

Evaluation of petroleum spray oils for control of the Asian citrus psylla, *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), in China

(Keywords: *Diaphorina citri*, petroleum oil, citrus greening disease)

D. J. RAE†, W. G. LIANG‡, D. M. WATSON†, G. A. C. BEATTIE† and M. D. HUANG‡

†New South Wales Agriculture, Biological and Chemical Research Institute, PMB 10 Rydalmere, New South Wales 2116, Australia

‡Guangdong Entomological Institute, 105 Xingang Road West, Guangzhou 510260, People's Republic of China

Abstract The efficacy of petroleum spray oil against citrus psylla was tested in Guangzhou, China. As the oil concentration increased from 0.25% to 1.0% there was a linear decrease in the number of psylla of each stage present on foliar shoots after 8 days. When psylla infested shoots were sprayed with equivalent oil concentrations, survival was significantly different between oil treatments. Psylla survival was also stage-dependent, with 1st–2nd instars being the most susceptible and eggs being the most tolerant to oil. Oil efficacy was compared to an organophosphate pesticide and an insect growth regulator, and all provided similarly effective control of psylla nymphs. Control of eggs was not achieved by any of the treatments as application of sprays was not correctly timed.

1. Introduction

Citrus greening disease is caused by two closely related strains (Asian and African) of gram-negative, phloem-limited bacterium-like organisms (BLO), which have recently been placed in the class Proteobacteria (Bové and Garnier, 1994). It is one of the most devastating citrus diseases of the world (Aubert, 1993) having destroyed around 50 million citrus trees since it was first described in China (Reinking, 1919), with most of this loss occurring in Southeast Asian countries (Aubert, 1989). The greening pathogen is readily transmitted by grafting and propagating with infected plant material and is lethal to most commercial citrus cultivars (Gottwald *et al.*, 1989). The Asian strain of the disease is also transmitted by the citrus psylla, *Diaphorina citri* (Kuwayama), in China, Southeast Asia, Nepal, India, Pakistan, the Arabian peninsular, Reunion and Mauritius (Bové, 1986; Aubert, 1987; Gottwald *et al.*, 1989) while the African strain is transmitted by the psylla *Trioza erythrae* (Del Guercio) in Africa south of the Sahara, Reunion and Mauritius (Bové, 1986). Citrus greening is much more serious in Asia than Africa as both the Asian strain of the disease and the vector are able to tolerate a much wider range of environmental conditions (Bové, 1986). Humidities in the range 45–75% are more suitable to *D. citri* than *T. erythrae* (Yang, 1989). Control of the disease requires a coordinated approach involving the establishment of disease-free citrus nurseries, eradication of infected plant material and effective vector control.

Many synthetic pesticides have been tested for use against *D. citri* including endrin, diazinon, parathion, malathion, methyl-demeton, thiometon, DDT (Kahn *et al.*, 1982), dimethoate, phosphamidon, monocrotophos (Bhagabati and Nariani, 1983), oxydemeton-methyl, phosalone, quinalphos (Batra *et al.*, 1990)

and phosmet (Huang *et al.*, 1987). Indiscriminate use of insecticides against *D. citri* has led to the disruption of natural enemies of other citrus pests such as the whitefly, *Aleuracanthus woglumi* Ashby (Rajak and Diwakar, 1987) and citrus red mite, *Panonychus citri* (McGregor) (Huang *et al.*, 1987). Other research efforts have concentrated on the biological control of *D. citri*. *Tamarixia radiata* (Waterson), a eulophid ectoparasite of *D. citri*, was successfully introduced into Reunion Island where it achieved excellent biological control in the late 1970s (Aubert, 1987). Since then it has also been successfully established in Taiwan (Chien *et al.*, 1988) and the Phillipines (Mercado *et al.*, 1991). This species is also found in southern China and levels of parasitism as high as 35.6–80% have been recorded in an orchard near Guangzhou (Liu, 1989).

Recent studies in Australia (Beattie *et al.*, 1995) and China (Rae *et al.*, 1996) have demonstrated that IPM compatible petroleum spray oils are able to provide as effective control of citrus leafminer, *Phyllocnistis citrella* Stainton, as traditionally used broad-spectrum pesticides. Citrus leafminer and citrus psylla both lay their eggs on the surface of young foliar shoots. Shoots are most favourable for psylla egg laying when they are 0.4–0.9 cm in length (C. T. Leong personal communication, 1996), and for leafminer when they are 1.4–5.1 cm in length (Ikemoto, 1972). It should therefore be possible for psylla and leafminer to be simultaneously controlled with a spray which is effective against both species. The current study was conducted to determine the effect of petroleum spray oils on *D. citri* oviposition rates and survival, and to compare the efficacies of three oil formulations with an organophosphate and an insect growth regulator.

2. Materials and methods

Experiments were conducted in October 1993, November 1993 and July 1995 at the Guangdong Entomological Institute in Guangzhou, southern China. One to 1.5 m tall calamondin trees, *Citrus madurensis* Loureoro, in 8 l pots were used as host plants. One week before trees were required they were pruned and lightly fertilized to encourage new growth suitable for *D. citri* colonization. *D. citri* were collected by aspirating adults from *Murraya paniculata* (L.) hedges growing in the Institute grounds in 1993, and from *M. paniculata* growing in the grounds of the nearby Zhongshan University in 1995.

†Current address: School of Horticulture, University of Western Sydney, Hawkesbury, Locked Bag 1, Richmond NSW 2753, Australia.

2.1. Experiment 1: the effect of oil on *D. citri* oviposition

On 25 October 1993, 15 calamondin trees, each with a similar number of foliar shoots (flushes), were chosen for this experiment. To ensure trees were psylla free prior to being treated, all foliar shoots were checked for the presence of psylla eggs and larvae using Zeiss prism loupes ($\times 8$), and any shoots with psylla removed and destroyed. There were five treatments representing concentrations of 0, 250, 500, 750 and 100 ml Caltex Lovis petroleum spray oil per 100 l water (see Rae *et al.* (1996) for technical specifications of the oil). Three trees were randomly allocated to each treatment and each tree labelled with a plastic card. Sprays were applied to run-off using small hand held sprayers. After the foliage had dried, trees were transferred to a large screen enclosure (4 m $\times$ 7 m $\times$ 3 m with 1 mm mesh) and placed in three rows using a completely randomized design. Pots were placed about 1 m apart so that the foliage from adjacent trees did not touch. About 500 adult female and male psylla collected on the same day were released evenly throughout the enclosure. The enclosure prevented dispersal of adult psylla to the nearby *M. paniculata* from which they were collected. After 8 days 20 flushes were haphazardly collected from each tree, placed in labelled bags and stored at 4°C. Flushes were examined using a Wild M8 stereo-microscope within 1 week of collection and the number of eggs and 1st–2nd, 3rd–4th and 5th instar nymphs recorded per flush.

For each tree the counts of psylla from 20 flushes were averaged to give a single value per tree. These data were normalized and variances stabilized using a log transformation [$\ln(x+1)$] and analysed using a factorial analysis of variance (ANOVA) (SPSS, 1995). The factors were oil treatment and psylla development stage (eggs and nymphs). All instars were pooled to give a total count because of low numbers of nymphs.

2.2. Experiment 2: the effect of oil on *D. citri* survival

On 8 November 1993, 75 citrus flushes infested with psylla eggs and nymphs were collected from the calamondin trees not used in experiment 1. Five flushes were randomly allocated to each of three replicates in five treatments. Concentrations of Caltex Lovis petroleum spray oil tested were as used in experiment 1. Each group of five flushes were examined using a Wild M8 binocular microscope and the number of psylla in each instar recorded. Flushes were then placed in labelled bottles and maintained with water. The oil treatments were applied to run-off using small hand held sprayers. Flushes were re-examined after 24 h to record psylla nymph survival and after seven days to record the number of unhatched eggs.

Survival data were normalized and variances stabilized using a square root transformation [$(x+0.5)^{0.5}$] and analysed using a factorial ANOVA. The factors were oil treatment and psylla development stage (eggs, 1st–2nd instars and 3rd–5th instars). Instars 3 to 5 were pooled because of low 5th instar numbers.

2.3. Experiment 3: a comparison of the effect of oils and conventional pesticides on *D. citri*

On 24 July 1995, 48 calamondin trees with a similar number of flushes were placed in three rows in a sunny position. Six treatments (Table 1) (four replicates of two trees each) were randomly allocated to the trees, and each tree labelled with a plastic card. Sprays were applied to run-off using small hand held sprayers with spray barriers to prevent spray drift. After the sprays had dried, about 800 adult psylla collected on the same day were released evenly throughout the block. A screened enclosure was not used in this instance as nearby *M. paniculata* were not suitable for psylla colonization. Sprays were re-applied on 29 July and about 500 adult psylla released. Nine days later 20 flushes were haphazardly collected from each tree, placed in labelled plastic bags and stored at 4°C. Flushes were examined using a Wild M8 stereo-microscope within 1 week of collection and the number of eggs and nymphs of each instar and the number of leaves per flush recorded.

Counts of psylla from 20 flushes were averaged to give a single value per tree. A 2-way ANOVA was used to test if there were any differences in the number of leaves per flush between treatments. No differences were found ($F_{5,18}=0.17$, ns). A reciprocal transformation [$1/(x+1)$] was applied to mean psylla counts per tree. A factorial ANOVA was used to analyse the data, with the factors being spray treatment and psylla development stage. Psylla development stages were defined as: eggs, 1st–2nd instars and 3rd–5th instars or eggs and 1st–5th instars. Linear contrasts were used to determine which treatments differed from the control and whether each of the oil treatments were as effective as the conventionally used pesticide treatments (Dunn–Sidak adjusted error rate for 11 contrasts=0.004 65).

3. Results

3.1. The effect of oil on *D. citri* oviposition

The number of psylla nymphs and eggs present on flushes 8 days after spraying differed significantly between oil treatments ($F_{4,20}=18.1$, $p<0.001$; Figure 1). The number of psylla in each

Table 1. Treatments and treatment concentrations tested

Treatment	Active ingredient per 100 l water	Formulation	Manufacturer
Control (water)			
omethoate	100 g	water soluble powder	Guangzhou Pesticide Factory, China
diflubenzuron	10 g	water soluble powder	Duphar B.V. Co., Holland
Lovis petroleum spray oil	500 ml	emulsive oil	Caltex Oil (Australia) Pty Ltd, Australia
D-C-Tron NR petroleum spray oil	500 ml	emulsive oil	Ampol Limited, Australia
Guangdong petroleum spray oil	500 ml	emulsive oil	Luoding Biochemical Plant, China

stage also differed significantly between treatments ($F_{1,20}=41.2$, $p<0.001$) but there was no interaction effect ($F_{4,20}=1.08$, ns; Figure 1). Polynomial trends analysis revealed that as oil concentration increased the number of psylla (eggs and nymphs pooled) on flushes decreased linearly ($F_{1,20}=72.04$, $p<0.001$; Figure 1).

3.2. The effect of oil on *D. citri* survival

There was a significant treatment by stage effect ($F_{8,30}=3.18$, $p=0.010$). Simple effects analysis revealed that for each psylla stage, survival differed significantly between treatments ($p<0.001$) and for each treatment, except the control, survival differed between stages ($p<0.001$). Although survival patterns were similar in each stage, survival was higher in eggs than in nymphs, and higher in older nymphs than 1st–2nd instars regardless of oil concentration (Figure 2).

3.3. A comparison of the effect of oils and conventional pesticides on *D. citri*

A comparison of the number of psylla between treatments revealed significant treatment by stage interactions using a model with three development stages (eggs, 1st–2nd instars and 3rd–5th instars; $F_{10,54}=8.06$, $p<0.001$) and a model with two development stages (eggs and all nymph instars; $F_{5,36}=11.29$, $p<0.001$). However, when the egg stage was removed from the former model and data re-analysed, there was no interaction effect ($F_{5,36}=1.94$, ns). These results indicate that while the psylla in both nymph age groups responded similarly to the treatments, psylla eggs responded in a different way (Figure 3). Simple effects analysis using the model with three development stages found no significant treatment differences for the number of eggs ($F_{5,54}=1.02$, ns) but highly significant differences for the number of 1st–2nd instars ($F_{5,54}=19.4$, $p<0.001$) and 3rd–5th instars ($F_{5,54}=21.8$, $p<0.001$). The number of 1st–2nd instars and 3rd–5th instars in all treatments differed significantly from the number in the control ($p<0.001$ for all contrasts). There were no significant differences between the oils and either omethoate or diflubenzuron in either the number

of 1st–2nd instars or the number of 3rd–5th instars. However, there was a tendency for the number of 1st–2nd instars on the 0.5% D-C-Tron NR treated trees to be greater than on omethoate treated trees, and for the number of 1st–2nd instars on the 0.5% D-C-Tron NR treated trees to be greater than on diflubenzuron treated trees ($p<0.05$).

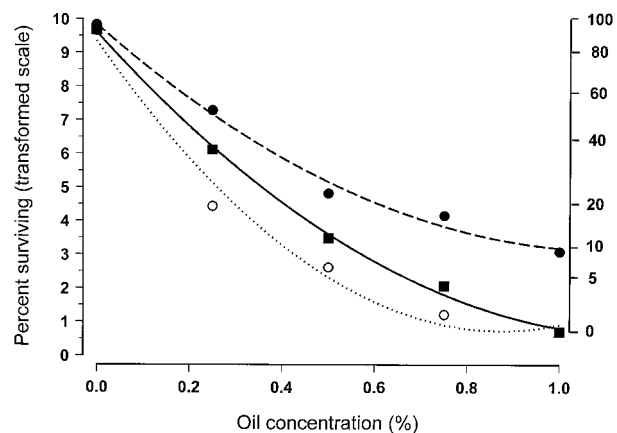


Figure 2. The effect of oil concentration on the survival of psylla eggs (●) 1st and 2nd instar nymphs (○) and 3rd to 5th instar nymphs (■).

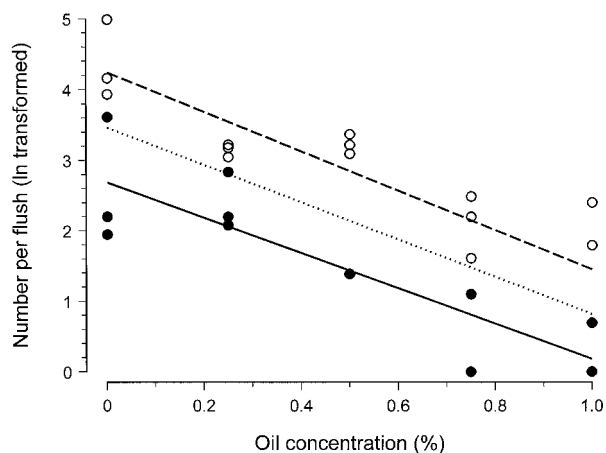


Figure 1. The effect of oil concentration on the number of psylla eggs (○—○; $y=4.24-2.79x$; $r^2=0.739$), nymphs (●—●; $y=2.68-2.50x$; $r^2=0.736$) and eggs and nymphs pooled (.....; $y=3.46-2.64x$) present after exposing oil sprayed calamondin trees to psylla for 8 days.

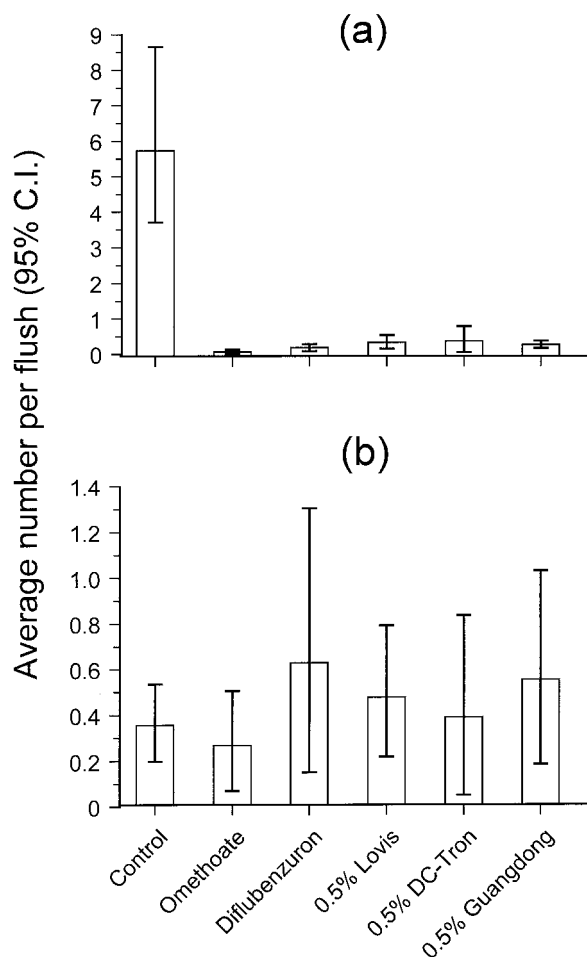


Figure 3. The effect of omethoate, diflubenzuron and three petroleum spray oils on the average number of psylla nymphs (a) and eggs (b) per flush.

4. Discussion

The use of oil to control citrus psylla is not a new concept. As early as 1919, spraying with fish oil–resin was recommended in India (Fletcher, 1919). Spraying with a crude oil emulsion was also recommended in India in 1945 (Pruthi and Mani, 1945) although it was considered more costly and not quite as effective as the alternative control at the time, tobacco decoction and resin compound. Since 1945 the effectiveness of mineral oils and other IPM compatible compounds against citrus psylla appears to have been forgotten. This probably results from a combination of phytotoxicity caused by the less refined oils used at that time, and the advent of synthetic broad-spectrum insecticides. Modern oils can still cause phytotoxicity, but with current oil formulations and correct use this is extremely rare.

Petroleum spray oils, or white oils have recently become increasingly important for the control of a wide range of insect pests of citrus. In Australia they are recommended for the control of armoured scales (red, yellow, purple, circular black and Glover's), soft scales (white wax, Chinese wax, pink wax, black, soft brown and citricola), mites (citrus red, rust and citrus rust) (Beattie, 1990) and citrus leafminer (Beattie and Smith, 1993). In Florida oils are used to control scales, mites and whitefly (Knapp, 1992), and in California, scales, mites, mealybugs, aphids, psylla and fruit-feeding Lepidoptera (Davidson et al., 1991).

It has generally been accepted that petroleum spray oil is most effective against small, immobile insects which are suffocated or drowned when covered with a thin film of oil. Psylla eggs and nymphs present on calamondin flushes did suffer significant mortality when sprayed with oil. Susceptibility to oil differed between stages with the youngest instars being most susceptible and eggs the most tolerant. However, this study clearly demonstrates that oil also reduced the resultant psylla populations when psylla free trees were sprayed prior to exposure to adult psylla. These reductions are presumably the result of oviposition deterrence. Oviposition deterrence as a result of oil deposits has also been demonstrated with citrus leafminer (Beattie et al., 1995; Rae et al., 1996), codling moth (Riedl et al., 1995) and whiteflies (Larew and Locke, 1990).

Petroleum spray oil provided as effective control of psylla nymphs as conventional insecticides when sprays were applied in a similar manner to commercial field applications. However, psylla eggs occurred in similar numbers on the control and all other treatments 9 days after the final spray was applied. This indicates an absence of both oviposition deterrence and egg mortality, which is inconsistent with the results of experiments 1 and 2. However, since psylla are able to utilize leaves as small as 0.4 cm in length for egg laying, and experiment 3 was conducted in mid-summer when foliar shoots were developing rapidly, we believe that the apparent inability to control eggs resulted from the long interval between the final spray and sampling. Any foliar shoots which were not yet present when the final spray was applied but were able to grow to a length of 0.4 cm or more prior to sampling would have been free from oil or insecticide and susceptible to psylla oviposition. In order to provide the best protection against transmission of greening disease, it appears that oil and other insecticide sprays would need to be applied more frequently than every 9 days during

summer in Guangzhou. If flushing is synchronous among trees, then 3–4 sprays may need to be applied on a 6–7 day interval. Temperature and the rate of psylla development would have to be taken into consideration to determine the frequency of sprays in other locations and during other seasons.

The application of multiple sprays of petroleum spray oil to citrus against leafminer during a flushing cycle results in similar material costs to a spray programme based on conventional insecticides (Rae et al., 1996). As citrus psylla and leafminer occur together on citrus flushes in most Asian countries, a spraying schedule using oils to simultaneously control both pests can now be developed. The use of oils has a number of advantages over conventional pesticides as they are less disruptive to natural enemies, insects do not develop resistance to them, they are non-toxic to vertebrates and they break down easily in the environment (Beattie and Smith, 1993) making their use far more sustainable in the long term.

Acknowledgements

We gratefully acknowledge The Australian Centre for International Agricultural Research (ACIAR), Ampol Limited, Caltex Oil (Australia) Pty Ltd and The Sciences and Technology Committee of Guangdong for financial support (ACIAR Project 9305) and Du Tongyuan, Liu Deguang, Li Ming Xiong Jinjun and Ding Yong for assistance with the collection of psylla and/or applying sprays.

References

- AUBERT, B., 1987. *Trioxa crytreae* Del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), the two vectors of citrus greening disease: biological control and possible control strategies. *Fruits*, 42, 149–162.
- AUBERT, B., 1989. Preventing citrus debilitating diseases for profitable crops in South East Asia. Intercountry UNDP-FAO regional project of governments of China, Indonesia, Malaysia, Philippines and Thailand for the control of citrus greening disease. UNDP-FAO Regional Project RAS/86/022 (FAO), pp. 19.
- AUBERT, B., 1993. Citrus greening disease, a serious limiting factor for citriculture in Asia and Africa. In E. Rabe (ed) *Proceedings of the IV World Congress of The International Society of Citrus Nurserymen* (Johannesburg: South African Citrus Nurserymen's Association), pp. 134–142.
- BATRA, R. C., SANDHU, G. S. and SHARMA, D. R., 1990. Relative efficacy of different organophosphorus insecticides for the control of psylla, *Diaphorina citri* Kuwayama. *Research and Development Reporter*, 7, 53–58.
- BEATTIE, G. A. C., 1990. Citrus petroleum spray oils. Agfact H2.AE.5 (NSW Agriculture and Fisheries), pp. 4.
- BEATTIE, G. A. C. and SMITH, D., 1993. Citrus leafminer, Agfact H2.AE.4. 2nd edition (NSW Agriculture), pp. 6.
- BEATTIE, G. A. C., LIU, Z. M., WATSON, D. M., CLIFT, A. D. and JIANG, L., 1995. Evaluation of petroleum spray oils and polysaccharides for control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *Journal of the Australian Entomological Society*, 34, 349–353.
- BHAGABATI, K. N. and NARIANI, T. K., 1983. Chemical control of citrus psylla, a vector of citrus greening disease. *Journal of Research, Assam Agricultural University*, 4, 86–88.
- BOVE, J. M., 1986. Greening in the Arabian peninsula: toward new techniques for its detection and control. *FAO Plant Protection Bulletin*, 34, 7–14.
- BOVE, J. M. and GARNIER, M., 1994. The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the Proteobacteria. *International Journal of Systematic Bacteriology*, 44, 379–386.
- CHIEN, C. C., CHIU, S. C. and KU, S. C., 1988. Biological control of citrus psylla, *Diaphorina citri*. 1. The introduction, augmentation and release of *Tamarixia radiata*. *Journal of Agricultural Research of China*, 37, 430–439.
- DAVIDSON, N. A., DIBBLE, J. E., FLINT, M. L., MARER, P. J. and GUYE, A., 1991. *Managing Insects and Mites with Spray Oils*, Publication No. 3347 (University of California) pp. 47.

- FLETCHER, T. B., 1919. Annotated list of Indian crop pests. In T.B. Fletcher (ed) *Proceedings of the Third Entomological Meeting, Pusa*, 1919. (Calcutta: Government Printer), pp. 33–313.
- GOTTWALD, T. R., AUBERT, B. and ZHAO, X. Y., 1989. Preliminary analysis of citrus greening (Huanglungbin) epidemics in the People's Republic of China and French Reunion Island. *Phytopathology*, 79, 687–693.
- HUANG, M. D., XIONG, J. J. and DU, T. Y., 1987. The selection for and genetical analysis of phosmet resistance in *Amblyseius nicholsi*. *Acta Entomologica Sinica*, 30, 133–139.
- IKEMOTO, T., 1972. Ecological studies on a field population of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Pyhlocnistidae), with special reference to spacial distribution pattern. *Japanese Journal of Applied Entomology and Zoology*, 16, 127–138.
- KAHN, K. M., NARKHEDE, S. S. and BORLE, M. N., 1982. Evaluation of some insecticides for the control of citrus psylla. *Pesticides*, 16, 28–29.
- KNAPP, J. L., 1992. 1992 *Florida citrus spray guide*, Florida cooperative extension service, Institute of Food and Agricultural Services (Gainesville: University of Florida), pp. 46.
- LAREW, H. G. and LOCKE, J. C., 1990. Repellancy and toxicity of a horticultural oil against whiteflies on chrysanthemum. *Hortscience*, 25, 1406–1407.
- LIU, Z. M., 1989. The population dynamics of citrus psylla and its ectoparasite. In D. J. Lin (ed) *Studies on the Integrated Management of Citrus Insect Pests* (Beijing: Academic Book and Periodical Press), pp. 165–175.
- MERCADO, B. G., PABELO, F., GAVARRA, M. R. and GONZALES, C. I., 1991. Population studies and biological control of *Diaphorina citri* Kuwayama – the insect vector of citrus greening disease in the Philippines. In K.E. Chung and S.B. Osman (eds) *Proceedings of the 6th International Asia Pacific Workshop on Integrated Citrus Health Management*, Kuala Lumpur, Malaysia, 24–30 June, 1991, pp. 105–117.
- PRUTHI, H. S. and MANI, M. S., 1945. *Our Knowledge of the Insect and Mite Pests of Citrus in India and Their Control*. Scientific Monograph no. 16, The Imperial Council of Agricultural Research (Calcutta: Government of India Press), pp. 42.
- RAE, D. J., WATSON, D. M., LIANG, W. G., TAN, B. L., LI, M., HUANG, M. D., DING, Y., XIONG, J. J., DU, D. P., TANG, J. and BEATTIE, G. A. C., 1996. Comparison of petroleum spray oils, abamectin, cartap and methomyl for citrus leafminer (Lepidoptera: Gracillariidae) control in southern China. *Journal of Economic Entomology*, 89, 493–500.
- RAJAK, R. L. and DIWAKAR, M. C., 1987. Kolshi problem in orange orchards of Vidarbha region (Maharashtra). *Plant Protection Bulletin Faridabad*, 39, 1–2.
- REINKING, O. A., 1919. Diseases of economic plants in Southern China. *Phillipine Agriculturalist*, 8, 109–135.
- RIEDL, H., HALAJ, J., KREOWSKI, W. B., HILTON, R. J. and WESTIGARD, P. H., 1995. Laboratory evaluation of mineral oils for control of codling moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, 88, 140–147.
- SPSS Inc., 1995. SPSS® for Windows™ Release 6.1 (Chicago: Illinois).
- YANG, Y., 1989. Influence of light, temperature and humidity on the development, reproduction and survival of citrus psylla. *Acta Ecologia Sinica*, 9, 348–354.