Conventional and reduced-risk fungicides to control postharvest Diplodia and Phomopsis stem-end rot on lemons

Article *in* Scientia Horticulturae · November 2017

DOI: 10.1016/j.scienta.2017.08.008

CITATIONS
4

READS
50

7 authors, including:

Luciana Cerioni
National University of Tucuman
18 PUBLICATIONS **182** CITATIONS
SEE PROFILE

Joseph Smilanick
United States Department of Agriculture
152 PUBLICATIONS **6,650** CITATIONS
SEE PROFILE

Jacqueline Ramallo
SA San Miguel
26 PUBLICATIONS **205** CITATIONS
SEE PROFILE

Viviana A Rapisarda
National University of Tucuman
47 PUBLICATIONS **533** CITATIONS
SEE PROFILE

Scientia Horticulturae 225 (2017) 783–787

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Research paper

Conventional and reduced-risk fungicides to control postharvest Diplodia and Phomopsis stem-end rot on lemons

Luciana Cerioni[a], Paula B. Bennasar[b], Daniela Lazarte[b], Milena Sepulveda[b], Joseph L. Smilanick[c], Jaqueline Ramallo[b], Viviana A. Rapisarda[a,*]

[a] Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT) and Instituto de Química Biológica, Facultad de Bioquímica, Química y Farmacia (UNT), Chacabuco 461, Tucumán T4000ILI, Argentina
[b] Laboratorio de Desarrollo e Investigación, SA San Miguel, Lavalle 4001, Tucumán T4000BAB, Argentina
[c] Retired USDA ARS San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA93648, United States

ARTICLE INFO

Keywords:
Diaphorte citri
Lasiodiplodia theobromae
Thiabendazole
Azoxystrobin
Pyrimetanil
Propiconazole

ABSTRACT

Stem-end-rot (SER), caused by *Lasiodiplodia theobromae* or *Diaporthe citri,* is an important postharvest disease in humid and warm citrus growing areas, such as the Northwestern region of Argentina. The commercial control of SER has been accomplished by applications of the systemic fungicide carbendazim (CARB). However, alternative chemicals for this fungicide are needed due to increasing restrictive regulations in overseas markets. The aim of this work was to evaluate the effectiveness of conventional and reduced-risk postharvest fungicides to control SER. *In vitro* tests show that *L. theobromae* was more resistant to fungicides than *D. citri*. In trials using artificially inoculated lemons, SER caused by *D. citri* (Phomopsis SER) was controlled by the fungicides evaluated. The conventional fungicides imazalil and thiabendazole (TBZ) were the most effective chemicals to control SER caused by *L. theobromae* (Diplodia SER), while other fungicides, as fludioxanil, azoxystrobin, pyrimetanil and propiconazole, were not effective. The best control of Diplodia SER was obtained by immersion for 60 s in 2000 mg/L of TBZ at pH 5 and 20 °C. In this condition, TBZ-residue loading on lemons was 3.0 mg-L^{-1}, which did not exceed the allowed maximum residue levels. TBZ applied in lemon commercial treatments is the best option to reduce SER caused by both pathogens. At this time, this is the unique suitable alternative to replace CARB treatment in Argentinean packinghouses.

1. Introduction

During the storage and marketing of citrus fruit, important economic losses can occur due to postharvest diseases such as green mold (caused by *Penicillium digitatum*), blue mold (*Penicillium italicum*), Diplodia stem-end rot (*Lasiodiplodia theobromae*), Phomopsis stem-end rot (*Diaporthe citri),* sour rot (*Geotrichum citri-aurantii*) and brown rot (*Phytophthora citrophthora or Phytophthora nicotianae*) (Eckert and Eaks, 1989). Green mold is the most important citrus decay and sour rot, although it is less common, causes significant losses in high rainfall years (Eckert and Eaks, 1989). Diplodia and Phomopsis stem-end rot (SER) are favored in production areas with high humid and subtropical conditions and when fresh fruit are exposed to long-term handling, storing, and transit time (Kucharek and Brown, 1992; Brown and Eckert, 2000; Zhang, 2007).

Argentina is a world leader in lemon production, and the 85% of this production is located in the Northwestern region (Tucumán) between 26 and 28°S of latitude (Federcitrus, 2016; Palacios, 2005). Because this production area is far 10,000–17,000 km from the overseas markets, fresh fruit requires from 25 to 40 d reaching the final consumers; this transit time is an important barrier that limits global competitiveness. Additionally, the humid and warm environmental conditions in Tucumán groves favor the infection and rapid development of Diplodia and Phomopsis SER (Palacios, 2005). Therefore, management of these two fungal postharvest diseases is central to maintain fruit quality for a long period of time.

Fungicide application in the citrus packinghouse is one of the most important steps for controlling of postharvest decay. The conventional fungicides such as thiabendazole (TBZ) and imazalil (IMZ) are important in order to minimize these citrus diseases. Without fungicides, overseas citrus trade would be significantly reduced (Adaskaveg et al., 2004; Smilanick et al., 2006). To control SER on oranges, TBZ and IMZ were used on the packinghouse in aqueous or wax treatments on fruit (Ismail and Zhang, 2004). Three newer fungicides, pyrimethanil, fludioxonil, and azoxystrobin, that are classified as reduced risk compounds, were approved by the US EPA for postharvest use on various

* Corresponding author.
E-mail addresses: vrapisarda@fbqf.unt.edu.ar, vrapisarda@gmail.com (V.A. Rapisarda).

http://dx.doi.org/10.1016/j.scienta.2017.08.008
Received 28 June 2017; Received in revised form 3 August 2017; Accepted 4 August 2017

fruit including citrus (Adaskaveg et al., 2004). Previous reports on oranges fruit indicate that fludioxonil has good activity against SER natural infections, unlike pyrimethanil or fludioxonil (Zhang, 2007, 2009, 2012).

In the packinghouses of Tucumán the commercial control of green mold, blue mold and sour rot is conducted by integrated procedures with fungicides such as IMZ, TBZ, propiconazole and pyrimethanil, among others (Palacios, 2005; Sepulveda et al., 2015). Moreover, the commercial control of SER has been performed with the systemic fungicides benomyl or CARB in a drench treatment. However, the use of chemicals to control SER generates several problems due to the restrictions in lemon industry on the applied chemical species and the allowed Maximum Residue Limits (MRL) in oversea markets (EFSA, 2014). For instance, CARB is not allowed in European Union, while TBZ, a fungicide of the same FRAC group of CARB, is still permitted. Hence, it is urgent to develop alternative technologies to control SER disease.

In our previous work, the partial control of SER by low toxicity salts as potassium sorbate, sodium bicarbonate and potassium phosphite on artificially inoculated lemons has been reported (Cerioni et al., 2013). The aim of the present study was to evaluate the effectiveness of conventional and reduced-risk fungicides to control Diplodia and Phomopsis SER.

2. Materials and methods

2.1. Chemical sources

Several postharvest fungicides were evaluated *in vitro* and *in vivo*. The fungicides and their sources were: imazalil (IMZ, Fungaflor 500 EC, 44.6% a.i.; Janssen PMP), thiabendozale (TBZ, Tecto 500SC, 42.92% a.i.; Syngenta); pyrimethanil (PYR, Mythos, 30% a.i.; Bayer cropSciencie); IMZ plus PYR (Philabuster® 400 SC, 20% imazalil and 20% pyrimethanil; Janssen PMP); procloraz (PCLO, Sportak® 45 EC, 42.1% a.i.; Bayer CropScience); propiconazole (PROP, TILT® 25 EC, 25.1% a.i.; Syngenta); difenoconazole (DIF, BOGARD®, 25.1% a.i; Syngenta); fludioxonil (FLUD, Scholar® 23SC, 23% a.i.; Syngenta); azoxystrobin (AZO, AMISTAR®, 25% a.i.; Syngenta); fludioxonil (FLUD) plus azoxystrobin (AZO) (Graduate A+, 20.6% fludioxonil and 20.6% azoxystrobin; Syngenta); carbendazim (CARB, Carbendazim 50 NUFARM, 50% a.i.; NUFARM S.A.); and guazatine (GUA, KENOPEL®, 50% a.i.). Other compounds and their sources were: 1) potassium phosphite (45.5% potassium phosphite, AFITAL Fosfito de Potasio, AgroEMCODI); and 2) both sodium bicarbonate (SBC, 99% a.i.) and potassium sorbate (KS, 99% a.i.) purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.2. Fungi

L. theobromae and *D. citri* were isolated from naturally decayed lemons in Tucumán (Argentina) and were grown on potato dextrose agar (PDA), at 28 °C for 7–14 d. Besides the morphology, cultural behavior and molecular characterization of both pathogens were done. 37 DNA were extracted from representative fungi isolates (10 from *L. theobromae and* 27 from *D. citri*). Primers ITS4 and ITS5 were used to amplify the internal transcribed spacer ITS1 and ITS2, including the 5.8 S region of rDNA (White et al., 1990). PCR products were purified and sequenced in both directions at CERELA-CONICET, Tucumán. The sequences were edited using the program sequencing analysis 5.3.1 and aligned using BLAST of the National Center for Biotechnology Information, National Institutes of Health USA (Bethesda, MD) with sequences representative isolates of each morphological group in GenBank. A 97% identity to *Lasiodiplodia theobromae* and 99% identity to *Diaphorte citri* were obtained.

2.3. Fruit

Eureka lemons [*Citrus limon* (L.) Burm] were collected from commercial orchards in Tucumán (Argentina) and stored at 7 °C and 90% RH. Lemons used in the study were free of postharvest treatment or coatings.

2.4. In vitro assays

TBZ, IMZ, PYR, IMZ plus PYR, and CARB were evaluated *in vitro*. These fungicides were suspended on PDA obtaining final concentrations of 1, 3 and 5 mg/L to prepare 9 mL plates. Mycelium plugs of each pathogen grown previously at 28 °C for 7–14 d, were placed on each Petri dish with the different fungicides concentrations. The colony diameters were measured after 5 d of incubation at 28 °C. As a control, mycelium of each pathogen was placed on PDA without fungicides.

2.5. Fruit inoculation

For *in vivo* fungicides evaluation, lemons were inoculated by a toothpick method, adapted from Cerioni et al. (2013). The technique was modified reducing the exposure times of the toothpicks to the pathogens and of the fruit to the contaminated toothpicks. Briefly, five portions of 5 × 5 mm diameter of mycelium plugs of each pathogen were placed on PDA. Fifty quill-type wooden toothpicks were added and incubated at 28 °C 24 h to ensure adequate contamination of the toothpicks. After that, these sticks were used to inoculate lemons. They were inserted approximately 1.5 cm diagonally downward into the stylar-end of the lemon fruit. Then the inoculated fruit were stored at 20 °C and 95% relative humidity (RH) for 24 h. The toothpicks were removed from the fruit before treatment with fungicides.

2.6. Fungicide treatments of inoculated lemons

L. theobromae and *D. citri* inoculated lemons were immersed for 60 s in 20 L of water (control) or 2000 mg-L^{-1} of TBZ, IMZ, PYR, IMZ plus PYR, PCLOZ, PROP, DIF, FLUD, AZO, FLUD plus AZO, GUA, or CARB solutions at 20 °C. The fruit were not rinsed after treatment and were stored for 4 d at 20 °C and 95% RH.

Similar tests were done to evaluate the effect of temperature increase at 40 °C of the fungicides TBZ, IMZ, PYR and IMZ plus PYR. These fungicides were evaluated in combination with 20 g/L KS or 30 g/L SBC. TBZ (2000 mg/L) was further evaluated with different immersion times or pH, and in mixture with 20 g/L of KP and 1000 mg/L PROP or PYR to control SER on *L. theobromae*-inoculated lemons.

2.7. Storage of fruit

After treatments, the fruit were stored at 20 °C and 95% HR. The infected fruit were counted after 4 d. The asymptomatic fruit were stored for 14 d before discharged.

2.8. TBZ residue analysis

Non-inoculated fruit were evaluated for TBZ residues with a GC-NPD chromatograph (Agilent model 7890, Agilent Technologies, CA, USA) in the Laboratory of Pesticide Residues of Estación Experimental Agroindustrial-Obispo Colombres, Argentina. Residues were reported as mg kg/FW.

2.9. Statistical analysis

Each treatment was applied to 5 replicates of 20 fruit each and the whole test was done twice. The analysis of variance (ANOVA) was applied on arcsine transformation of the square root of the proportion of infected lemons in respect to total inoculated fruit for each

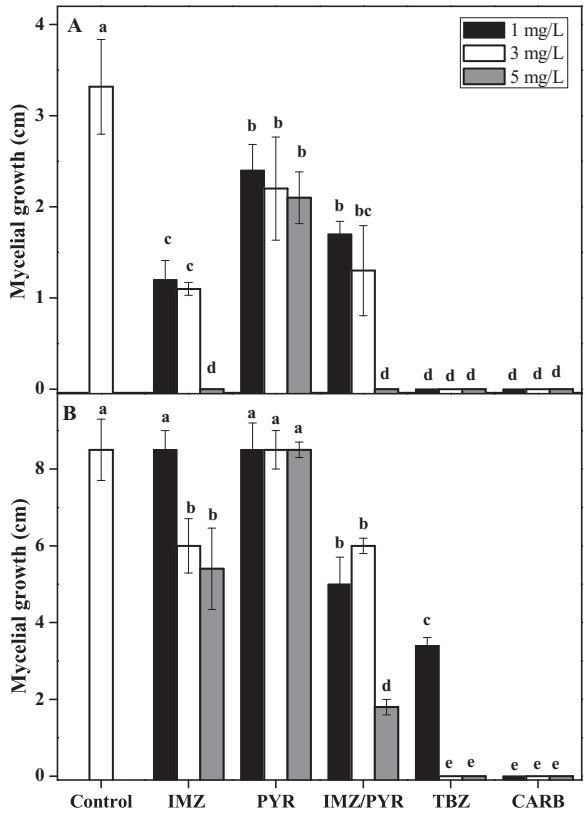

Fig. 1. *In vitro* effect of fungicides on mycelial growth. *Diaphorte citri* (A) and *Lasiodiplodia theobromae* (B) growth on PDA plates in the presence of different concentrations of IMZ, PYR, IMZ plus PYR, TBZ or CARB. The control plates were prepared without fungicides. Different letters on columns indicate they differ significantly according to Tukey's test (P ≤ 0.05).

treatment. Tukey's HSD ($p < 0.05$) tests were used to separate means. All analyses employed statistical software (with Statistix 9.0 Analytical Software 2008 for Windows; USA).

3. Results and discussion

3.1. Effect of postharvest fungicides on mycelial growth of L. theobromae and D. citri

The effect of fungicides on growth of *L. theobromae* and *D. citri* was different. *D. citri* was more sensitive than *L. theobromae* to all the assayed fungicides (Fig. 1). PYR was the less effective fungicide to inhibit the mycelial growth of both pathogens, while CARB and TBZ were the most effective. However, considering these two benzimidazoles, *L. theobromae* was able to grow with TBZ at the lower assayed concentration. Either IMZ or Philabuster® (combination of IMZ and PYR) at 5 mg/L inhibited the mycelial growth of *D. citri*, while only inhibited growth of *L. theobromae* by 25 or 75%, respectively. With Philabuster®, a synergistic effect of IMZ and PYR was observed. The novelty of our study is the comparison of the action of different fungicides on the two etiological agents causing SER, by using local fungal isolates of lemon. Similar results were shown in the few previous reports on the topic (Zhang 2007, 2009, 2012) in which IMZ, PYR and TBZ were evaluated on the growth of *L. theobromae* (previously named as *Diplodia natalensis*) isolated in Florida from sweet citrus.

3.2. Control of SER on lemons by postharvest fungicides

Conventional and alternative postharvest fungicides were evaluated on lemons that were artificially inoculated with both pathogens. The inoculation method was optimized from Cerioni et al. (2013) to increase the reproducibility assays to test chemical compounds. TBZ, IMZ, PYR, PCLO, PROP, FLUD, AZO, GUA and CARB were evaluated to control Phomopsis SER (Fig. 2). All fungicides evaluated, except for PCLOR and PYR (with an incidence of 30%), were effective to control this disease.

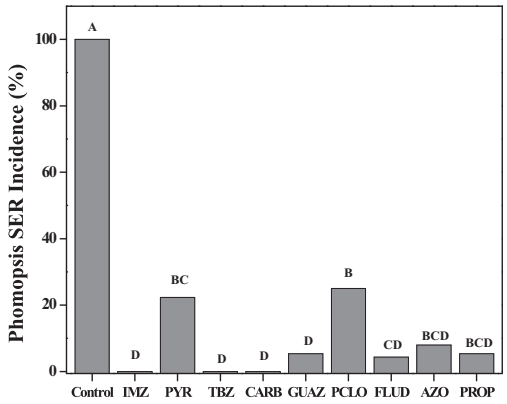

Fig. 2. Effect of fungicides on Phomopsis SER control. Inoculated lemons were immersed in TBZ, IMZ, PYR, PCLOZ, PROP, FLUD, AZO, GUA, PROP or CARB solutions at 2000 mg/L. Analysis of variance was applied to arcsine of the square root of the proportion of infected lemons. Actual average values are shown. Values followed by different letters on columns are significantly different by Tukey's HSD (P ≤ 0.05).

For Diplodia SER, TBZ, IMZ and IMZ plus PYR were the most effective, while FLUD, AZO, GUA, PYR, DIF, PROP, CARB, FLUD plus AZO or PCLOZ had incidence values of between 30 and 80% (Fig. 3). Note that, in the case of *in vivo* assays, the combination of IMZ plus PYR (Philabuster®) did not improve the effectiveness of IMZ against Diplodia SER. Phomopsis SER was easier to control than Diplodia SER for all the fungicides evaluated. FLUD, AZO and PYR were effective to control Phomopsis SER but they were unable to control Diplodia SER. It is remarkable that the combination FLUD/AZO (Graduate A+) had a better Diplodia SER control than the individual compounds. The new fungicides FLUD, AZO y PYR, classified as reduced risk compounds, were approved by the US EPA for their postharvest use on various fruits including citrus (Adaskaveg et al., 2004; Zhang, 2003; 2007). Previous reports showed that FLUD was effective to control Diplodia SER when it was applied on naturally infected oranges (Zhang, 2007). In our experimental conditions, Diplodia SER was only controlled by the conventional fungicides TBZ and IMZ. Fig. 4 shows the incidence of Diplodia SER after treatments with IMZ, CARB, TBZ, PYR, and IMZ plus

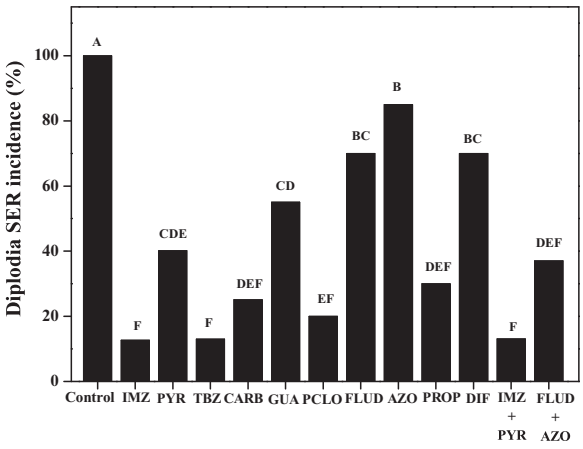

Fig. 3. Effect of fungicides on Diplodia SER control. Inoculated lemon were immersed in TBZ, IMZ, PYR, IMZ plus PYR, PCLOZ, PROP, DIF, FLUD, AZO, FLUD plus AZO, GUA or CARB solutions at 2000 mg/L. Analysis of variance was applied to arcsine of the square root of the proportion of infected lemons. Actual average values are shown. Values followed by different letters on columns are significantly different by Tukey's HSD (P ≤ 0.05).

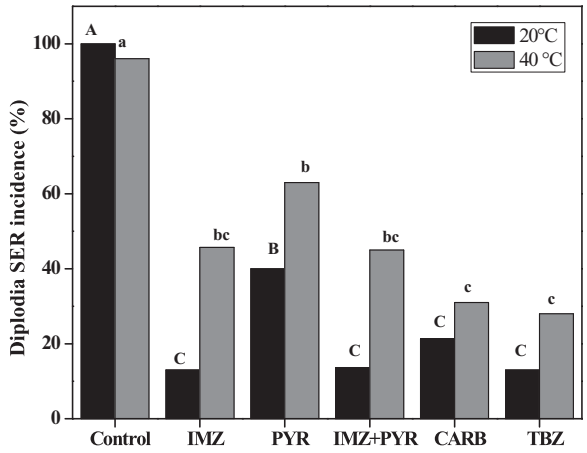

Fig. 4. Effect of temperature increase to control Diplodia SER. Inoculated lemons were immersed in 20 or 40 °C solutions of TBZ, IMZ, PYR, IMZ plus PYR or CARB at 2000 mg/L. Analysis of variance was applied to arcsine of the square root of the proportion of infected lemons. Actual average values are shown. Values followed by different letters on columns are significantly different by Tukey's HSD ($P \leq 0.05$).

Table 1
Diplodia SER incidence among 'Eureka' lemons after treatments with fungicides and salts.

	Incidence (% ± SD)		
Treatments	–	KS (20 g/L)	SBC (30 g/L)
–	100.0 ± 0.0a	40.0 ± 7.0b	84.0 ± 15.9a
IMZ (2000 mg/L)	12.7 ± 8.9a	28.9 ± 10.2a	16.0 ± 8.0a
TBZ (2000 mg/L)	13.0 ± 10.3a	27.1 ± 13.0a	10.0 ± 3.a
PYR (2000 mg/L)	40.2 ± 11.2a	27.9 ± 10.3a	nd
IMZ/PYR (2000 mg/L)	13.1 ± 7.0a	52.1 ± 11.9b	nd

nd: not determined. Values within a row followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

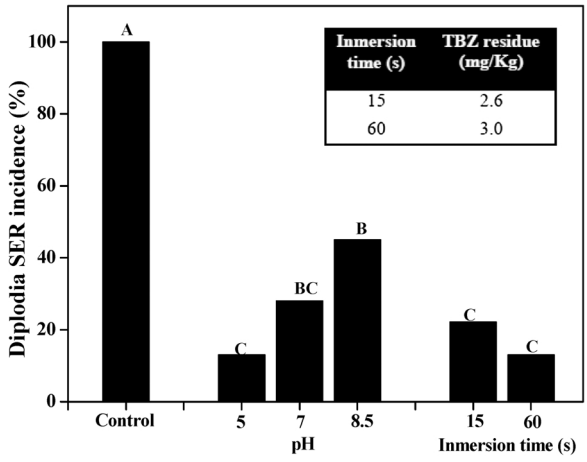

Fig. 5. Effect of TBZ for the control of Diplodia SER on lemons. Inoculated lemons were immersed in solutions of 2000 mg/L TBZ at different pH or immersion time. Analysis of variance was applied to arcsine of the square root of the proportion of infected lemons. Actual average values are shown. Values followed by different letters on columns are significantly different by Tukey's HSD ($P \leq 0.05$). Insert in the figure depicts the residue values after treatment at pH 5 for the indicated times.

PYR at 20 or 40 °C. When the fungicide solution temperature was increased, the control of Diplodia SER decreased. Besides, the addition of inorganic or organic salts (20 g/L KS or 30 g/L SBC) to the fungicide solutions did not improve the Diplodia SER control in respect to fungicides alone (Table 1). In previous report by Cerioni et al. (2013), potassium sorbate was moderately effective to control Diplodia SER and the temperature increase did not affect its effectiveness.

3.3. TBZ performance to control Diplodia SER on artificially inoculated lemons

IMZ, as TBZ, controlled Diplodia SER, although this compound is reserved for other decay management in order to minimize the risk of resistance development mainly on *Penicillium* populations. Thus, TBZ was further evaluated to control Diplodia SER at three pH values (5, 7, or 8.5) and two immersion times (15 or 60 s) (Fig. 5). As the pH increased, the effectiveness of TBZ decreased. The best treatment to control Diplodia SER was fungicide solution at pH 5 and 60 s of immersion. TBZ residue loading on lemons (insert in Fig. 5) was between 2.6 and 3.0 mg-Kg^{-1}, values that did not exceed the maximum residue levels. When TBZ was evaluated in combination with other fungicides and the inorganic salt KP its performance did not improve, with Diplodia SER incidence percentage values of 20.0 ± 14.1 for TBZ/PYR/KP and 15.0 ± 8.0 for TBZ/PROP/KP.

It has been reported that TBZ is effective for Diplodia SER control on oranges in Florida (Ismail and Zhang, 2004). In commercial orchards, it is usually applied in drenchers and in packinghouse line both in water (1000 mg/L) and wax (2000 mg/L) sprays. In Argentinean packinghouses, TBZ is only applied in sprays, in preselection and production packingline.

L. theobromae is a saprophyte fungus that completes its life cycle on dead stems and wood of citrus trees in groves. On fruit in groves, fungal colonization remains latent and does not causes fruit decay before harvest. Infections develop after harvest especially under conditions of high temperature and elevated relative humidity that occur mainly during ethylene degreening treatments (Kucharek and Brown, 1992). The level of control accomplished by postharvest treatments with TBZ could be improved with some preharvest practices. In preliminary tests, we observed that head and lateral pruning reduced melanose and SER symptoms, while the removal of internal dead branches did not affect the SER incidence (data not shown). This insight should be considered for the design of an integrated pre- and postharvest management to control Diplodia SER and other postharvest diseases.

4. Conclusions

To our knowledge, this is the first report where an artificial inoculation method was used to evaluate chemical fungicides against SER disease in lemon fruit. All the fungicides evaluated were more effective to control Phomopsis SER than Diplodia SER. The reduced-risk products as FLUD, AZO, PROP or PYR reduced Phomopsis SER incidence, but they did not control Diplodia SER. The application of conventional fungicide TBZ in commercial treatments remains the best option to reduce SER caused by both pathogens on lemons in the Argentinean packinghouses.

Acknowledgements

We acknowledge San Miguel S.A for providing lemons. This work was supported by grants from the Agencia Nacional de Promoción Científica y Técnica (ANPCyT-MINCYT), and the Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT).

References

Adaskaveg, J.E., Kanetis, L., Soto-Estrada, A., Förster, H., 2004. A new era of postharvest decay control in citrus with the simultaneous introduction of three new 'reduced-risk' fungicides. Proc. Int. Soc. Citricult. 10, 999–1004.

Brown, G.E., Eckert, J.W., 2000. Diplodia stem-end rot. In: Timmer, L.W., Gernsey, S.M., Graham, J.H. (Eds.), Compendium of Citrus Diseases. APS Press, St. Paul,MN, USA, pp. 43–44.

Cerioni, L., Sepulveda, M., Rubio-Ames, Z., Volentini, S.I., Rodríguez-Montelongo, L., Smilanick, J.L., Ramallo, J., Rapisarda, V.A., 2013. Control of lemon postharvest diseases by low-toxicity salts combined with hydrogen peroxide and heat. Postharvest Biol. Technol. 83, 17–21.

EFSA (European Food Safety Authority), 2014. Reasoned opinion on the review of the

existing maximum residue levels (MRLs) for thiophanate-methyl and carbendazim according to Article 12 of Regulation (EC) No 396/2005. EFSA J. 12 (3919). http://dx.doi.org/10.2903/j.efsa.2014.3919. (118 pp.).
Eckert, J.W., Eaks, I.L., 1989. Postharvest disorders and diseases of citrus fruit. In: In: Reuter, W., Calavan, E.C., Carman, G.E. (Eds.), The Citrus Industry Vol. 5. University of California Press, Berkeley, CA, USA, pp. 179–260.
Federcitrus (Federación Argentina de Citrus), 2016. La Actividad CitrícolaArgentina-Año 2016. Federcitrus; (Federación Argentina de Citrus), Bs. As., Argentina, pp. 1–16. www.federcitrus.org.
Ismail, M., Zhang, J., 2004. Post-harvest citrus diseases and their control. Outlooks Pest Manage. 15, 29–35.
Kucharek, T.A., Brown, G.E., 1992. Stem-End Rot. Fact Sheet PP-149. Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
Palacios, J., 2005. In: Talleres Gráficos Alfa Beta S.A (Ed.), Origen de los Agrios. Citricultura, Bs. As., Argentina, pp. 58–60.
Sepulveda, M., Cuevas, I.I., Smilanick, J.L., Cerioni, L., Rapisarda, V.A., Ramallo, J., 2015. Improvement in imazalil treatments in commercial packinglines to control green mold on lemon fruit. Sci. Hort. 192, 387–390.
Smilanick, J.L., Mansour, M.F., Gabler, F.M., Goodwine, W.R., 2006. The effectiveness of pyrimethanil to inhibit germination o: *Penicillium digitatum* and to control citrus green mold after harvest. Postharvest Biol. Technol. 42, 75–85.
White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press Inc, New York, pp. 315–322 (©W°).
Zhang, J., 2003. New Fungicide Registrations and Their Impact on Post-Harvest Decay Control of Citrus. Florida Cooperative Extension Service, Packinghouse Newsletter No. 198. IRREC, University of Florida, Fort Pierce, FL, USA, pp. 1–2.
Zhang, J., 2007. The potential of a new fungicide fludioxonil for stem-end rot and green mold control on Florida citrus fruit. Postharvest Biol. Technol. 46, 262–270.
Zhang, J., 2009. Evaluation for the potential of a new fungicide pyrimethanil for post-harvest Diplodia stem-end rot control on Florida citrus fruit. Proc. Fla. State Horticult. Soc. 122, 350-353.
Zhang, J., 2012. Comparative effects of postharvest fungicides for Diplodia Stem-end Rot control of Florida citrus. Proc. Fla. State Horticult. Soc. 125, 243–247.