Hymenoptera: Encyrtidae) are well-known mealybug parasitoids. Both are proven biological control agents of _Planococcus ficus_ (Signoret) (Hemiptera: Pseudococcidae) in vineyards. These parasitoids are affected by some pesticides used for the control of ants (Hymenoptera: Formicidae) and _P. ficus_ in vineyards. To establish which of the vineyard pesticides is more toxic to parasitoids, pesticide bioassays were carried in the laboratory using adult and pupal parasitoids. Fipronil and α-cypermethrin caused significant acute toxicity of both parasitoids. Low mortality was recorded for all these pesticides for parasitoids emerging from mummies indicating that the mummy case was an effective barrier to pesticides for parasitoids. Buprofezin, mancozeb and an insecticidal soap were not toxic to parasitoids in both bioassays. Some pesticides have far-reaching negative impacts on parasitoids of orchard and vineyard arthropod pests. A refinement on pest management strategies regarding method and timing of application of pesticides where parasitoids constitute part of the pest management program is essential.

Use of pesticides in integrated pest management (IPM) depends in part, on knowledge of the effects of pesticides on beneficial insects like natural enemies and pollinators. The knowledge allows the use of strategies that minimise the disruptive effect of pesticides, such as use of selective compounds and reduced rates or proper timing of applications (Hassan *et al.*, 1994; Williams & Price, 2004). Direct impacts of pesticides due to direct contact with toxins are manifested as short-term mortality or relatively long term sublethal effects, which generally have the greatest impact on natural enemies' life span, fecundity and ability to locate hosts (Desneux *et al.*, 2007).

Anagyrus species near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae) are tiny solitary koinobiont endoparasitoids of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Islam & Copland, 1997). These parasitoids have potential for use in augmentative release programs for suppression of vine mealybug in Western Cape Province vineyards (Whitehead, 1957; Walton & Pringle, 1999). Effective use of *A.* sp. near *pseudococci* and *C. perminutus* in the augmentative release programs will depend on timing parasitoid releases so that the disruptive effects of pesticides are minimised. Previous researchers noted a negative effect on parasitoid performance of mass released *C. perminutus* in Western Cape Province vineyards due to injudicious application of pesticides during release periods (Walton & Pringle, 1999). Because releases of parasitoids are made after pesticide applications, an understanding of the direct effects of pesticide residues on these two parasitoids is critical for the development of appropriate guidelines for timing of releases.

Work has been done on impacts of field weathered pesticides residues on *Aphelinus mali Haldeman*, a parasitoid of wooly apple aphid, *Eriosoma lanigerum* (Hausmann) in apple orchards (Heunis & Pringle, 2003) and in citrus orchards on *C. perminutus* (formerly *Pauridia peregrine* Timb) (St. Leger Searle, 1963; Hattingh & Tate, 1995). There is limited information on impacts of pesticides on parasitoids in vineyards yet some, like chlorpyrifos, fipronil, α-cypermethrin, among others, are used against vine mealybugs and ants.

Several pesticides were found compatible with natural enemies in apple and citrus orchards in South Africa (Wakgari & Giliomee 2001; Heunis & Pringle 2003). Very little is known about direct effects of pesticides used in vineyards in South Africa on *A.* sp. near *pseudococci* and *C. perminutus.* A better understanding of these impacts could lead to development of strategies that reduce the disruptive effects of the pesticides in commercial vineyards.

There is a growing concern on health and environmental problems caused by heavy reliance on pesticides used against ants and mealybugs. Pesticides are used based on their efficacy and/or cost rather than their potential impacts. The presence of pesticide residues in fruit and wine results in rejection incidences on the international markets such as the USA and Taiwan (Urquart, 1999; Page, 2001) and buyer prerequisites in the UK and Western Europe outlets such as Sainsbury, Tesco, Asda and Marks and Spencer) imposing strict limits on pesticide residues. In South Africa, the scheme for Integrated Production of Wine (IPW) (http://www.ipw.co.za) has set down standard guidelines on the application and timing of pesticides to reduce the health and

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environmental risks associated with pesticide residues. Table 1 summarises the toxicological properties of some of the pesticides used in vineyards against ants and mealybugs.

This investigation focused on evaluating the effects of direct contact with pesticide residues on leaf tissue since parasitoids mostly come in contact with leaves during their search for mealybug hosts, feeding, mating and resting (Longley & Jepson, 1997; Stapel *et al.*, 2000). Topical application of pesticides on mummies containing parasitoid pupae determined the impact of ingestion of pesticide residues upon adult exit from the mummy.

The objective of these bioassays was to assess impact of pesticide residues on acute mortality of *A.* sp. near *pseudococci* and *C. perminutus*. This would lead to the refinement of timing of parasitoid releases to reduce pesticide-induced mortality of parasitoids in vineyards.

This investigation utilised only the egg-laying individuals for each parasitic wasp as the biocontrol effect is from females (egg laying individuals). For the arrhenotokous *A.* sp. near *pseudococci* only females were used and for the thelytokous *C. perminutus*, all individuals were used (Avidov *et al.*, 1967; Islam & Copland, 1997; Ceballo & Walter, 2005).

MATERIALS AND METHODS

Continuous exposure to residues

Parasitoids were exposed to pesticide residues on treated glass plates for 24 hours over a range of doses and replicated five times. Exposure chambers consisted of two pesticide treated glass plates (10cm x 10cm) fitted to a Munger cell (10cm x 10cm x 2cm internal measurements) with six holes (0.8cm diameter) through the side of the walls for ventilation. The holes were covered with fine gauze using a non-toxic adhesive (Universal Silicon, Global sealants South Africa). One hole was left uncovered for introduction of parasitoids. After the introduction of parasitoids, the hole was plugged with cotton wool soaked in 50% honey-water solution, a food source for the parasitoids. For each of five replicates, six Munger cells were assembled, (Hassan, 1992; Hassan *et al.*, 1994) consisting of five dose rates and a blank of distilled water as a control treatment. After trial runs (range finders), α-cypermethrin was tested from 1/32 times to ½ times

TABLE 1

Toxicological characteristics of some pesticides used in vineyards and orchards against ants and mealybugs.

Pesticide	**Type/Application ***	**Mode of action**	**Comment(s)**
Buprofezin	Insect growth regulator Contact/ stomach poison Foliar application	Effective against nymph stages of whitefly, scale and mealybug by inhibiting chitin biosynthesis, i.e. kills insect upon molting. Suppresses oviposition of adults and reducing egg viability (Izawa *et al.*, 1985).	Compatible with IPM programs utilising parasitic wasps and predators such as lacewings, mites, spiders except vedalia beetles (James, 2004).
Imidacloprid	Chloro-nicotinyl Systemic Soil application as a drench	Affects the nervous system by blocking the post synaptic acetyl cholinesterase receptors (Stenersen 2004; Buckingham *et al.*, 1997.	Affects beneficials that feed on nectar.
Fipronil	Phenyl pyrazole- chemicals with herbicidal effect. Contact and stomach poison and moderately systemic Foliar application	Disrupts insect central nervous system via the gamma-amino butyric acid (GABA) regulated chloride channel, i.e. binds to the GABA receptor (Stenersen 2004; Jepson 1989).	Affects some beneficials Incompatible with many IPM programs due to long residual activity.
α-cypermethrin	Synthetic Pyrethrin (pyrethroid) Contact and stomach poison. Racemic mixture of two of the four *cis-* isomers comprising cypermethrin Foliar application	Highly active broad-spectrum insecticide Affects the nervous system by blocking the sodium pump during nerve transmission (Stenersen 2004).	Not compatible with many IPM programs due to long residual activity.
Mancozeb	Ethylene bisdithio carbamate (EBDC) protectant fungicide Foliar application as dust or wettable powder.	Enzyme inactivation (Stenersen 2004; Jepson 1989; Krieger *et al.*, 2001).	Compatible with IPM programs
Borax and citrus oil	Pesticide, fungicide, miticide, biorational contact pesticide. Foliar application	Biorational contact pesticide with broad spectrum control of foliar pests and diseases Immediate knockdown effect. Kills on contact by physically disrupting the target organisms' lipid membrane rendering the organism susceptible to desiccation by the environment. Effective on various stages of pest (eggs, nymphs, larvae and adults) (Krieger *et al.*, 2001).	Compatible with many IPM programs because it has no documented residual effect. Can be mixed with pyrethroids as a wetting agent or as a tank adjuvant.

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the recommended field dose for both parasitoids, buprofezin ¼ to four times (*C. perminutus*) and eight to 128 times (*A.* sp. near *pseudococci*), fipronil 1/8 to double (*C. perminutus*) and ¼ to four times (*A.* sp. near *pseudococci*) and mancozeb and the insecticidal soap, eight to 128 times for both parasitoids.

The glass plates were thoroughly cleaned with a detergent, rinsed with distilled water and then air-dried. A stock solution of the highest dose was prepared for each pesticide (depending on the range established). Serial dilutions with distilled water were then performed to give doses representing lower doses for each parasitoid species- pesticide combination as shown in Table 2.

The aqueous solutions/suspensions of pesticides were applied onto the glass plates using a standard laboratory Potter's Spray Tower (Burkhard Manufacturing Co., Ltd., Hertfordshire, UK) (Potter, 1952) with 2ml of each dose rate at a pressure of approximately 50kPa (7.25lb in^{-2}) delivering approximately 0.02ml liquid cm^{-2} for each glass slide.

The spray tower was thoroughly cleaned and flushed with acetone and distilled water between treatments. Pesticides were applied in order of increasing dose rate. Each time fresh solutions/suspensions were made, i.e. chemical solutions were not stored.

After application, the glass plates were air-dried for 10-15 min. The Munger cells were then assembled with treated glass surfaces facing inwards. Twenty parasitoids were carefully released into each cell through the uncovered hole using a special aspirator. One-day-old *C. perminutus* and one to two-day old female *A.* sp. near *pseudococci* were used. The Munger cells were connected to a manifold, which split the air stream to each of the six cells. To minimise pesticide vapour in the cells, the whole system was ventilated with humidified air (70±5% RH) using a small aquarium pump connected to the main rubber tube. The complete system was maintained in an environment chamber at 25±0.5°C with a 12:12 (L: D) photoperiod.

Parasitoids were checked 6, 12, 18 and 24 hours after introduction. They were regarded as dead when they did not move (after 10 seconds) upon disturbance. A magnifying lens (Optivisor-Donegan Optical Co. USA) was used to examine the parasitoids. Dose-mortality data were adjusted for control mortality using Abbott's formula (Abbott 1925) and Probit analyses performed with POLO-PC program (LeOra Software 1987) to obtain dose-response statistics (Finney 1971; Robertson *et al.*, 2007).

Topical bioassays of field rate pesticides on parasitoid pupae

Anagyrus species near *pseudococci* and *C. perminutus* mummies were exposed to pesticide residues to measure their susceptibility to pesticides and to investigate the role of the mummy case as a barrier to pesticides. Vine mealybugs of appropriate developmental stages were exposed to *C. perminutus* adults and fertilised females of *A.* sp. near *pseudococci*. After mummification, 20 mummies with each type of parasitoid were placed on a sticky tape. The tapes were placed on glass plates, which were sprayed with the recommended field dose rate for the pesticides using a standard Potter's spray tower (protocol described above). The sticky tapes were air dried for one hour then sprinkled with fine soil to prevent emerging parasitoids from coming in contact with the pesticide residues and from getting stuck on the adhesive. The tape was placed in ventilated Petri dishes (9.6cm diameter) and kept under controlled conditions (70±5% RH, 25±0.5°C with a 12:12 (L: D) photoperiod) in an environment chamber. This experiment was replicated five times for each pesticide and parasitoid species.

Parasitoid emergence was checked daily between 14:00 and 15:00 hours. Emerged parasitoids were placed in ventilated vials supplied with 50% honey-water solution. Abbott's correction formula was used to adjust for control mortality. Repeated measures ANOVA followed by Tukey's HSD test was performed in the computer program STATISTICA v.7 (Stat-Soft, South Africa) on parasitoid emergence data.

TABLE 2

Pesticides tested on *Anagyrus* species near *pseudococci* and *Coccidoxenoides perminutus* adults with formulations, target pests and range of doses tested.

Pesticide (active ingredient (a. i.))	Formulation		Field rate	Target pest	Dose rates tested (ml/L)
	Trade name*	Grams pure a.i.			
Buprofezin	Applaud SC	400g/L	60ml/100L (0.6ml/L)	*Planococcus ficus* (Vine mealybug)	0.15; 0.3; 0.6; 1.2; 2.4; 4.8; 9.6; 19.2; 38.4; 76.8
α-cypermethrin	Fastac SC	100g/L	250ml/100L (2.5ml/L)	Formicidae (Ants)	0.0781; 0.1563; 0.3125; 0.625; 1.25
Fipronil	Regent SC	200g/L	10ml/100L (0.1ml/L)	Formicidae (Ants)	0.0125; 0.025; 0.05; 0.1; 0.2; 0.4
Mancozeb	Dithane M45 WP	800g/kg (80%)	200g/100L (2g/L)	*Plasmopara viticola* (Downy mildew)	16; 32; 64; 128; 256
Insecticidal soap (borax and orange oil)	Wet-Cit EC	Borax 10g/kg Orange oil 50g/kg	50ml/100L (0.5ml/L)	*Planococcus ficus* (Vine mealybug)	4; 8; 16; 32; 64

*SC=soluble concentrate; WP = Wettable power; EC = Emulsifiable concentrate.

TABLE 3

Probit parameters of dose responses of *Anagyrus* sp. near *pseudococci* to various doses of different pesticide residues during a 24-hour bioassay.

Pesticide	**Field dose rate (ml/L)**	**χ^2 (d.f)**	**LC_{50} (ml/L)**	**95% fiducial limits**	**LC_{90} (ml/L)**	**95% fiducial limits**
α-cypermethrin	2.5	1.79 (3)	0.25	0.187 to 0.317	3.28	1.95 to 7.57
Fipronil	0.1	0.92 (3)	0.15	0.138 to 0.169	0.344	0.3 to 0.41
Buprofezin	0.6	8.94 (3)	31.8	19.29 to 54.16	126	68.13 to 753
Mancozeb*	2	2.76 (3)	–	–	–	–
Insecticidal soap	0.5	0 (3)	–	–	–	–

*LC values of Mancozeb and insecticidal soap were not established for *A.* sp. near *pseudococci*

TABLE 4

Probit parameters of dose responses of *Coccidoxenoides perminutus* to various doses of different pesticides residues during a 24-hour bioassay.

Pesticide	**Field dose rate (ml/L)**	**χ^2 (d.f)**	**LC_{50} (ml/L)**	**95% fiducial limits**	**LC_{90} (ml/L)**	**95% fiducial limits**
α-cypermethrin	2.5	0.96 (2)	0.19	0.154 to 0.227	0.96	0.744 to 1.345
Fipronil	0.1	10.91 (3)	0.026	0.014 to 0.039	0.083	0.052 to 0.254
Buprofezin	0.6	2.43 (3)	2.59	2.08 to 3.78	10.11	5.92 to 32.92
Mancozeb	2	0.56 (3)	86.78	66.1 to 116.5	1217.15	646.9 to 3492.2
Insecticidal soap	0.5	6.06 (3)	29.57	19.5 to 44.97	106.8	63.29 to 410.5

RESULTS

Continuous exposure to pesticide residues

Population responses to pesticides for *A.* sp. near *pseudococci* and *C. perminutus* were significantly different since none of the 95% fiducial limits overlapped for the two parasitoid species (Tables 3 and 4) (Robertson *et al.,* 2007).

For *A.* sp. near *pseudococci*, the LC_{50} value for fipronil was 1.5 times larger than the field dose rate. However, fipronil is one of the most persistent pesticides making it toxic to parasitoids over a long period of time (Stenersen, 2004). α-cypermethrin LC_{50} was 10 times lower than the field dose rate for the same parasitoid. Although LC_{50} and LC_{90} values for Mancozeb and insecticidal soap were estimated for *A.* sp. near *pseudococci*, no estimates of the 95% fiducial limits could be made and therefore not given (Table 3) implying that there was no correlation between dose rate and mortality for these two pesticides.

For *C. perminutus*, the field dose rates were higher than the LC_{50} values for α-cypermethrin and fipronil by 13 times and five times, respectively. These results indicate that these two pesticides were the most toxic of those tested on *A.* sp. near *pseudococci* and *C. perminutus*. The hypotheses of equality ($\chi^2_{df=2}$=17.4813 and $\chi^2_{df=2}$=365.7; $p\leq0$, respectively) and parallelism ($\chi^2_{df=1}$=9.3027; p=0.002 and $\chi^2_{df=1}$ =10.4753; p=0.001, respectively) of probit regression lines for α- cypermethrin and fipronil were rejected.

Buprofezin, mancozeb and the insecticidal soap were not toxic to parasitoids within their recommended field rates although at high doses *C. perminutus* was more affected by these pesticides compared to *A.* sp. near *pseudococci*). For buprofezin, mancozeb and the insecticidal soap, the hypothesis that probit regression lines were equal was rejected ($\chi^2_{df=2}$= 123.6, 340.28 and 196.28, respectively; $p\leq0$ in all cases) while that of parallelism was accepted ($\chi^2_{df=1}$ =0.021; p=0.963, $\chi^2_{df=1}$ =0.2965; p=0.586 and $\chi^2_{df=1}$ =3.0392; p=0.081, respectively).

TABLE 5

The mean number of days to emergence and number of emerged parasitoids after topical pesticide treatments of 10 day old mummies (n=20).

Pesticide Treatment	***A.* sp. near *pseudococci***		***C. perminutus***	
	Days to emerge†	**Emerged**	**Days to emerge†**	**Emerged**
Water	7.1^a (0.33)	14.4 (0.20)	5.2^a (0.21)	16.5 (0.23)
α-cypermethrin	5.5^a (0.09)	12.5 (0.25)	6.0^a (0.11)	15.1 (0.93)
Buprofezin	12.7^b (0.23)	14.1 (0.20)	11.8^b (0.28)	15.7 (0.51)
Fipronil	7.5^a (0.12)	12.5 (0.40)	5.4^a (0.15)	14.5 (0.60)
Mancozeb	7.1^a (0.52)	14.1 (0.4)	6.2^a (0.66)	15.1 (0.25)
Insecticidal soap	6.3^a (0.53)	13.6 (0.33)	7.0^a (0.46)	14.9 (0.44)

†Means in columns with different letters denote significant difference at 95% confidence limits.

±SE in parenthesis.

Topical application of field rate pesticides on parasitoid pupae

Days to parasitoid emergence after treatment with pesticides significantly differed between treatments ($F_{(5,24)} = 24.48$; $p \leq 0.001$) with buprofezin causing a significant delay in emergence by almost a week relative to other treatments (Table 5). Days to emergence did not differ significantly between species ($F_{(1,58)} = 1.02$; $p = 0.3167$).

Significantly, more *C. perminutus* emerged than *A.* sp. near *pseudococci* ($F_{(10,46)} = 6.514$; $p \leq 0.001$). No significant differences were found between treatments for *C. perminutus* ($F_{(5,24)} = 0.6842$; $p = 0.6399$). *A.* sp. near *pseudococci* mortality due to fipronil and α-cypermethrin was significantly higher than the other treatments ($F_{(5.24)} = 19.604$; $p \leq 0.05$).

DISCUSSION AND CONCLUSION

Mortality rates due to insecticide residues on glass plates in cells provide an indication of impact of pesticide residues on parasitoids. However, the field situation with pesticide residues on vine foliage is likely to be lower. Longley & Jepson (1997) indicated a difference in bioavailability due to pesticide residues becoming bound with the epicuticular layers on leaf surfaces, amongst other factors. The toxicity calculations obtained in this investigation pertain to glass plates as substrates and may therefore differ from results obtained using natural substrates such as leaves. Additionally, insects in the field can shelter in places where pesticide residues may not reach them, for example, parasitised vine mealybug can hide under the bark or crevices subsequently protecting the developing parasitoids. Results may also vary due to insect generation, sex, species and size of parasitoids.

Fipronil is used to control ants in vineyards. Control of ants in mealybug-infested vineyards allows *A.* sp. near *pseudococci* access to mealybug that they would not otherwise access in the presence of ants. Mgocheki and Addison (2009) demonstrated that the main ant species present in Western Cape vineyards significantly affects this parasitoid.

α-cypermethrin and fipronil caused high mortality of the parasitoids, therefore, may not be compatible with IPM programs utilising parasitoids for vine mealybug control, unless these pesticides are used in containerised low toxic baits, or applied to an area of the vine not utilised by parasitoids, such as the stem. Walton and Pringle (1999) also found cypermethrin to be very toxic to *C. perminutus* and discouraged full cover application of this pesticide during augmentative release periods.

Observations showed that *A.* sp. near *pseudococci* died as they gnawed an exit hole with their mandibles through the dorsal portions of the mummies treated with fipronil and α-cypermethrin. The resulting partial emergence indicated the high degree of toxicity of these two pesticides. Chewing an exit hole presented a risk for parasitoids ingesting the pesticides, which are stomach poisons. In the field, *A.* sp. near *pseudococci* could be poisoned by feeding on contaminated mealybug honeydew, an important food source for parasitoids. Consequently, parasitoid mortality could result or their performance adversely affected (Wang *et al.*, 2008). Mortality of parasitoids at the time of emergence has been documented for adults of aphid parasitoids (Hsieh & Allen, 1986; Krespi *et al.*, 1991; Lingren *et al.*, 1991; Islam & Copland, 1997; Walton & Pringle, 1999). Low mortality of *C. perminutus* when exposed to fipronil and α-cypermethrin could be due to a different mechanism of exiting the mummy case. They push to crack open the mummy case instead of chewing an exit hole subsequently avoiding ingestion of poison. Mortality rates were low across treatments indicating that the mummy case is indeed, an efficient barrier to pesticides. From this investigation, the adult stage of parasitoid was more vulnerable to pesticides than the juvenile stages developing in the mummies.

Timing of insecticide application is very crucial given the continued conventional high volume spraying in commercial vineyards. The use of economic injury levels (EIL) and economic thresholds (ET) for pests pays no regard to the role of natural enemies, therefore some adaptations to population dynamics of important parasitoid species is required. Pesticide treatments can be restricted to periods of low activity of the vulnerable stages of parasitoids (adults), for example, early spring treatments. The limited persistence of active ingredients such as α-cypermethrin may be exploited to achieve selectivity (Elzen, 1989). If only the insensitive stages of parasitoids within mummified mealybugs are exposed to treatment, the more sensitive adults may be protected (Metcalfe, 1980). Stem application of pesticides in hot spots later in the season minimises risk to parasitoids, which by this time will have a large prey population to achieve maximum parasitism rates, and provides areas where parasitoids can shelter.

Although buprofezin and the insecticidal soap showed little negative impact on parasitoids in the laboratory, in vineyards, these two pesticides can reduce populations of parasitoids indirectly by reducing populations of VMB (Grafton-Cardwell *et al.*, 2006). In South Africa, the use of buprofezin has been warned against especially when utilising coccinellid predators as main natural enemies for mealybugs and cottony cushion scale (Hattingh & Tate, 1995). Exposure of mealybug mummies containing parasitoid pupae to buprofezin showed a delayed emergence of adults. This may interfere with the phenological synchrony between mealybugs and their parasitoids resulting in reduced ability of the parasitoids to regulate mealybug populations. The reproductive statistics of *A. pseudococci* (Güleç *et al.*, 2007) and *C. perminutus* (Davies *et al.*, 2004) suggest that these biological control agents will be able to complete more generations than the mealybug within the field temperatures, thus, they are both effective against *P. ficus* where pesticides are used selectively.

α-cypermethrin and fipronil were very toxic vineyard pesticides to mealybug parasitoids while buprofezin, mancozeb and insecticidal soap did not cause any significant mortality at the recommended field rates. *A.* sp. near *pseudococci* adults were more robust and resilient to pesticides than *C. perminutus*, possibly due to their larger size. Although the insecticidal soap and buprofezin caused no significant parasitoid mortality, they can impact on parasitoids indirectly by reducing the host (mealybugs) population. Timing of pesticide application is very important regarding the vulnerable stages of parasitoids. *C. perminutus* are released as pupae while *A.* sp. near *pseudococci* are released as adults. This affects the choice of parasitoid and timing of augmentative release regarding breakdown of pesticides on plant surfaces.

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