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F1 Coffee Hybrids: Combining High Productivity with Genetic Resistance to Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae)

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Abstract

To identify *Coffea arabica* genotypes with enhanced resistance to the coffee berry borer (CBB), *Hypothenemus hampei*, two wild accessions (C306 and C534) and their derived hybrids were evaluated. Four F1 genotypes—H1 (CU1842 × C306), H2 (CX2385 × C534), H3 (CX2385 × C306), and H4 (CX2848 × C534)—were generated and subsequently mass-propagated in vitro via somatic embryogenesis. In the original F1 hybrids, laboratory bioassays using artificial coffee diets showed that while CBB mortality in susceptible controls (Var. Caturra and maternal lines) did not exceed 15%, paternal lines induced 31–49%, and hybrids H1 and H3 reached 26%. Population suppression was further quantified in infested parchment coffee, where these hybrids exhibited intermediate CBB numbers between parents. Subsequent field evaluations in Caldas, Colombia, confirmed that H1 and H3 maintained CBB populations up to 43% lower than maternal lines ($p < 0.05$). To verify the stability of the resistance trait after clonal multiplication, a five-year field study was conducted in Risaralda, Colombia, using in vitro-propagated clones. These regenerated hybrids (H1, H2, and H3) demonstrated stable phenotypic expression, achieving up to a 70% reduction in CBB populations in the field compared to commercial varieties. Specifically, H1 and H3 clones induced significantly higher insect mortality (up to 47%) and superior population suppression. Furthermore, by manifesting hybrid vigor, cumulative production was significantly higher in all four hybrids than in commercial controls, with H3, H1, and H4 exhibiting the highest yields. Based on the dual criteria of useful heterosis for yield and stable pest resistance, these results identify the most promising hybrid combinations which integrate elite agronomic traits with stable resistance, providing a strategic genetic resource for sustainable coffee production under climatic change conditions.

Keywords: pest; genetic control; plant breeding; susceptibility; yield; adaptation; coffee

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1. Introduction

As the primary threat to Colombian coffee production, the Coffee Berry Borer (CBB) (*Hypothenemus hampei*) remains a significant challenge. This scolytid beetle exhibits a

cryptic life cycle, developing entirely within the coffee fruit, which compromises both bean quality and economic yield. In the field, colonizing females penetrate the fruit to oviposit directly into the endosperm; upon hatching, the larvae feed on the seed tissue. Current management of this pest relies on Integrated Pest Management (IPM) strategies, which combine cultural practices—specifically the removal of ripe fruits—with biological and chemical interventions.

Several authors suggest that CBB uses a complex mixture of volatile compounds to recognize fresh coffee berries [1–3] which has enabled the development of behavioral control strategies such as mass trapping using alcohol-based attractants [4]. Currently, most of the IPM control strategies based on biological and chemical control are implemented once the insect is attacking and penetrating the berries, before it enters the seed endosperm. A completely resistant coffee variety in which the insect does not cause damage to the seed would involve either the lack of recognition by the CBB or the presence of a highly toxic component within the seeds that kills the adult CBB once it starts to penetrate the fruit.

Although the search for sources of resistance within the genetic resources of *Coffea* L. has been conducted, no coffee genotypes with complete resistance to this pest. have been identified. However, these explorations have led to the identification of both wild genotypes and diploid species that, despite allowing insect entry, interfere with its biology [5]. The development of populations derived from these genotypes has shown a significant reduction in the total number of insect stages originating from a founding female, which, in general terms, could ultimately lead to a decrease in the insect population [6,7]. Additionally, other studies in *C. canephora* lines have revealed varying degrees of susceptibility to the CBB. These findings demonstrate a strong genetic effect on the percentage of CBB infestation, which ultimately impacts yield—an affect that amplifies as the coffee tree age [8].

These genotypes are considered relatively less susceptible than the other evaluated materials. This characteristic is referred to in this study as host plant resistance-specifically antibiosis [9], although the term does not imply a complete absence of insect damage to the seed. Instead, antibiosis in this context represents an intermediate level of resistance [10]. This approach focuses on reducing the pest fitness by exerting negative effects on CBB (e.g., survival, growth, development) [9], rather than mere tolerance [11,12]. Consequently, this mechanism serves as a robust genetic tool for long-term population control within coffee agroecosystems.

The use of coffee varieties that are less susceptible to the CBB, whereby the number of insect generations decreases over time, coupled with outstanding agronomic traits, offers a highly suitable alternative for coffee growers. This approach can reduce the economic damage caused by insect attacks while increasing yields, thereby minimizing the reliance on external chemical inputs and agrochemicals in the crop.

Although breeding programs aimed at developing varieties with this characteristic are currently underway [7], their main limitation is the time required for trait fixation, which can take up to 25 years. To address this, the use of H1 hybrids emerges as a promising tool for this species, as this generation contains a comprehensive and complex genetic combination from both parents and exhibits superior performance driven by heterosis [13].

In theory, this greater genetic potential also increases the likelihood of adaptation to a wide range of environments [14]. Consequently, identifying coffee cultivars that can adapt to different environments while maintaining stable, high-yielding, and high-quality production has become a priority for breeders, especially in the context of climate change [15]. This objective, together with conferring resistance to major pests and diseases, presents a new challenge, particularly in an era where pest and disease behavior is shifting due to climate variability.

In coffee cultivation, the first F1 hybrids used commercially were released in early 2008, and since then, new varieties have been made available at a handful of nurseries in Central America [16]. In general, farmers have rated F1 coffees highly for most of their agronomic qualities, including productivity and disease resistance [17]. In Colombia, the potential use of F1 hybrids has also been highlighted, showing good agronomic performance and achieving yields at least 40% higher than the parent plants under two contrasting light conditions [13]. However, the specific resistance mechanisms of these F1 hybrids against CBB, and how this resistance operates from the seed level to multi-year field conditions, have not yet been characterized.

Despite these general observations, a fundamental knowledge gap persists regarding their entomological protection. Prior to this study, while the overall agronomic potential of these hybrids was recognized, their direct resistance to CBB and their subsequent field performance after mass propagation remained entirely unknown. Characterizing whether these materials maintain a remarkable population suppression in the field is critical to opening the road for a novel genetic strategy for insect control.

To address this knowledge gap, the primary objective of this study was to evaluate host plant resistance to CBB in four F1 hybrids—H1 (CU1842 × C306), H2 (CX2385 × C534), H3 (CX2385 × C306), and H4 (CX2848 × C534) [13,18] and their respective parental lines established at the Cenicafé Naranjal station (Caldas, Colombia). The evaluation followed a comprehensive three-stage approach: (1) assessing antibiosis-related biological parameters using artificial diets formulated with ground coffee from the hybrid seeds; (2) performing direct laboratory evaluation on intact seeds to determine their inhibitory effect on insect development and survival; and (3) conducting multi-year field evaluations of fresh berries and established trees under natural and controlled infestation to validate population suppression.

Additionally, due to the limited availability of these F1 hybrids in the Colombian Coffee Collection and their heterozygous nature, seed-based reproduction or sexual reproduction was restricted to avoid the loss of key traits through genetic segregation in the F2 generation. Therefore, the second objective of this study was to propagate the F1 hybrid materials in vitro via somatic embryogenesis. This biotechnological pathway enables the large-scale generation of plantlets for field transplantation at the Cenicafé—La Catalina station (Risaralda, Colombia), aiming to evaluate the resulting clones for CBB resistance stability and agronomic performance over a five-year period, under field conditions.

Through this approach, this research aimed to identify improved hybrid coffee materials that combine high agronomic potential with stable antibiosis resistance to CBB. The integration of efficient mass propagation techniques, which ensure genetic fidelity and phenotypic uniformity, is discussed as a sustainable genetic strategy for the coffee industry. These hybrids can offer a critical adaptation tool under a changing climate while simultaneously reducing the economic and environmental costs associated with chemical control.

2. Materials and Methods

The experimental framework of this study was structured in a sequential, three-phase workflow to systematically evaluate host-plant resistance to the coffee berry borer (CBB) and its stability after clonal propagation. First, F1 coffee hybrids were generated and evaluated under controlled laboratory and field conditions to characterize their initial phenotypic resistance and population suppression potential (Sections 2.1–2.4). Second, the selected superior F1 materials were propagated via in vitro somatic embryogenesis to generate clonal lines (Section 2.5). Third, these in vitro-propagated hybrids were established in the

field for a multi-year validation to confirm both the temporal stability of their CBB resistance mechanisms and their long-term agronomic performance and yield (Sections 2.6–2.9).

To ensure a uniform challenge and eliminate the environmental variability of natural populations, all bioassays and field trials were conducted under artificially standardized and controlled CBB infestation conditions.

Given the multi-stage approach of this research, the number of biological, experimental, and field replicates was customized for each evaluation phase, as detailed in the respective sections below.

2.1. Coffee Hybrids and Experimental Site Description

The coffee hybrids were obtained through manual pollination by crossing three Var. Castillo pure lines: CU1842, CX2385, and CX2848 [19] as female parents with two wild Ethiopian accessions selected from the Colombian Coffee Germplasm: CCC306 (also identified as E286) and CCC534 (also identified as E554) [18]. For the purposes of this study, CCC306 and CCC534 are referred to as C306 and C534, respectively. These hybrids were initially evaluated for their agronomic potential in 2011 under two contrasting light conditions [13,18].

The experimental plots were located at the Cenicafé-Naranjal experimental station in Chinchiná, Caldas, Colombia (04°58′ N, 75°39′ W). The site is situated at an elevation of 1400 m above sea level (m a.s.l), with an average annual temperature of 21.4 °C and a mean relative humidity of 68%.

Four selected hybrids H1 (CU1842 × C306), H2 (CX2385 × C534), H3 (CX2385 × C306), and H4 (CX2848 × C534), along with their paternal (C306, C534) and maternal genotypes (CU1842, CX2385, and CX2848), were stumped in 2017. Following a regrowth period between 2019 and 2021, CBB resistance evaluations were performed. For this purpose, berries from these plants were collected and processed by removing the peel and pulp. The washed seeds were then sun-dried until they reached a moisture content of approximately 40% to 50%. These seeds were either used to formulate coffee-based artificial diets or evaluated directly to determine their inhibitory effects on CBB population dynamics.

2.2. Preparation of Artificial Coffee Diets and Evaluation of Insect Development

For the preparation of the artificial coffee diets, the methodology reported by Giraldo-Jaramillo et al. [20] was followed. Parchment coffee seeds (50% moisture content) collected in 2020 and 2021 from genotypes H1, H3, and H4, their respective parents, and *C. arabica* Var. Caturra were used.

The diets were prepared in 24-well plates (Techno Plastic Products 920024) (Techno Plastic Products AG, Trasadingen, Switzerland), with each well containing 3–4 mL of diet. *H. hampei* eggs from an insect colony were collected; 24 h post-oviposition, six eggs were placed in each well. Two plates were prepared for each genotype, providing a total of 48 independent experimental units (wells) per treatment and a total of 288 evaluated insects per genotype. The plates were incubated in the dark at a constant temperature of 25 °C, the optimal growth temperature for the insect [20,21]. The initial relative humidity of the diets was 50%, decreasing to 30% by the end of the experiment.

For each treatment, the total number of insects that hatched from the eggs in the 48 wells (across the two plates) was counted. Over a 35-day period, the number of live and dead insects was recorded daily in each well. The proportion of mortality was evaluated using a generalized linear model (GLM) with a binomial distribution and a logit link function, implemented in SAS version 9.4 (TS1M1; SAS Institute Inc., Cary, NC, USA) using the PROC GENMOD procedure. The model incorporated the genotype (material) as a fixed categorical factor. Adjusted means and their 95% confidence intervals were estimated on

the original response scale (proportion) using the ILINK option. Multiple comparisons with Tukey-adjusted *p*-values were applied to control for Type I error.

2.3. Bioassay of CBB Population Development on Infested Coffee Seeds

After obtaining the dry parchment coffee seeds (50% moisture content) from genotypes H1, H2, and H3, their respective parents, and *C. arabica* var. Caturra, only healthy, defect-free seeds were selected for the bioassay.

Each seed was placed inside a borosilicate vial (0.9 cm diameter × 3.4 cm height). A single adult CBB female, approximately 35 days old, was introduced into each vial following previously reported procedures [22,23]. Each seed-insect pair constituted an independent experimental unit, providing at least 135 biological replicates per genotype (treatment).

Following infestation, the vials were incubated at 25 °C. After 30 days, the seeds were dissected under a stereoscope at 10× magnification to record the number of insects at each developmental stage. The primary variable of interest was the average total number of CBB stages per treatment. Prior to analysis, assumptions of normality and homogeneity of variances were verified using Shapiro–Wilk and Levene's tests, respectively. The effect of the genotype on the total number of stages was analyzed via Analysis of Variance (ANOVA) using the PROC GLM procedure in SAS. Treatment means were compared using the Least Significant Difference (LSD) test, and 95% confidence intervals for the means were estimated using the LINES and TABLE options (LINES/TABLES).

2.4. Field Evaluation of CBB Resistance and Population Suppression at Naranjal Station

To characterize the initial phenotypic resistance and population suppression potential of the original F1 hybrid evaluations were conducted in August 2019 at the Cenicafé-Naranjal experimental station using field-planted hybrids. These plants had regrown following the stumping (zoqueo) performed in 2017. The evaluation included the maternal lines (CU1842, CX2385, and CX2848), paternal lines (C306 and C534), and their respective crosses (H1, H2, H3, and H4).

For each genotype, 10 trees with the highest fruit load were selected. On each tree, the branch with the highest number of berries and greatest developmental uniformity was chosen as the experimental unit (10 independent field replicates per treatment). All small or immature berries were removed to leave a selection of 50 to 70 berries at approximately 120 days of development. These berries were artificially infested with CBB at a 2:1 (insect:berry) ratio using entomological tulle sleeve cages. After 24–48 h, infestation was verified; any CBB individuals that failed to penetrate the fruits were removed, and the cages were then placed back on the branches.

Sixty days post-infestation, the infested berries from each treatment were harvested, and the insect population was quantified under a stereoscope (10× magnification). Each fruit was opened to count the number of eggs, larvae, pupae, and adults in each seed. To reduce experimental unit variability, the average of total number of insects and CBB stages per seed was calculated for each treatment and replicate.

The database was then summarized by treatment and replicate, and the means and standard errors for the variables total stages and live adults were estimated for each genotype. Prior to modeling, assumptions of normality and homogeneity of variances were verified using Shapiro–Wilk and Levene's tests, respectively, and the data met both criteria without transformation. A one-way analysis of variance (ANOVA) was conducted to compare the mean number of live adults between treatments corresponding to parental genotypes and hybrids of each family. The model was implemented using the PROC GLM procedure in SAS, and differences between the genotypes of each family were identified using Duncan's test ($\alpha = 0.05$).

2.5. Propagation of F1 Hybrids by In Vitro Culture

For in vitro propagation of the F1 materials by somatic embryogenesis and plantlets generations, the methodology reported by Aguilar et al. [24] with modification, as shown in Figure 1 was followed.

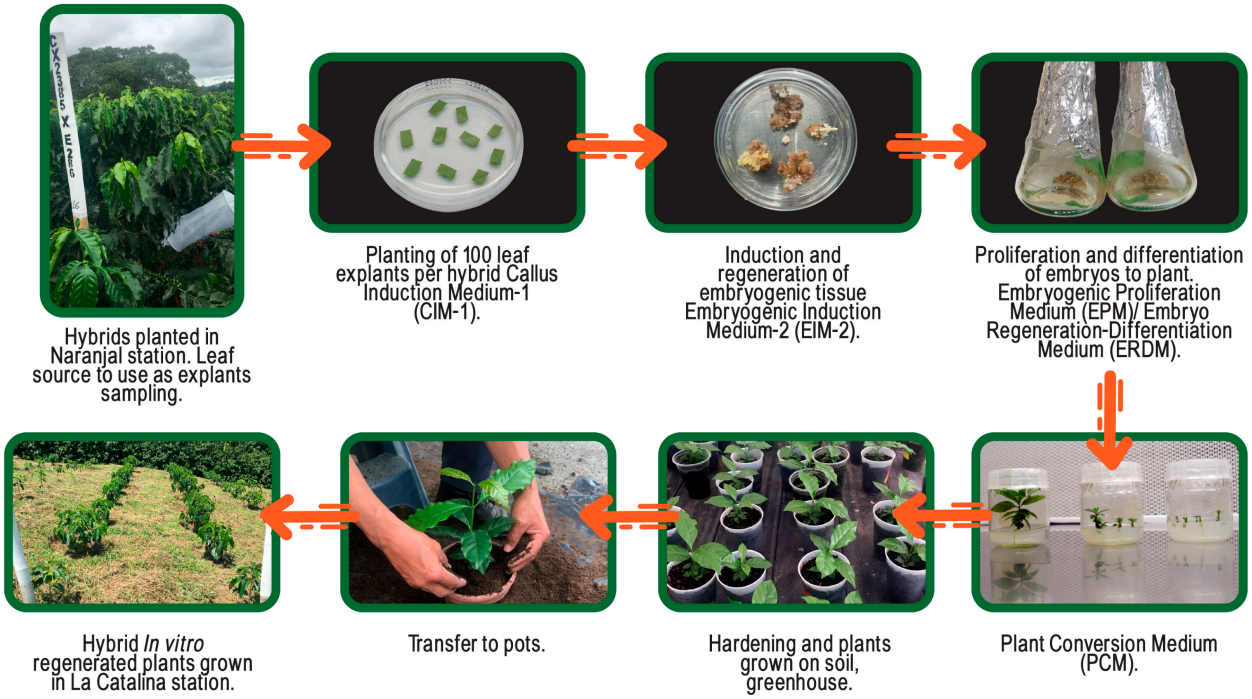

Figure 1. Regeneration process of coffee hybrids by in vitro culture.

From January to March 2015, leaves were collected from a single plant of each of the four F1 hybrids (H1, H2, H3, and H4) at the Naranjal station (Caldas) [18]. These mother plants were selected based on their superior phenotype and high productivity. For each selected plant, 5 to 10 young leaves were taken from the second pair of primary or secondary branches.

In the laboratory, under a laminar flow hood, the leaves were disinfected by washing with liquid soap and water. Subsequently, five leaves per plant were immersed in 500 mL of a 2.5% sodium hypochlorite solution for 15 min under constant stirring at 40 rpm. After removing the solution, three 5 min rinses with sterile deionized water were performed. Following the final rinse, the water was removed under a laminar flow hood. The leaves were dried with sterile paper towels and partitioned into 1 cm^2 explants, yielding between 10 and 20 segments per leaf. Each explant included a portion of the secondary vein, while the midrib and leaf margins were discarded.

For each hybrid, 100 explants were placed in **solid Callus Induction Medium-1 (CIM-1)** with NPC macro and micro nutrients [25] supplemented with 3% sucrose (*w*/*v*) (PhytoTechnology Laboratories, Lenexa, KS, USA), 0.5 mg/L 2,4-D (Sigma-Aldrich, St. Louis, MO, USA), 1.0 mg/L 2iP (Sigma-Aldrich, St. Louis, MO, USA), 15 mg/L Thiamine-HCl (Sigma-Aldrich, St. Louis, MO, USA), 1.0 mg/L Pyridoxine (Sigma-Aldrich, St. Louis, MO, USA), 1.0 mg/L Nicotinic acid (Sigma-Aldrich, St. Louis, MO, USA), 130 mg/L m-Inositol (Sigma-Aldrich, St. Louis, MO, USA), 0.3% GelRite (*w*/*v*) (PhytoTechnology Laboratories, Lenexa, KS, USA). Placing ten explants per Petri dish, and incubated under dark conditions at 24 °C. After one month, the explants were subculture onto **solid Embryogenic Induction Medium-2 (EIM-2)** [26] with some modifications: ½MS (Murashige and Skoog 1962) (PhytoTechnology Laboratories, Lenexa, KS, USA) supple-

mented with 3% sucrose (*w*/*v*), 0.42 mg/L KI (PhytoTechnology Laboratories, Lenexa, KS, USA), 60 mg/L Adenine Sulphate (PhytoTechnology Laboratories, Lenexa, KS, USA), 0.5 mg/L 2,4-D, 8 mg/L 6-BAP (PhytoTechnology Laboratories, Lenexa, KS, USA), 20 mg/L Thiamine-HCl, 200 mg/L m-Inositol, 20 mg/L Glycine (Sigma-Aldrich, St. Louis, MO, USA), 40 mg/L Cysteine (Sigma-Aldrich, St. Louis, MO, USA), 200 mg/L Casein hydrolysate (Sigma-Aldrich, St. Louis, MO, USA), 800 mg/L Malt extract (PhytoTechnology Laboratories, Lenexa, KS, USA), 0.3% GelRite (*w*/*v*). Cultures were monitored every 15 days, and if contamination exceeded 30%, new leaves were collected. The embryogenic induction on EIM 2 began after four to six months on some explants. Once embryogenic tissue was obtain, it was subcultured into **liquid Embryogenic Proliferation Medium (EPM)** [25] with some modifications: ½MS (Murashige and Skoog 1962) supplemented with 1.5% sucrose (*w*/*v*), 0.42 mg/L KI, 2.0 mg/L 2,4-D, 2.0 mg/L Kinetin (Sigma-Aldrich, St. Louis, MO, USA), 5 mg/L Thiamine-HCl, 0.5 mg/L Nicotinic acid, 0.5 mg/L Pyridoxine, 50 mg/L m-Inositol, 100 mg/L Cysteine, 100 mg/L Casein hydrolysate, 200 mg/L Malt extract. The embryogenic tissue was maintained for approximately four months, with subcultures performed every 15 days. To induce differentiation, the tissue was transferred to a **liquid Embryo Regeneration-Differentiation Medium (ERDM)**: ½ MS (Murashige and Skoog 1962) supplemented with 1.5% sucrose (*w*/*v*), 2850 mg/L KNO3, 0.42 mg/L KI, 0.05 mg/L NAA, 0.5 mg/L 6-BAP, 10 mg/L Thiamine-HCl, 100 mg/L m-Inositol, 370 mg/L Cysteine. Tissues in ERDM were subcultured every 15 days. Once somatic embryos were fully formed, they were transferred to a **solid Plant Conversion Medium (PCM**): ½MS (Murashige and Skoog 1962) supplemented with 2.0% sucrose (*w*/*v*), 0.42 mg/L KI, 10 mg/L Thiamine-HCl, 100 mg/L m-Inositol, 1.0 mg/L Nicotinic acid, 0.7% GelRite (*w*/*v*). All media have adjusted pH = 5.6 with KOH 0.1 N/HCl 0.1 N (Sigma-Aldrich, St. Louis, MO, USA), autoclave during 15 min at 15 psi.

2.6. Acclimatization and Field Establishment of F1 Hybrids at the La Catalina Experimental Station

Once the in vitro-derived plantlets developed six pairs of true leaves in the PCM medium, they were transferred to soil in plastic cups for greenhouse acclimatization. After a three-month hardening period, the plants were transplanted into larger pots. Following an additional three months of growth, the materials were ready for field transfer.

In April 2018, these in vitro-propagated F1 hybrids—consisting of 50 plants per line—were established at the La Catalina experimental station (Pereira, Risaralda, Colombia; 04°45′ N, 75°44′ W). The field planting was conducted to evaluate the performance of these materials under field conditions following their in vitro propagation.

2.7. Field Validation of Resistance Stability in In Vitro Propagated Coffee Hybrids at La Catalina Station

To evaluate the temporal and environmental stability of the characterized resistance, the in vitro propagated hybrid materials (H1, H2, H3, and H4), the maternal line CX2848, and plants obtained by seed from commercial varieties (Var. Cenicafé 1 and Var. Castillo Naranjal) were evaluated. All materials were six months old and had been maintained under greenhouse conditions prior to field establishment. In April 2018, the plants were transplanted at the Cenicafé-La Catalina experimental station, located in the municipality of Pereira, Risaralda, Colombia, at 1321 m a.s.l., with an average temperature of 21.9 °C and 82% relative humidity.

The plants were established following a randomized complete block design with seven blocks (plots). The seven genotypes were randomly distributed within each block, with eight plants per genotype. Each plot contained all seven genotypes; each genotype

was planted in a single row of eight plants with a within-row spacing of 1.0 m, providing a total of 56 field plants per genotype (7 blocks per 8 plants per block) as structural replicates.

The distance between rows was 1.5 m, and a 3.0 m isolation strip separated each block.

Throughout the evaluation period (2018–2023) at the La Catalina experimental stations, all plots were maintained under standard agronomic practices for Colombian technical coffee production. Soil fertilization was performed twice a year, with nutrient dosages adjusted according to periodic soil chemical analysis. Weed management followed an integrated framework, utilizing selective mechanical mowing to maintain ground cover and prevent competition.

Regarding entomological evaluations, all CBB infestations were carried out artificially under controlled experimental conditions. General pest management across the experimental sites relied strictly on standard Integrated Pest Management (IPM) frameworks established by Cenicafé—emphasizing cultural practices such as regular sanitation and frequent harvesting—ensuring that the background insect population remained regulated without compromising the accuracy of the controlled host-plant resistance assays.

In May 2020, twenty-six months after field establishment, the CBB resistance of the in vitro-derived materials was evaluated through controlled artificial infestations using entomological sleeve cages. For each genotype, 7 to 10 trees were selected across the seven blocks in a randomized layout. Within each tree, one or two branches containing berries of susceptible age were chosen as the experimental units, yielding a range of 7 to 10 independent field replicates (infested branches) per genotype depending on branch availability.

Fruit infestations were performed following the same standardized procedure described for the Naranjal station. Sixty days post-infestation, the infested berries from each treatment were harvested, and the CBB population was quantified under a stereoscope (10× magnification). Each fruit was dissected to count the number of eggs, larvae, pupae, and adults per seed.

The mean total population (all developmental stages) and the mean number of adults per seed were determined for each genotype. A descriptive analysis was initially performed, followed by a Generalized Linear Mixed Model (GLMM) using the PROC GLIMMIX procedure in SAS to evaluate the effect of the genotypes on the number of live adults.

Given that the response variable consisted of count data, a Poisson distribution with a log link function was specified in the model. To account for the experimental design, Blocks were treated as a random effect. Treatment-adjusted means (Least Squares Means) were compared using Scheffé's multiple comparison test ($\alpha = 0.05$). Given the use of a generalized linear mixed model with a Poisson distribution, traditional assumptions of normality and variance homogeneity were not required. Instead, model adequacy and the absence of overdispersion were verified by analyzing Pearson and deviance residual plots.

2.8. Assessment of CBB Population Development on Artificial Diets and Infested Dry Parchment Seeds of In Vitro Propagated Coffee Hybrids

To confirm the stability and reproducibility of the resistance mechanisms after clonal propagation via somatic embryogenesis, additional laboratory bioassays were conducted using the clones established at La Catalina. Antibiosis was evaluated using artificial coffee diets prepared from the seeds of each genotype. CBB development, including larval survival and developmental rates, was recorded as described previously in Section 2.2. The proportion of dead individuals was analyzed using a Generalized Linear Model (GLM) with a binomial distribution and logit link function, implemented via PROC GENMOD in SAS. The model included the coffee material as a fixed categorical factor. Adjusted means and their 95% confidence intervals were estimated on the original response scale (proportion) using the ILINK option. Multiple comparisons were performed using Tukey's test with adjusted *p*-values to control for Type I error.

Additionally, an evaluation of CBB population development was performed using infested dry parchment coffee seeds, following the methodology detailed previously in Section 2.3. A descriptive analysis was conducted, followed by a Generalized Linear Mixed Model (GLMM) using the PROC GLIMMIX procedure in SAS to evaluate the effect of each treatment on the number of live adults. Since the response variable consisted of count data, a Poisson distribution with a log link function was specified. Treatment-adjusted means were compared using Scheffé's method. For both bioassays, because generalized linear models (GLM and GLMM) with Binomial and Poisson distributions were implemented, standard parametric assumptions of normality and homoscedasticity were not applicable. Model goodness-of-fit and dispersion behaviors were systematically checked using deviance and Pearson residual diagnostics.

2.9. Agronomic Performance and Yield of In Vitro Propagated Hybrids at La Catalina Station

In the seven hybrid plots, agronomic traits were monitored over a five-year production cycle (2020–2024, 60 months). The following variables were evaluated: tree height, number of nodes (branch crosses), stem diameter, and coffee yield (measured as kg/tree of fresh berries).

Tree height and the number of nodes were recorded semi-annually (January and June of each year). Yield data were obtained by weighing mature fresh berries harvested every 15–20 days throughout the production period.

For the statistical analysis of agronomic traits, a descriptive analysis was performed for each evaluation date. To assess the effect of genotypes, an Analysis of Variance (ANOVA) was conducted for each time point. The experimental error was estimated from the variation among plots nested within genotypes. To identify superior materials, significant differences between the hybrids and the commercial control (Var. Castillo Naranjal) were determined using Dunnett's test at a 5% significance level.

2.10. Meteorological Data Collection

Weather was monitored throughout the 5-year evaluation period (2018–2023) to characterize the environmental conditions at the experimental site. Daily temperature (°C) and total precipitation (mm) data were obtained from La Catalina meteorological station, managed by the National Coffee Research Center (Cenicafé). The parameters analyzed included annual precipitation, as well as the lower, upper, and overall mean temperatures, as these are the primary environmental drivers influencing the biology and development of the CBB and plant growth. These historical datasets were verified and retrieved from the official Agroclima platform (https://agroclima.cenicafe.org/) and cross-referenced with the corresponding Annual Meteorological Reports (Anuarios Meteorológicos Cafeteros, 2018–2023) published by Cenicafé.

3. Results

To facilitate tracking the evaluation sequence, the results of this study are presented under a progressive line of reasoning. Data advance sequentially from highly controlled laboratory assays (artificial diets and intact seeds) to natural infestation pressures under field conditions, concluding with the multi-year performance of the clonally propagated hybrids.

3.1. Effect of Artificial Diets Derived from Hybrid Seeds on CBB Development

The impact of the different artificial diets on CBB mortality is summarized in Table 1, using seeds from the original materials established at the Naranjal station. Var. Caturra was the most susceptible genotype, with mortality rates not exceeding 10%, a value consistent with the natural mortality of insects in these diets. The parental lines exhibited contrasting

responses: the C306 genotype (paternal) induced the highest CBB mortality ($p < 0.0001$) estimated between 42.9% and 54.4%. Conversely, the maternal lines CU1848 and CX2385 showed lower mortality rates, below 22.2%. The hybrids generally exhibited an intermediate response, with estimated mortalities ranging from 20.7% to 32.5%. According to Tukey's test, these hybrids differed significantly from both parents ($p < 0.0247$ and $p < 0.05$).

Table 1. Effect of artificial diets derived from hybrid materials, their parental lines, and *C. arabica* Var. Caturra on the mortality of CBB.

Evaluation	Genotype	CBB Percentage Mortality (Mean)	*	Standard Error	Lower Mean	Upper Mean
1	Var. Caturra	6.3	d	1.4	4.0	9.7
	CU1842	14.9	c	2.1	11.3	19.5
	CX2385	15.4	bc	2.2	13.4	22.2
	C306	48.6	a	2.9	42.9	54.4
	H1 (CU1842 × C306)	25.4	b	2.6	20.7	30.7
	H3 (CX2385 × C306)	27.1	b	2.6	22.3	32.5
2	CX2848	15.1	b	2.0	10.9	19.3
	H4 (CX2848 × C534)	15.2	b	2.9	11.0	19.5
	C534	30.7	a	2.7	25.2	36.2

* For each evaluation. Mortality Mean followed by different letters indicate statistically significant differences (Tukey–Kramer test at the 5% significance level).

Regarding the paternal line C534, mortality was estimated between 25% and 36%. In this case, the hybrid H4 showed a statistical response similar to the maternal line, with 15% mortality (Table 1, Evaluation 2), differing significantly only from the male parent ($p < 0.001$).

3.2. Evaluation of CBB Population Development on Infested Coffee Seeds

Regarding the seed infestation bioassays, the average total CBB population (all developmental stages) and the 95% confidence intervals for the H3 family and the control are presented in Table 2. Similar to the results observed in the artificial diets, Var. Caturra was the most susceptible genotype, supporting the highest insect development with an average of 29 to 34 stages per seed. The maternal line CX2385 showed significantly lower susceptibility than Var. Caturra. maintaining population levels between 21 and 25 individuals per seed. The hybrid H3 exhibited an intermediate resistance response, with a 25% reduction in total population compared to the maternal line, and 20% more individuals than the paternal line (C306). These differences were statistically significant according to the Least Significant Difference (LSD) test at 5%.

Table 2. Effect of coffee seeds obtained from hybrid materials H3, their parents and control *C. arabica* Var. Caturra in the development of the CBB.

Genotype	CBB Total Stages/Seed (Mean)	*	Lower Mean	Upper Mean
Var. Caturra	31.7	a	29.7	33.7
CX2385	23.0	b	20.7	25.4
H3 (CX2385 × C306)	18.5	c	16.5	20.4
C306	13.7	d	13.4	13.9

* LSD grouping Total stages means with the same letter are not significant different may have a footer.

Table 3 details the mean CBB populations for the H1 and H2 families. A consistent trend of intermediate population levels in the hybrids compared to their parents

was observed (ANOVA. $p < 0.05$), with significant differences between the maternal and paternal lines.

Table 3. Effect of coffee seeds obtained from hybrid materials, their parents in the development of the CBB.

Evaluation	Genotype	Number of Observations	CBB Total Stages/Seed (Mean)	*	Lower Mean	Upper Mean
1	CU1842	161	21.2	a	19.7	22.7
	H1 (CU1842 × C306)	143	18.6	b	17.1	20.1
	C306	155	12.3	c	11.1	13.5
2	CX2385	139	18.8	a	17.4	20.2
	H2 (CX2385 × C534)	147	14.0	b	12.8	15.2
	C534	148	12.2	c	11.1	13.4
3	CX2848	138	16.0	a	14.4	17.6
	H4 (CX2848 × C534)	135	14.8	a	13.2	16.4
	C534	143	12.6	b	11.4	13.7

* For each evaluation-LSD grouping means with the same letter are not significant different.

For the H2 hybrid family, an initial evaluation in 2020 showed no significant differences in the total number of stages between the hybrid and its parents. However, a detailed analysis of the stages (Figure 2) revealed that both the hybrid and the paternal line (C534) had significantly higher counts of eggs and larvae, which were the predominant stages. In contrast, in Var. Caturra and the maternal line (CX2385), the predominant stages were pupae and adults. This indicates that population development is delayed in the hybrids and the paternal line compared to the maternal line and the susceptible control. This assay was repeated in 2021 (Table 3) with seeds dissected 10 days later. In this second evaluation, the hybrid exhibited a population density similar to the male parent, differing significantly from the maternal line according to the LSD test at 5%.

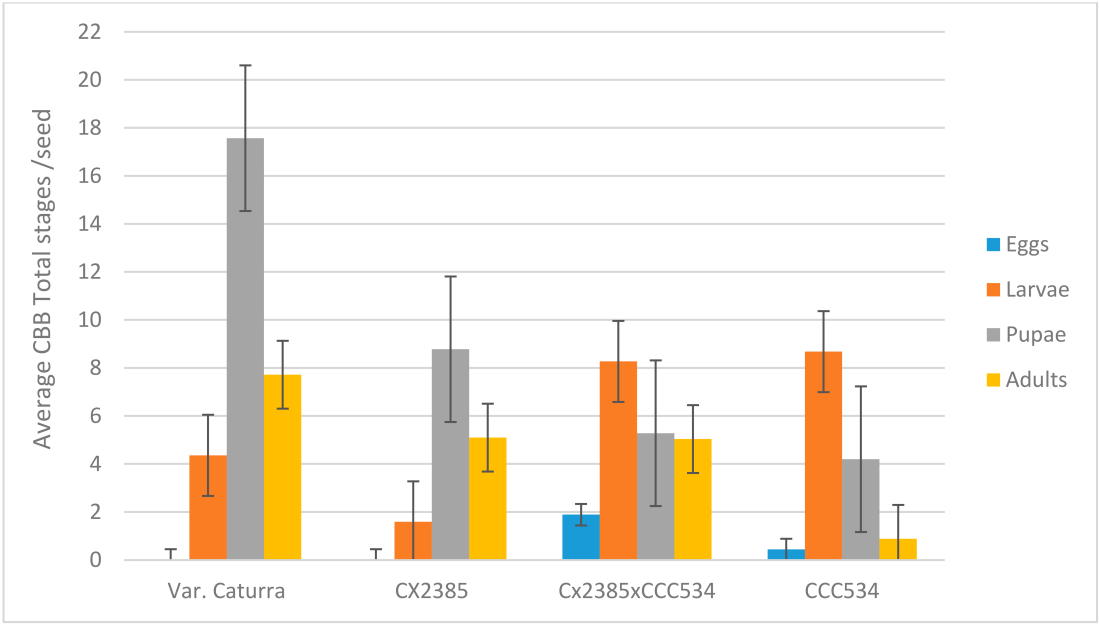

Figure 2. Distribution of Mean and Standard Error (±SE) CBB developmental stages in seeds of F1 hybrids (H2), parental lines, and the susceptible control *C. arabica* Var. Caturra.

For the H4 hybrid, two evaluations were conducted. While the first showed no significant differences, the second evaluation (Table 3) revealed that the hybrid behaved

similarly to the maternal line and differed significantly from the male parent (ANOVA. $p < 0.05$). This trend is consistent with the results obtained in the artificial diet evaluations.

3.3. Assessment of Host-Plant Resistance to H. hampei Under Natural Field Infestation

A descriptive analysis of CBB population development across the different genotypes is presented in Figure 3, showing the total number of insect stages per fruit. Table 4 displays the data for the total number of adults, which was the variable that provided the clearest differentiation between treatments.

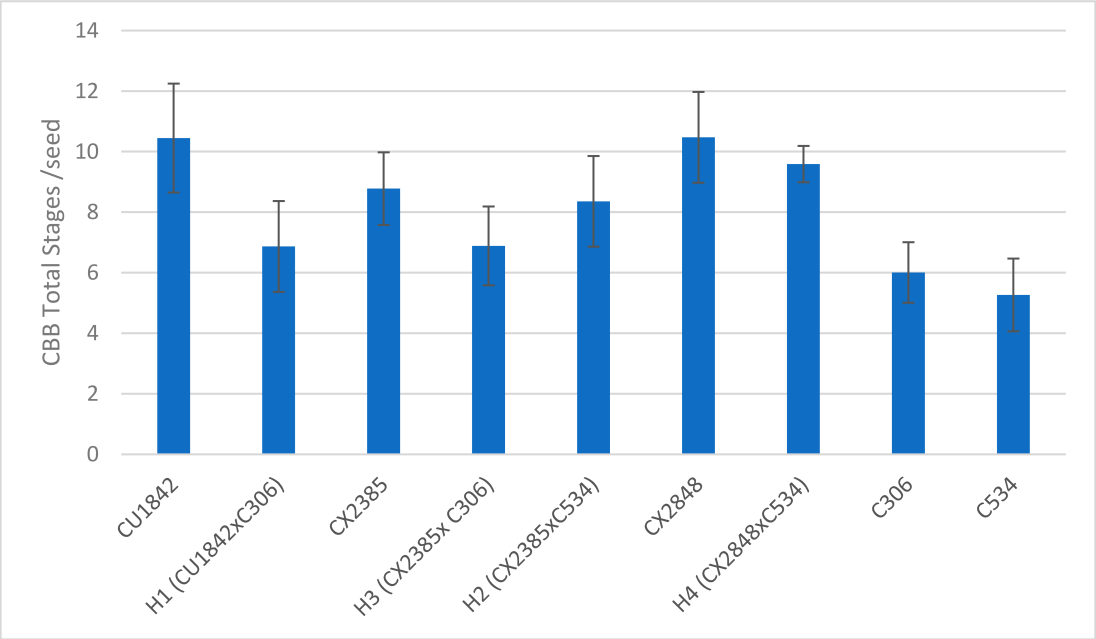

Figure 3. Mean total CBB population across different coffee genotypes under field conditions at Naranjal Station. Bars represent the total number of developmental stages per fruit, and error bars indicate the standard error (±SE).

Table 4. Mean and standard error (±SE) of CBB adults per seed in coffee hybrid lines and their parental lines, 60 days post-infestation under field conditions.

Family	Genotype	Total CBB Adult Stages/Seed (Mean)	*	Standard Error
1	CU1842	4.4	a	0.59
	H1 (CU1842 × C306)	2.5	b	0.38
	C306	1.6	b	0.11
3	CX2385	3.2	a	0.34
	H3 (CX2385 × C306)	2.2	b	0.28
	C306	1.6	b	0.11
2	CX2385	3.2	a	0.34
	H2 (CX2385 × C534)	3.0	a	0.41
	C534	1.7	b	0.19
4	H4 (CX2848 × C534)	4.3	a	0.33
	CX2848	3.3	b	0.42
	C534	1.7	c	0.19

* For each family, Means with the same letter are not significantly different (Duncan's test ($\alpha = 0.05$).

The paternal genotypes exhibited the lowest CBB populations, followed by hybrid lines H1 and H3, which showed similar performance. These results indicate that the resistance levels observed in laboratory bioassays (diets and seeds) are consistently maintained under

field conditions at the Naranjal station. This alignment between in vitro and in vivo data suggests that the antibiosis traits identified in the laboratory are stable phenotypic markers for CBB population suppression.

The Duncan's Multiple Range Test performed on the adult stage population revealed significant differences between the parental lines. The wild paternal materials (C306 and C534) exhibited the lowest adult populations, with an average of 1.6 adults per fruit. In contrast, the maternal lines (CU1842, CX2385, and CX2848) showed significantly higher infestation levels, with approximately 50% more adults (averaging 3.5 adults per fruit).

When comparing the adult population of each hybrid with its respective parents, the following patterns were observed: for the H1 and H3 families, hybrids showed an adult stage population statistically similar to the resistant male parent ($F = 11.37$, $p < 0.05$), reaching levels 43% and 31% lower than those of their maternal lines, respectively (Table 4). Conversely, for the H2 and H4 families ($F = 0.0068$), the adult population differed from the paternal line and was more similar to the maternal line, indicating higher susceptibility.

3.4. In Vitro Propagated Hybrids

3.4.1. Field Validation of CBB Resistance and Genetic Stability in In Vitro Propagated Coffee Hybrids at La Catalina Station

The adult stage populations of CBB developed in the in vitro propagated hybrid, along with the results of the Scheffé test, are presented in Table 5. The hybrids H1, H3, and H2 differed significantly from the commercial varieties, showing up to 70% fewer individuals in the adult stage population.

Table 5. Mean number of live CBB adults in in vitro propagated hybrid genotypes and commercial varieties, 60 days post-infestation under field conditions (La Catalina Station).

Genotype	CBB Total Adult Stages/Seed (Mean)	*	Standard Error
CX2848	3.62	a	0.575
Var. Castillo Naranjal	2.95	a	0.294
Var. Cenicafé 1	2.55	ab	0.333
H4 (CX2848 × C534)	1.46	bc	0.157
H2 (CX2385 × C534)	0.93	c	0.087
H3 (CX2385 × C306)	0.92	c	0.135
H1 (CU1842 × C306)	0.85	c	0.114

* Means with the same letter are not significantly different. Scheffe test results.

The significant reduction in the adult population across these three hybrid lines suggests that CBB resistance is stably expressed under the environmental conditions of the La Catalina station. These findings confirm that the suppressive effect on the insect's reproductive success, initially identified in laboratory bioassays, remains effective in clonally propagated materials evaluated under field pressure.

3.4.2. Evaluation of CBB Mortality on Artificial Diets (La Catalina Station)

The mortality rates of CBB larvae reared on artificial diets prepared with in vitro propagated hybrid seeds are summarized in Table 6. Hybrids H1 and H3 induced the highest mortality levels, reaching 41.3% and 32.9%, respectively ($p < 0.0001$). according to Tukey's adjusted multiple comparison test.

In previous evaluations using seeds from the Naranjal station, the H4 hybrid showed a mortality rate of 15%, a value similar to the maternal lines. In the evaluations conducted at La Catalina, the hybrids H2 and H4 (both derived from the C534 paternal line) exhibited mortality rates between 22% and 23%. Although these rates did not differ significantly

from the maternal line CX2848, both hybrids maintained lower CBB populations compared to the commercial controls.

Table 6. Mortality percentage of CBB populations in artificial diets prepared from in vitro propagated coffee hybrids at La Catalina station.

Genoptype	CBB Percentaje Mortality (Mean)	*	Standard Error	Lower Mean	Upper Mean
H1 (CU1842 × C306)	41.67	a	2.598	36.68	46.83
H3 (CX2385 × C306)	32.78	a	2.474	28.12	37.8
H4 (CX2848 × C534)	22.78	b	2.21	18.74	27.4
H2 (CX2385 × C534)	21.67	bc	2.171	17.71	26.22
CX2848	17.78	bcd	2.015	14.16	22.08
Var. Cenicafe 1	14.17	cd	1.838	10.93	18.16
Var. Castillo Naranjal	12.78	d	1.76	9.707	16.64

* Mortality Mean followed by different letters indicate statistically significant differences (Tukey–Kramer test at the 5% significance level).

Specifically, the analysis showed that H2 differed significantly from Var. Castillo Naranjal, while H4 exhibited significant differences compared to both Castillo Naranjal and Cenicafé 1. These results identify a reduction in CBB population levels in the hybrid materials relative to the commercial varieties currently available on the market.

The results for hybrid seeds infested with CBB are presented in Table 7. After 30 days of infestation, the total number of individuals (eggs, larvae, pupae, and adults) per seed was quantified for each genotype. Analysis via a generalized linear mixed model (GLMM) confirmed that the four hybrids differed significantly from the commercial varieties.

Table 7. Effect of coffee seeds obtained from hybrid materials and commercial Varieties planted at La Catalina) in the development of the CBB.

Genotype	CBB Population (Mean)	*	Standard Error	Lower Mean	Upper Mean
Var. Cenicafe1	27.9	a	0.43	27.04	28.73
Var. Castillo Naranjal	24.3	b	0.40	23.48	25.05
CX2848	21.8	c	0.38	21.08	22.57
H2 (CX2385 × C534)	21.2	c	0.38	20.48	21.96
H4 (CX2848 × C534)	21.1	c	0.38	20.42	21.90
H3 (CX2385 × C306)	16.7	d	0.33	16.05	17.36
H1 (CU1842 × C306)	15.3	d	0.32	14.73	15.99

* Population Means with the same letter are not significantly different. Scheffe test results.

Hybrids derived from the paternal line C306 (H1 and H3) exhibited the lowest populations, with 15 and 17 insects per seed, respectively. This represents a population decrease of up to 45% compared to the commercial variety Cenicafé 1 (28 insects per seed).

Meanwhile, hybrids from the paternal line C534 (H2 and H4) performed similarly to the maternal line CX2848 (22 individuals/seed). Although the reduction was less pronounced than in the C306 hybrids, a population decrease of approximately 25% was still observed when compared to the Var. Cenicafé 1 control.

3.4.3. Agronomic Performance and Yield of In Vitro Propagated Hybrids at La Catalina Station

Seven plots were established at the La Catalina Station; however, data from plot number three were excluded from the analysis due to some mealybug infestation.

The cumulative average of the primary branch crosses for each genotype after 60 months are presented in Figure 4. Furthermore, the cumulative average production

at 24–36, 48, and 60 months is shown in Figure 5. Table 8 details the total cumulative production after 60 months, including the statistical analysis based on mean yield and standard error and Dunnett's test at a 5% significant level.

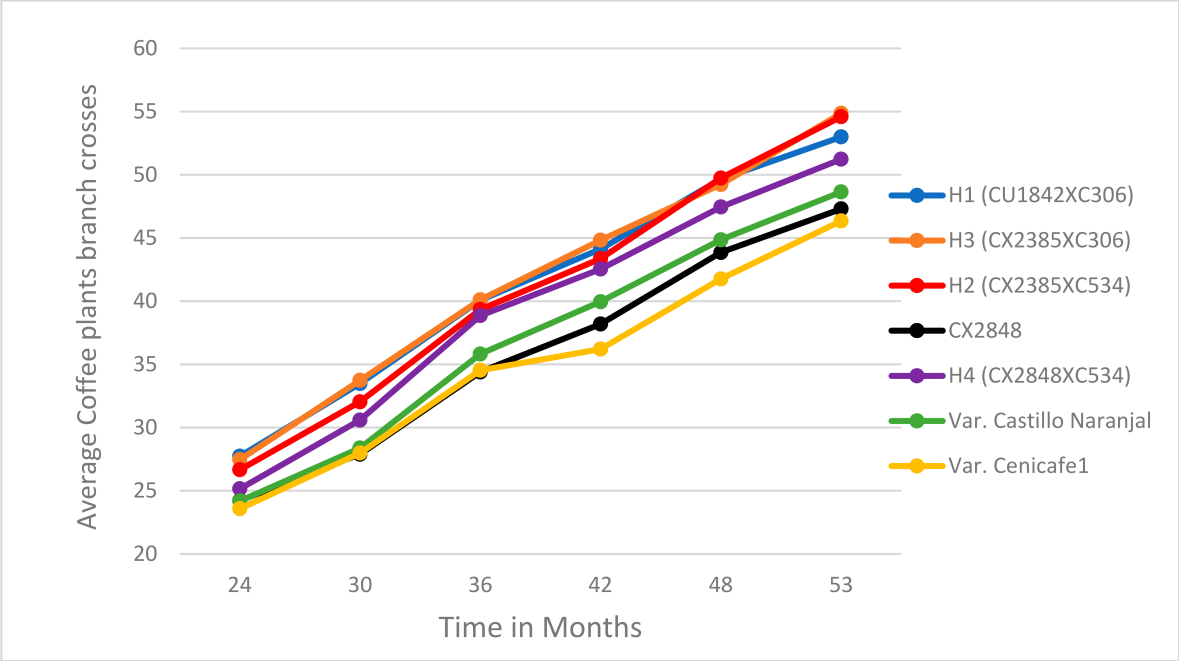

Figure 4. Cumulative average number of primary branch crosses in hybrid materials and commercial controls at the La Catalina station, evaluated over a 60-month period.

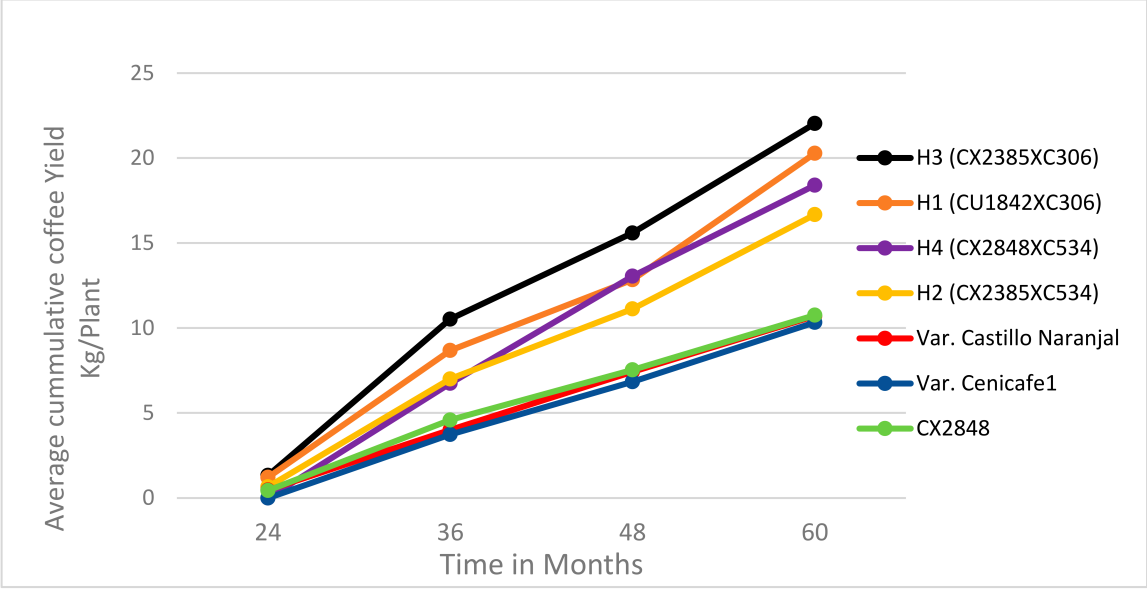

Figure 5. Cumulative Coffee Yield in the hybrid materials and controls planted at La Catalina station, over 60 months.

Initial growth rates differed significantly across all materials in terms of plant height (Appendix A Table A1), and the number of primary branches, with distinct architectural differences emerging in the early years. In general, the hybrids grew faster and remained taller until month 42. After this period the plant heights of all genotypes began to converge, showing no differences by the end of the study and by months 53 and 60.

Table 8. Comparison of yield of hybrid genotypes and commercial materials planted at La Catalina experimental station after 60 months.

Treatments	Cumulative Coffee Yield (Mean)	*	Standard Error
CX2848	10.8		0.096
H1 (CU1842 × CCC306)	20.3	*	1.524
H4 (CX2848 × CCC534)	18.4	*	1.086
Cenicafe1	10.3		0.682
H2 (CX2385 × CCC534)	16.7	*	1.189
H3 (CX2385 × CCC306)	22.0	*	1.959
Castillo Naranjal	10.7		0.684

* Indicate significant difference compared to treatment Var. Castillo Naranjal (commercial variety) according to Dunnett's test at 5% significance level.

Regarding the number of primary branch crosses, the hybrids demonstrated accelerated growth; at 36 months, they reached a node count equivalent to what the commercial varieties only achieved between 42 and 48 months (Figure 4). The hybrids outperformed the commercial varieties in yield from the beginning of the assay. At 36 months, the hybrids showed a significantly higher yield than the controls. Between 36 and 48 months, the hybrids maintained a production level superior to the commercial varieties, a trend that continued through to 60 months (Figure 5). By the end of the experiment, the controls showed an average cumulative production of 10.5 kg/plant, while the hybrids yielded between 16.7 and 22.0 kg/plant. The highest-producing hybrids were H3 and H1, followed by H4 and H2 (Table 8). Results Summary, Hybrid Characteristics Appendix A Table A1.

3.5. Environmental Data Analysis

The meteorological data recorded at La Catalina station between 2018 and 2023 showed distinct patterns for temperature and precipitation (Appendix A Figure A1). The temperature parameters displayed low variability across the 6-year period. The overall mean temperature remained stable between 21.0 °C and 22.0 °C, while the lower and upper annual means fluctuated minimally, ranging from 17.0 °C to 18.0 °C and 26.5 °C to 27.5 °C, respectively. In contrast, annual precipitation exhibited higher variability throughout the evaluation period. Rainfall ranged from a minimum of approximately 1900 mm in 2020 and 2023 to a maximum peak of 2933 mm in 2022.

4. Discussion

4.1. Rationale for Hybrid Crosses and Genetic Background

Hybrids are widely accepted in agriculture, primarily due to their advantages in increasing production, adapting to adverse weather conditions, and enhancing resistance to pests and diseases. In coffee cultivation, hybrid development has focused on improving agronomic traits such as adaptation, yield, quality, chemical composition, and disease resistance [27–33]. However, their large-scale implementation has been hindered by the lack of efficient mass-multiplication mechanisms and the higher initial cost of in vitro plants compared to those obtained from seed [16,32]. Despite these challenges, the potential of hybrids to transform the industry continues to be recognized.

The hybrid crosses in this study were initially designed to evaluate performance regarding yield, shade adaptation, cup quality traits inherited from paternal lines, and the expression of heterosis (hybrid vigor) in the F1 generation [13,18]. The Var. Castillo lines used as maternal parents are characterized by their resistance to coffee rust (*Hemileia vastatrix*), high productivity, and superior cup quality.

While the wild paternal lines were selected for their excellent organoleptic profiles, and at least one (C534) had previously been reported as less susceptible to CBB [23], our

results identified an additional wild *C. arabica* accession, C306, which also exhibits high levels of antibiosis resistance to this pest. The identification of C306 as a source of resistance is particularly relevant, as it broadens the genetic base for breeding programs aimed at pest population suppression without compromising cup quality.

Consequently, this study is the first to demonstrate in coffee how the exploitation of heterosis can be strategically combined with resistance traits against major pests. This specific combination of agronomic vigor and active pest suppression represents a novel strategy that distinguishes these materials from past breeding efforts, strengthening integrated pest management (IPM) systems in coffee cultivation.

4.2. Multi-Stage Screening Validation and Bioassay Evaluation

The validation of results was conducted in three steps: using artificial seed-based diets, seeds infested with CBB under laboratory conditions, and fruits infested under field conditions. In the first step, CBB eggs were placed in the diet, allowing direct observation of the effects of different genotypes on the larvae that hatched and consumed the material. This method facilitated the identification of mortality rates and growth delays. In the second step, individual seeds were infested with a single female CBB, which deposited eggs within the seed. After 30 days, the seeds were opened to determine the total population and assess the genotypes' effect on insect development by measuring the reduction in the number of individuals reaching adulthood. Finally, in the third step, fruits attached to the branches were infested under field conditions. Similar to the laboratory seeds, the impact of the fruit on the insect population was evaluated; this time, accounting for environmental variables and the whole-plant response. Through this approach, the agronomic potential of the hybrids—as previously reported by Bertrand et al. [13]—was validated under real-world environmental conditions. This comprehensive, multi-stage validation aligns with modern sustainable crop protection paradigms, which emphasize that precise evaluation of host–pathogen or host–insect interactions requires standardized multi-method screening assays to accurately quantify tissue damage and pest progression across different developmental layers [34].

The results from the artificial diet assays indicate that, compared to Var. Caturra, the evaluated hybrids exhibit significant host-plant resistance to CBB by restricting its population growth, while Var. Caturra served as a highly susceptible control—supporting optimal insect development with a low baseline mortality rate of 6.3%—the hybrid-based diets effectively impaired the biological fitness of the pest. This contrast suggests that the chemical composition of the hybrid seeds contains natural inhibitory compounds that disrupt the CBB life cycle.

In the maternal progenitors CU1842, CX2385, and CX2848, CBB mortality did not exceed 15%. However, in the resulting hybrids, mortality rates reached up to 27%. While these maternal lines were originally selected for rust resistance and agronomic traits, they also exhibited inherent low susceptibility to CBB compared to Var. Caturra. Ultimately, the diet bioassays revealed that the two hybrids sharing the C306 paternal lineage exerted the strongest inhibitory effect on the insect, demonstrating enhanced resistance compared to both their maternal parents and the commercial controls.

4.3. Differential Performance and Characterization of Hybrid Families

The paternal line C306 was identified as the most resistant to CBB across all evaluation methods, including artificial diets and seed assays. Consequently, the hybrid materials derived from this parent (H1 and H3), as well as their in vitro-propagated clones, exhibited significant host-plant resistance, adversely affecting CBB development under both laboratory and field conditions. These hybrids consistently maintained lower CBB infestation

levels compared to commercial controls. Notably, in trials conducted at the Naranjal station (Caldas), these specific hybrids showed a substantial population reduction compared to their maternal parents, with up to 43% fewer individuals per seed.

While the paternal line C534 also exhibits host-plant resistance, its suppressive effect was lower than that of C306, as observed in the diet bioassays. The phenotypic expression of the hybrids derived from C534 varied: H4 behaved similarly to its maternal parent in both laboratory and field settings, whereas H2 demonstrated enhanced resistance compared to the maternal line, reflecting the successful incorporation of paternal defensive traits into these genotypes. Laboratory evaluations using infested seeds revealed that H2 induces a delay in insect development, providing evidence of a mechanism of antibiosis present in the endosperm.

The hybrids H1, H2, and H3, which were in vitro propagated and established at the La Catalina station (Risaralda), exhibited significantly lower CBB population levels under field conditions compared to commercial materials obtained from seed, such as Var. Cenicafé 1, Var. Castillo, and the maternal line CX2848 used as control. This field validation represents a major advancement over historical resistant lines, as these hybrids showed up to a 70% reduction in adult CBB populations compared to the commercial varieties.

Under laboratory conditions using artificial diets, H1, H3, and H4 caused the highest CBB mortality rates, differing significantly from the commercial controls. Subsequent seed/insect population evaluations confirmed that hybrids derived from the C306 paternal line (H1 and H3) supported the lowest CBB populations, with only 15–17 individuals per seed. This represents a 40–45% reduction in insect population compared to the other materials. Furthermore, while the C534-derived hybrids (H2 and H4) showed intermediate results, they still demonstrated a significant reduction in insect populations relative to the commercial varieties. The data demonstrate a strong correlation between laboratory bioassays and field observations, confirming that the host-plant resistance identified in the hybrids remains genetically stable and is robustly expressed under natural infestation pressure. This consistent performance suggests a synergistic interaction between the defensive traits of the hybrids and the environment, which further constrains the colonization and growth of the CBB population. The 70% reduction in adult populations observed at the La Catalina station underscores the high efficacy of these clonally propagated hybrids as a cornerstone for integrated pest management (IPM) strategies.

4.4. Mechanisms Driving Antibiosis

Preliminary gene expression analyses in these hybrid materials revealed the upregulation of defense-related genes shortly after CBB attack on fresh berries. Key identified transcripts include isoprene synthase, patatin (lipid acyl-transferase), and trypsin inhibitors, all of which are highly expressed during the early stages of infestation. These molecular signatures closely mirror the defense response observed in *Coffea liberica* berries [35], a species characterized by its innate resistance to CBB.

Crucially, both the morpho-molecular evaluations of *C. liberica* accessions in Colombia [36] and functional transcriptomic analyses [35] confirm that host-plant defense against this pest is driven by active metabolic pathways and chemical antibiosis, rather than physical or macro-morphological traits such as fruit or endosperm hardness. This molecular activation accounts for the hybrids' inhibitory effect on insect development and explains their observed phenotypic resistance.

While further studies are required to fully characterize the specific signaling networks underlying this genetically driven suppression, these preliminary observations provide a valuable foundation to complement traditional agronomic evaluations.

The antibiosis observed in our bioassays strongly suggests that this molecular upregulation triggers protective chemical profiles in the resistant genotypes. In coffee, volatile sesquiterpenes like beta-caryophyllene and farnesene variants critically disrupt the host-seeking and colonization behavior of the coffee berry borer [37–39]. Utilizing such endogenous terpenoids aligns with modern eco-compatible pest management, where natural compounds—such as farnesal—are increasingly leveraged to develop targeted, bioclean alternatives to synthetic pesticides [37]. These insights offer a broader, biochemical perspective on the mechanisms driving pest population suppression in coffee agroecosystems.

A sustainable reduction in the number of CBB individuals per seed has profound implications for long-term insect population dynamics. As fewer individuals successfully complete their life cycle within the hybrid berries, the net reproductive rate (R_0) decreases across successive generations. This inhibitory effect is primarily driven by increased mortality during immature stages (eggs, larvae, and pupae), which effectively prevents a significant portion of the population from reaching adult stage and reproductive maturity. This confirms antibiosis as the driving mechanism of host-plant resistance in these hybrids, rather than tolerance or non-preference alone.

In contrast, highly susceptible varieties such as Var. Caturra provide an optimal nutritional environment that supports high survival rates and rapid population expansion. In these susceptible materials, each new generation emerges with a stable or increasing number of individuals, progressively exacerbating infestation levels. However, the hybrid materials evaluated in this study disrupt this cycle by limiting the pest's ability to thrive. This cumulative reduction in CBB populations leads to a progressive decline in infestation pressure, ultimately mitigating the economic losses associated with the pest. These findings highlight the potential of these hybrids as a foundational and sustainable component of integrated pest management (IPM), significantly enhancing crop resilience.

Determining the biological and reproductive fitness of CBB populations across the specific agroecological zones intended for hybrid cultivation is essential. This allows for a precise quantification of the combined effects of hybrid genotypes and abiotic factors, particularly temperature, on the insect's life cycle. As demonstrated by Giraldo-Jaramillo et al. [20], temperature is a primary driver of the potential number of CBB generations per year. The introduction of these coffee hybrids could generate a synergistic effect, where genetic resistance and environmental temperature fluctuations interact to further suppress insect populations. Such an additive impact would be particularly valuable in coffee-growing regions where climate variability already influences pest pressure, providing a more robust and resilient production system.

4.5. Agronomic Performance, Heterosis, and Physiological Trade-Offs

The five-year evaluation (2018–2023) of these hybrids demonstrated outstanding agronomic performance, characterized by a strong expression of hybrid vigor. During the initial growth phase, hybrids H1, H3, and H4 exhibited significantly greater plant height until month 42; however, by month 53, all seven genotypes reached a similar height equilibrium.

A more distinctive advantage was observed in the primary and secondary branching (crosses). All four hybrids outperformed both the commercial varieties and the maternal line CX2848 in branch production. Notably, by month 36, the hybrids had already developed a number of branches that commercial materials typically do not achieve until 42 to 48 months. This accelerated architectural development suggests that these hybrids can reach full productive capacity significantly earlier than conventional varieties.

The cumulative coffee yield was significantly higher in the evaluated hybrids compared to the commercial controls. The highest productivity was observed in H3, H1, and H4.

While the commercial varieties, including Var. Castillo, reached a cumulative production of 10 kg/plant after 60 months, the hybrids achieved this same threshold between 36 and 48 months, demonstrating an accelerated onset of productivity. Furthermore, at the end of the 60-month evaluation period, H3 and H1 doubled the total coffee production of the commercial materials, underscoring their superior yield potential.

This co-expression of high productivity and stable CBB resistance highlights a pivotal achievement in coffee breeding, as it addresses the traditional physiological trade-off between defense and yield. Typically, the metabolic cost of synthesizing secondary metabolites, such as trypsin inhibitors and volatile sesquiterpenes, can reduce agronomic performance. However, the exploitation of heterosis in these F1 hybrids overrides this fitness cost. Hybrid vigor grants the materials enhanced metabolic efficiency, allowing them to allocate resources simultaneously toward accelerated architectural development (early branching) and robust biochemical antibiosis without compromising cup quality or yield.

Furthermore, the environmental conditions during the 5-year evaluation period (2018–2023) highlighted a clear contrast between thermal stability and precipitation variability. The low variability in mean temperature provided a constant and predictable thermal environment that is highly favorable for the continuous biological development of the coffee berry borer (CBB). In contrast, the substantial multi-year fluctuations in annual precipitation reflect the alternation of distinct climatic phases typical of this Colombian coffee-growing region. Despite this marked variability in water availability, the consistently stable expression of resistance traits in the evaluated hybrids underscores their robust agronomic performance and genetic stability across diverse agroecological scenarios. These results highlight the hybrids' capacity for rapid adaptation across diverse Colombian coffee-growing regions, offering a robust biological tool to mitigate the risks of climate change and meet future production goals under shifting environmental conditions.

4.6. Future Perspectives and Integration into Precision IPM Frameworks

This study introduces an integrated phenotyping framework for identifying CBB resistance within the coffee germplasm cultivated in Colombia. Our findings suggest that additional wild accessions within the Colombian Coffee Germplasm Bank at Cenicafé may also harbor undiscovered defensive traits. This highlights a critical need for high-throughput screening programs to characterize and incorporate these genetic resources into precision breeding efforts, ultimately enhancing the durable resistance of future coffee cultivars against this pest.

Ultimately, the integration of these hybrids—distinguished by high productivity, rust resistance, and intrinsic CBB resistance—into a comprehensive Integrated Pest Management (IPM) framework incorporating volatiles, traps, agroecology, and biological control [40] will drive the transition toward a more sustainable and environmentally resilient coffee industry. As highlighted in modern molecular breeding frameworks, implementing insect-resistant hybrids provides significant economic benefits by reducing chemical input costs and ensures environmental sustainability by minimizing pesticide runoff. This reduced chemical dependency directly enhances compatibility with biological control approaches by preserving natural predators within the agroecosystem [41].

By drastically suppressing overall pest populations in the field, these materials inherently enhance the efficacy of standard frequent harvesting and biological controls, preventing economic thresholds from being breached under commercial conditions.

Following the development and evaluation of these hybrids, the next objective is to implement a mass propagation system utilizing somatic embryo maturation in RITA® temporary immersion bioreactors. This cost-effective technology will enable large-scale clonal

propagation, ensuring the volume of plant material required for extensive commercial evaluation, while facilitating the standardization of plantlet production, the optimization of acclimatization protocols, and the refinement of nursery transplantation techniques.

5. Conclusions

In this study, four F1 coffee hybrids—H1 (CU1842 × C306), H2 (CX2385 × C534), H3 (CX2385 × C306), and H4 (CX2848 × C534)—were successfully generated and clonally propagated via somatic embryogenesis. At least three of these genotypes demonstrated significant host-plant resistance to the coffee berry borer (CBB). Hybrids H1 and H3 emerged as the most effective lines, inducing up to 47% mortality and a 50% population reduction under laboratory conditions, while consistently maintaining a high antibiosis effect and insect suppression in the field. This was followed by H2, which expressed a stable intermediate resistance under field conditions. These hybrids were established in the field within a representative Colombian coffee-growing region and evaluated over a five-year period, during which they consistently maintained the stable expression of the resistance traits inherited from their parental lines.

Overall, Population reductions reached 25% to 45% in laboratory conditions and 50% to 70% under field conditions. This level of suppression could demonstrate a significant impact on population dynamics by limiting the reproductive success of subsequent generations. Furthermore, by expressing hybrid vigor, these materials displayed superior agronomic traits with a yield potential capable of doubling the coffee production compared to current commercial varieties. These findings establish a solid foundation for the commercial release and integration of F1 hybrids with specific resistance to CBB. Due to their high productivity and robust performance, these materials show strong potential for adaptation to diverse agroecological zones, providing a strategic genetic resource for coffee production under climate change scenarios.

Ultimately, the utilization of CBB-resistant hybrids offers a sustainable genetic solution. Although this resistance is not absolute or complete, it significantly decreases the insect population, making insect control combined with other strategies more effective and more environmentally friendly. This approach reduces reliance on chemical insecticides, thereby lowering Integrated Pest Management (IPM) costs while enhancing environmental stewardship and farmer profitability. The future adoption of these materials by coffee growers will be instrumental in scaling these biological and economic benefits across the industry.

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Abbreviations

The following abbreviations are used in this manuscript:

CBB	Coffee Berry Borer
IPM	Integrated Pest Management
ANOVA	Analysis of variance
m a.s.l.	metros above sea level
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model

Appendix A

Table A1. Hybrid Characteristics: Results Summary. Data were obtained from plants evaluated over a 5-year production cycle (2019–2024) planted at the La Catalina station (Pereira). Comparisons were made with respect to the controls, Cenicafé 1 and Castillo Naranjal varieties.

Genotypes	ID	% CBB Mortality in Diets (*1)	% Reduction in CBB Populations Compare to Control (Laboratory Seed Assay) (*2)	% Reduction in CBB Populations on Seeds under Field Conditions (*3)	Cumulative Yield Kg/Plant (*4)	Number of Crosses or Nodes /Plant (*4)	Plant Height Cm
CU1842 × C306	H1	40 *	50 *	70 *	20.3 *	53.0 *	257.3
CX2385 × C534	H2	22 *	30 *	67 *	16.7 *	54.86 *	253.1
CX2385 × C306	H3	30 *	50 *	67 *	22.0 *	54.0 *	261.5
CX2848 × C534)	H4	22 *	30 *	46 *	18.4 *	51.23 *	260.2
Var Cenicafé and Var. Castillo Naranjal	Susceptible Controls	10–15			10.5	47.5	252.3

Asterisks (*) indicate significant differences compared to the control—95% confidence intervals. *1 ANOVA $p < 5$; *2 Test Tukey–Kramer; *3 Test Scheffe; *4 Test Dunnett.

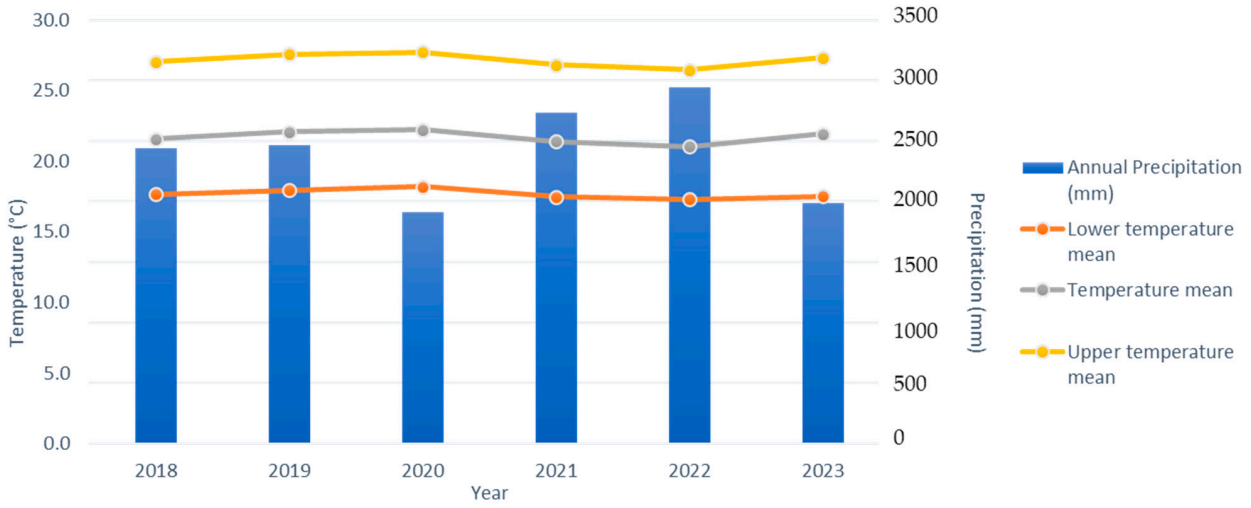

Figure A1. Meteorological conditions recorded at La Catalina station (Cenicafé) during the 6-year evaluation period (2018–2023). Bars represent cumulative annual precipitation (mm, right y-axis). Lines represent the annual upper, lower, and overall mean temperatures (°C, left y-axis).

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