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Risk Assessment of Ozone Fumigation Under Vacuum to Control Potential Infestation of Coffee Berry Borer and Coffee Leaf Rust in Green Coffee Beans Imported Into Hawaii

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Abstract

Studies were conducted with ozone gas fumigation under vacuum as a methyl bromide alternative against life stages of coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), and the urediniospores of coffee leaf rust (CLR), *Hemileia vastatrix* Berkeley & Broome (Basidiomycota: Pucciniales) in green coffee, *Coffea spp*. L. Fumigation with 10,000 ppm O_3 gas under –25.4 mm Hg vacuum1 at 13.0 ± 3.0°C for 6.0 h killed all CBB larvae, pupae, and adults, but did not kill all CBB eggs (~15% survival). Mortality of CLR urediniospores was 100% within the first hour of the 6-h fumigation. Ozone fumigation had no adverse effects on coffee quality. Results indicated that CBB adult hitchhikers may be the only target life stage of quarantine concern, and additional studies focused on this stage. CBB adult survival and reproduction decreased significantly at moisture contents ≤20%, and F_1 generation survival did not occur in green coffee at moisture contents ≤15%. As the international standard for green coffee moisture content is 9–12%, adult CBB should not survive or reproduce in exported dry green coffee. Standard industry processing of harvested coffee cherries to the green coffee stage using either mechanical- or sun-drying eliminated CBB infestations from the field. A systems approach is recommended for exporting green coffee to control CBB and CLR that includes eliminating CBB life stages with standard processing methods, reducing moisture content to 9–12% to prevent egg deposition, survival or reproduction, and O_3 fumigation to ensure quarantine security against potential CBB adult hitchhikers.

Key words: ozone, disinfestation, phytosanitary treatment, fumigation, quarantine pest

Coffee berry borer, (*Hypothenemus hampei* Ferrari) (Coleoptera: Curculionidae: Scolytinae), and coffee leaf rust, (*Hemileia vastatrix* Berk. & Broome) (Pucciniales), the two most destructive pests of coffee (*Coffea arabica*L.) (Gentianales: Rubiaceae) production worldwide, are found in all coffee-growing areas including Puerto Rico and Hawaiian Islands (Portilla and Grodowitz 2018). The most

recent introduction of coffee berry borer was reported in Oceania (Papua New Guinea) (ICO 2017–2018), which seriously threatens Australia's coffee industry. The most recent introduction of coffee leaf rust was reported in October 2020 in Hawaii (hdoa.hawaii.gov). Coffee is the world's most important agricultural commodity, with an estimated retail value of 70 billion U.S. dollars (Talhinhas et al. 2017). It is crucial for the economy of more than 60 countries and the main source of income for more than 100 million people (Hoffmann 2014; ICO 2016). The economic damage to coffee caused by coffee berry borer results from infested immature berries that drop to the ground; weight loss in beans processed from berries that do not drop caused by adult and larval tunneling and feeding (resulting in lower production yields by weight); damaged beans containing frass, mold, and remnants of the brood killed during processing that adversely affect organoleptic properties (coffee flavor and aroma); increased susceptibility to invasion by secondary pathogens that further degrade the beans; and conspicuous damage that results in rejection of beans for export and decreased value (Hernandez-Paz and Sanchez De-Leon 1972). Coffee berry borer damage to a bean in a coffee berry results in a distinctive blue-green staining that significantly reduces its market value (McNutt 1975, Waterhouse and Norris 1989).

Talhinhas et al. (2017) noted that coffee leaf rust causes losses of one to two billion U.S. dollars annually and is one of the main limiting factors of Arabica coffee production worldwide. Coffee leaf rust damage consists of reduced photosynthetic capacity of infected leaves and premature defoliation (leaf drop) associated with high infection levels, reduced vegetative and berry growth, reduced berry size, and eventual starvation of roots and shoots resulting in dieback with increasing losses of production each consecutive year ranging from 30 to 80% in major epidemics and about 15% annually under normal infestation conditions (Becker et al. 1975; Becker and Kranze 1977; Kushalappa and Eskes 1989a, b). In some cases, coffee leaf rust can kill the coffee plant after heavy, chronic infestation (Steinman 2001).

Coffee berry borer survival and reproduction are dependent on a high moisture environment (Portilla and Street 2006, 2008), and an extended dry season can reduce coffee berry borer infestations due to its sensitivity to humidity levels (Baker et al. 1994). Most studies identify coffee berry borer as a field pest, not a stored product pest (Haarer 1962, 1970; Hernandez-Paz and Sanchez De-Leon 1972; Baker 1984; Baker et al. 1989, 1992, 1994), as processing results in a moisture content too low for coffee berry borer to reproduce or its life stages to survive (Waterhouse and Norris 1989). Coffee berry borer cannot feed on green coffee and cannot survive at a moisture content <30% (Trujillo et al. 1995), and hence it typically does not attack coffee after the beans have been processed to the green coffee stage. Coffee processing is the single most important factor in eliminating all coffee berry borer life stages and results in green coffee devoid of the pest (originating from infested berries) before export (Haarer 1962). Hernandez-Paz and Sanchez De-Leon (1972) reported that coffee berry borer life stages could survive the wet-processing of coffee until the crop undergoes dry-processing in mechanical dryers or on cement floors exposed to the sun. Adults of coffee berry borer can pose a quarantine risk, however, as a hitchhiker in green coffee imports, on the bags of coffee, or in shipping containers.

Coffee companies in Hawaii import green coffee beans from the U.S. mainland for blending and roasting with locally grown coffee, and this imported green coffee (25.5 million lb in 2017–2018) is the foundation for the roasting, blending, and brewing sectors of the Hawaii coffee industry. Green coffee is not shipped directly to Hawaii from the coffee growing and exporting areas of the world, but instead is shipped to ports on the U.S. mainland, where it is fumigated with methyl bromide or treated with moist heat to kill potential infestations of coffee berry borer life stages and coffee leaf rust urediniospores. However, neither of those treatments as currently proposed (USDA-APHIS 2006) is efficacious against these two major pests of concern that currently are devastating coffee farms across Hawaii.

Ozone is a highly oxidizing gas with insecticidal activity, and it is a potential alternative to conventional fumigants such as methyl bromide as a quarantine treatment for controlling surface pests on fresh horticultural commodities (Leesch et al. 2007, Hollingsworth and Armstrong 2005, Mahroof et al. 2018) and for dry agricultural products where free moisture is not an issue (Leesch 2003). The ozone molecule (O_3) has been given "generally recognized as safe" (GRAS) status and is approved by the USDA for processing of organic foods (Food and Drug Administration 2001). Ozone is highly reactive and damages the cell membranes of organisms by causing oxidative stress. Vitali and Valdenassi (2019) mentioned that ozone can oxidize with all kinds of materials, and the percentage of inactivation for bacteria, fungus, and virus can be total. At levels that kill insects, damage varies greatly by commodity and treatment conditions (Hollingsworth and Armstrong 2005, Mahroof et al. 2018, Lemic et al. 2019). Coffee berry borer is like a surface pest because it bores into the coffee berry and tunnels in the seed, and therefore it is exposed to the same atmosphere as occurs outside the seed.

We report here the results of fumigation efficacy studies using 10,000 ppm O_3 under −25.4 mm Hg vacuum at 13.0 ± 3.0°C for 6.0 h of O_3 to control coffee berry borer life stages and coffee leaf rust urediniospores, studies to determine the effects of O_3 fumigation on the organoleptic properties of green coffee, studies to elucidate the effects of green coffee moisture level on coffee berry borer survival and reproduction, and a pest risk analysis to determine the impact of coffee processing on coffee berry borer infestation in coffee berries that are processed and dried to green coffee beans.

Materials and Methods

Ozone Application

A commercial fumigation technology, called Ozofume (http://www.ozofume.com), that was developed by Tahoe Food Technology, Inc. (Sparks, NV) was used in this investigation. An experimental 31.6-liter fumigation chamber built by Tahoe Food Technology to study the potential use of O_3 fumigation with or without vacuum was used in all tests. The experimental chamber was fitted with a corona discharge O_3 gas generator (Model CD12, Clearwater Tech Inc., San Luis Obispo, CA) and an O_3 gas analyzer (Model HA-100-GTP-12, Ozocan Corp., Scarborough, Ontario, Canada) used to monitor and maintain the selected O_3 gas set-point (e.g., 10,000 ppm by volume in air).

All research with coffee berry borer and coffee leaf rust had to be done outside of Hawaii because, at that time, the quarantine status of the target pests and the potential danger of in-state research to the Hawaii coffee industry was in place. Therefore, ozone fumigation studies with coffee berry borer were carried out at ARS-San Joaquin Valley Agricultural Sciences Center, Parlier, CA; coffee leaf rust studies were carried out by the Tahiti Department of Plant Protection, Ministry of Agriculture, Livestock, and Forestry, Pape'ete; green coffee moisture content levels on coffee berry borer survival and reproduction were done in cooperation with ARS-Jamie Whitten Delta States Research Center, Stoneville, MS; and coffee berry borer pest-risk analysis

for processed green coffee was done in cooperation with the University of Florida Tropical Research and Education Center, Homestead, FL, and Cenicafé, Chinchiná, Colombia. The coffee quality test using roasted coffee, i.e., with beans free of any coffee berry borer or coffee leaf rust, was done in cooperation with the University of Hawaii at Manoa, Department of Tropical Plant and Soil Sciences.

Based on preliminary experimental data and O_3 fumigation reports in the literature (e.g., Weavers and Wickramanayake 2001), a fumigation schedule consisting of 10,000 ppm O_3 under –32 mm Hg vacuum at 13.0 ± 3.0°C for 6.0 h was selected for testing against coffee berry borer and coffee leaf rust. The O_3 concentration, amount of vacuum, and fumigation time were selected with the belief that it would control coffee berry borer life stages but without knowing its potential efficacy against the more tolerant coffee leaf rust urediniospores. Available literature indicated that coffee leaf rust urediniospores would be very difficult to kill based on known chemical and fungicidal resistance (Kushalappa and Eskes 1989a). Choosing a single O_3 treatment schedule allowed for more extensive testing of important biological features such as efficacy against different coffee berry borer life stages and the effects on quality of multiple coffee varieties, as well as non- O_3 studies on green coffee moisture content and coffee berry borer survival.

Coffee Quality Tests

Two independent coffee quality tests were done to determine whether fumigation at 10,000 ppm O_3 under –25.4 mm Hg vacuum at 13.0 ± 3.0°C for 6.0 h adversely affected the organoleptic properties of green coffee that was roasted and brewed after fumigation. Green coffee processed from berries harvested from plantations in Hawaii was used to test coffee types grown in the state, and green coffee representing the broad range of coffee types imported into the U.S. were used to test samples of green coffee imported into Hawaii for blending and roasting with Hawaii-grown coffees. Additional samples of green coffees representing the broad range of coffee types were fumigated using the standard methyl bromide fumigation schedule for green coffee imported into Hawaii to test for quality differences between O_3-fumigation versus methyl bromide-fumigation. Hawaii-grown coffees were used only as an initial test subject for quality.

O_3 Quality Tests with Hawaii-Grown Green Coffees

Green coffee beans (1.0 kg each) of the two major coffee cultivars grown in Hawaii, 'Yellow Caturra' and 'Red Catuai' were obtained from commercial growers and brought to USDA PBARC (Hilo, Hawaii). One-half (500 g) of each coffee cultivar sample was fumigated with 10,000 ppm O_3 under –25.4 mm Hg vacuum at 13.0 ± 3.0°C for 6.0 h. The other one-half (500 g) of each coffee cultivar sample was not treated (control). Treated and control samples were shipped by air within 24 h for quality tests to observe for significant differences in flavor or aroma. For this blind test, a group of professional coffee tasters from Royal Coffee were used as in the Triangle Test evaluations.

The treated and untreated green beans were roasted to a medium roast, ground to a "filter" coffee grind, and brewed in a Braun coffee maker. Each brewed coffee sample was held in a sterile stainless-steel thermos until samples of the brewed coffee were poured for individual panelists. In each set of samples, the treated coffee was compared by the panelists with an untreated control. A Triangle Test (Gacula and Singh 1984) was used to determine whether the coffees were significantly different from each other. In the Triangle Test, each panelist received either two control samples and one treated sample or two treated samples and one control sample. Each sample was coded, and samples were randomly presented to each panelist. The panelists were asked to pick out the one sample that was different from the other samples based on difference of flavor and/or aroma. Panelists consisted of Ad hoc volunteer professional or student food technologists for each Triangle Test comparison, and each Triangle Test comparison was replicated six times using the same panelists.

O_3 and Methyl Bromide Quality Tests with Green Coffees Imported into the U.S.

Green beans of 'Kenyan', and 'Mexican', both washed (wet-processed) coffees, 'Ethiopian', an unwashed (dry-processed) coffee, and Sumatran a semi-washed coffee, were selected on the basis of quality, distinctive flavor, and representation of the wide range of green coffee beans currently available in the marketplace. The green coffees used in the quality tests, with the exception of Mexican (Chiapas), were grown using standard production methods, which may or may not have included the use of pesticides.

Eighteen samples (4.5 kg) of each coffee variety (Kenyan, Mexican, Ethiopian, and Sumatran) were shipped by courier service from Royal Coffee, Emeryville, CA to Sparks, NV where they were fumigated by Tahoe Food Technology with either 10,000 ppm O_3 at –30.5 cm Hg of vacuum at 13. 0 ± 2.0°C for 6.0 h (6 samples), or 48 mg/liter methyl bromide for 8-h at ambient temperature and relative humidity and normal atmospheric pressure (6 samples) in a commercial O_3-fumigation chamber or MB-fumigation chamber, respectively. Six samples (4.5 kg) of each coffee variety were held as untreated controls. The moisture content of each sample (treated and control) was measured before and after fumigation to determine whether the fumigation affected moisture content.

The treated and controls samples were returned to Royal Coffee for nondestructive observation for changes in color, texture, or odor, as well as any other obvious differences between treated and untreated beans. The treated and control samples were stored under commercial conditions for 3 or 6 wk before roasting, brewing, and sensory evaluation. After that, 2.3-kg subsamples of treated and control green beans for each coffee variety were roasted identically in a small commercial coffee roaster. During roasting, beans were observed for noticeable changes in appearance, aroma, and other roasting characteristics.

For the sensory evaluation test, the treated and control coffee beans were ground identically and used in Triangle Tests to observe for significant differences in flavor or aroma. Triangle Tests were done separately for each treated and control coffee type (Kenyan, Mexican, Ethiopian, or Sumatran) in a research sensory evaluation analysis room with separate booths for each panelist. Lighting in the room was red to mask any color differences so panelists would compare differences only in flavor or aroma. Seven or eight professional coffee tasters from Royal Coffee were used as in the Triangle Test evaluations.

In the Triangle Tests, each panelist received three 147-ml drinking glasses containing coffee that was brewed from 8.0 g of ground coffee beans mixed with 130 ml boiling water and then allowed the coffee to be cooled between 48 and 60°C at room temperature. Allowing the brewed coffee to cool to room temperature kept potential differences from being masked by heat. In each Triangle Test for each coffee type, two drinking glasses contained treated coffee and one drinking glass contained untreated coffee, or two drinking glasses contained untreated coffee and one drinking glass contained treated coffee. Panelists were required to select the coffee that was different (treated or untreated), and then rate the quality factors for making their selection. The Triangle Tests for each coffee type was

replicated three times. The assessment sensory evaluation was determined based on acidity, body, and flavor.

Triangle Test results were analyzed for statistical differences in the panelists' ability to identify O_3-treated coffee (i.e., the probability that the panelists could distinguish between the treated and control coffee more times than by chance alone), and for the sensory acceptability of the coffee (i.e., did the sensory difference, if a difference was perceived by the panelist, reduce the coffee quality and marketability).

Statistical Analysis for Coffee Quality Test

A generalized linear mixed model was used for the analysis, using SAS PROC GLIMMIX (SAS 2007). This method was chosen in order to handle the binomial response variable ("correct" or 1 versus "incorrect" or 0) from a judge correctly identifying the mismatched observation on a given replication in a triangle tasting. Coffee variety and weeks stored were fixed effects in one trial and treatment comparison and weeks stored in the other. In both cases, judges and replications were treated as random effects. The Kendall Rogers correction was used to adjust degrees of freedom. Estimates of the proportion of correct ratings were also obtained along with 95% confidence intervals for each combination of week and variety or treatment. Since a probability of 1/3 is expected from guessing correctly, a test was performed for the null hypothesis of 1/3 or less correct versus the alternative of greater than 1/3 correct. Since six tests were being performed at a time, a Sidak adjustment for multiplicity was used.

O_3 Fumigation Against Coffee Berry Borer

To determine the efficacy of O_3 to control coffee berry borer, efficacy tests were performed at USDA SJVASC (Parlier, CA) with coffee berry borer eggs, larvae, pupae, or adults treated with 10,000 ppm O_3 under −30.5 cm Hg of vacuum at 13.0 ± 2.0°C for 6.0 h in an experimental 31.6-liter Ozofume fumigation chamber provided by Tahoe Food Technology. Coffee berry borer for fumigation efficacy tests were received biweekly from the rearing-laboratory colony operated by the Biological Control of Pests Research Unit (BCPRU) USDA-ARS-JWDSRC located at Stoneville, MS. Additionally, BCPRU also provided wet parchment coffee beans Castillo variety at 45% moisture content for infestation by coffee berry borer for use in the efficacy fumigation tests.

Green parchment coffee beans were infested with coffee berry borer females at a rate of three females per bean by placing the beans in a single layer (suture side up) in small (50.0 mm diameter × 14.0 mm depth) (20 beans) and large plastic petri dishes (85.0 mm diameter × 14.0 mm depth) (60 beans). Each petri dish had a 5- to 10-mm diameter hole that was covered with screen to allow air exchange. The outer edge of the screen was sealed with Parafilm (Pechiney Plastic Packaging Company, Asheville, NC) to prevent coffee berry borer from escaping while maintaining the humid environment within the dish. Four to six of large Research dishes were prepared for each test and fumigated (treated insects). Two of the smaller Research dishes were prepared for each test but were not fumigated (untreated control insects). The untreated controls were used to estimate the number of coffee berry borer of each life stage in the treated petri dishes. The infested beans were held at 24 ± 1°C and 70% RH for predetermined times necessary to obtain the target developmental stages for testing. The developmental times were 7 to 14 d, 22 to 25 d, or 30 to 35 d to obtain the larval, pupal, or adult stages, respectively. Although some eggs were always present, the egg stage was not a targeted stage in this study.

Test insects were pre-conditioned overnight to the treatment temperature (13 ± 1°C) before they were fumigated with 10,000 ppm O_3 gas under −30.5 cm Hg vacuum for 6.0 h at 13 ± 3°C. The fumigation efficacy test was repeated until a minimum estimated treated population ≥35,000 coffee berry borer at each developmental stage (larvae, pupae, and adults) were treated, observed, and evaluated. This required 22 separate fumigations over a 13-month period.

Treated and control insects were held at 27 ± 1°C and 60 ± 5% RH after each fumigation was completed, and mortality evaluations were made 1 to 7 d after treatment. Each treated and control coffee bean was thoroughly dissected under a microscope at ≥10x magnification and the numbers of coffee berry borer survivors at each life stage were recorded. Insects were considered dead when no movement was observed after prodding. Observations of the controls were done on the same day as the treatment or 1 d after treatment to determine the estimated number of each coffee berry borer life stage in the treatment.

O_3 Fumigation Against Coffee Leaf Rust

To determine the efficacy of O_3 to control coffee leaf rust uridiniospores, tests were performed in Pape'ete, Tahiti consisting of fumigating pieces of coffee leaves infected with mature coffee leaf rust lesions with 10,000 ppm O_3 under −30.5 cm Hg of vacuum at 13. 0 ± 2.0°C for 6.0 h. Heavily infested leaves with coffee leaf rust were harvested from backyard coffee plants in Pape'ete and brought to the laboratory where they either were used immediately in fumigation tests or stored in closed plastic bags in darkness at 24 ± 2.0°C to maintain urediniospore viability for use in fumigation tests within 24 h. Infected leaves that were >24-h old were replaced with fresh materials to ensure maximum urediniospore viability.

Four to five infected coffee leaves that contained many lesions with mature urediniospores were cut into pieces with a sterile scalpel blade. One-half of the leaf pieces were randomly selected and placed in plastic petri dishes for fumigation. The remaining leaf pieces were held as controls (untreated) in petri dishes until the fumigation was completed.

Efficacy tests were performed using an experimental 31.6-liter Ozofume fumigation chamber provided by Tahoe Food Technology. The petri dishes containing leaf pieces for fumigation were placed inside the fumigation chamber and treated with 10,000 ppm O_3 under −30.5 cm Hg of vacuum at 13. 0 ± 2.0°C for 15.0, 22.5, 30.0, 45.0, 60.0, or 300. 0 min. Fumigation tests at each selected time were replicated three times.

After the fumigation, urediniospores were harvested from lesions on the treated or control pieces and their germination enumerated by methods similar to those described by DeJong et al. (1987). A sterile scalpel blade was used to collect ≈ 1 mg samples of urediniospores from each lesion. The urediniospore samples were suspended in 50 µliters of sterile distilled water, from which a sample containing ≈ 3,000 urediniospores was placed on a sterile glass microscope slide. The microscope slides were placed on a wetted, expanded-polystyrene sheet (1.0-cm thick) inside a plastic humidification chamber (≈ 0.35m^3) and incubated in darkness at 24 ± 2.0°C. Two or more slides were prepared from each fumigation treatment.

After 24 h, a glass coverslip was placed on each slide containing treated or control urediniospores and the slides were examined by light microscopy at 200 or 400x magnification. Twenty fields each containing 15 to 35 urediniospores were examined and the total numbers of germinated and ungerminated urediniospores were recorded. Treated urediniospores were examined twice, once after 24 h and again after 48 h to account for any O_3-related delay in germination. Urediniospores were recorded as germinated if the germ tube length exceeded the diameter of the urediniospore (Kushalappa and Eskes 1989a, b).

Green Coffee Moisture Content Studies

Parchment Coffee Beans as a Rearing Media

Two separate studies were performed to determine the effects of green coffee moisture content on the survival and reproduction of coffee berry borer.

Experiment 1

Two varieties of parchment coffees (*C. arabica*) were used in the bioassays. Parchment beans were shipped by air from their respective production and processing areas of Cenicafé, Chinchiná, Colombia (Castillo), or Kona, Hawaii (Red Catuai). The coffee from Colombia was wet parchment with a moisture content of 45%, and the coffee from Hawaii was dry parchment with a moisture content of 10%. Both the wet and dry parchment coffees were treated by 24-h immersion in a 0.2% Benomyl (*syn*. Benlate, Du Pont Chemical, Wilmington, DE) solution (20.0 g Benomyl/10. 0 l of sterile distilled water) to prevent mold and mildew formation on the beans that could interfere with coffee berry borer survival and reproduction. After the Benomyl treatment, the treated beans underwent dehydration (wet parchment) or further rehydration (dry parchment).

Experiment 2

Aliquots of the Benomyl-treated wet parchment coffee from Colombia were dehydrated to obtain beans with selected moisture content of 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, or 45.0% using an incubator (Isodem 500 Series, Model 516 D, Fisher Scientific, Pittsburg, PA) set at 50°C. Aliquots of the Benomyl-treated dry parchment coffee from Hawaii were soaked in sterile distilled water for ≈3 d until the beans were rehydrated to a moisture content of 45%, and then dehydrated identically to the wet parchment beans from Colombia to obtain 15, 20, 25, 30, 35, or 40% moisture content. The respective drying or water-soaking and drying processes resulted in parchment beans from Colombia or Hawaii with moisture content of 10, 15, 20, 25, 30, 35, 40, or 45%. Samples consisting of five beans were selected randomly from each aliquot and the moisture content was measured with a Denver IR-50 moisture analyzer (Denver Instruments, Denver, CO). Dehydrating wet parchment and rehydrating dry parchment to the selected moisture content allowed comparison of the two methods of obtaining the moisture content to ensure that the method used did not impact coffee berry borer survival or reproduction.

Insect Colonies

Coffee berry borer females used in all tests were reared according to the methods described by Portilla (1999) using the modified Cenibroca diet (Portilla and Streett, 2006) in 32-cell rearing trays (14.6 cm wide by 55.1 cm long by 1.1 cm deep) with a round bottom (Tillman et al. 1997), which were modified and molded in the laboratory by using polyvinyl chloride plastic (15.0-cm wide × 20.0-mm gauge) (American Mirrex Corp., Delaware City, DE). Active recently emerged coffee berry borer female adults were used to infest the parchment coffee beans used in the moisture content survival and reproduction tests.

Effects of Green Coffee Moisture Content on Coffee Berry Borer Survival and Reproduction

Coffee beans (n = 200) for each moisture content were placed in individual clear plastic rearing containers (15.0-cm diameter by 2.5-cm deep, Pioneer Plastic #170-C, Eagan, MN) secured with a lid that had a single hole (0.5-cm diameter) covered with fine muslin fabric to prevent coffee berry borer adults from escaping while maintaining the moisture content level (Portilla and Streett 2007, 2008). Coffee berry borer adult females (n = 800) were added to each plastic rearing container and the containers were held at 25°C, 75% RH, and a 0:24 h (L:D) photoperiod for 8, 30, or 45 d before observations were made to monitor for coffee berry borer survival and reproduction. Eight days were allowed to elapse from the time the infestation of beans at each moisture content began to the first observation to provide adequate time for the coffee berry borer females to begin laying eggs. Two additional containers of Columbian parchment coffee at 45% moisture content were infested with 200 females as checks to assess coffee berry borer quality, viability, and fecundity.

Eight, 30, or 45 d after infestation, 50 coffee beans were randomly selected from each moisture content treatment and dissected under a dissecting microscope (6.3x magnifications) to observe for number of infested beans and the presence and number of coffee berry borer life stages. Eggs were identified as viable or dead based on the presence of embryonic development or lack thereof, using the dissecting microscope. Uninfested beans in each sample were used to determine that the moisture content had been maintained during the holding period.

Any coffee berry borer frass and/or coffee dust created by foundress female boring activity was left undisturbed in the rearing containers after the 50-bean samples were removed for the 8- or 30-d observations. Removal of the 50-bean samples left 150 beans after the 8-d observations and 100 beans after the 30-d observations. However, proportional numbers of coffee berry borer females were not removed, which resulted in an increasing coffee berry infestation pressure as time progressed to the final observation at 45 d. Therefore, the test was biased toward coffee berry borer survival and reproduction, especially at the 30- and 45-d sample observations. In this study, coffee berry borer survival and reproduction tests at 10, 15, 20, 25, 30, 35, 40, or 45% moisture content with observations at 8, 30, and 45 d were replicated three times.

Effects of Green Coffee Moisture Level on Coffee Berry Borer F_1 Generation Survival

A second study was performed to determine the effect of moisture content on coffee berry borer reproduction and survival when the foundress females were removed (e.g., eliminated by efficacious fumigation) after an adequate period to provide for egg deposition in the coffee beans.

Wet parchment and dry parchment beans were dehydrated or water-soaked and dehydrated, respectively, as previously described to obtain beans at 10, 15, 20, and 25% moisture content. Six of each 25-bean aliquots were taken from each moisture content and placed in individual plastic rearing containers. One hundred female coffee berry borer adults were added to each rearing container, and the containers were held as previously described for 6 d. Two additional rearing containers for each moisture content were prepared identically except that no coffee berry borer females were added. The uninfested beans were used for monitoring the moisture content at 6 and 30 d.

At 6 d, one uninfested aliquot at each moisture content was used for moisture analysis to ensure that each selected moisture content had been maintained, and all foundress females were removed from each of the infested aliquots. The 6-d holding period provided adequate time for the females to infest the beans with eggs. All infested and uninfested aliquots were held for 30 d as previously described. Removal of the adult females ensured that only F_1 coffee berry borer

would be present in the infested aliquots at the end of the 30-d holding period.

At 30 d, the remaining uninfested aliquots at each moisture content were used to ensure that each selected moisture content had been maintained and all parchment coffee beans in each of the infested aliquots were dissected as previously described to observe for coffee berry borer life stages. In this study, coffee berry borer survival and reproduction tests at 10, 15, 20, or 25% moisture content with observations 30 d after the end of a 6-d infestation period and removal of all female adults were replicated three times.

Statistical Analysis for Coffee Moisture Content Studies

In the first series of tests to determine the effects of moisture content on coffee berry borer survival and reproduction on green coffee at 10, 15, 20, 25, 30, 35, 40, or 45% moisture content, data on untransformed mean numbers of the total number of insects (all life stages) were subjected to ANOVA followed by means separations using a Tukey HSD test ($P = 0.05$). Descriptive statistics were calculated for each life stage at 8, 30 and 45 d after infestation. In the second series of tests to determine moisture content on survival and reproduction of the coffee berry borer F_1 generation 30 d after exposure to green coffee at 10, 15, 20, or 25% moisture content to adult females for 6 d before removing the females, data on untransformed mean numbers of each life stage were subjected to ANOVA followed by means separations using a Tukey HSD test ($P = 0.05$). Descriptive statistics were calculated for each life stage at 30 d after infestation for coffee berry borer populations in the Hawaii-grown or Colombia-grown green coffee. All statistical analyses were performed using SAS system software (SAS Institute 2007, 2013).

Coffee Berry Borer Risk Analysis for processed Green Coffee

To augment the literature and provide a substantial regulatory basis for determining the target coffee berry borer life stages that may be found in green coffee after processing to 9–12% moisture content, the effects of standard commercial wet-processing, including both mechanical and sun drying, on coffee berry borer survival were tested during June to August, 2006, at the Central Experimental Station Naranjal, Cenicafé, Chinchiná, Colombia through a SCA with the University of Florida Tropical Research and Education Center. The Experimental Station Naranjal grew coffee in field plots for experimental use and had a small-scale commercial coffee processing facility.

Coffee used in all tests was Castillo variety, the major coffee variety produced in Colombia due to its resistance to coffee leaf rust (Alvarado and Moreno 2005). To today, after 13 yr, Castillo variety is still resistance both to leaf rust and coffee berry disease and 45% of the Colombia's coffee is Castillo (Perfect Daily Grind 2017). Field samples (25.0 kg) of ripe coffee berries were harvested by hand from ten field plots known to be heavily infested with coffee berry borer. Subsamples (1.0 kg berries) were randomly selected from each 25.0-kg sample and the berries were dissected to determine the estimated population of coffee berry borer eggs, larvae, pupae, and adults in the field samples.

The field samples (now 24.0-kg each) were divided into ten subsamples. Five subsamples were selected for processing using mechanical drying and the remaining five subsamples were used for processing using sun-drying (common method to dry parchment coffee using sun and wind). Each 24.0-kg sample was process individually through a standard washing and flotation process to remove debris, immature berries, and infested berries, followed by depulping in a demucilager machine, sieving, and washing to clean the coffee seed and result in "parchment" coffee.

One-half of the 24.0-kg field samples were sun-dried for 12 h to ≈45% moisture content, resulting in "humid" parchment coffee, from which 1.0 kg was removed from each 24.0-kg sample of humid parchment to observe for coffee berry borer infestation. The remaining 23.0-kg samples were sun-dried for an additional 5 d to a moisture content of 10–12%, resulting in "dry" parchment coffee. A 1.0-kg sample was taken randomly from each 23.0-kg dry parchment sample 1 d after the end of the drying process to observe for live coffee berry borer life stages. The remaining 22.0-kg dry parchment samples held for 30 d before they were used to determine whether coffee berry borer had survived and reproduced in the of sun-dried coffee.

The remaining 23.0-kg samples that were not sun dried were mechanically dried for 17 h at 50°C and resulted in mechanically-dried parchment coffee with a moisture content of 10–12%. The mechanically-dried parchment coffee was held at 22°C. Subsamples of 100 mechanically-dried parchment beans were selected at 1 d and at 30 d. Each of the beans in the 100-bean subsamples was selected based on observation for distinctive damage that indicated the possibility of coffee berry borer infestation and dissected to observe for coffee berry borer life stages. The 30-d holding period at 22°C was to allow any viable life stages present to feed on the beans and cycle into the next generation. Pest risk tests to determine the effects on coffee berry borer survival for mechanically-dried or for sun-dried processed coffee were replicated five or eight times, respectively.

Results

Coffee Quality Tests

Quality Tests with Hawaii-Grown Green Coffees

In the preliminary Triangle Taste tests that compared untreated Yellow Caturra and Red Catuai with O_3-fumigated Yellow Caturra and Red Catuai the panelists were unable to determine any significant difference (data not shown).

Quality Tests with Green Coffees Imported Into the U.S.

Moisture content levels of all coffee samples fumigated with O_3 or methyl bromide remained within ±0.081% from their corresponding controls, which indicates that the conditions of our test fumigations (regardless of fumigant) had little or no effect on moisture content.

Table 1 shows, for each combination of variety and week, estimates of proportion of correct ratings, an estimate of its standard error, 95% confidence intervals, and degrees of freedom associated with the mean. Also shown are results from a t-test to compare the proportion correct to 1/3 as expected by chance and the Sidak correction for multiplicity. After the correction was applied, none of the estimated proportion correct differs significantly from chance expectation. Another indication that none of the estimates differ from that expected by chance can be seen from the fact that the 95% confidence intervals always include 0.333 (Table 1).

As with the variety data, there was no significant difference in proportion of correct selection of the mismatched coffee sample due to weeks of storage, treatment comparison or the interaction. Table 2 shows the results for each treatment comparison at each week similar to the data in Table 1 for variety. Again, there are no significant differences from the proportion expected by chance by either the t-test or the confidence interval method.

Table 1. Statistical estimates from Triangle Tests[a] comparing untreated (control) versus O_3-fumigated coffee varieties

				95% CI					
Variety	Week	Estimate p-correct[b]	Estimate StdErr	Lower	Upper	DF	t value	One-tail $P > t$	Sidak Adjustment[c]
Ethiopian	3	0.326	0.1167	0.143	0.584	54.02	–0.0630	0.525	0.989
Kenyan	3	0.425	0.1250	0.209	0.674	46.72	0.7362	0.233	0.796
Sumatran	3	0.575	0.1249	0.326	0.791	46.61	1.9387	0.029	0.163
Ethiopian	6	0.326	0.1167	0.143	0.584	54.02	–0.0630	0.525	0.989
Kenyan	6	0.525	0.1266	0.285	0.755	45.42	1.5178	0.068	0.345
Sumatran	6	0.375	0.1217	0.175	0.630	49.42	0.3466	0.365	0.935

[a]Sensory evaluation of difference testing involving the simultaneous presentation of three coded samples, two of which are identical.
[b]Adjustment of a *P*-value of a single significant test.
[c]Test to achieve an overall significant level of α.

Table 2. Statistical estimates from Triangle Tests[a] comparing untreated (control) versus O_3-fumigated versus methyl bromide-fumigated coffee varieties

				95% CI					
Comparison	Week	Estimate *P*_correct	Estimate StdErr	Lower	Upper	DF	t value	One-tail $P > t$[b]	Sidak adjustment[c]
O3 vs MeBr	3	0.237	0.1145	0.075	0.543	16.26	–0.8429	0.794	1.000
ctl vs MeBr	3	0.427	0.1420	0.173	0.726	11.53	0.6596	0.261	0.837
ctl vs O3	3	0.475	0.1452	0.202	0.764	11.67	0.9778	0.174	0.682
O3 vs MeBr	6	0.379	0.1377	0.146	0.686	11.85	0.3349	0.372	0.939
ctl vs MeBr	6	0.378	0.1379	0.145	0.686	11.95	0.3244	0.376	0.941
ctl vs O3	6	0.378	0.1379	0.145	0.686	11.95	0.3244	0.376	0.941

[a]Sensory evaluation of difference testing involving the simultaneous presentation of three coded samples, two of which are identical.
[b]Adjustment of a *p*-value of a single significant test.
[c]Test to achieve an overall significant level of α.

O_3 Fumigation Against Coffee Berry Borer

A total of 22 separate fumigations were performed resulting in combined estimated treated populations of 126,081 coffee berry borer individual including eggs, larvae, pupae, and adults (Table 3). The 14.6% survival in the treated eggs demonstrates that the coffee berry borer egg is the most O_3-tolerant life stage. Although fumigation with 10,000 ppm O_3 gas under –30.5 cm Hg vacuum for 6.0 h at 13 ± 3°C will provide quarantine security against coffee berry borer larvae, pupae, and adults, other ameliorative factors must be applied to ensure quarantine security against coffee berry borer eggs. The results of the fumigation efficacy test instigated research on the effects of moisture content on the survival and reproduction of coffee berry borer in green coffee and development of a systems approach to controlling coffee berry borer in green coffee.

O_3 Fumigation Against Coffee Leaf Rust

Table 4 shows the results of efficacy tests against coffee leaf rust urediniospores using 10,000 ppm O_3 at –30.5 cm Hg of vacuum at 13. 0 ± 3.0°C for 15.0, 22.5, 30.0, 60.0, or 300.0 min. The data in Table 4 represent actual counts of individual live and dead urediniospores, not estimated treated populations. The data indicate that the 10,000 ppm O_3 at –30.5 cm Hg of vacuum at 13.0 ± 3.0°C for 6.0 h is 5.0 h longer than needed to kill all coffee leaf rust urediniospores.

Our observations found that bleaching of the leaf sections occurred within 15.0 min after initiating fumigation, and extreme bleaching of both the leaf sections and the orange coffee leaf rust lesions occurred by 22.5 min. At 60 min, the coffee leaf rust lesions had turned brown and appeared "burned". Microscopic examination of urediniospores that had been fumigated for 60 min found large numbers of urediniospores with ruptured walls and/or without internal contents. After fumigation for 300 min, urediniospores were completely disintegrated.

Green Coffee Moisture Content Effects on Coffee Berry Borer

Effects of Green Coffee Moisture Content on Coffee Berry Borer Survival and Reproduction

In the first series of tests, the effect of country of origin (Colombia-grown versus Hawaii-grown) and whether the green coffee was dehydrated or rehydrated, respectively, to obtain the desired moisture content (10, 15, 20, 25, 30, 35, 40, or 45%) was not significant ($F = 0.7$; df = 1, 2; $P = 0.40$). Therefore, data for the total number of coffee berry borer from the Colombia and Hawaii bean populations was pooled for means separations analysis of the effect of moisture level at each time after infestation. Although the country of origin by moisture content interaction was not significant ($F = 1.4$; df = 3, 2; $P = 0.24$), the effect of moisture content on survival and reproduction was highly significant.

The block effect (days after infestation) was significant ($F = 22.5$, df = 3, 2; $P < 0.0001$), and the effect of green coffee moisture content was significant for 8, 30, and 45 d after infestation (Table 5). In general, the number of coffee berry borer reproducing and surviving on green coffee at moisture content levels between 25 and 45% was similar, and then coffee berry borer numbers declined rapidly as the moisture content was reduced from 20% to15%, and from 15 to 10%. At 10% moisture content, the number of coffee berry borer

was greatly reduced and only a small number of live eggs or larvae were found (Figs. 1 and 2).

The coffee berry borer F_1 generation at 8 d (Figs. 1A and 2A) consisted of eggs and larvae only because of the time interval between exposure to adult females and observation for the F_1 generation was too short for larvae to pupate or adult emergence. Foundress females produced the greatest number of eggs at moisture content ≥20%, with egg deposition and larval population decreasing significantly <25% moisture content (Table 5). Larval eclosion from eggs decreased rapidly at <25% moisture content with little or no eclosion occurring at moisture content ≤ 15% (Figs. 1A and 2A), which demonstrates the highly significant ($F = 273.4$; df = 3, 2; $P < 0.001$) negative impact of low moisture content on coffee berry reproduction.

Observations at 30 d (Figs. 1B and 2B) found egg deposition decreased significantly at all moisture content levels with no egg deposition at 10% moisture content in the Hawaii-grown coffee and 3. 8 ± 0.2 (mean ± SEM) total eggs and larvae in the Colombia-grown coffee (Table 5). Eggs observed in coffee at moisture content ≤ 15% were not viable and larvae observed at moisture content ≤15% appeared undersized and unhealthy compared with stronger, healthier larvae in coffee at moisture content ≥20%. Larvae, pupae and adults comprise the greatest proportion of the F_1 generation population at moisture content ≥25% in Colombia-grown coffee (Fig. 1B) and moisture content ≥20% in Hawaii-grown coffee (Fig. 2B). However, no pupae or adults were observed in either Hawaii- or Colombia-grown coffee at moisture content ≤15% (Figs. 1B and 2B), which demonstrates the negative impact of low moisture content on coffee berry borer survival beyond the larval stage.

Little or no egg deposition was observed at 45 d for moisture content ≤30% (Figs. 1C and 2C), which was unexpected. Although we can speculate that the older foundress females may produce fewer eggs by the end of the 45-d holding period, the combined populations of foundress female and the (now mature) F_1 generation adults (males and females) should have resulted in more egg deposition than was observed. The 45-d observations found no F_1 generation adults in the Hawaii-grown coffee (Fig. 2C) at moisture content ≤15%, or in Colombia-grown coffee at 10% moisture content (Fig. 1C), which was similar to our observations at 30 d. However, several pupae and adults (Table 5) were found at moisture content = 15% in Colombia-grown coffee (Fig. 1C).

Effects of Green Coffee Moisture Level on Coffee Berry Borer F_1 Generation Survival

Our second series of tests were designed to observe for the effects of low (10, 15, 20, or 25%) moisture content levels on coffee berry borer survival and reproduction after the source of the infestation (adult females) is removed. Because the females deposited eggs for 6 d before they were removed, these tests mimic a potential situation following an O_3 fumigation in which all larvae, pupae, and adults are killed but some eggs survive. In these tests, the country of origin (Colombia-grown or Hawaii-grown) and whether the green coffee was dehydrated or rehydrated, respectively, to obtain the desired moisture content (10, 15, 20, or 25%) was significant for the mean number of larvae ($F = 20.4$; df = 3, 2; $P < 0.001$), pupae ($F = 20.9$; df = 3, 2; $P < 0.001$), and adults ($F = 26.1$; df = 3, 2; $P < 0.001$) 30 d after the green coffee was exposed to adult females. Therefore, unlike the data from Colombia-grown and Hawaii-grown coffees in the first series of tests, ANOVA was done on the coffee berry borer survival and reproduction data for Colombia-grown and Hawaii-grown coffees separately (Table 6).

For the coffee berry borer F_1 generation on Colombia-grown green coffee, the effect of moisture content was not significant for eggs ($F = 3,7$; df = 3, 2; $P = 0.12$), but was significant for larvae ($F = 175.7$; df = 3, $P < 0.001$), pupae ($F = 133.8$; df = 3, 2; $P < 0.0001$)

Table 3. Survival of coffee berry borer stages fumigated with 10,000 ppm O_3 under –30.5 cm Hg of vacuum for 6.0 h at 13.0 ± 3.0°C

CBB stages	Fumigated CBB stages		
	Total number[a]	Total number survival	Survival (%)
Eggs	5,919	863	14.6
Larvae	46,746	0	0
Pupae	39,924	0	0
Adults	35,492	0	0

[a]Each total number represent actual amount of treated population, that represents a total of 22 separate fumigations.

Table 5. Mean number of coffee berry borer (all life stages) per 25 coffee beans at 10, 15, 20, 25, 30, 35, 40, or 45% moisture content levels 8, 30 or 45 d after infestation

% moisture content	Days after infestation		
	8	30	45
10	3. 8 ± 0.2**c**	0. 5 ± 0.5**b**	0. 8 ± 0.5**c**
15	13. 5 ± 0.2**c**	7. 8 ± 2.5**b**	10. 0 ± 1.7**bc**
20	84.3 ± 24.7**c**	50. 5 ± 7.2**b**	69. 8 ± 3.2**bc**
25	283.7 ± 66.0**bc**	254. 5 ± 41.8**ab**	191. 3 ± 27.0**ab**
30	438.8 ± 45.6**ab**	361. 8 ± 18.5**a**	265. 8 ± 6.5**a**
35	556.2 ± 37.8**ab**	523. 7 ± 84.0**a**	302. 2 ± 61.5**a**
40	573. 2 ± 96.5**a**	439. 3 ± 93.7**a**	292. 8 ± 61.5**a**
45	458.8 ± 51.2**ab**	414. 3 ± 77.3**a**	255. 0 ± 16.7**a**

Mean (±SE) in the same column followed by the same letter were not significantly different at $P > 0.05$.

Table 4. Survival of coffee leaf rust urediniospores fumigated with 10,000 ppm O_3 under –25.4 cm Hg of vacuum at 13.0 ± 3.0°C for 15.0, 22.5, 30.0, 60.0, or 300.0 min

Fumigation time (min)	Control urediniospores			Fumigated urediniospores		
	Total number[a]	Number germinated	% survival	Total number	Number germinated	% survival
15.0	1,160	390	33.66	3,018	224	7.42
22.5	630	227	36.03	1,185	32	2.70
30.0	1,002	246	24.55	2,772	2	0.07
60.0	1,638	552	33.70	3,547	0	0
300.0	2,234	624	27.93	6,031	0	0

[a]Numbers of urediniospores represent actual counts, not estimated treated populations.

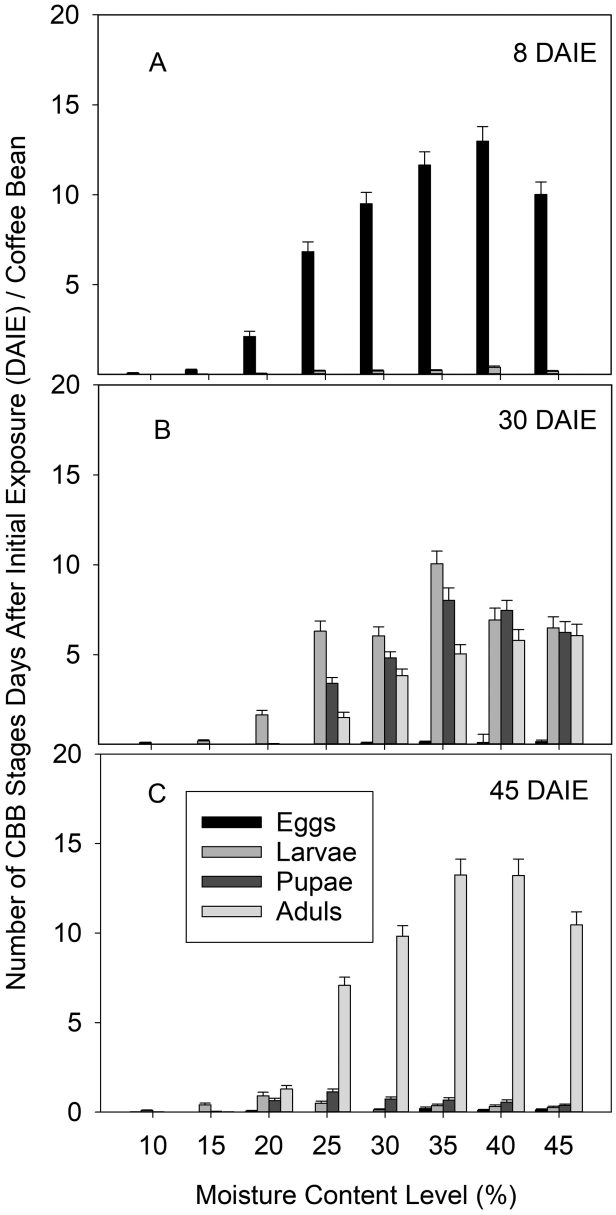

Fig. 1. Survival and reproduction of coffee berry borer on Colombian-grown green coffee at 10, 15, 20, 25, 30, 35, 40, or 45% moisture content 8, 30, and 45 d after initial exposure (DAIE) to adult females.

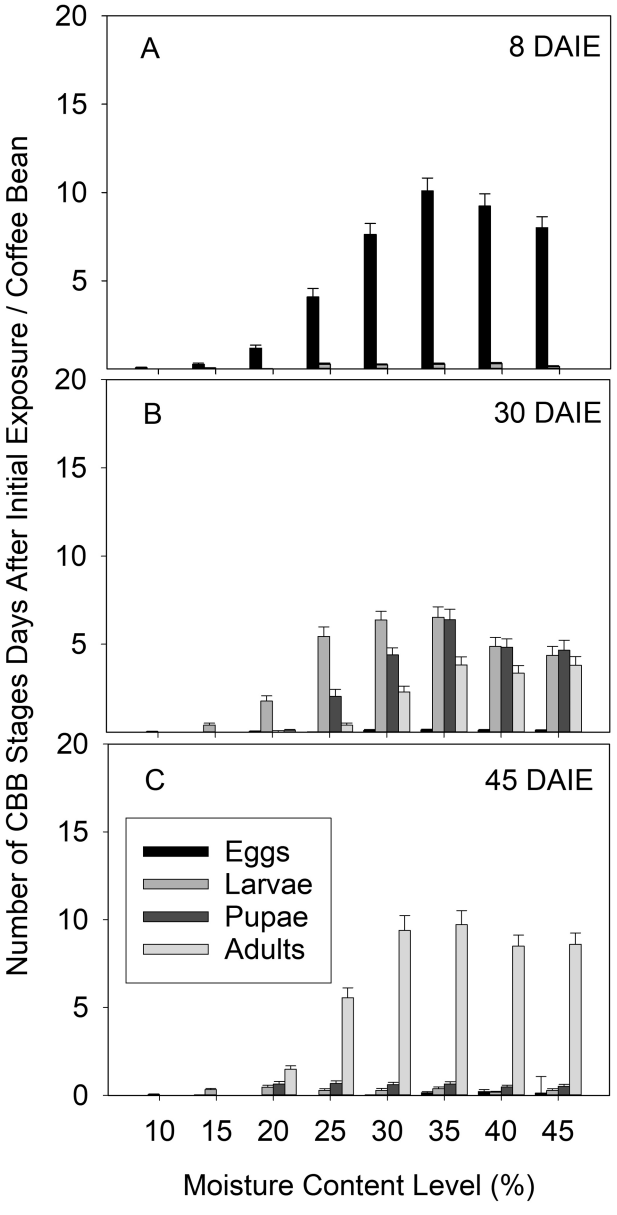

Fig. 2. Survival and reproduction of coffee berry borer on Hawaii-grown green coffee at 10, 15, 20, 25, 30, 35, 40, or 45% moisture content 8, 30, and 45 d after initial exposure (DAIE) to adult females.

and adults (F = 64.8; df = 3, 2; P < 0.0001) (Fig 3A). For the coffee berry borer F_1 generation on Hawaii-grown green coffee, the effect of moisture content was not significant for eggs (F = 2.01; df = 3, 2; P = 0.1104), but was significant for larvae (F = 147.19; df = 3, 2; P < 0.0001), pupae (F = 185.22; df = 3, 2; P < 0.0001) and adults (F = 135.20, df = 3, 2; P < 0.0001) (Fig. 3B).

Coffee Berry Borer Risk Analysis for Processed Green Coffee

The data from the risk analysis (Table 7) follows the standard wet processing of harvested coffee berries through the processing activities and shows the reduction in live coffee berry borer infestation by the two different drying methods. Specifically, Table 7 shows the percent live coffee berry borer life stages found in field samples, in harvested coffee berries before processing samples, and in samples of humid-parchment before silo or sun-drying is 87. 34 ± 3.11% (SE). Observations at 1 d after the completion of processing and mechanical-drying or sun-drying found 0% or 1. 60 ± 0.81% coffee berry borer survival, respectively. Observations at 30 d after the completion of processing and mechanical-drying or sun-drying found 0% survival for both drying methods (Table 7). Although the processing steps before drying, such as water cleaning, flotation, soaking, and removal of skin and pulp may play a part in reducing infested berries, there was no reduction in percent live (survival) for semi-processed humid-parchment coffee. Therefore, all the processing steps before drying have little or no effect on coffee berry borer mortality.

Discussion

Prior to 1998, Hawaii allowed the entry of unroasted coffee into the State from the U.S mainland if it had been fumigated with methyl bromide to lessen the risks of introduction of coffee pests including

Table 6. Mean number of coffee berry borer life stages per 25 coffee beans 30 d after infestation at four moisture content levels

% moisture	Eggs	Larvae	Pupae	Adults	Total
10	0.09 ± 0.04**a**	0. 13 ± 0.05**c**	0.0**b**	0.0**b**	0. 22 ± 0.09**c**
15	0. 06 ± 0.02**a**	0. 50 ± 0.09**c**	0.0**b**	0.0**b**	0. 56 ± 0.09**c**
20	0. 05 ± 0.04**a**	3. 33 ± 0.12**b**	0. 27 ± 0.06**b**	0. 04 ± 0.01**b**	3. 69 ± 0.11**b**
25	0. 01 ± 0.01**a**	8. 36 ± 0.56**a**	9. 99 ± 1.06**a**	2. 21 ± 0.71**a**	15. 52 ± 1.22**a**

Mean (±SE) in the same column followed by the same letter were not significantly different at $P > 0.05$

coffee berry borer and coffee leaf rust (House of Representative Thirtieth Legislature, State of Hawaii 2020). The Bill For And ACT, 2021 noted that in 1998, the USDA banned the importation of unroasted coffee into Hawaii and Puerto Rico. In 2005, The State of Hawaii requested a modification to permit the importation of methyl bromide-fumigated unroasted coffee into Hawaii. In 2006 the USDA did not approve the request (USDA-APHIS 2006). Despite of the denial, Hawaii continued to allow entry of methyl bromide-fumigated unroasted coffee, and in 2010, the coffee berry borer was reported in Hawaii with devastating economic impacts on coffee farmers in the years since (House of Representative Thirtieth Legislature, State of Hawaii 2020). In October 2020, the State of Hawaii USDA confirmed the coffee leaf rust from plant samples collected on Maui, Hawaii (hdoa.hawaii.gov). All these suggest that other safety measurements different that the standard methyl bromide (USDA-APHIS 2006) or heat treatments at 315°F (Hansen and Johnson 2007, Hollingsworth et al. 2013) would be required to kill 100% of coffee berry borer and coffee leaf rust urediniospores populations to prevent their movement into other Hawaiian Islands.

In the present study, observations were limited to experiments of a single concentration of 10,000 ppm O3 gas under –25.4 mm Hg vacuum1 at 13. 0 ± 3. 0°C for 6 h. Based in preliminary results, this concentration killed 100% population of larvae, pupae, and adults of coffee berry borer after 6 h of exposure and 100% of urediniospores of coffee leaf rust during the first hour. Although, 6 h were not enough to kill coffee berry borer eggs, population of eggs in unroasted green coffee beans, if any, will be unfertile based on our results. The present study is the first report of a postharvest ozone treatment for coffee berry borer and coffee leaf rust and its possible effect on the coffee quality.

It is well known that the high standards for Hawaiian coffee (extra fancy, fancy, and number one) are not comparable to the global average with values ranging from US$30 to $ 120/lb (Aristizabal et al. (2017). Therefore, maintaining this high quality is crucial for the Hawaiian coffee industry and its coffee producers. Our first experiment demonstrated that the professional coffee quality judges consistently remarked that they could not tell the differences between coffee treatments (controls versus O_3 fumigation versus MB fumigation versus 3- or 6-wk holding period). Additionally, the judges indicated that correct choices were either by chance alone or due to other factors, such as slight differences in preparation, roasting, or temperature of coffee samples that were not quality defects and would not be detected by the consumer. The data indicate that the professional coffee quality judges found no deleterious effects from O_3 fumigation of green coffee within the test parameters given for a relatively severe O_3 concentration and fumigation time. The data further indicate there are no deleterious effects from methyl bromide fumigation of green coffee, which was expected after more than five decades of green coffee imports into Hawaii fumigated with methyl bromide without loss of quality (House of Representative Thirtieth Legislature, State of Hawaii 2020). That no differences were observed between O_3-fumigated and MB-fumigated green coffee

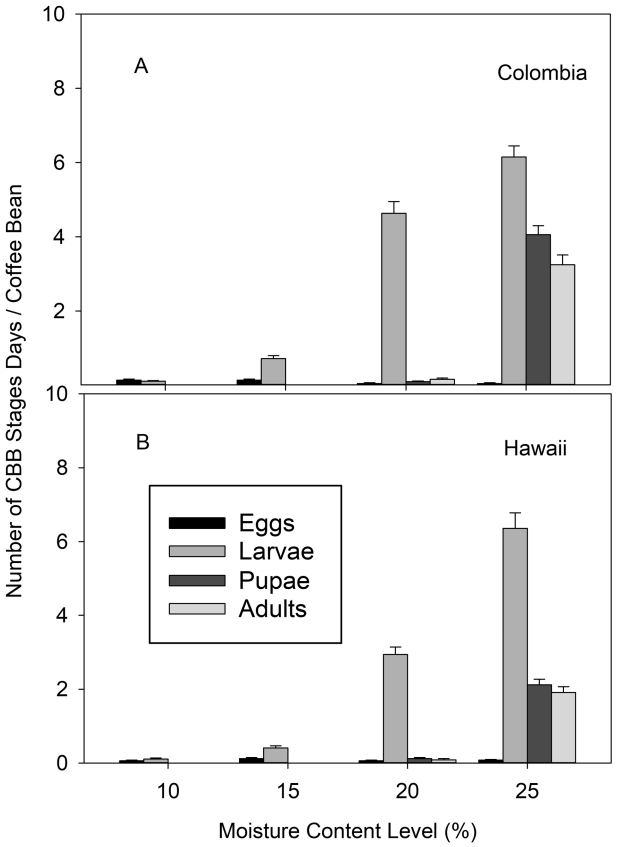

Fig. 3. Survival and reproduction of coffee berry borer on Colombian and Hawaii grown green coffee at 10, 15, 20, or 25% moisture content 30 d after a 6-d after initial exposure (DAIE) to and removal of adult females.

further supports the data that O_3 fumigation should not adversely affect coffee quality if approved as a quarantine treatment against coffee berry borer and coffee leaf rust. These results could be comparable to the results of Kim et al. (1999) and Guzel-Seydim et al. (2003), who reviewed the use of ozone in the food industry, listing a series of fruits and vegetables treated with ozone. They reported that most of the treated products were found to have increased shelf-life and notably, no trace of ozone was found.

Alternative approaches to disinfest coffee of coffee berry borer have been reported by Hollingworth et al. (2013) and Follett (2018) using freezing temperatures and irradiation, respectively. Hollingworth et al. (2013) reported 100% mortality in adults and larvae of coffee berry borer after 48 h of exposure to –15°C. Follett (2018) demonstrated that irradiation to coffee berry borer adults at 100 Gy sterilized females. While these treatments have potential, their efficacy against coffee leaf rust and effect on coffee quality is unknown. The current study showed not adversely effects of coffee quality after ozone fumigation treatment at 10,000 ppm O_3 gas

Table 7. Mean percent coffee berry borer infestation and live insects (all stages) in the field and in harvested coffee berries sampled before, during and after processing to dry-parchment coffee using either mechanical-drying or sun-drying

Sampling sequence during coffee processing system	Coffee berry borer (CBB) life stages		
	Total	% live	% infestation
Field samples to determine field infestation rate of coffee berries before harvest	188.77 ± 26.96	84.62 ± 2.92	9.06 ± 1.38
Harvested coffee berry samples before processing	500.70 ± 60.68	97.07 ± 0.71	18.02 ± 4.72
Semi-processed samples of humid-parchment coffee before silo-drying	132.20 ± 30.55	84.66 ± 5.54	11.12 ± 1.57
Semi-processed samples of humid-parchment coffee before sun-drying	224.13 ± 35.03	84.59 ± 3.58	12.58 ± 5.34
Processed samples of dry parchment coffee 1 d after completion of processing and mechanical-drying	52.0 ± 6.49	0	0
Processed samples of dry parchment coffee 1 d after completion of processing and sun-drying	41.0 ± 11.08	1.60 ± 0.81	3.38 ± 1.82
Processed samples of dry parchment coffee 30 d after completion of processing and mechanical-drying	29.00 ± 7.20	0	0
Processed samples of parchment coffee 30 d after completion of processing and sun-drying	68.00 (25.75)	0	0

under –25.4 mm Hg vacuum1 at 13.0 ± 3. 0°C for 6 h. At that concentration, 22 time exposures over 125,000 individuals of coffee berry borer including larvae, pupae, and adults were killed (100% mortality) and 14% of egg population from over 5,000 eggs survived. However, the data from the two phases of our third experiment showing reduced survival and reproduction of coffee berry borer depending on moisture content level agrees with the preponderance of literature (Haarer 1962, 1970; Hernandez-Paz and Sanchez De-Leon 1972, Baker 1984, Baker et al. 1989, 1992; Baker and Barrera 1993), indicating that coffee berry borer is a field pest and not a stored-product pest. Therefore, the presence of viable eggs of coffee berry borer in unroasted coffee beans is moisture limited and not possible. Coffee berry borer reproduction and survival requires moisture content levels of 20% or more (Baker and Barrera 1993, Portilla and Bustillo 1995, Portilla and Streett 2006, 2008) and green coffee at the international standard of 9–12% moisture content cannot support coffee berry borer survival or reproduction as was demonstrated in this study. None of the eggs found at 10, 15, 20, or 25% moisture content were viable regardless of the presence of adult coffee berry borer in both the Hawaii and Colombia-grown coffees at 20 and 25% moisture content. Larvae found in both Hawaii- and Colombia-grown coffees at 10 or 15% appeared undersized and unhealthy compared to larger and healthier-appearing larvae in coffees at 20 or 25% moisture content. The results for both Colombia-grown and Hawaii-grown coffees further demonstrate the inability of a coffee berry borer F_1 generation in green coffee at moisture content levels ≤15%. Moreover, the foundress females in these tests remained on the coffee for the entire 45-d test duration, which resulted in an increasing infestation pressure from both the original adults and from gravid F_1 females. The results of these tests demonstrated that decreasing coffee moisture content below 20% significantly limits or eliminates egg production and/or viability, and significantly decreases or prevents survival of the F_1 generation, regardless of the infestation pressure provided by our test conditions. Our data from the coffee berry borer risk analysis for processed green coffee also corroborated that moisture content is crucial for coffee berry borer's reproduction and survival. It was clearly observed that fresh harvested coffee berries with 87% coffee berry borer infestation, had 0% or 1.60% survival after 1 d of mechanical-drying or sun-drying processing, respectively, and 0% survival for both drying methods at 30 d after processing.

Coffee leaf rust is the most damaging disease agent in global coffee production. Despite efforts to restrict the spread of this disease via quarantine and other biosecurity control, this pathogen is spreading rapidly to coffee plants around the world (Nagarajan and Singh 1990, Isard et al. 2005, Viljanen-Rollinoon et al. 2007, Bebber et al. 2016). Due to the recent introduction of coffee leaf rust into Hawaii (October 2020) and to prevent the further dispersal of coffee leaf rust and coffee berry borer in the state, in March of 2021, the State of Hawaii prohibited the importation of unroasted coffee into the State and authorized the Department of Agriculture to adopt rules to allow for the importation of "partially" roasted coffee into the State (House of Representative 13 Legislature). To date, there is not established a quarantine treatment for coffee leaf rust or coffee berry borer. However, based on our results coffee leaf rust can be killed with ozone treatments at 10,000 ppm O_3 gas under –25.4 mm Hg vacuum1 at 13.0 ± 3. 0°C, and contrary to our expectations, urediniospores of coffee leaf rust were found to be easily killed, with no survival at 60 min and complete disintegration 300 min (6 h) after exposure. This information confirmed the fungicidal effect of O_3 that has been documented on a wide variety of spores and vegetative cells (Guzel-Seydim et al. 2003). In general, our results suggest that treatment at 10,000 ppm O_3 gas under –25.4 mm Hg vacuum1 at 13.0 ± 3. 0°C, could be considered as a quarantine treatment for both coffee berry borer and coffee leaf rust without affecting coffee quality. Further investigation needs to be done to evaluate the feasibility of this method at commercial scale application.

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