

# Comparative evaluation of fungicide effectiveness in controlling coffee leaf rust in Hawaii

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**Abstract:** Coffee leaf rust (CLR), *Hemileia vastatrix*, is a disease that has severely impacted the major coffee growing areas of the world. The discovery of CLR in Hawaii in October 2020 was devastating news to local growers, as it quickly spread to all coffee-growing areas, threatening the second-highest-valued crop in the State. Although the fungicide Priaxor Xemium had been approved as a Section 18 emergency exemption, there was still an urgent need for true systemic fungicides to provide sustained control of the disease. The study's overall objective was to test the efficacy of various systemic fungicides against CLR in field conditions. During the coffee growing season for two years, various fungicides were tested on two farms in the Kona district of Hawaii. Treated trees showed an 84% reduction in CLR incidence compared to untreated trees in Year 1 and a 79% reduction in Year 2 by the end of the study. Treated trees also had 12% and 38% more total leaves per branch than untreated trees in Years 1 and 2, respectively. All fungicide treatments provided excellent or good control of CLR compared to the untreated control under the climate of Hawaii.

**Keywords:** *Coffea arabica*; disease incidence; Hawaii coffee industry; integrated pest management

Hawaii has a history of growing coffee commercially for more than 200 years because of the consistent temperature and weather. As one of the very few states and territories in the US that cultivates coffee, Hawaii grows coffee as a major economic industry. In the year 2022–2023, there were 7 000 acres of coffee in Hawaii (2.8% decrease from the previous year) and with a yield of over 25 mil. pounds (9.7% decrease from the previous year) (United States Department of Agriculture 2023). Introducing coffee leaf rust (CLR, *Hemileia vastatrix*) altered the previous status of Hawaii as one of the few regions without CLR, which had been the case before 2020 (Ramírez-Camejo et al. 2022). CLR

was first identified by Berkeley in 1869 as a basidiomycete fungus belonging to the Pucciniaceae family (Zambolim 2016) during Berkeley's examination of dried leaves from *Coffea arabica* plants that originated from Sri Lanka. The fungus produces spores called urediniospores, dispersed by wind, rain, or physical contact (Bowden et al. 1971). These spores can infect new coffee leaves and initiate the disease cycle. When urediniospores land on susceptible coffee leaves, they germinate and form specialized structures called appressoria and urediniospores, which may germinate *in situ*, producing basidia and basidiospores. The optimal temperature range for developing *H. vastatrix*, is around 21–25 °C,

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and the presence of water is essential for its growth (Zambolim 2016). Temperature plays a critical role in rust development, as temperatures below 15 °C can impede spore germination and hinder growth, while temperatures above 35 °C can slow fungal growth (Gichuru et al. 2021). CLR attacks leaves and causes yellow spots on the upper leaf surface. As these spots enlarge over time, severe defoliation occurs, resulting in decreased plant growth and fruiting, eventually leading to death (Avelino et al. 2015). CLR threatens coffee businesses, family farms, and communities in Hawaii. Villarreyana et al. (2020) highlighted the detrimental impact of coffee leaf rust on small farms with limited education and training in Nicaragua, emphasizing the higher losses suffered by these farmers.

Kona coffee is a cultural and agricultural icon of the Kona district on the Big Island of Hawaii, as most coffee farms are located in this area. The unique combination of fertile volcanic soil, ideal elevation, and favourable climate in the Kona region creates the perfect conditions for growing high-quality coffee beans. Coffee farms in Kona can be found at various elevations, each contributing to the unique characteristics of the coffee beans grown in those areas. The elevation at which coffee is cultivated affects factors such as temperature, rainfall, sunlight exposure, and soil composition, which influence the incidence of CLR (Matovu et al. 2013; Ehrenbergerova et al. 2018; Garedew et al. 2019). In October 2020, CLR was first detected and reported on Maui (Keith et al. 2022). The consistent warm wind and seasonal rain provide an ideal CLR spreading path and breeding ground (Nutman et al. 1963). Additional detections of CLR were reported on neighbour islands: November 2020 on Hawaii

Island, December 2020 on Lanai, January 2021 on Oahu, June 2021 on Molokai, and July 2021 on Kauai (Aristizábal & Johnson 2022; Keith et al. 2022).

The use of modern fungicides to manage CLR is a continuous process, with new formulations and improved application strategies being developed since the 1940s world widely (Bock 1962; Waller 1982; Zambolim 2016; Sera et al. 2022). The performance of these fungicides on CLR can vary based on factors such as climate conditions, application timing, dosage, and resistance management strategies (Gichuru et al. 2021; Sera et al. 2022). However, it is important to note that due to their specific mode of action, the targeted pathogen can develop resistance if not effectively managed (Honorato et al. 2015; Zambolim 2016). In Brazil, the main coffee-producing country, 62.8% of the registered CLR fungicides were triazoles and strobilurins, and 95.6% were systemic (Capucho et al. 2013). The tested fungicides in this study were selected from various chemical groups (Table 1). Mancozeb, on the other hand, is a non-systemic or contact fungicide. It remains on the surface of treated plant tissues and inhibits fungal growth upon direct contact.

To manage this fungal disease, currently, Hawaiian growers can apply some copper-based fungicides and biological products, but these are typically only effective as protectants when rust infections are lower than 5% (Zambolim 2016). In collaboration with BASF and the Hawaii Department of Agriculture, the Hawaii coffee growers successfully obtained a Section 18 emergency exemption for Priaxor Xemium fungicide (Kawabata & Nakamoto 2021). There is still an urgent need for true systemic fungicides to provide sustained disease control throughout the year and build a sound IPM

Table 1. The chemical groups of tested fungicides and their mode of action according to the Fungicide Resistance Action Committee (FRAC)

	Fungicides	Chemical group: mode of action
Systemic	Azoxystrobin; Pyraclostrobin; Picoxystrobin	Strobilurins – inhibit mitochondrial respiration in fungal cells
	Cyproconazole; Difenoconazole	Triazoles – inhibit the biosynthesis of ergosterol, a vital component of fungal cell membranes
	Benzovindiflupyr; Fluxapyroxad	SDHI (Succinate dehydrogenase inhibitors) – disrupt energy production in fungal cells by targeting succinate dehydrogenase
	Inpyrfluxam	Carboxamides – inhibit fungal cell growth by interfering with lipid biosynthesis
	Pyraziflumid	Pyrazole – target fungal mitochondria
Non-systemic/contact	Mancozeb	Mancozeb – a broad-spectrum contact fungicide that acts through multiple mechanisms, including enzyme disruption and oxidative stress induction

program to manage fungicide resistance. As a result, testing different pesticides for coffee trees in Kona at different farms and elevations can be a valuable approach. The study's overall objective was to test the efficacy and crop safety of various systemic fungicides on coffee plants in field conditions for the management of CLR in Hawaii. This study is also the earliest field research investigation into the effects of systemic fungicides to control CLR on coffee trees in Hawaii.

## MATERIAL AND METHODS

**Sites and treatments.** In this study, we conducted research on two coffee farms in Kona. In 2021, plots were established at Full Moon Coffee farm (19°34' 24.83" N, 155°56'22.29" W elevation about 380 m) planted with *Coffea arabica* var. *typica*, the predominant variety grown in Kona. This variety is highly susceptible to coffee leaf rust (Van der Vossen 2009). The plots were arranged in a randomized complete block design, with eight treatments plus a control, each replicated three times. The three replicates were established across a sloped gradient in the coffee field, with treatments randomized within each block. Tree spacing within the row was 1.2 m, and the distance between rows was 1.8 m. The standard commercial spacing for coffee farms is typically 1.2 m between trees and 3.7 m between rows. Because the spacing at this farm was unusually close, the standard commercial spacing was used to calculate the application rates. Tree heights ranged 1.5–2.1 m. There were five trees per plot, and efficacy and crop safety data were collected from the inner three trees within each plot to avoid potential edge effects from adjacent plots. Plots were naturally infected with CLR. The manufacturers supplied seven fresh test substances for use in the Year 1 trial. The fungicides that had the highest and lowest efficiency in Year 1 were tested again on a different farm in the second year.

The Year 2 study was conducted in the Kona Hills coffee farm (19°26'33.94" N, 155°52'26.54" W elevation: 560 m), where *Coffea arabica* var. *typica* grows. Pyraziflumid and inpyrfluxam (292 mL/ha) were applied again, guided by the outcomes observed in the Year 1 trial, while the other two treatments, cyproconazole and azoxystrobin, were newly included. Notably, these two chemicals are the same active ingredients (AIs) as the Quad-

risXtra, a combination of azoxystrobin plus cyproconazole. Plots were set up and laid out following the same method as Year 1, except each plot contained 6 trees.

Treatments were applied to the entire plot using a gasoline-powered Solo® backpack mist blower set at full throttle with the nozzle orifice set at 3 (orifice settings ranged 1–4). Crop growth stages during the applications were flowering and fruiting. Application details for both years are listed in Table 2. On each spray date, the sprayer was calibrated immediately before application. On both farms, the coffee plots were maintained following similar normal commercial practices.

**Treatment performance and crop safety evaluation.** Crop safety was evaluated pre-treatment and approximately 7 and 14 days after each spray application in both years. The middle three (in 2021) and four (in 2022) trees were assessed in each plot on each evaluation date. Coffee plants were evaluated for evidence of stunting. Disease incidences were evaluated at the start of the trial on May 3, 2021, and June 23, 2022 (pre-treatment) and subsequently approximately every 30 days. The last observation was done in October in Year 1 and November in Year 2. In both years, four randomly selected branches were marked on each tree (two tagged branches/side of a tree). The trees were partitioned into two halves based on their foliage area – top and bottom canopies. On each side, one branch from the upper and one branch from the lower section of the tree were selected. On each evaluation date, rust incidence was evaluated by counting the total number of leaves per marked branch and the number of leaves infected by rust on that branch. Percent rust incidence is calculated by dividing the number of infected leaves per branch by the total number per branch. The criteria to determine if leaves were infected was the presence of rust pustules on the undersides of the leaf. Leaves with pale yellow spots were not counted as infected. The previously marked branches were consistently monitored for incidence, and the number of leaves per branch was recorded during each evaluation date. Defoliation was calculated by determining the difference in leaf quantity per branch between evaluation dates. The percentage of leaf changes was calculated by dividing the difference in leaf quantity per branch between the evaluation date and pre-treatment by the leaf quantity per branch before the treatment. Yield data was not collected in this study.

Table 2. The treatment list and application methods follow the manufacturer's requirements

Trade name	Active ingredient	Rate (mm/ha)	GPA (L/ha)	Adjuvant (product & rate)	Dates of application
<b>Year 1 (2021)</b>					
Untreated control	N/A	N/A	N/A	NA	N/A
QuadrisXtra®	Azoxystrobin + Cyproconazole	753	561	no adjuvant	5/5, 6/21, 8/5
Aprovia® Top	Benzovdiflupyr + Difenconazole	987	561	no adjuvant	5/5, 5/12, 5/19, 5/26
Priaxor® Xemium®	Fluxapyroxad + Pyraclostrobin	522	561	Widespread Max 6 mL/12.8 L	5/5, 6/4
Excalia™	Inpyrfluxam	292	561	no adjuvant	5/5, 6/21, 8/5
Excalia™	Inpyrfluxam	146	561	no adjuvant	5/5, 6/21, 8/5
Pyraziflumid 20 SC	Pyraziflumid	497	561	no adjuvant	5/5, 5/12, 5/19, 5/26
Aproach® Prima	Picoxystrobin + Cyproconazole	146	561	no adjuvant	5/5, 6/21, 8/5
Dithane® F-5 Rainshield®	Mancozeb	11 225	561	no adjuvant	5/5, 6/4, 7/2
<b>Year 2 (2022)</b>					
Untreated control	N/A	N/A	N/A	N/A	N/A
Pyraziflumid 20 SC	Pyraziflumid	497	935	no adjuvant	6/30, 7/7, 7/14, 7/21
Excalia™	Inpyrfluxam	292	935	no adjuvant	6/30, 8/15, 9/29
Alto 100SL	Cyproconazole	402	935	no adjuvant	6/30, 7/28
Abound	Azoxystrobin	1 133	935	no adjuvant	6/30, 7/7, 7/14, 7/21, 7/28

**Statistical analysis.** Graphs and statistical analysis were generated and performed using SAS JMP14 pro software (version 14.3.0). Coffee leaf rust incidence among treatments was evaluated on non-transformed data using the non-parametric Kruskal-Wallis each pair comparison. Wilcoxon comparison was used because the data was not normally distributed. Pearson's correlation test was used to see any correlation between the CLR incidence and total leaves per branch. The significance level was set at 0.05. A general linear mixed model was utilized to assess variations in CLR-induced damage between the upper and low canopy levels. In this analysis, different treatments were assigned as random factors. The canopy strata were designated as fixed factors and nested under each treatment. The percentage of leaves compared the benefit of mixed AIs on defoliation change throughout only the Year 1 study.

## RESULTS

**Year 1 incidence.** On May 3, 2021, pre-treatment, the average per cent CLR incidence was less than 2.3% in all plots, and there were no significant dif-

ferences between treatments (Kruskal-Wallis Tests,  $P = 0.07$ ) (Figure 1). From May 5, 2021 to October 25, 2021, rust incidence increased progressively in the untreated plots. All fungicide treatments had less rust incidence during this period than untreated plots. The last spray application of inpyrfluxam for these plots was later in the season, on October 5, 2021, compared to the other treatments where spray applications stopped in late May or early June. Most of the treatment's lowest incidences were in either July or August. On October 12, 2021, all fungicide treatments still had significantly less rust incidence than untreated plots (Kruskal-Wallis Tests,  $P < 0.0001$ ). By the end of the study, incidence in the untreated plots increased to 66%; meanwhile, among all the treated groups, picoxystrobin + cyproconazole and pyraziflumid were the only two treatments in that rust incidence exceeded 20%. The fungicides that showed a mean of highest (pyraziflumid) and lowest (inpyrfluxam) incidence were selected to be tested in the second year again.

**Year 2 incidence.** Throughout the research period, rust incidence increased progressively in the untreated plots, from around 2% to over 50% by the end of the experiment (Figure 2). All fun-

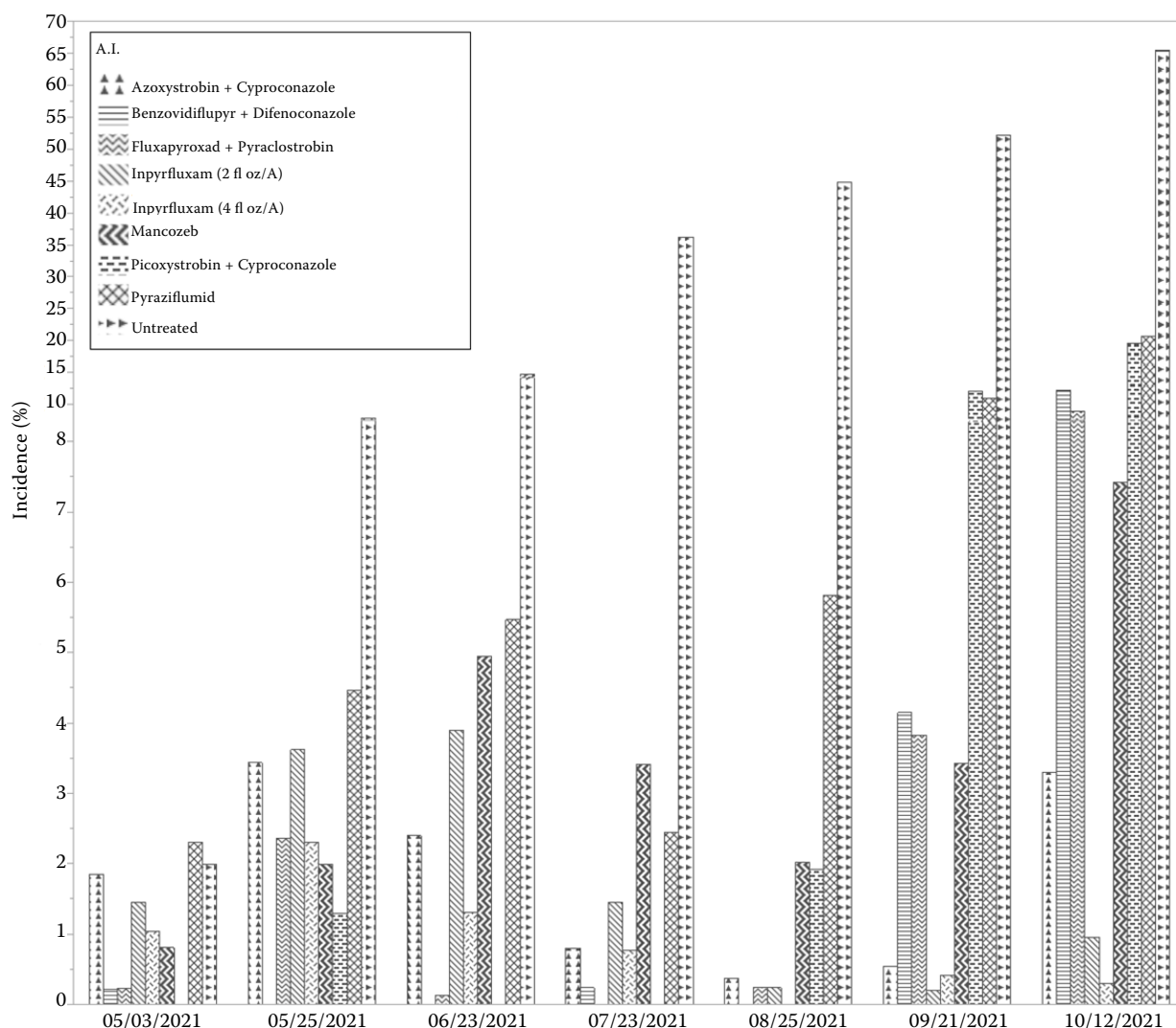


Figure 1. The percentage of incidence changed across months under different treatments

Different patterns of the bar indicate coffee plots under different fungicide treatments; the Y-axis breaks and changes increment at 8%

gicide treatments demonstrated a significantly lower rust incidence two months into the experiment than the untreated plots. Among these treatments, pyraziflumid showed the highest (Kruskal-Wallis Tests,  $P < 0.001$ ) incidence when compared to other groups, with a peak (10.2%) in August and a mean of 7.11% overall. Inpyrfluxam at 292 mL/ha showed the lowest incidence, with a mean of 0.52%.

**Impact of canopy strata on CLR infection.** Both the incidence and total number of leaves per branch were significantly impacted by the canopy strata and treatments (Table 3). The upper canopy showed a significantly higher leaf count with a lower incidence rate. Different treatments were set as the random factor significantly impacted both incidence and number of leaves.

Among all the different chemicals, most showed a higher incidence of CLR disease in the low canopy of the plants compared to the upper canopy. This decreased total leaf counts due to increased leaf shedding in the lower canopy region (Figure 3). Of all the treatments, only the chemicals cyproconazole and benzovindiflupyr + difenoconazole exhibited an opposite trend compared to the other treatments regarding total leaves and incidence, respectively.

**Defoliation.** Changes in the total number of leaves per branch over time can serve as an indicative measure of defoliation resulting from CLR infection. In Year 1, in addition to effectively controlling CLR incidence, the four fungicides containing a mixture of two AIs demonstrated an advantageous effect of increased total leaves after the study

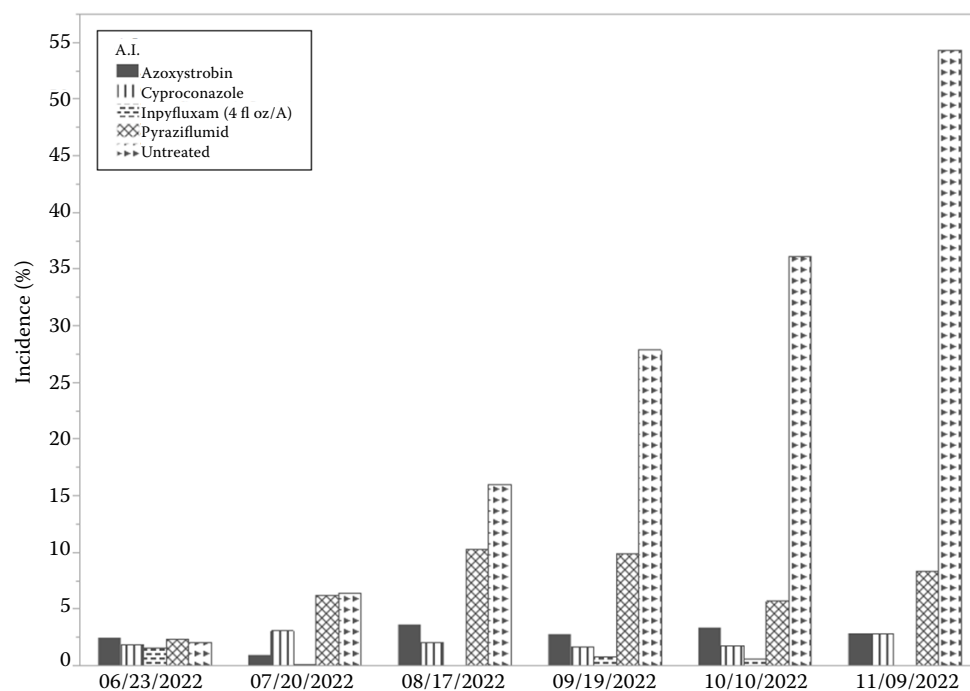


Figure 2. The percentage of incidence changed across months under different treatments. Different patterns of the bar indicate coffee plots under different fungicide treatments.

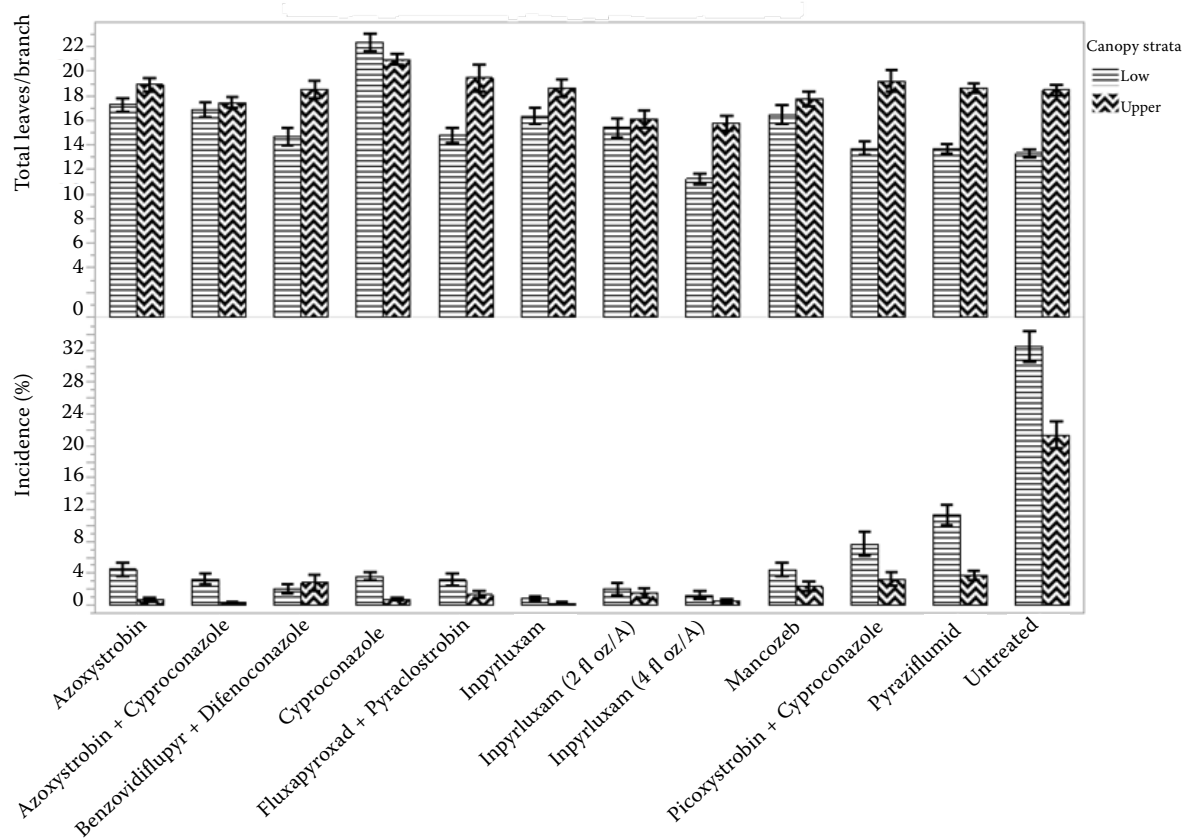


Figure 3. The total leaves/branch differences and incidence between low (L) and upper (U) canopy across different treatments.

Each error bar is constructed using a 95% confidence interval of the mean; dash lines represent data from the upper canopy, and solid lines represent data from the lower canopy.



Table 3. The significant difference in mean CLR incidence and total leaves per branch between upper/low canopy and among different fungicide treatments using a general linear mixed model

General linear mixed model	Impact significance of canopy strata	Upper canopy		Low canopy	Impact significance of treatments
Incidence	$F = 11.39; P < 0.0001$	4.6%	<	8.6%	Wald $P = 0.02$
Total leaves/branch	$F = 20.49; P < 0.0001$	18.4	>	15.3	Wald $P = 0.027$

Canopy strata were set as a fixed factor, and treatments were set as a random factor; mean incidence and total leaves were calculated using data from both years

Wald – significance of treatments

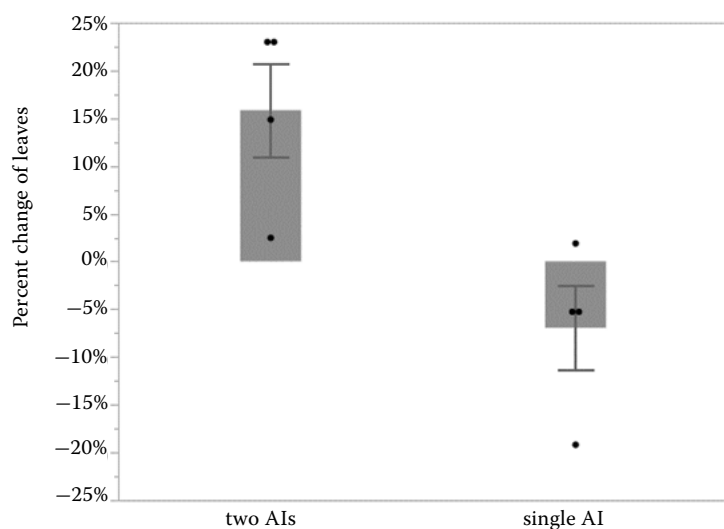


Figure 4. Change of total leaves number per branch compared between fungicides containing two active ingredients (AIs) and those with a single AI

The percentage change was determined by calculating the difference in the number of leaves per branch at the beginning and end of the study; each data point on the graph represents a different fungicide chemical; a positive number indicates an increase in leaves, while a negative number represents a decrease

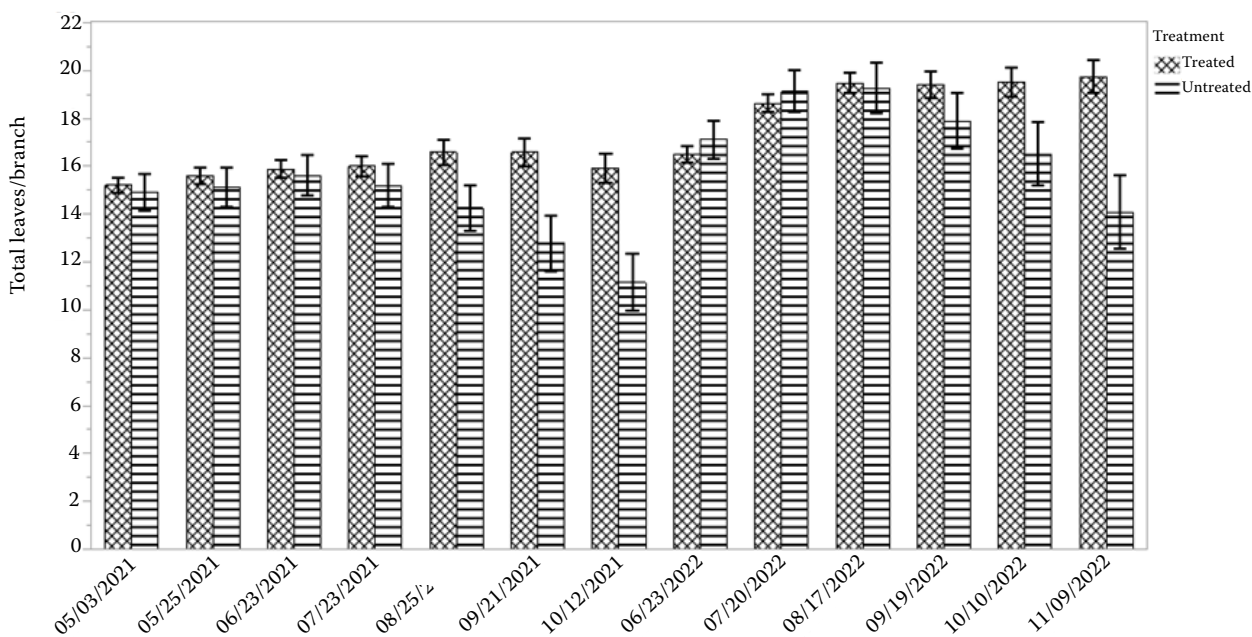


Figure 5. The mean change ( $\pm$  SE) of total leaves per branch in all treated and untreated coffee trees in 2021 and 2022. Error bars indicate the standard error; besides, there was a noticeable negative correlation between incidence and total leaves per branch in both 2021 (Pearson's correlation  $F_{1,2194} = 35.4, P < 0.0001$ ) and 2022 ( $F_{1,1430} = 167.28, P < 0.0001$ )

in comparison to the initial stage (Figure 4). The average increase in total leaves per branch under the treatment of two AIs was 16%, while the average decrease in total leaves under the treatment of a single AI was negative 7%.

During the entire study period, it was observed that the untreated trees exhibited a significant decrease in leaf numbers compared to the initial count and overall treated trees (Figure 5). The number of leaves on these trees declined continuously from August in both years. By the end of the study, the untreated trees showed 25.5% and 17.5% leaves lost in 2021 and 2022, respectively. In contrast, the mean leaves of all treated trees showed a 4.6% and 19.4% increase in 2021 and 2022, respectively.

## DISCUSSION

Even though these fungicides targeting CLR have been tested and employed in other coffee cultivation regions (Honorato et al. 2015; Sera et al. 2022), their efficacy in Hawaii must be evaluated in local conditions. All fungicides assessed in this study exhibited no adverse effects on the coffee crop, as evidenced by the absence of phytotoxicity. Furthermore, this investigation revealed variations in the efficacy of fungicides when comparing formulations containing a single AI versus those containing a mixture of AIs, namely azoxystrobin + cyproconazole, fluxapyroxad + pyraclostrobin, and picoxystrobin + cyproconazole. In 2021, it was observed that fungicides with a mixture of AIs effectively controlled CLR and demonstrated a positive impact on leaf growth (total leaves per branch). Because of necessary pesticide rotation due to potential rust resistance (Zambolim 2016), in 2022, the AIs (azoxystrobin and cyproconazole) from QuadrisXtra were tested individually and showed good control of CLR for both AIs. However, different from the results of 2021, all the treated plots showed an increase in total leaves per branch when the study ended, indicating possible ecological factors impacting the incidence of CLR in these two farms.

The soil properties did not significantly impact the CLR incidence (Ehrenbergerova et al. 2018). One possible environmental impact could be elevation, as the higher elevation contributed to a lower CLR incidence, as also noticed in another study done in the same area (Aristizábal et al. 2022), though their difference was insignificant. In this study, the farm's elevation in Year 1 is approxi-

mately 185 m lower than in Year 2. According to research conducted by Avelino et al. (2012), there was a lower incidence of CLR at higher elevations due to comparatively lower temperatures. Furthermore, both Belachew et al. (2020) from Ethiopia and Bigirimana et al. (2012) from Rwanda demonstrated a substantial negative association between the incidence of CLR and elevation.

In this study, two concentrations of inpyrfluxam were evaluated in 2021 to assess their effectiveness in reducing damage caused by CLR. The results indicated that applying 292 mL/ha concentration provided a notable benefit by controlling the incidence of CLR. However, it is important to note that the statistical difference between the high and low concentrations was insignificant. In a previous study conducted by Júnior et al. in 2015, various fungicide concentrations within the recommended range were investigated, demonstrating comparable effectiveness in controlling CLR. It was observed that higher concentrations of the fungicide significantly prolonged the re-sporulation period in comparison to lower and medium concentrations. Based on these findings, it is suggested that future research should explore this perspective further. Specifically, it would be beneficial to investigate the effects of different fungicide concentrations on their long-term effectiveness and phytotoxicity.

The coffee growing season in this study immediately followed the first CLR detection in Hawaii; thus, the CLR incidence rate was relatively low at the beginning. Upon completing the experiment, we observed that the untreated trees in this study were severely impacted in a remarkably short time, with an incidence rate of over 60%. These results were similar to another recent study conducted in Kona, Hawaii, which showed the highest incidence of over 50% in their high-elevation plots during September and October (Aristizábal & Johnson 2022). Similar to other areas, CLR infections in the low canopy of the tree were observed to a greater extent of CLR damage compared to the leaves located in the upper section in this study (Silva-Acuña et al. 1999; Ward et al. 2017; Alvarado-Huamán et al. 2020). Additionally, some studies have suggested that CLR can indirectly lead to defoliation by causing leaf abscission (Brown et al. 1995; Yirga 2020), which was also observed in this study. The result of the mixed model test also showed the important impact of different treatments on the incidence and total leave between different canopy layers. Over-



all, the symptoms observed in coffee trees infected with CLR in Hawaii were similar to those observed in other regions, suggesting that the response to CLR in Hawaii is similar to that in other regions.

No phytotoxicity was noted in any coffee plots throughout the trial period. Coffee trees were healthy, producing new leaves, and the fruit load was normal. No stunting was observed in any of the plots. All fungicides tested in this study were safe for the coffee crop and effectively controlled coffee leaf rust. All products would serve as strong potential candidates for domestic registration on coffee and integration into an IPM program for disease management. Strategic rotation of systemic fungicides with copper and biological products has the potential to provide season-long control of coffee leaf rust in well-managed coffee orchards.

## CONCLUSION

The results of this study demonstrate the potential for several fungicide products to control CLR under Hawaiian growing conditions effectively. No phytotoxicity issues were observed with any of the fungicides evaluated, indicating they are crop-safe options. While single active ingredient products like azoxystrobin and cyproconazole provided good CLR control, the multi-ingredient formulations such as QuadrisXtra performed particularly well, controlling disease and promoting leaf growth. Environmental factors like elevation influenced CLR incidence, with higher elevation farms experiencing lower CLR incidence and higher total leaves per branch among untreated trees. This aligns with previous research on the impact of microclimate conditions on CLR development.

Overall, the observed symptom expression and disease progression patterns mirrored those documented in other coffee-growing regions. The fungicides investigated represent promising candidates for registration and incorporation into integrated CLR management programs for Hawaii's coffee industry. Importantly, as the first study evaluating systemic fungicide products for CLR management under Hawaiian conditions following the recent introduction of this devastating disease, this research provides crucial insights into effective chemical control strategies tailored to the coffee agroecosystems in Hawaii and potentially in coffee production regions with similar growing conditions. In future studies, we plan to include yield and detailed

coffee crop safety data to comprehensively evaluate agronomic and economic impacts. Additionally, incorporating micro-climate information, such as temperature, humidity, and rainfall, would be valuable for comparing and contextualizing these data.

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