Citrus R&T (e-ISSN 2236-3122)
Citrus Research & Technology, v. 38, n. 1, 2017
http://dx.doi.org/10.4322/crt.ICC044

Artigo/Article

Does fungicide run-off from citrus delay leaf litter decomposition?

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SUMMARY

Leaf litter is a major inoculum source for citrus diseases such citrus black spot caused by *Phyllosticta citricarpa*, and greasy spot caused by *Mycosphaerella citri*. In order to reduce this inoculum source, the efficacy of urea, dolomitic lime, a commercial compost accelerator, and an organic mulch, was assessed for enhanced leaf decomposition and reduction in sporocarps. However, due to the potential for run-off from high volume fungicide applications to disrupt leaf decomposition and microbial antagonism, the amendments were compared with and without simulated fungicide run-off. Mature green leaves of *Citrus sinensis* were removed from trees and placed inside mesh bags before being pinned to the orchard floor. The amendments were applied, and then simulated run-off from a typical citrus black spot fungicide program (copper, mancozeb, azoxystrobin) was applied. Leaf degradation was assessed every 2-3 weeks by visual ratings and dry weight. No direct effects on sporocarps could be observed due to insufficient infection. The results showed that the organic mulch was the most effective at enhancing decomposition, while there was significantly ($P < 0.05$) less decomposition in the presence of fungicide run-off.

Index terms: fungi, dolomite, pesticide.

O fungicida atrasa a decomposição da palhada de folhas de citros?

RESUMO

A palhada de folhas de citros é uma importante fonte de inóculo para doenças cítricas como mancha preta de citros (MPC) causada por *Phyllosticta citricarpa* e mancha graxa causada por *Mycosphaerella citri*. A fim de reduzir esta fonte de inóculo, avaliou-se a eficácia da ureia, calcário dolomítico, um acelerador comercial de adubo e uma cobertura orgânica (*mulch*), para melhorar a decomposição e redução de esporos. No entanto, devido ao potencial de escorrimento de fungicidas em aplicações de alto volume em interromper a decomposição da folha e o antagonismo microbiano, as alterações foram comparadas com e sem aplicação simulada de fungicida. As folhas verdes maduras de *Citrus sinensis* foram removidas das árvores e colocadas dentro de bolsas de malha antes de serem presas ao chão do pomar. Os tratamentos foram aplicados e, em seguida, simulou o escoamento de um programa típico de fungicida para MPC (cobre, mancozeb, azoxistrobina). A degradação das folhas foi avaliada a cada 2-3 semanas, avaliando a massa seca. Não foram

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observados efeitos diretos sobre os esporos devido à insuficiência da infecção. Os resultados mostraram que o *mulch* orgânico foi o mais efetivo para melhorar a decomposição, havendo diminuição significativa (P <0,05) na presença de escorrimento de fungicida.

Termos de indexação: fungos, calcário dolomítico, pesticidas.

INTRODUCTION

Citrus leaf litter in orchards is a source of inoculum for several fungal diseases including citrus black spot caused by *Phyllosticta* (*Guignardia*) *citricarpa* (McAlpine) Van der Aa (McAlpine, 1899; Kiely, 1948; Van der Aa, 1973) and greasy spot caused by *Mycosphaerella citri* (Whiteside) (Whiteside, 1970). Leaf litter is also an inoculum source for diseases in other tree crops; one particularly well studied example being apple scab caused by *Venturia inaequalis* (Cooke) G. Winter (Gadoury et al., 1984). For all these examples, ascospores of the pathogen are released from leaf litter and are a source of airborne inoculum (Kiely, 1948; Whiteside, 1970; Gadoury et al., 1984). For this reason, various forms of leaf litter management have been investigated for their potential to reduce inoculum, and hence, reduce disease. The application of grass mulch over the orchard floor has been demonstrated to reduce the incidence of citrus black spot in South Africa (Schutte & Kotze, 1997). Mechanical forms of leaf litter management such as shredding have been shown to be effective in reducing *V. inaequalis* inoculum and apple scab incidence in apple orchards (Holb, 2007; Sutton et al., 2000). However, as applying mulch or shredding leaves can require additional equipment and labour costs, orchard operators often express interest in leaf litter management approaches that utilise existing equipment such as herbicide boom sprayers or fertiliser spreaders. Promising amendments complementing this desire include urea, $CaCO_3$ and dolomitic lime forms, and commercial compost accelerators (Rodrigues et al., 2016).

In general, the aim of amendments such as urea, dolomitic lime, and compost accelerators is to promote microbial activity leading to increased leaf decomposition and/or antagonise the pathogens directly (Crosse et al., 1968; Green et al., 2006; Condron et al., 1993; Bengtsson et al., 2006). The application of these amendments to manage leaf litter inoculum sources has been evaluated in a number of studies in tree crops with promising results for reducing inoculum (Sutton et al., 2000; Mondal & Timmer, 2003; Mondal et al., 2007; Bellotte et al., 2009; Spotts et al., 1997), but in some cases significant improvements in disease control were not observed (Von Diest et al., 2016). However, it has been shown that the fungicides used to control diseases in apples, for example, can have negative impacts on non-target microbial populations in leaves and leaf litter (Walter et al., 2007; Andrews & Kenerley, 1979). Rates of leaf decomposition can therefore be reduced as a result of these altered microbial communities (Duarte et al., 2008; Rasmussen et al., 2012). In citrus orchards, fungicides are routinely used for the control of diseases such as citrus black spot (CBS) and greasy spot. Consequently, attempts to stimulate leaf decomposition through enhanced microbial activity in response to amendments such as urea may be counteracted by fungicide run-off from trees.

In some citrus production areas in Australia and South Africa, fungicide run-off is significant due to the adoption of high fungicide application volumes (>7,000 L/ha) that exceed the theoretical canopy retention volume of mature citrus of 2,300 L/ha (Beattie et al., 1989; Chapman et al., 1981; Cunningham & Harden, 1998a, 1998b; Van Zyl et al., 2013; Fourie et al., 2009). As fungicide programs for the control of citrus black spot, for example, typically incorporate monthly fungicide applications during the first 20-24 weeks of fruit development (Baldassari et al., 2006; Kotze, 1981; Wager, 1952; Miles et al., 2004; Agostini et al., 2006; Schutte et al., 2003), it is highly likely that citrus leaf litter under these circumstances is readily exposed to the fungicides being applied. Three of the most commonly used fungicides in citrus disease management are various copper-based formulations, dithiocarbamates and strobilurins (Schutte et al., 2003, 2012; Miles et al., 2004, 2005; Makowski et al., 2014). As these fungicides have efficacy against a wide range of fungal genera (Hewitt, 1998), off-target effects from run-off of these fungicides on microbial communities in leaf litter are a concern.

Leaf litter management in citrus orchards is considered a cultural practice which may improve the control of diseases such as citrus black spot. However, consideration needs to be given to the potential for other orchard practices, such as high volume fungicide application, to disrupt leaf litter management strategies. In this study we aim to investigate: 1) the efficacy of leaf litter amendments for enhancing leaf litter decomposition in citrus orchards in Queensland, Australia; 2) the efficacy of leaf litter amendments for directly reducing *Phyllosticta* sporocarp development in leaf litter; and 3) determine the impact of fungicide run-off on amendment efficacy and leaf litter decomposition. Addressing these aims will greatly assist citrus producers to determine the value of adopting leaf litter strategies for citrus disease control.

MATERIAL AND METHODS

Experiment 1

In order to determine the effect of leaf litter amendments and fungicide run-off on leaf litter decomposition and sporocarp development, urea, calcium carbonate, and a commercially available compost accelerator, were applied to leaf litter and compared over time to untreated leaf litter. The effect on leaf decomposition of fungicide run-off from routine high-volume fungicide applications for CBS was also investigated by duplicating the application of urea, calcium carbonate, the compost accelerator and the untreated control in the presence of simulated fungicide run-off.

Attached mature citrus leaves were harvested on the 10th December 2014 from sweet orange (*Citrus sinensis* (L.) Osbeck) trees in an orchard in Mundubbera, Queensland (-25.596598, 151.305108). The collected leaves were pooled, randomised through agitation, then 15 harvested leaves were arbitrarily assigned to each treatment in four replicates, and eight sampling times. The batches of 15 leaves were then laid out evenly in poly-mesh bags (35 mm × 23 mm) which were pinned to the ground under the canopy of trees in an orchard adopting a minimal fungicide regime (low frequency and volumes of application). One bag for each of the eight treatments was pinned under each tree. The bags evenly surrounded the trunk of each tree at a distance of 50 cm from the trunk, with the position of the treatments around the trunk determined using a random number generator. When applying the amendments, a 50 cm × 50 cm quadrat was placed around the bag to be treated, and the amendments applied to the entire area of the quadrat. Urea (46% N, Richgro, Australia) was applied at a rate of 23.3 kg/ha (20.81 lbs/acre) in a carrying volume of 467.5 L/ha (50 gal/acre). Dolomitic lime (14% Ca, 8% Mg, Richgro, Australia) was applied dry at a rate of 2,000 kg/ha (1,785 lbs/acre). The compost accelerator, Actizyme (proprietary enzymatic ingredients, Aware Environmental Products Pty Ltd, Australia), was applied at a rate of 40 kg/ha (35.69 lbs/acre) in a higher carrying volume of 2,600 L/ha (277 gal/acre) in order to best suspend the pelletised product. Control leaves were not treated. The amendments were applied immediately after placement of the mesh bags, and again 7 days later due to a period of stormy weather (~70 mm over 7 days).

Simulated amounts of fungicide run-off from typical fungicides used for controlling CBS (copper, mancozeb, and then azoxystrobin) were applied to the duplicated mesh bags during the experiment. A maximum, worst case, potential run-off amount of 7,700 L/ha (823 gal/acre) was used on the basis of the canopy retention volume for mature citrus being 2,300 L/ha (Cunningham & Harden, 1998b), and high-volume application rates of 10,000 L/ha. Therefore, the fungicides were applied directly to the ground in a carrying volume of 7,700 L/ha. Fungicide run-off was applied to the mesh bags using a knapsack sprayer, and the bags treated using the quadrat as previously mentioned. Simulated run-off applications of 0.675 g/L cuprous oxide (Red Copper WG, Melpat International Pty Ltd, Australia), 1.5 g/L mancozeb (Penncozeb 750 DF, NuFarm Ltd, Australia), and 0.1 mL/L azoxystrobin (Amistar 250 SC, Syngenta, Australia) were applied on the 18th December 2014, 22nd January 2015, and 4th February 2015, respectively.

The first seven samplings were conducted fortnightly, with the final sample collected at a 14 week interval when leaves were almost completely degraded. At each sampling time, four replicate sets of mesh bags were collected and visually rated for their state of decomposition and inspected for sporocarp development. Visual assessment of leaf litter decomposition was undertaken using the rating scale of Mondal et al. (2007) with a minor modification: 0 = dead, not decomposed, leaf firm; 1 = not decomposed, flexible, still intact; 2 = leaf slightly decomposed, no loss of lamina; 3 = moderately decomposed, some loss of lamina; 4 = moderately decomposed, considerable loss of lamina; 5 = highly decomposed, skeletonized leaves; and 6 = no recognisable leaf. A second rating scale that was customised for Queensland conditions was also used whereby: 0 = green, intact, flexible; 1 = brown, dry, curled; 2 = laminar loss commencing <25% area; 3 = moderate laminar loss 26-50% area; 4 = high laminar loss 51-75% area; 5 = fully decomposed/skeletonised >75% area; and 6 = no recognisable leaf. The estimated density of sporocarps was determined according to Mondal & Timmer (2003) whereby: 0 = none, 1 = 1 to 5%, 2 = 6 to 10%, 3 = 11 to 15%, 4 = 16 to 20%, 5 = 21 to 25%, 6 = 26 to 30%, 7 = 31 to 35%, 8 = 36 to 40%, 9 = 41 to 45%, and 10 = >50% leaf area covered with sporocarps. After visual assessments, the dry weight of leaf tissue in each mesh bag was determined after drying at 50°C for 48 hours.

Experiment 2

In order to confirm the findings from experiment 1, a second experiment was conducted using largely the same methods as experiment 1 but with minor modifications. In addition to the four leaf amendments applied previously, sugar cane mulch (Rocky Point Mulching, Australia) was applied at the rate of 18 t/ha (8 t/ac). Attached mature citrus leaves were harvested on the 2nd December 2015, and leaves placed in mesh bags as described previously. The leaf amendments were applied once on the 17th December 2015. The simulated run-off applications of 0.675 g/L cuprous oxide, 1.5 g/L mancozeb, 0.1 mL/L azoxystrobin, and 1.5 g/L mancozeb were applied on the 17th December 2015, 7th January 2016, 9th February 2016, and 4th March 2016, respectively. Leaf samplings were conducted approximately every three weeks from week two to week 16, and leaf decomposition was assessed as previously described.

Statistical analysis

In order to compare treatment effects in each experiment, the mean visual ratings of degradation for each bag were analysed using residual maximum likelihood (REML) which allows the inclusion of smoothing splines in the model for investigating the presence of a non-linear response over time. The observed non-linear response in mean degradation over time was then modelled using an exponential curve.

Dry weight data were subjected to Analysis of Variance (ANOVA) with the fixed factors of amendment × fungicide run-off × time. Where the time factor was found to explain a large majority of the variance in the analysis, the area under the curve (AUC) was calculated for the dry weight values over time using the formula as previously described (Akinsanmi et al., 2007; Campbell & Madden, 1990). The leaf litter decomposition constant (*k* value) was also determined using the formula as previously described (Yue et al., 2016; Olson, 1963). The area under the curve and *k* value were then subjected to ANOVA with the fixed factors amendment × fungicide run-off.

Where a significant main effect or interaction was found ($p<0.05$), pairwise comparisons are made using Fisher's 95% least significant difference (LSD). All analyses were performed using GenStat for Windows 16th Edition (VSN International, United Kingdom).

RESULTS

The results of the REML analysis of the two visual assessment methods found the time effect to be significant ($P<0.05$), with decomposition ratings increasing as time increased, as expected. This increase was non-linear as decomposition rate slowed towards the end of the experiments (data not shown). The amendment factor was only found to be significant in experiment 2, whereby the mulch amendment was found to significantly ($P<0.05$) increase the decomposition ratings compared to the other amendments. The fungicide run-off factor was, however, found to be significant in both experiments for both rating scales, with the addition of fungicide run-off resulting in significantly ($P<0.05$) lower decomposition ratings (Table 1). Significant interactions were only observed in experiment 2, with the amendment × time interaction being significant ($P<0.05$). This interaction was explored by the fit of the data to an exponential model, with the model accounting for 90.5% and 82.7% of the variance for the Mondal and customised rating scales, respectively. Figure 1 shows the fitted exponential model to the customised rating scale data. Visual assessments of the prevalence of sporocarps in the leaf litter could not be meaningfully analysed due to low levels of leaf infection (data not shown).

Analysis of the leaf litter dry weight data by ANOVA for experiment 1 found the fungicide run-off and time factors to be significant for the dry weight data, and only the fungicide run-off factor to be significant for the area under curve data (Table 2). In both cases the addition of simulated fungicide run-off resulted in significantly ($P<0.05$) higher mean dry weights and area under the curve of dry weight (Table 3). In experiment 2, the amendment, fungicide run-off and time factors were significant for the dry weight, and amendment and fungicide run-off factors were significant for the area under curve data (Table 2). No interactions between factors were significant. Within the amendments, mulch was the only amendment to significantly ($P<0.05$) reduce the mean dry weight and area under the curve compared to the control (Table 3). As in experiment 1, the addition of simulated fungicide run-off resulted in significantly higher mean dry weight and area under the curve. For both experiments, the time

Table 1. Results of REML analysis of visual ratings of the effect of fungicide run-off on leaf litter decomposition ratings[a]

	Mondal[b]	Customised[c]
Experiment 1		
No run-off	4.38 a	3.81 a
Run-off	4.30 b	3.70 b
P	0.032	0.034
95% LSD	0.07	0.100
Experiment 2		
No run-off	4.52 a	4.00 a
Run-off	4.35 b	3.79 b
P	<0.001	0.007
95% LSD	0.07	0.11

[a]Mean values followed by the same letter are not significantly different ($P<0.05$); [b]Mean leaf litter decomposition rating based on Mondal et al. (2007) where: 0 = dead, not decomposed, leaf firm; 1 = not decomposed, flexible, still intact; 2 = leaf slightly decomposed, no loss of lamina; 3 = moderately decomposed, some loss of lamina; 4 = moderately decomposed, considerable loss of lamina; 5 = highly decomposed, skeletonized leaves; and 6 = no recognisable leaf; [c]Mean leaf litter decomposition rating based on a customised scale where 0 = green, intact, flexible; 1 = brown, dry, curled; 2 = laminar loss commencing <25% area; 3 = moderate laminar loss 26-50% area; 4 = high laminar loss 51-75% area; 5 = fully decomposed/skeletonised >75% area; and 6 = no recognisable leaf.

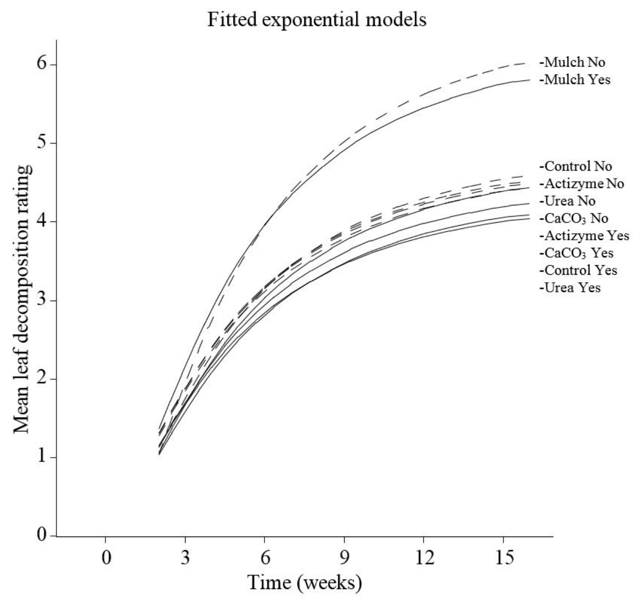

Figure 1. Exponential plots of mean leaf litter decomposition over time assessed using a customised rating scale from experiment 2, following the application of various amendments (mulch, urea, $CaCO_3$, actizyme, and an untreated control) in the presence (yes) and absence (no, dashed lines) of fungicide run-off. Mean leaf litter decomposition rating based on a customised scale where 0 = green, intact, flexible; 1 = brown, dry, curled; 2 = laminar loss commencing <25% area; 3 = moderate laminar loss 26-50% area; 4 = high laminar loss 51-75% area; 5 = fully decomposed/skeletonised >75% area; and 6 = no recognisable leaf.

Table 2. ANOVA of leaf dry weight, area under the curve of leaf dry weight (AUC), and decomposition rate constant (*k* value) from experiments 1 and 2

Factor	df	Mean square			F pr.		
		Weight	AUC	*k* value	Weight	AUC	*k* value
Experiment 1							
Amendment (A)	3	1.3867	126.47	2.024	0.223	0.140	0.524
Time (T)	7	66.9029	-	-	<0.001	-	-
Run-off (R)	1	13.3271	832.83	5.802	<0.001	0.001	0.152
A × T	21	1.0365	-	-	0.350	-	-
A × R	3	0.1555	9.25	2.936	0.920	0.929	0.366
T × R	7	0.9912	-	-	0.396	-	-
A × T × R	21	0.2403	-	-	1.000	-	-
Experiment 2							
Amendment (A)	4	8.7171	176.880	41.284	<0.001	<0.001	<0.001
Time (T)	5	2.1319	-	-	0.013	-	-
Run-off (R)	1	102.7151	53.754	1.723	<0.001	0.003	0.524
A × T	20	0.2783	-	-	0.507	-	-
A × R	4	0.3563	6.870	4.104	0.392	0.272	0.429
T × R	5	0.4399	-	-	0.261	-	-
A × T × R	20	0.3212	-	-	0.515	-	-

Table 3. ANOVA results for the amendment, fungicide run-off and time factors for mean leaf litter dry weight, and area under the curve for leaf litter dry weight in the presence of various amendments and simulated fungicide run-off onto *Citrus sinensis* leaf litter in an orchard in Mundubbera, Queensland[a]

Amendment	Experiment 1		Experiment 2	
	Dry weight (g)	AUC	Dry weight (g)	AUC
Control	3.2	58	2.7 a	31 a
Urea	3.1	55	2.5 a	29 a
CaCO3	3.4	60	2.7 a	31 a
Actizyme	3.4	64	2.5 a	29 a
Mulch	-	-	1.7 b	19 b
95% LSD	n.s.	n.s.	0.2	2
Fungicide run-off				
Yes	3.5 a	64 a	2.5 a	29 a
No	3.0 b	54 b	2.3 b	27 b
95% LSD	0.2	6	0.1	1
Time (weeks)				
2	5.4 a	-	5.4 a	-
4	4.3 b	-	-	-
5		-	2.9 b	-
6	4.0 b	-	-	-
7		-	2.2 c	-
8	3.8 b	-	-	-
10	2.6 c	-	1.6 d	-
12	2.6 c	-	-	-
13		-	1.1 f	-
14	2.4 c	-	-	-
16		-	1.3 e	-
28	0.7 d	-	-	-
95% LSD	0.5	-	0.3	-

[a]Means followed by the same letter are not significantly different ($P<0.05$); n.s. = not significant.

factor was observed to result in the leaf litter dry weight to decline with increasing time as expected (Table 3). The k values in both experiments were not significant ($P<0.05$) except for the amendment factor in experiment 2 (Table 2). In this case, the k value for the mulch amendment was significantly higher than all the other treatments (data not shown).

DISCUSSION

In this study we aimed to determine the efficacy of various leaf litter amendments for promoting leaf litter decomposition and/or *Phyllosticta* sporocarp development, as well as determine any effects of fungicide run-off from high volume fungicide applications. Our results have shown that significant ($P<0.05$) reductions in leaf litter decomposition were consistently observed in the presence of simulated fungicide run-off. The efficacy of the different leaf amendments was generally low, and/or inconsistent between seasons. However, the most effective amendment for significantly ($P<0.05$) increasing leaf decomposition was the sugar cane mulch amendment. While the cane mulch was only assessed in one season, the visual decomposition and dry weight measures were markedly more favourable for promoting decomposition than observed for the other amendments in either experiment. The ability to determine any direct effects on *Phyllosticta* sporocarps was limited due to low levels of leaf infection for assessment. Our findings indicate that under Queensland conditions reducing fungicide run-off to leaf litter may be more beneficial to promoting leaf litter decomposition than applying urea, dolomitic lime or a compost accelerator.

Managing off-target pesticide losses is an important issue for horticulture. Off-target losses have been associated with negative impacts on plants, insects and fungi (de Jong et al., 2008). In citrus specifically, studies have shown accumulation of several heavy metals in soil from agrichemical use including Cu and Mn which are key elements in Cu-based fungicides and mancozeb (Kelepertzis, 2014; Fan et al., 2011; Hewitt, 1998). Accumulation of these elements/fungicides in horticultural soils has been associated with declines in soil microflora and microfauna (Zhou et al., 2013; Al-Assiuty et al., 2014; Seguin et al., 1983). However, to our knowledge our study is the first to show a measurable reduction in leaf litter decomposition in a citrus orchard associated with the application of simulated fungicide run-off, probably resulting from disruptions to soil microflora and microfauna. Of concern is that measurable differences in decomposition were observed in our study within the 16-28 week lifespan of our experiments, whereas most citrus trees in the Queensland region are currently 13 years old or greater (Hancock, 2014). The level of soil exposure to fungicide run-off after 13 years in orchards using high volume application methods is likely to be significant. Further investigation of the long term impacts of fungicide run-off on leaf litter decomposition and associated aspects of soil biology are warranted.

The efficacy of urea, dolomitic lime and Actizyme was generally low and inconsistent in our study. This is consistent with findings from Florida that show a reduction of *M. citri* inoculum with urea and dolomitic lime applications, but generally not significant increases in leaf litter decomposition (Mondal et al., 2007; Mondal & Timmer, 2003). However, in Brazil increases in leaf litter decomposition were observed with similar amendments (Bellotte et al., 2009). A likely reason for differences among the studies are climatic differences between study regions. In particular, rainfall and/or humidity are likely to be important. For example, additional irrigation of citrus leaf litter in Florida was one of the most effective treatments for promoting decomposition (Mondal et al., 2007). The increased decomposition in the sugar cane mulch amendment in our study was also probably the result of higher moisture provided by the organic mulch (Faber et al., 2001; Fidalski et al., 2010). Interestingly, other relevant differences between study regions are evident when comparing the two visual decomposition rating scales in our study. While our results from using the two rating scales did not differ substantially, the rating scale from Florida suggests a different sequence of decomposition than that of the customised scale we developed specifically for our Queensland study site (Mondal et al., 2007). The most notable difference being that leaves in Queensland turn brown and curl very early in decomposition, while leaves in Florida remain flexible. This would suggest a drier overall climatic situation in Queensland relative to Florida, and therefore differences in decomposition.

Our study has provided evidence that fungicide run-off from high volume fungicide spraying for the control of citrus black spot may be contributing to the preservation of leaf litter which itself is an important inoculum source of the causal fungus. Furthermore, our study has shown that leaf litter amendments such as urea are not likely to be of significant benefit to Queensland citrus growers managing citrus black spot. Instead, it is recommended that growers aim to reduce fungicide run-off using lower volume application equipment, and/or consider organic mulching, to more effectively promote leaf litter decomposition.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Gavin Ford for the use of his orchard, and Dan Papacek and the team at Bugs For Bugs for their assistance and use of their facilities. We are grateful for the financial support provided by the University of Florida through the Citrus Research and Development Foundation and Horticulture Innovation Australia

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Received: November 15, 2016
Accepted: August 09, 2017