Crop Protection 42 (2012) 1–9

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses

Gerhardus C. Schutte [a,*], Charl Kotze [a], J. Gideon van Zyl [b], Paul H. Fourie [a,b]

[a] Citrus Research International, P.O. Box 28, Nelspruit 1200, South Africa
[b] Department of Plant Pathology, Stellenbosch University, Stellenbosch 7602, South Africa

ARTICLE INFO

Article history:
Received 1 December 2011
Received in revised form
2 April 2012
Accepted 8 April 2012

Keywords:
Copper fungicides
Fluorometry
Photomacrography
Residues
Retention

ABSTRACT

High volumes of copper hydroxide, cuprous oxide and copper oxychloride were sprayed under natural conditions onto mature orange trees to compare the retention on citrus leaves and fruit over a period of 56 days by means of copper residue analyses and a spray deposition assessment protocol using fluorometry, photomacrography and digital image analyses. Rainfall and increase in fruit size were also recorded to determine if it had an influence on weathering of copper residues. Initial retention following application of the different fungicides differed on Valencia leaves and fruit: applications with cuprous oxide retained significantly more copper residue and fluorescent pigment, while copper hydroxide retained higher copper and pigment levels on Navel leaves and fruit. Nonetheless, persistence of copper residues deposited by the three copper fungicide formulations was similar and decreased at the same tempo during both seasons; initially a fast reduction (48 and 60% for year one and two respectively) in residue during the first 14 days followed by a more gradual decline (41 and 24% for year one and two respectively) from 14 to 56 days. The loss of copper residues was attributed to weathering (days after treatment), fruit growth and cumulative rainfall as these factors were inversely correlated with copper residue levels (Pearson's $r = -0.840$, -0.722 and -0.733 respectively). A 76% and 90% correlation was observed between the copper residue analyzed and the quantitative fluorescent pigment measurements on mature leaves and fruit, respectively; showing that fruit is more reliable for fluorometry analyses and that this technique proved to be an effective tool for spray deposition and persistence assessment of copper fungicides. All copper formulations tested at these registered rates at 35-day spray intervals were effective in controlling *Guignardia citricarpa*.

1. Introduction

Many fungicides and insecticides are applied as high-volume sprays to citrus trees in South Africa (Grout, 1997) and the quantity and quality of the subsequent deposit is dependent on the physico-chemical nature of the spray mixture and plant surfaces while weathering, rapidly growing fruit and persistence are the main limiting factors that will determine sustained efficacy (Rudgard et al., 1990; Van Zyl et al., 2010a). In the past, fluorometry and colorimetry on artificial targets such as paper, mylar, film, glass or metal were used to measure the amount of spray deposition on leaves, fruit and twigs (Yates and Akesson, 1963; Himel, 1969; Solie and Gerling, 1985). These methods included visual assessment and chemical residue recovery techniques from artificial surfaces. However, deposition research should be done on biological targets in their natural environment, since deposition on artificial surfaces does not accurately simulate deposition on natural targets (Holownicki et al., 2002). Visual deposition assessment was greatly improved by adding fluorescent dyes to the spray mixture, followed by illumination of deposits under black (UV-A) light (Furness et al., 2006a, 2006b). However, as visual deposition in the latter case was dependent on human discretion, it lacked quantitative measuring (Jiang and Derksen, 1995). Several bioassay and chemical residue recovery techniques were performed on citrus, some of them were done with (Whitney et al., 1988, 1989; Salyani and McCoy, 1989; Juste et al., 1990; Albrigo et al., 1997, 2005; Timmer et al., 1998; Orbovic et al., 2007) or without (Vicent et al., 2007) the overall assessment of the quantity of spray deposits on either leaves or fruit, but not both at the same time. Furthermore, residue levels alone do not give a good indication of application quality such as uniformity of spray distribution on fruit or leaves (Holownicki et al., 2002).

In South Africa, copper fungicides are recommended as high-volume foliar sprays for the control of citrus black spot (CBS) (*Guignardia citricarpa* Kiely) (Wager, 1950; Kiely, 1965; Kotzé, 1981;

* Corresponding author. Tel.: +27 13 759 8042; fax: +27 13 744 0578.
E-mail address: ts@cri.co.za (G.C. Schutte).

0261-2194/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2012.04.015

Schutte et al., 1997) and Alternaria brown spot (*Alternaria alternata* (Fr.:Fr.) Keissl pv. *citri*) (Swart et al., 1998) at 28–35-day spray intervals or as a corrective measure for copper deficiency (Du Plessis, 1968). At present, full cover fungicide/insecticide spray application to citrus trees in South Africa involves applications of 10,000–16,000 L ha^{-1} (Grout, 1997), which exceeds the estimated spray retention level of 2300 L ha^{-1} for mature citrus trees (Cunningham and Harden, 1998, 1999). Fourie et al. (2009) demonstrated the detrimental effect of run-off on spray deposition and subsequent biological efficacy in laboratory spray trials.

In the USA, low-volume foliar sprays of 1168–4662 L ha^{-1} with 21-day intervals in spray programme were used for the development and testing of a recommendation system to schedule copper hydroxide sprays for disease control. However, with the low dosage of copper hydroxide tested and 21 day spray intervals, control of melanose [*Phomopsis citri* H. Fawcett non (Sacc.) Traverso & Spessa] was poor, especially on inner and top canopy fruit in grapefruit trees that did not receive the desired copper deposits for disease control. This can possibly be attributed to the low spray volumes used. Furthermore, rainfall and fruit surface expansion was recorded to determine copper residue loss but these measurements were not directly related to copper loss. No other copper formulations other than copper hydroxide were evaluated to develop the system (Albrigo et al., 2005).

Frequent usage of copper fungicides can lead to copper stippling, darkening of wind-scars and insect damage on fruit (Brodrick, 1970; Albrigo et al., 1997; Schutte et al., 1997) and accumulation of copper in soils if used extensively (Alva and Graham, 1991). These factors are restricting on continuous use of copper fungicides. It is not known when copper levels decline sufficiently after application to allow a second application and prevent side-effects such as stippling. Copper fungicides have a long residual period of activity against conidia of *P. citri*, but are not readily redistributed to newly formed tissue (Whiteside, 1977). Formulation and adjuvants added to the copper formulations might also influence the amount of copper retained after spray application as possibly also its persistence on the plant surface (Rich, 1954; Orbovic et al., 2007; Vicent et al., 2007; Van Zyl et al., 2010a, 2010b). Knowledge of copper residue breakdown on fruit and leaves will assist in compiling effective spray programs with copper fungicides for the control of citrus diseases.

In order to study the optimization of the fungicide spray application on grapevines and citrus, a spray assessment protocol was developed using fluorometry, photomicrography and digital image analyses (Brink et al., 2004, 2006; Fourie et al., 2009; Van Zyl et al., 2010a, 2010b). Spray retention is traced through the use of SARDI (South Australian Research and Development Institute) Yellow Fluorescent Pigment (Furness et al., 2006a). Microscopic measurements have indicated that particle size in the pigment ranged from 0.5 to 9.0 μm (J.G. van Zyl, unpublished results), which is equivalent to that of certain copper hydroxide formulations (Orbovic et al., 2007), thus suggesting that this pigment is ideally suited to trace deposition of copper fungicides.

The aims of this study were to determine CBS control by cuprous oxide, copper oxychloride and copper hydroxide sprays, and to determine retention and persistence of these formulations on citrus leaves and fruit using fluorometry and residue analyses under natural conditions.

2. Materials and methods

2.1. 2008–2009 field trial

A uniform Valencia orange [*Citrus sinensis* (L.) Osbeck] orchard on 'Cleopatra' mandarin rootstock (*Citrus reshni*) at Crocodile Valley Citrus Co., Nelspruit, was selected. The trees were 17 years old and approximately 4 m high. The rows ran directly east to west. A randomized design with three single-tree plots per treatment was used with guard trees between the plots. Trees were selected for uniformity in canopy density and tree size. Fungicides were applied on 13 November 2008 to the point of run-off with a trailer-mounted, high-volume, high-pressure (2500–3000 kPa) sprayer with two hand-held spray guns. The fungicides tested included copper hydroxide (Kocide 2000, 53.8% WG; DuPont de Nemours International Societe Anonyme, Halfway House, South Africa), copper oxychloride (Demildex, 85% WP; Delta Chemicals, Meyerton, South Africa) and cuprous oxide (Nordox 86% WG; Avima, Kenmare, South Africa).

Registered rates of 0.765 g active ingredient (a.i.) l^{-1} cuprous oxide, 1.076 g a.i. l^{-1} for copper hydroxide and 1.70 g a.i. l^{-1} water for copper oxychloride were used. Hundred milliliters SARDI Yellow Fluorescent Pigment (40% EC; South Australian Research and Development Institute, Loxton SA 5333 Australia) per 100 L water was added to the spray mixture of each copper formulation. For eight weeks, 20 fruit (every 14 days) and 20 fully expanded mature leaf samples (every seven days) were randomly picked between 1 and 2 m above ground height from the outside circumference from each of the three replicate trees.

2.2. 2009–2010 field trial

The above-mentioned trial was repeated the following season and a uniform Navel orange (*C. sinensis*) orchard on 'Cleopatra' mandarin rootstock (*C. reshni*) at Crocodile Valley Citrus Co., Nelspruit, was selected. The trees were 21 years old and 4–5 m high. The rows ran directly north to south. A randomized design with three single-tree plots per treatment was used with guard trees between the plots. The same fungicides, spray application and assessment protocols were used as described above. Fungicides were applied on 21 October 2009.

2.3. Efficacy of copper hydroxide, copper oxychloride and cuprous oxide against citrus black spot

Two Valencia orange (*C. sinensis*) orchards on 'Rough lemon' and 'Volckameriana' rootstocks at Crocodile Valley Citrus Co., Nelspruit, with uniform trees were selected for the 2007–2008 and 2009–2010 field trials, respectively. Five single-tree plots per treatment were randomized and spray programs commenced in mid-October before the onset of the first summer rain as previously recommended (McOnie, 1964). Trees in orchards were selected for uniformity in canopy density and tree size. Fungicides were applied with the same spray machine as mentioned before and sprayed to the point of run-off, each receiving approximately 33 L of spray material per tree per application. Guard trees were located between plots within rows. Spray programs were based on 5-week intervals at recommended dosages (dates of application and fungicide concentrations are listed in Table 1). At fruit maturity in July, CBS severity was rated on 100 fruits per tree according to a 3-point index that was described previously (McOnie and Smith, 1964): 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analyzed by analysis of variance (ANOVA) and Fisher's Least Significance Difference (LSD) test ($P = 0.05$).

2.4. Deposition assessment and copper residue analysis

For deposition assessment, 20 sprayed leaves and fruit were illuminated using a Labino Mid-light (UV-A; ≈365 nm) and digital photos were taken of upper and lower surfaces of leaves and fruit