

Tank mix application of copper hydroxide either with cyproconazole or pyraclostrobin fungicides reduced the control of coffee leaf rust

Gabriela Alves da Costa^a, Jaciele Beserra Lira^a, Rejane do Livramento Freitas-Lopes^b, Ueder Pedro Lopes^{a,*}

^a Unidade Acadêmica de Garanhuns, Universidade Federal Rural de Pernambuco, 55292-272, Garanhuns, PE, Brazil

^b Departamento de Agronomia, Universidade Federal Rural de Pernambuco, 52171-900, Recife, PE, Brazil

ARTICLE INFO

Keywords:

Hemileia vastatrix
Chemical control
Disease management
Strobilurin
Triazole

ABSTRACT

Coffee leaf rust (CLR) is a major disease that occurs in all coffee-growing regions, and chemical control is the most effective strategy to control the disease. Usually, this control is based on the application of contact and/or systemic fungicides to the foliage of the plants, and a very common practice is the use of tank-mix solution of fungicides to reduce the costs of the application. The aim of this study was to evaluate the efficacy of mixtures of systemic fungicides (triazoles and strobilurins) and copper hydroxide on the germination of uredospores of *Hemileia vastatrix* and on the control of CLR. The results showed that the effectiveness of a fungicide can be affected when used in mixtures with another fungicide, resulting in ineffective disease control. When copper hydroxide was combined with cyproconazole, the inhibition of uredospore germination was lower than when copper hydroxide was used alone. For both fungicides pyraclostrobin and cyproconazole, effectiveness in the control of CLR was reduced with the presence of copper hydroxide in the mixture, and this loss in efficacy may be associated with the occurrence of alkaline hydrolysis of the fungicides when they are mixed with copper hydroxide. The findings of this study demonstrate that tank mix application of copper hydroxide either with cyproconazole or pyraclostrobin fungicides reduces the control of CLR, so this practice should be avoided by growers.

1. Introduction

Coffee leaf rust (CLR) caused by *Hemileia vastatrix* Berk. et Br. is a major disease that occurs in all regions where coffee is cultivated (Zambolim, 2016; Talhinhos et al., 2017). The disease causes leaves to fall prematurely, resulting in the death of branches and low photosynthesis rates, which ultimately reduce the coffee yield in the following year by 35–50% (Zambolim, 2016; Waller et al., 2007). In recent years, severe epidemics of the disease in Central and South America, particularly in Nicaragua, Ecuador, El Salvador, Panama, and Honduras, have led to significant losses (Avelino et al., 2015).

The management strategies to CLR are based on the use of resistant cultivars and chemical control. However, most genotypes cultivated in Brazil are susceptible to CLR, which makes chemical control the most effective strategy for controlling the disease in this country (Zambolim, 2016). Usually, this control is based on spraying contact and/or systemic fungicides on the foliage of the plants (Zambolim et al., 2002). In Brazil,

CLR control is accomplished using four to five applications of contact fungicides, especially copper-based fungicides (copper oxychloride and hydroxide, cuprous oxide and bordeaux mixture), which are sprayed at 30-day intervals throughout the growing season. Additionally, systemic fungicides belonging to the DMI (demethylation inhibitors) group, including triazoles, are applied alone or in commercial mixtures with strobilurin fungicides (QoI - Quinone outside Inhibitors) (Souza et al., 2011; Lopes et al., 2013; Lopes et al., 2014; Honorato et al., 2015).

The combined or alternate use of systemic and copper-based fungicides also has the advantage of providing copper to the plants, in addition to reducing the risk of selecting fungicide-resistant rust populations (Souza et al., 2011; Zambolim, 2016). Coffee growers commonly use copper-based and systemic fungicides in mixtures in a tank to reduce the costs of the application. However, little is known about the possible effect of these mixtures, and guidelines for agrochemical mixtures in tanks do not exist in Brazil (Gazziero, 2015). Thus, the aim of this study was to evaluate the efficacy of mixtures of systemic

* Corresponding author.

E-mail address: ueder.lopes@ufrpe.br (U.P. Lopes).

<https://doi.org/10.1016/j.cropro.2019.104856>

Received 16 October 2018; Received in revised form 22 April 2019; Accepted 18 June 2019

Available online 19 June 2019

0261-2194/© 2019 Published by Elsevier Ltd.

Table 1

Fungicides and rates used to examine the effect of fungicide mixtures on the germination of *H. vastatrix* uredospores and on the coffee leaf rust control.

Treatment	Commercial product	Commercial rate (g or mL L ⁻¹) ^a
Control (without fungicide)		
Copper hydroxide	Kocide® WDG Bioactive (Du Pont)	5.0
Pyraclostrobin	Comet® 250 EC (BASF)	1.76
Cyproconazole	Alto 100 (Syngenta)	1.88
Copper hydroxide +	Kocide® WDG Bioactive +	5.0 +
Pyraclostrobin	Comet® 250 EC	1.76
Copper hydroxide +	Kocide® WDG Bioactive +	5.0 +
Cyproconazole	Alto 100	1.88

^a Rate recommended by the MAPA (2018) to control coffee leaf rust.

fungicides (triazoles and strobilurins) and copper hydroxide on the germination of uredospores of *H. vastatrix* and on the control of CLR.

2. Methodology

Leaves containing pustules of CLR were collected in May and August of 2016, from growing areas of coffee cv. Catuaí Vermelho IAC 144, without previous fungicide application, in the state of Pernambuco, Brazil. The uredospores were removed from the leaves with the aid of gelatin capsules one day before use and were maintained at 4 °C to ensure viability.

The viability of the *H. vastatrix* uredospores was assessed before use in plant inoculation. For this process, 50 µL of a suspension of uredospores (1.0×10^6 conidia. mL⁻¹) were spread on the surface of five Petri dishes (9 mm in diameter) containing agar medium (2%), and the dishes were kept at 25 °C in the dark for 12 h. In each plate 100 conidia were evaluated, with the aid of an optical microscope. When the length of germ tube was larger than the total length of the uredospore, it was considered to have germinated. The percentage of uredospore germination was obtained by the mean of the five plates.

To evaluate the effect of the mixture of fungicides on uredospore germination, in vitro experiments were performed in a completely randomized design with six treatments (Table 1) and five replicates. Each experimental unit consisted of one Petri dish (9 mm in diameter). The commercial fungicides were dissolved in water to obtain the recommended concentration of use (MAPA, 2018). Then, 1 mL of the suspension of uredospores (1.0×10^6 conidia. mL⁻¹) was transferred to 5 mL of each fungicide suspension (treatment), and 200 µL of this suspension were transferred to the Petri dishes containing agar medium (2%). The plates were kept at 25 °C in the dark for 12 h. The percentage of germination was assessed by analyzing 250 uredospores from each plate with a light microscope, as described previously.

Coffee seeds (cv. “Catuaí Vermelho IAC 144”) were sown in a moist sand bed until germination (60 days). After this period, the plants were transplanted to plastic pots (2 L) containing a mix of soil, cow manure and sand (2.5:1:0.5 ratio). Coffee plants were fertilized with 25 mL of a nutrient solution containing NPK (10:8:10 g.L⁻¹) at seven days after transfer and then every seven days thereafter until the end of the experiment. The experiments were performed in a greenhouse using three-month-old coffee plants in a completely randomized design with six treatments (Table 1) and three replicates. Each experimental unit consisted of three plastic pots, each containing one coffee plant. The two youngest pairs of expanded leaves of each plant were inoculated with a suspension of uredospores (1.0×10^6 conidia. mL⁻¹). The inoculated plants were maintained under controlled conditions (25 °C in the dark and relative humidity of approximately 100%) for 48 h. After this period, the plants were transferred to a greenhouse at 22 ± 3 °C, where

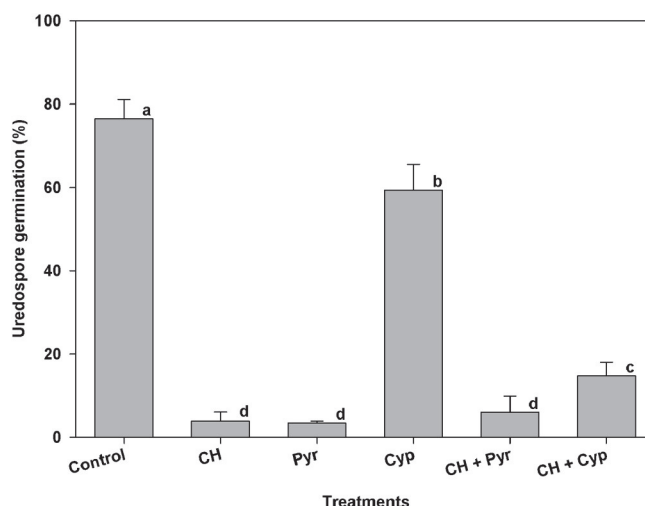


Fig. 1. Germination of uredospores submitted to treatments with different mixtures of fungicides. Control - No fungicide; CH - Copper hydroxide; Pyr - Pyraclostrobin; Cyp - Cyproconazole; CH + Pyr - Copper hydroxide + Pyraclostrobin; CH + Cyp - Copper hydroxide + Cyproconazole. Columns followed by the same letters do not differ by Tukey's test at 5% significance. The lines outside the columns represent the standard deviation. Each value represents the mean of ten replicates.

they remained for 60 days. At twenty days after inoculation, the fungicides and the mixtures were sprayed in the abaxial and adaxial surfaces of the leaves until runoff, with a manual backpack sprayer configured to apply a volume of 400 L. ha⁻¹. The CLR severity was assessed weekly until 42 days after fungicide application (DAFA) using a diagrammatic scale with six severities (2.5, 5, 10, 20, 40, and 80%) of CLR (Capucho et al., 2011). For each experimental unit (three plants), the mean severity was obtained, and the data were used to calculate the Area Under Rust Progress Curve (AURPC), according to Shaner and Finney (1977).

The pH value of the fungicide solutions was measured immediately after preparation and prior to application to the plants with the aid of a pH meter (Luca-210, Lucadema, Brazil). The pH of the water used to prepare the fungicide solutions was also assessed. The procedure was performed three times for each solution, and the mean was obtained. To verify precipitate formation, 200 mL of each fungicide solution were transferred to a glass beaker and maintained at 25 ± 2 °C. Visual evaluations were performed immediately and at 30 and 60 min after the preparation.

The data for uredospore germination of the two experiments were submitted to Cochran's test for homogeneity of variance (Gomez and Gomez, 1984) and then were pooled for subsequent analysis. The percentage of uredospore germination and AURPC were submitted to analysis of variance (ANOVA) and verification of the assumptions of normality, homogeneity and independence of the experimental error. The means of all treatments were compared by Tukey's test ($P \leq 0.05$) using SAS version 9.0 (SAS Institute Inc., 2004).

3. Results and discussion

The efficacy of the fungicides on the in vitro germination of *H. vastatrix* uredospores was evaluated, and 76.4% of germination was observed in the control treatment without fungicide (Fig. 1). The highest inhibitory effect was observed with the fungicides copper hydroxide and pyraclostrobin with a reduction in germination of approximately 95% when used alone and 92% when combined. The copper hydroxide + cyproconazole mixture led to a reduction in germination of 80%, whereas the treatment with cyproconazole alone was the least effective, reducing germination by approximately 22%.

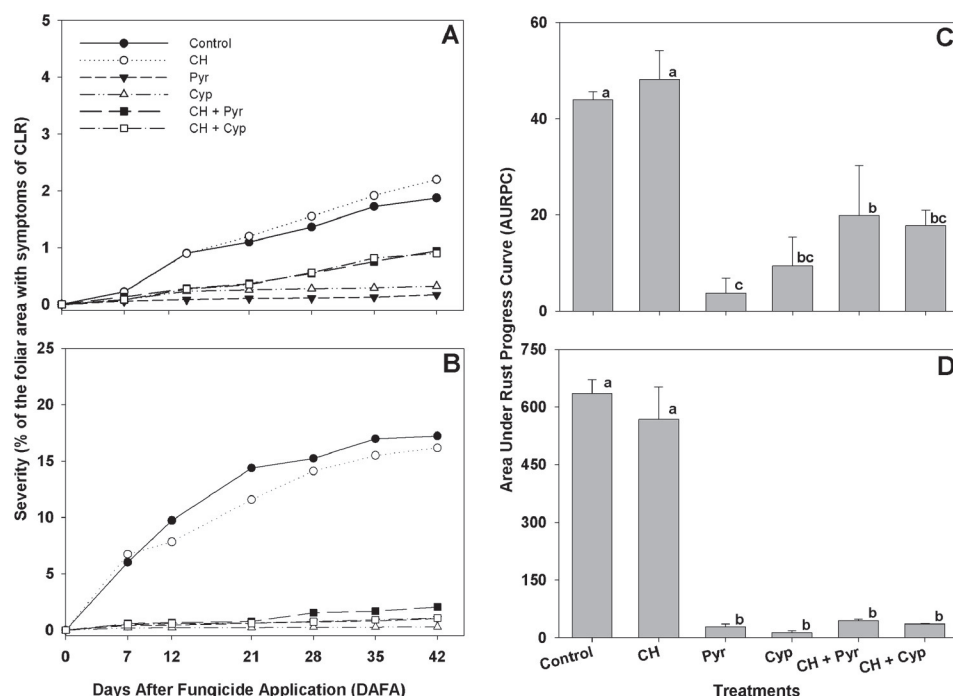


Fig. 2. Progress of coffee leaf rust severity and Area Under the Rust Progress Curve (AURPC) of plants to which fungicides were applied in experiments I (A and C) and II (B and D). Control - without fungicide; CH - Copper hydroxide; Pyr - Pyraclostrobin; Cyp - Cyproconazole; CH + Pyr - Copper hydroxide + Pyraclostrobin; CH + Cyp - Copper hydroxide + Cyproconazole. Columns followed by the same letters do not differ by Tukey's test at 5% significance. The lines outside the columns represent the standard deviation. Each value represents the mean of three replicates.

The in vivo efficacy of the fungicides for controlling CLR was evaluated in coffee plants artificially inoculated with uredospores of *H. vastatrix*. In general, the severity of CLR was low, and the maximum value at 42 DAFA was 1.9% and 17% in the control treatment (no fungicide) in experiment I and II, respectively (Fig. 2 A, B). In experiment I, the severity of CLR was very low, which may be associated with the quality of the inoculum, since it was obtained from coffee plants in the field. Indeed, the percentage of germination of the uredospores used in experiment I was approximately 30%, whereas the uredospores used in experiment II had up to 70% germination. The application of copper hydroxide had no effect on reducing the severity of the disease since the maximum severity reached 2.2% and 16% in experiment I and II, respectively. However, when the plants were sprayed with the fungicides cyproconazole or pyraclostrobin alone, the severity was considerably reduced. For the treatment with cyproconazole, the severity did not exceed 0.3% in both experiments, and for pyraclostrobin, the values were 0.15% and 1% in experiment I and II, respectively. The copper hydroxide + pyraclostrobin mixture also reduced the severity to 1.0% and 2% in experiment I and II, respectively. Similarly, the copper hydroxide + cyproconazole mixture led to a reduction in CLR severity to 0.9% and 1% in experiment I and II, respectively.

The AURPC followed the severity of CLR in the greenhouse experiments, having higher values in experiment II (Fig. 2 C, D). In both experiments I and II, the treatments with the fungicides cyproconazole and pyraclostrobin either alone or in combination with copper hydroxide showed AURPC values lower than those of the control treatment. The plants that received the application of copper hydroxide alone had AURPC values that did not differ from the control treatment. In experiment I, the AURPC values were reduced by approximately 91.0, 79.0, 55.0, and 59.0% in the treatments with pyraclostrobin, cyproconazole, copper hydroxide + pyraclostrobin, and copper hydroxide + cyproconazole, respectively, compared to that of the control. In experiment II, these values were reduced by 95.0, 98.0, 93.0, and 94.0%, respectively, with no significant difference among treatments. As verified in experiment I, the application of pyraclostrobin alone was more efficient in reducing the severity of CLR (approximately 91%) than the treatment in combination with copper hydroxide (55%), which demonstrates a reduction in the efficacy of control with the presence of copper

Table 2

pH values of the fungicide solutions used in the experiments, measured immediately after preparation.

Treatment	pH value		
	Exp. I	Exp. II	Mean
Control (without fungicide)	7.4 ^a	7.2	7.3
Copper hydroxide	10.0	9.0	9.5
Pyraclostrobin	7.4	7.0	7.2
Cyproconazole	4.6	4.00	4.3
Copper hydroxide + Pyraclostrobin	9.9	8.9	9.4
Copper hydroxide + Cyproconazole	10.0	9.0	9.5

^a Values are the mean of three measures for each solution.

hydroxide. This effect was also verified with cyproconazole, although the difference among treatments was not significant. In this case, a numerical difference was observed with an AURPC value approximately two times greater when cyproconazole was combined with copper hydroxide than when cyproconazole was used alone. Similar results were obtained in experiment II. Even with no significant difference among treatments, the AURPC values were numerically greater in the presence of copper hydroxide for both pyraclostrobin (approximately 1.5 times) and cyproconazole (approximately 3 times), demonstrating the loss of efficacy of control when using the fungicides combined with copper hydroxide.

The loss of efficacy of the fungicides could be associated with physical and chemical changes in the mixtures. Indeed, the evaluation of pH values indicated that the presence of copper hydroxide led to an alteration in the pH of the mixture (Table 2). The pH values for pyraclostrobin and cyproconazole alone averaged 7.2 and 4.3, respectively. However, when combined with copper hydroxide, the mixtures showed pH values of 9.4 and 9.5, respectively, which were similar to the pH value of copper hydroxide alone (9.5). Despite the drastic changes in pH, there was no precipitate formation in the mixtures.

In this study, the highest inhibitory effect on uredospore germination was observed with the fungicides copper hydroxide and pyraclostrobin alone or combined. However, when copper hydroxide was combined with cyproconazole, the inhibition of uredospore germination was lower than when copper hydroxide was used alone, which may be associated

with chemical incompatibility (Azevedo, 2015). This result demonstrates that the protective effect of copper hydroxide applied prior to an infection of *H. vastatrix* may be negatively affected when combined with cyproconazole. However, in artificially inoculated plants, the application of copper hydroxide was not effective for CLR control. The most effective control was achieved when pyraclostrobin was used alone, although the treatment with cyproconazole was also effective. Copper hydroxide is a contact fungicide that acts mainly by inhibiting the germination of uredospores on the surface of susceptible tissues, leading to the death of the protoplasm. Thus, it is a protective fungicide and should be applied preventively before the infectious process begins to ensure disease control (Silva and Behlau, 2018). Pyraclostrobin is a systemic fungicide that acts by restricting fungal development within leaf tissues. This characteristic can explain its effect on both uredospore germination and the early stages of the infectious process. Finally, cyproconazole acts by inhibiting the biosynthesis of ergosterol and thus is ineffective in inhibiting germination (Mueller et al., 2013; Oliver and Hewitt, 2014).

Overall, the results showed that the effectiveness of a fungicide can be affected when used in mixtures with another fungicide, resulting in ineffective disease control. For both fungicides pyraclostrobin and cyproconazole, the effectiveness of CLR control was reduced with the presence of copper hydroxide in the mixture. As verified by analyzing the pH value of the mixtures, a possible reason for this loss of efficacy is the occurrence of alkaline hydrolysis in the fungicides when they are mixed with copper hydroxide. In this case, the fungicides exposed to a pH greater than 7.0 may have been hydrolyzed and rendered ineffective. This has been demonstrated for azoxystrobin, which degradation under alkaline (pH 9) condition is faster than under neutral (pH 7) or acidic (pH 4) condition (Singh et al., 2010).

Our findings demonstrate that tank mix application of copper hydroxide either with DMI or QoI fungicides reduces the control of coffee leaf rust. Although this practice is very common in coffee production areas, it should be avoided, since it may compromise the effectiveness of disease control.

4. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Avelino, J., Cristancho, M., Georgiou, S., Imbach, P., Aguilar, L., Bornemann, G., Läderach, P., Anzueto, F., Hruska, A.J., Morales, C., 2015. The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. *Food Secur.* 7, 303–321. <https://doi.org/10.1007/s12571-015-0446-9>.
- Azevedo, L.A.S., 2015. *Misturas de tanque de produtos fitossanitários: teoria e prática*, first ed. IMOS Gráfica e Editora, Rio de Janeiro.
- Capucho, A.S., Zambolim, L., Duarte, H.S.S., Vaz, G.R.O., 2011. Development and validation of a standard area diagram set to estimate severity of leaf rust in *Coffea arabica* and *C. canephora*. *Plant Pathol.* 60, 1144–1150. <https://doi.org/10.1111/j.1365-3059.2011.02472.x>.
- Gazziero, D.L.P., 2015. Misturas de agrotóxicos em tanque nas propriedades agrícolas do Brasil. *Planta Daninha* 33, 83–92. <https://doi.org/10.1590/S0100-83582015000100010>.
- Gomez, K.A., Gomez, A.A., 1984. *Statistical Procedures for Agricultural Research*, second ed. Wiley, New York.
- Honorato Jr., J., Zambolim, L., Lopes, U.N., Lopes, U.P., Duarte, H.S., 2015. DMI and QoI fungicides for the control of coffee leaf rust. *Austral. Plant Pathol.* 44, 575–581. <https://doi.org/10.1007/s13313-015-0373-4>.
- Lopes, U.P., Zambolim, L., Souza Neto, P.N., Souza, A.F., Capucho, A.S., Rodrigues, F.A., 2013. Effect of foliar application of potassium silicate on the progress of coffee leaf rust. *Trop. Plant Pathol.* 38, 547–551. <https://doi.org/10.1590/S1982-56762013000600012>.
- Lopes, U.P., Zambolim, L., Souza Neto, P.N., Duarte, H.S.S., Ribeiro, J.I., Souza, A.F., Rodrigues, F.A., 2014. Silicon and triadimenol for the management of coffee leaf rust. *J. Phytopathol.* 162, 124–128. <https://doi.org/10.1111/jph.12166>.
- MAPA, 2018. Ministério da Agricultura, Pecuária e Abastecimento. <http://www.agricultura.gov.br/>. (Accessed 3 April 2018).
- Mueller, D.S., Wise, K.A., Dufault, N.S., Bradley, C.A., Chilvers, M.I., 2013. *Fungicides for Field Crops*. APS Press, St. Paul.
- Oliver, R.P., Hewitt, H.G., 2014. *Fungicides in Crop Protection*, second ed. CAB, Boston.
- SAS Institute Inc, 2004. *Statistical Analysis System (SAS)*, version 9.0.
- Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67, 1051–1056.
- Silva Jr., J.G., Behlau, F., 2018. Controle químico. In: Amorim, L., Rezende, J.A.M., Bergamin Filho, A. (Eds.), *Manual de Fitopatologia: princípios e conceitos*. Agronômica Ceres, Ouro Fino, pp. 239–260.
- Singh, N., Singh, S.B., Mukerjee, I., Gupta, S., Gajbhiye, V.T., Sharma, P.K., Goel, M., Dureja, P., 2010. Metabolism of 14C-azoxystrobin in water at different pH. *J. Environ. Sci. Health* 45, 123–127. <https://doi.org/10.1080/03601230903471910>.
- Souza, A.F., Zambolim, L., Jesus Jr., V.C., Cecon, P.R., 2011. Chemical approaches to manage coffee leaf rust in drip irrigated trees. *Austral. Plant Pathol.* 40 (3), 293. <https://doi.org/10.1007/s13313-011-0046-x>.
- Talhinhas, P., Batista, D., Diniz, I., Vieira, A., Silva, D.N., Loureiro, A., Tavares, S., Pereira, A.P., Azinheira, H.G., Guerra-Guimarães, L., Várzea, V., Silva, M.D.C., 2017. The coffee leaf rust pathogen *Hemileia vastatrix*: one and a half centuries around the tropics. *Mol. Plant Pathol.* 18, 1039–1051. <https://doi.org/10.1111/mpp.12512>.
- Waller, J.M., Bigger, M., Hillocks, R.J., 2007. *Coffee Pests, Diseases and Their Management*. CAB International, Oxfordshire.
- Zambolim, L., 2016. Current status and management of coffee leaf rust in Brazil. *Trop. Plant Pathol.* 41, 1–8. <https://doi.org/10.1007/s40858-016-0065-9>.
- Zambolim, L., Vale, F.X.R., Costa, H., Pereira, A.A., Chaves, G.M., 2002. Epidemiologia e controle integrado da ferrugem do cafeeiro. In: Zambolim, L. (Ed.), *O estado da arte de tecnologias de produção de café*. Editora UFV, Viçosa, pp. 369–450.