Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i8.3600

Research note

Antibiotics, nutrients and thermotherapy as control methods for Huanglongbing (HLB)

Hadassa Yuef Martínez-Padrón[1]
Criseida Alhelí Sáenz-Pérez[2]
Francisco A. Paredes-Sánchez[3]
Verónica Herrera-Mayorga[3]
Raúl Rodríguez-Herrera[4]
Eduardo Osorio-Hernández[2,§]

1 Subdirección de Enseñanza e Investigación-Hospital Regional de Alta Especialidad de Ciudad Victoria 'Bicentenario 2010'. Libramiento Guadalupe Victoria S/N, Área de Pajaritos, Ciudad Victoria, Tamaulipas, México. CP. 87087. Tel. 834 161024. (hadassayufo@gmail.com).

2 División de Estudios de Postgrado e Investigación-Facultad de Ingeniería y Ciencias-Universidad Autónoma de Tamaulipas. Centro Universitario Adolfo López Mateos, Ciudad Victoria, Tamaulipas, México. Tel. 834 3181721, ext. 2111. (alhe-saenz@outlook.com).

3 Unidad Académica Multidisciplinaria Mante-Universidad Autónoma de Tamaulipas. El Mante, Tamaulipas, México. CP. 89840, (faparedes@docentes.uat.edu.mx; evherrera@docentes.uat.edu.mx).

4 Departamento de Investigación en Alimentos-Facultad de Ciencias Químicas-Universidad Autónoma de Coahuila. Boulevard Venustiano Carranza, esquina con Ing. José Cárdenas Valdés, Col. República, Saltillo, Coahuila, México. CP. 25280. Tel. 844 4161238. (raul.rodriguez@uadec.edu.mx).

Autor para correspondencia: eosorio@docentes.uat.edu.mx.

Abstract

Huanglongbing (HLB), also known as greening disease or citrus greening, is a bacterial disease that affects citrus worldwide. This disease causes yellowing of the leaves, deformities in the fruits and eventually, the death of the tree. Currently, there is no cure for Huanglongbing; therefore, control focuses on preventing and mitigating the disease. The present research aimed to discuss the different control methods that are currently applied to control Huanglongbing, among which antibiotics, nutrients, and thermotherapy stand out. Antibiotics have been shown to be effective in controlling Huanglongbing in controlled studies. Nevertheless, their widespread use is limited due to concerns about antibiotic resistance. On the other hand, the application of certain nutrients, such as zinc and phosphorus, has shown promising results in improving the tolerance of citrus to Huanglongbing. These nutrients strengthen the plant's immune system and make it more resistant to infection. Finally, thermotherapy has proven to be an effective method to eliminate the Huanglongbing bacteria. Controlling Huanglongbing in citrus requires an integrated approach that combines different methods, such as the use of specific antibiotics, nutrient application, and thermotherapy. Continued research is needed to develop more effective, sustainable, and economically viable control methods to protect this important agricultural industry.

Keywords:

Candidatus Liberibacter, *Diaphorina citri,* Huanglongbing.

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v15i8.3600

Candidatus Liberibacter is the bacterium associated with Huanglongbing (HLB), which is the most destructive disease of citrus trees, causing the loss of millions of trees in almost all citrus-growing areas of the world; in Florida, United States of America, it has reduced the citrus area by 40% and production by 49%, causing a loss of one billion dollars (da-Graça *et al*., 2016). The first records of HLB disease date back to the early eighteenth century in India; likewise, in 2004, the bacterium was reported in Brazil and in 2005, in South Florida. HLB has reduced citrus production in many countries; for example, in the United States of America, it has reduced production by 81% and killed more than 60 million citrus trees in Africa and Asia (Huang *et al*., 2018; Shimwela *et al*., 2018).

Worldwide, Mexico is the sixth largest producer of citrus fruits, with a production of 6 634 000 t (da-Graça *et al*., 2016), behind China (32 705 000 t) (Song *et al*., 2018), Brazil (16 555 000 t) (Shimwela *et al*., 2018), India (9 755 000 t) (Huang *et al*., 2018), the United States of America (7 829 000 t) (da-Graça *et al*., 2016) and Spain (6 882 000 t) (Liu *et al*., 2019); however, recently, citrus production in Mexico has been affected by the presence of pests and diseases, among which Huanglongbing (HLB) or 'yellow dragon' stands out (Robles-González *et al*., 2017; Robles-González *et al*., 2018).

HLB is transmitted by two different routes, insects (*Diaphorina citri* and *Trioza erytreae*) (Figure 1) and through the use of infected grafts, which is why it has been classified as a systemic disease; the lack of HLB-resistant cultivars and the rapid spread of this disease has caused its control to become difficult in many countries (Song *et al*., 2018). Populations of *Diaphorina citri* show seasonal dynamics, with peaks of abundance during the warmest and driest months of the year (25 °C and 30 °C) (da-Graça *et al*., 2016).

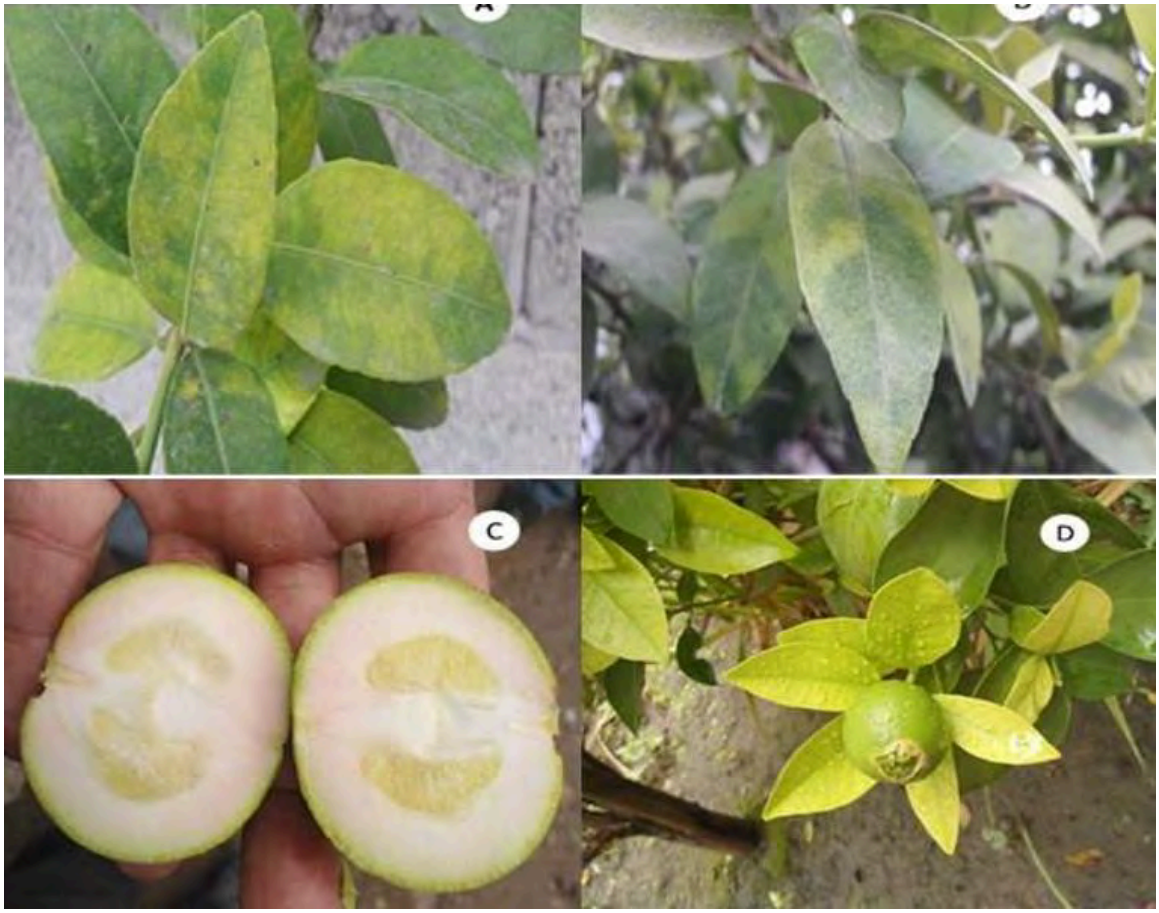
Figure 1. *Diaphorina citri,* vector of Candidatus Liberibacter (A and B).

The fruits with this disease are smaller (Figure 2), production decreases, there is seed abortion, uneven color of the peel, decrease in total soluble solids, calyx spot and fruit abortion; consequently, over the years, it will cause the tree to die in a period of 8 years; this time could vary depending on the type of crop, the age of the tree, its nutrition, etc. (Li *et al*., 2019).

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i8.3600

Figure 2. Symptoms of HLB, leaves with irregular and asymmetrical spots, diffuse mottling (A and B); smaller fruit, pericarp thickening (C) and calyx staining (D).



The symptoms of HLB disease vary depending on the age of the tree and cultivar; in some cases, they can be confused with nutritional disorders; however, the main symptoms are asymmetric chlorosis in the leaf (irregular spots), yellow veins with obstruction of vascular bundles, chlorotic patterns, deficient in green or with a slight spot (green islands) (Pereira *et al*., 2011; Liu *et al*., 2019) (Figure 3).

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v15i8.3600

Figure 3. Symptoms caused by zinc deficiency, commonly confused with symptoms caused by HLB (A, B, C and D).

The aim of this paper is to discuss the different control methods that are currently applied to control HLB.

Antibiotics, control methods for Huanglongbing (HLB)

The antibiotics used to control the disease are diverse and the applications are mostly by injection into the trunk. Studies by Zhang *et al*. (2011) concluded that ampicillin at 200 mg L^{-1} showed moderate efficacy for the control of *Candidatus* spp. in oranges, reducing the incidence of the disease by 50% and severity by 30%. Nonetheless, no significant effect on fruit quality was observed.

On the other hand, Vincent *et al*. (2022) conducted a research where they examined the effects of a variety of adjuvants on the absorption of oxytetracycline and streptomycin by foliar application. They also compared the efficiency of foliar application of oxytetracycline and streptomycin with trunk injection. They included extremely low levels of oxytetracycline and streptomycin in leaves that were coated during foliar application, indicating that neither streptomycin nor oxytetracycline were successfully administered systemically by foliar application, even after mixing with adjuvants (Zhang et al., 2014; Shin et al., 2016; Habiba et al., 2018; Ghosh et al., 2018).

DOI: https://doi.org/10.29312/remexca.v15i8.3600

Among other treatments used against diseases of bacterial and phytoplasmic origin, oxytetracycline has been used successfully in emergency situations; in addition, Slinsky (2016) proposes good practices (such as proper irrigation, fertilization, and weed control to maintain the health of trees and make them more resistant to diseases) in the management of varieties tolerant or resistant to bacteria as an efficient method of control. A practice like this represents an effective method for HLB control (Yang *et al*., 2016; Archer *et al*., 2023).

Nutrients, a control option for Huanglongbing (HLB)

Mineral nutrients are necessary for the growth and development of plants and microorganisms and are important factors in plant-pathogen interactions (da-Graça *et al*., 2016). Nutrient deficiencies or excesses in plants can affect their susceptibility to this disease through the metabolic changes of plants, thus creating a more favorable environment for the development of the disease (Gilani *et al*., 2018).

Zinc is an essential micronutrient that is involved in various physiological processes in plants, including defense against disease. It has been shown that the application of zinc can reduce the incidence and severity of HLB in citrus trees (Habiba *et al*., 2018). Boron is another important micronutrient that plays a crucial role in the health of citrus plants.

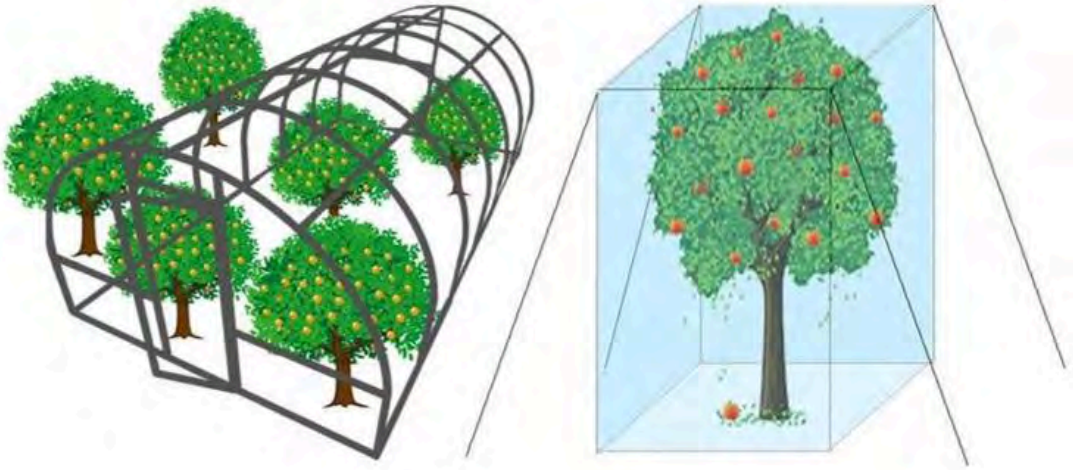
The application of boron can improve nutrient absorption, strengthen cell walls, and increase resistance to stress, which can help plants better tolerate HLB (Gilani *et al*., 2018). Calcium is an essential macronutrient for the structural development and physiological function of citrus plants. The application of calcium can improve the integrity of cell walls and increase resistance to stress, which can help plants better tolerate HLB (Ahmad *et al*., 2011; Gilani *et al*., 2018; Zhang *et al*., 2018; Killiny *et al*., 2019).

Temperature, a factor in reducing the incidence of HLB

Thermotherapy acts on the bacterium *Candidatus* spp. in two main ways: cell damage: elevated temperatures can damage the cell membrane of the bacterium, leading to the loss of its ability to reproduce and survive; and the denaturation of enzymes: enzymes essential for the metabolism of the bacterium are denatured when exposed to elevated temperatures, which interrupts their functioning and leads to the death of the bacterium (Fan *et al*., 2016; Hussain *et al*., 2018). Abdulridha *et al*. (2018) indicated that continuous thermal exposure at 55 °C was sufficient to eliminate or significantly reduce the titer of *Candidatus* spp. in citrus seedlings affected by HLB; in contrast, Yang *et al*. (2016) mentioned that in healthy two-year-old plants of *Citrus paradisi* Macfad infected with *Candidatus* spp. exposed to temperatures of 40 °C, 42 °C or 45 °C in growth chambers at a constant relative humidity of 85% and alternating (Figure 4).

DOI: https://doi.org/10.29312/remexca.v15i8.3600

Figure 4. Structures for thermotherapy treatments in HLB-infected trees.



Conclusions

Currently, methods of controlling the bacteria are effective, but they do not guarantee that the plant is free from contracting the disease again, so the application of antibiotics, integrated nutrient management, and thermotherapy do not guarantee trees completely free of the pathogen.

The attack and spread of the bacterium is worrying, particularly in Mexico, added to the lack of or little research to control the disease in the plant, so the disease continues to spread, decreases production considerably and with it, the economic losses are significant. It is necessary to continue with the research work to determine the best control method focused on the control of the bacterium and the vector, promoting the use of tolerant and resistant cultivars to this disease.

Bibliography

1 Abdulridha, J. A.; Ampatzidis, Y. C.; Ghaterhsamani, S. S. and Ehsani, R. R. 2018. Mobile thermotherapy system for treating HLB-infected *citrus* trees utilizing hot water and steam. Assabe. 1800513(2018):1-7. Doi: 10.13031/aim201800513.

2 Ahmad, K. K.; Sijam, K. K.; Hashim, H. H.; Rosli, Z. Z. and Abdu, A. A. 2011. Field assessment of calcium, copper and zinc ions on plant recovery and disease severity following infection of Huanglongbing (HLB) disease. Afr. J. Microbiol. Res. 5(28):967-4979. Doi: 10.5897/AJMR11.236.

3 Archer, L. L.; Kunwar, S. S.; Alferez, F. S.; Batuman, O. O. and Albrecht, U. U. 2023. Trunk injection of oxytetracycline for huanglongbing management in mature grapefruit and sweet orange trees. Phytopathology. 113(6):1010-1021. https://doi.org/10.1094/PHYTO-09-22-0330-R.

4 da-Graça, J. V.; Dohuan, G. W.; Halbert, S. E.; Keremane, M. L.; Lee, R. F.; Vidalakis, G. A. and Zhao, H. L. 2016. Huanglongbing: an overview of a complex pathosystem ravaging the world's citrus. J. Integr Plant Biol. 58(4):373-387. Doi: 10.1111/jipb.12437.

5 Fan, G. C.; Xia, Y. l.; Lin, X. J.; Hu, H. Q.; Wang, X. D.; Ruan, Ch. Q.; Lu, L. M. and Liu, B. 2016. Evaluation of thermotherapy against Huanglongbing (*Citrus* greening) in the greenhouse. J. Integr Agri. 15(1):11-119. Doi: 10.1016/S2095-3119(15)61085-1.

6 Ghosh, D. K.; Kokane, S. S.; Kumar, P. P.; Ozcan, A. A.; Warghane, A. R.; Motghare, M. F.; Santra, S. S. and Sharma, A. K. 2018. Antimicrobial nano-zinc oxide-2S albumin protein

DOI: https://doi.org/10.29312/remexca.v15i8.3600

formulation significantly inhibits growth of "*Candidatus* Liberibacter asiaticus" in plants. Plos One. 13(10):1-20. Doi: 10.1371/journal.pone.0204702.

7 Gilani, K. A.; Nas, S.; Aslam, F. and Gurley, W. 2018. A comparison of zinc, phosphorous and potassium levels in leaves and fruit pulp of healthy and Huanglongbing affected *Citrus* cultivars. J. Plant Physiol Pathol. 7(1):1-8. Doi: 10.4172/2329-955X.1000192.

8 Habiba, H. M. F.; Uddin, J. B. G.; Lisa, A. K.; Islam, M. A. and Sikdar, B. 2018. Identification and molecular characterization of *Candidatus* Liberibacter asiaticus (*Citrus* Huanglongbing disease pathogenic agent) and its control by plant extracts in Bangladesh. Eur. J. Med. Plants. 23(2):1-13. Doi: 10.9734/EJMP/2018/40298.

9 Huang, M. A.; Roose, M. L.; Yu, Q. Q.; Du, D. S.; Yu, Y. Y.; Zhang, Y. C.; Deng, Z. C.; Stover, E. S. and Gmitter, F. G. 2018. Construction of high-density genetic maps and detection of qtls associated with Huanglongbing tolerance in *Citrus*. Front. Plants Sci. 9(1694):1-16. Doi: 10.3389/fpls.2018.01694.

10 Hussain, M. A.; Akutse, K. S.; Lin, Y. Y.; Chen, S. S.; Huang, W. W.; Zhang, J. J.; Idrees, A. A.; Qiu, D. D. and Wang, L. L. 2018. Susceptibilities of *Candidatus* Liberibacter asiaticus-infected and noninfected *Diaphorina citri* to entomopathogenic fungi and their detoxification enzyme activities under different temperatures. Microbiol. Open. 7(6):e00607-1-5. Doi: 10.1002/mbo3.607.

11 Killiny, N. A.; Etxeberria, E. E.; Ponce, F. A.; González, B. P.; Flores, R. T. and Ponce, C. L. 2019. Laser-induced breakdown spectroscopy (LIBS) as a novel technique for detecting bacterial infection in insects. Sci. Rep. 9(2449):1-7. Doi: 10.1038/s41598-019-39164-8.

12 Li, J. J.; Li, L. L.; Pang, Z. Z.; Kolbasov, V. V.; Eshani, R. E.; Carter, E. W. and Wang, N. W. 2019. Developing *citrus* Huanglongbing management strategies based on the severity of symptoms 2 in HLB-endemic citrus-producing regions. Phytopathol. 109(4):582-592. Doi: 10.1094/phyto-08-18-0287-r.

13 Liu, X. L.; Zheng, Y. Y.; Wang-Pruski, G.; Gan, Y. U.; Zhang, B. X.; Hu, Q. A.; Du, Y. A.; Zhao, J. Z. and Liu, L. A. 2019. Transcriptome profiling of periwinkle infected with Huanglongbing ('*Candidatus* Liberibacter asiaticus'). Eur. J. Plant Pathol. 153(3):891-906. Doi: 10.1007/s10658-018-01607-9.

14 Pereira, F. M. V.; Milori, D. M. B. P.; Pereira-Filho, E. R.; Venâncio, A. L.; Russo, M. D. S. T. and Cardinali, M. C. D. B. 2011. Laser-induced fluorescence imaging method to monitor citrus greening disease. Comput. Electron. Agric. 79(1):90-93. Doi: 10.1016/j.compag.2011.08.002.

15 Robles, G. M. M.; Orozco, S. M.; Manzanilla, R. M. A.; Velázquez, M. J. J.; Medina, U. V. M. and Sánchez, S. E. 2018. Experiencias con huanglonbing en limón mexicano en el estado de Colima, México. *Citrus* Res. Technol. 39(1):2.12. Doi: https://Doi.org/10.4322/crt.16518.

16 Robles-González, M. M.; Orozco-Santos, M.; Manzanilla-Ramírez, M. A.; Velázquez-Monreal, J. J. y Carrillo-Medrano, S. 2017. Efecto del HLB sobre el rendimiento de limón mexicano en Colima, México. Revista Mexicana de Ciencias Agrícolas. 8(5):1101-1111. Doi: https://doi.org/10.29312/remexca.v8i5.111.

17 Shimwela, M. M.; Schubert, T. S.; Albritton, M. M.; Halbert, S. E.; Jones, D. J.; Sun, X. X.; Roberts, P. D.; Singer, B. H.; Lee, W. S.; Jones, J. B.; Ploetz, R. C. and Bruggen, A. H. C. 2018. Regional spatial temporal spread of citrus huanglongbing is affected 1 by rain in Florida. Phytopathol. 108(12):1420-1428. Doi: 10.1094/PHYTO-03-18-0088-R.

18 Shin, K. A.; Ascunce, M. S.; Narouei-Khandan, H. A.; Sun, X. X.; Jones, D. D.; Kolawole, O. O.; Goss, E. M. and Van Bruggen, A. H. C. 2016. Effects and sites and effects of penicillin injection in Huanglongbing affected grapefruits trees. Crop Prot. 90(1):106-116. Doi: 10.1016/j.cropro.2016.08.025.

DOI: https://doi.org/10.29312/remexca.v15i8.3600

19 Slinsky, S. L. 2016. The use of bactericides in plant agriculture with reference to use in *Citrus* to mitigate HLB. bactericide project manager, *Citrus* Research and Development Foundation Inc. 1-5 pp.

20 Song, X. X.; Peng, A.; Cui. Y. Y.; Ling, J. J.; Cheng, B. B. and Zhang, L. L. 2018. Characterization and pathogenicity of a *Paecilomyces variotii* isolate infecting the Asian *Citrus* psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), in China. Int. J. Pest Manag. 66(1):1-6. Doi: 10.1016/j.jip.2008.03.007.

21 Vincent, C. I.; Hijaz, F. F.; Pierre, M. M. and Killiny, N. N. 2022. Systemic uptake of oxytetracycline and streptomycin in huanglongbing-affected *citrus* groves after foliar application and trunk injection. Antibiotics (Basel, Switzerland). 11(8):1092-101 https://doi.org/10.3390/antibiotics11081092.

22 Yang, C. C.; Powell, C. A.; Duan, Y. V.; Shatters, R. G.; Lin, Y. T. and Zhang, M. W. 2016. Mitigating citrus huanglongbing via effective application of antimicrobial compounds and thermotherapy. Crop Prot. 84(1):150-158. Doi: 10.1016/j.cropro.2016.03.013.

23 Zhang, M. D; Guo, Y. F.; Powell, C. A.; Doud, M. S.; Yang, C. S. and Duan, Y. B. 2014. Effective antibiotics against '*Candidatus* Liberibacter asiaticus' in HLB-affected *citrus* plants identified via the graft-based evaluation. Plos One. 9(11):1-11. Doi: 10.1371/journal.pone.0111032.

24 Zhang, M. C.; Powell, C. A.; Zhou, L. B.; He, Z. S.; Stover, E. S. and Duan, Y. S. 2011. Chemical compounds effective against the citrus Huanglongbing bacterium '*Candidatus* Liberibacter asiaticus' in planta. Phytopathol. 101(9):1097-1103. Doi: 10.1094/PHYTO-09-10-0262.

25 Zhang, M. A.; Yang, C. Y.; Powell, C. A.; Wang, J. L.; Huang, Y. A. and Avery, P. B. 2018. Field evaluation of integrated management for mitigating *Citrus* Huanglongbing in Florida. Front. Plant Sci. 9(1890):1-24. Doi: 10.3389/fpls.2018.01890.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i8.3600

Antibiotics, nutrients and thermotherapy as control methods for Huanglongbing (HLB)

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 November 2024
Date accepted: 01 December 2024
Publication date: 06 January 2025
Publication date: Nov-Dec 2024
Volume: 15
Issue: 8
Electronic Location Identifier: e3600
DOI: 10.29312/remexca.v15i8.3600

Categories

Subject: Research note

Keywords:

Keywords:

Candidatus Liberibacter

Diaphorina citri

Huanglongbing

Counts

Figures: 4

Tables: 0

Equations: 0

References: 25

Pages: 0