
From the laboratory to the field: contrasting effects of multi-trophic interactions and agroforestry management on coffee pest densities

Author A. V. Teodoro

Author T. Tschardtke

Author A.-M. Klein

Abstract Only few factors influencing pest populations can be studied in the laboratory, but many population-driving factors interact in the field. Therefore, complementary laboratory and field approaches are required for reliable predictions of real-world patterns and processes. Laboratory and field experiments with the red spider mite, *Oligonychus ilicis* McGregor (Acari: Tetranychidae), and the coffee leaf miner, *Leucoptera coffeella* Guerin-Meneville (Lepidoptera: Lyonetiidae), on coffee plants, *Coffea arabica* L. (Rubiaceae), were combined to study the relative importance of biotic interactions, including resource preferences and natural-enemy impact, and habitat factors, such as agroforestry type and management intensity, on coffee pest densities. In the laboratory, leaf discs cut from undamaged coffee plants were significantly preferred by red spider mites over those from plants infested with conspecific mites, leaf rust pathogens [*Hemileia vastatrix* Berkeley & Broome (Uredinales)], or coffee leaf miners, resulting in higher reproductive success. Similarly, undamaged plants were preferred by coffee leaf miners over red spider mite-infested plants. However, in the field, red spider mite densities were positively correlated with coffee leaf miner and leaf rust densities, thereby contrasting with laboratory predictions. Hence, our study suggests that the importance of resource preferences and fitness expected based on laboratory experiments was suppressed by environmental conditions in the field, though other unassessed biotic interactions could also have played a role. Furthermore, intensified agroforestry was characterized by higher red spider mite densities, whereas densities of its major natural enemy, the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae), were not related to agroforestry management. Densities of coffee leaf miner and its main natural enemy, a eulophid parasitoid (Hymenoptera), were not affected by management practices. In conclusion, patterns found in the laboratory did not hold for the field, emphasizing the difficulties of extrapolating small-scale experiments to larger spatial scales and the need to combine both approaches.

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Effects of crop management patterns on coffee rust epidemics

Author J. Avelino

Author L. Willocquet

Author S. Savary

Abstract The effects of crop management patterns on coffee rust epidemics, caused by *Hemileia vastatrix*, are not well documented despite large amounts of data acquired in the field on epidemics, and much modelling work done on this disease. One main reason for this gap between epidemiological knowledge and understanding for management resides in the lack of links between many studies and actual production situations in the field. Coffee rust epidemics are based on a seemingly simple infection cycle, but develop polycyclic epidemics in a season and polyetic epidemics over successive seasons. These higher-level processes involve a very large number of environmental variables and, as in any system involving a perennial crop, the physiology of the coffee crop and how it affects crop yield. Crop management is therefore expected to have large effects on coffee rust epidemics because of its immediate effect on the infection cycle, but also because of its cumulative effect on ongoing and successive epidemics. Quantitative examples taken from a survey conducted in Honduras illustrate how crop management, different combinations of shade, coffee tree density, fertilization and pruning may strongly influence coffee rust epidemics through effects on microclimate and plant physiology which, in turn, influence the life cycle of the fungus. We suggest there is a need for novel coffee rust management systems which fully integrate crop management patterns in order to manage the disease in a sustainable way.

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The effect of light intensity on incomplete resistance of coffee to *Hemileia vastatrix*

Author A. B. Eskes

Abstract Resistance of coffee to race II of *Hemileia vastatrix* was tested in different environments at light intensities (LI) from 17 to 100% of total outdoor radiation. Nine treatments, in which three levels of LI before inoculation were combined with three levels of LI after inoculation, were applied to seedlings of the susceptible cv. Mundo Novo. Higher LI before inoculation induced a significant increase in lesion density, whereas the opposite was observed for treatments after inoculation. Maximum differences in lesion density were threefold. The interaction between pre- and post-inoculation treatments was also significant. Necrosis of lesions occurred under extremely high LI after inoculation. Genotypes of the Icatu population and of *Coffea canephora* cv. Kouillou, which varied in disease level in the field, were tested in different environments, constant LI being

applied before and after inoculation. Most genotypes were more resistant at low LI than at high LI, paralleling the results obtained for the control cv. Mundo Novo. With cv. Kouillou, sporulating lesion density, latency period and reaction type were significantly affected by LI and genotype. The interaction between LI and genotypes was significant for sporulating lesion density and reaction type, mainly because the most resistant genotype was not affected, or affected in opposite direction, by LI. Environment affected the expression of the resistance gene SH4. Observations on a segregating F2 population indicated dominant gene action in the greenhouse (low LI) and incomplete dominant to nearly recessive gene action in the nursery (high LI). Incomplete dominance was expressed by heterogeneous to susceptible reaction types of heterozygote plants (SH4sH4), under high LI. Some ecological and breeding aspects of the observed effect of LI on resistance to coffee leaf rust are discussed.

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Evidence for hyperparasitism of coffee rust (*Hemileia vastatrix*) by the entomogenous fungus, *Lecanicillium lecanii*, through a complex ecological web

Author J. Vandermeer

Author I. Perfecto

Author H. Liere

Abstract The entomogenous fungus, *Lecanicillium lecanii* is hyperparasitic on *Hemileia vastatrix*, the cause of coffee leaf rust in the laboratory, and has frequently been observed attacking it in the field. The existence of a complex ecological web involving the spatially clustered mutualism of an ant (*Azteca instabilis*) with a scale insect (*Coccus viridis*), where the scale insect was infected by *L. lecanii*, prompted a search for a spatial correlation between the attack of *L. lecanii* on the scale insect and the incidence of rust in a commercial coffee crop. A weak but statistically significant effect of hyperparasitic control of coffee rust was observed on two distinct scales: in a 45-ha plot and on a scale of approximately 10 m. It was concluded that this effect was linked to an indirect effect of the ant–coccid mutualism, where *L. lecanii* was a parasite of the coccid.

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Control of coffee rust in Kenya by fungicides

Author R.W. Rayner

Abstract The results are reported of a series of four factorial field trials carried out at Ruiru, Kenya, to determine the effect on the control of coffee rust (*Hemileia vastatrix* B. et Br.) of the main variables associated with the application of copper fungicides, i.e. concentration, volume per acre, leaf surface to which applied, time of application, and types of fungicide and application used. Rust control increased with log concentration, there being evidence for a linear relationship. As neither asymptotic nor optimal values occur the optimum for commercial practice must be determined by economics and possible phytotoxic effects. Control also increased with volume per acre, but adjusting the amount of spray residue via concentration was more effective and less wasteful of fungicide than via volume. Upper-surface application was as effective as lower in one trial at all concentrations, and in another at the higher concentrations. It is considered that liberation of fungicide from upper surface residues by rain, and the presence of thick aggregations of these residues may be of importance for rust control. Differences in rust control following the use of three spraying machines were correlated with the differences in amount of spray residue on the leaves, there being a linear relation to log residue. For the same rust control, a mist-blower with swirl-plate atomisation required the greatest expenditure of fungicide per acre and a pneumatic hand-sprayer, closely approached by a mist-blower with spinning-cage atomisation, the least. However, to obtain as heavy residues and as good rust control as with the hand-sprayer, higher concentrations were necessary. At the same copper content of spray fluid no differences in rust control by Burgundy mixture, cuprous oxide (as 'Perenox') and copper oxychloride (as 'Blitox') were found. Some observations on the fungicides fermete, cuprous oxide in oil and captan were made. Captan did not increase foliage density. Evidence that this is directly influenced by copper sprays is presented. For reducing the amount (but not the percentage) of rust attack March and April sprays were most effective, January least, and October was intermediate. The earlier sprays reduced the effect of later ones. Reduction of inoculum in January to March did not reduce the July-August rust peak. The implications are discussed.

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Biological control of coffee rust by antagonistic bacteria under field conditions in

Brazil

Author F. Haddad

Author L. A. Maffia

Author E. S. G. Mizubuti

Author H. Teixeira

Abstract Rust (*Hemileia vastatrix*) is the most important coffee disease in Brazil. Organic coffee production has increased in the country and a research program aimed to develop alternatives to chemicals for disease control was required. Seven bacterial isolates, isolated from organic coffee plantings and selected in greenhouse tests, were evaluated under commercial organic crop conditions in 2005 (Experiment 1) and 2005/2006 (Experiment 2), in Machado, MG, Brazil. Ten treatments consisting of the seven bacterial isolates, copper hydroxide, calcium silicate and water were applied as three or four monthly sprays in Experiment 1 or 2, respectively. Rust severity and incidence were evaluated monthly. In Experiment 1, the sprays started in January when rust incidence was 23.8%, and none of the treatments reduced rust progress significantly. In Experiment 2, the sprays began in November 2005, when rust incidence was approximately 7.5%. There were significant differences ($P < 0.0001$) between treatments regarding maximum incidence and severity (as assessed in June, 2006), the rate of increase of the incidence between November 2005 and June 2006 and for the areas under disease progress curves for both rust incidence and severity. Lower values for these treatments were obtained in the plots treated with copper hydroxide or *Bacillus* sp. isolate B157, and intermediate values with the *Pseudomonas* sp. isolate P286. In a third experiment conducted in 2007 in Ervália, MG, isolates B157 and P286 were also evaluated; isolate B157 reduced rust intensity as effectively as copper hydroxide. Isolate B157 is considered a potential biocontrol agent for coffee rust for organic crop systems in Brazil.

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Shade over coffee: its effects on berry borer, leaf rust and spontaneous herbs in Chiapas, Mexico

Author L. Soto-Pinto

Author I. Perfecto

Author J. Caballero-Nieto

Abstract The objective of this research was to determine the relationships between different ecological features of shade and the incidence of coffee berry borer, coffee leaf rust and spontaneous herbs in rustic coffee plantations in Chiapas, Mexico. Thirty-six 10 m by 10 m plots were established within coffee plantations. The following variables were measured or estimated: number of vegetation strata, percent canopy cover, direct, diffuse and total sunlight below the canopy, plant species richness and diversity, shade tree/shrub density, altitude, aspect, basal area, yields, percentage of coffee berry borer (*Hypothenemus hampei* Ferr), percentage of coffee leaf rust (*Hemileia vastatrix* Berk & Br.), percentage of spontaneous herb cover and the presence of paths and runoffs. Results showed a complex agroforestry system, composed of five strata. Coffee berry borer and coffee leaf rust incidence averages were 1.5% and 10.1%, respectively. Average spontaneous herb cover was 34.1%. Coffee leaf rust percentage correlated positively with the coffee berry borer. Number of strata of shade vegetation correlated negatively with leaf rust, while the presence of paths correlated positively with the leaf rust. Species richness and diversity correlated negatively to broad-leaf-herb cover and the presence of runoffs correlated positively to this last variable. Shade tree density (> 10cm d.b.h.) correlated negatively to linear-leaf-herb cover. Percentage of shade cover, light, coffee density, aspect, stand age, basal area and yields were not correlated to pest, disease and weeds. Results support the ecological theory that postulates that diversity and structural complexity in mixed plant systems maintain a healthy system.

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Copper contamination of soil and vegetation in coffee orchards after long-term use of Cu fungicides

Author J. Loland

Author B. R. Singh

Abstract The repeated use of Cu fungicides to control coffee berry disease can result in increased Cu content in soils and vegetation and thus raising the pollution levels and a concern for potential effects on human health. Therefore a field survey of coffee orchards of the Kilimanjaro and Arusha regions of Tanzania, where Cu fungicides are frequently used, was conducted to determine the possible Cu contamination of soils and vegetation. Soil samples were collected from 0–5, 5–15 and 15–30 cm depth at a distance of 0.5 m from the trunk of coffee trees. Plant leaf samples of beans and maize crops intercropped with coffee trees as well as leaves of coffee trees, were also collected at the same time and places. Soil and plant samples were collected both from small farms and commercial estates. Copper in the soil samples was extracted with CaCl₂ (CCu), whereas for the total Cu, samples were digested with aqua regia (HNO₃ and HCl(1:3) (ACu)). Plant

samples were drying ashed and digested with HNO₃. Copper concentration in the extracted and digested solutions was determined with atomic absorption spectrophotometry. A significant Cu enrichment of the soils was observed and the Cu concentration in the 0–5 cm soil depth was about three times higher than in the 15–30 cm depth. A large variability in the soil Cu concentration between fields of the same farm and within the same orchard was observed. The differences in the total Cu concentration (ACu) between small farms and commercial estates were not statistically significant, but the extractable Cu (CCu) was higher and soil pH was lower in the estates of the Kilimanjaro region than at small farms. The soil organic carbon (SOC) content increased significantly with increasing altitudes, and it was also higher on small farms than on commercial estates. The SOC content showed a close and positive correlation with total Cu. At soil pH below 5.3, the CCu fraction increased steeply. Bean plants from coffee fields showed a high concentration of Cu, suggesting a possible Cu toxicity problem in bean plants. Coffee leaves also showed extremely high Cu concentration.

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Low-volume spraying to control coffee leaf rust in Kenya

Author I. D. Firman

Author J. A. N. Wallis

Abstract In a 2-year trial on arabica coffee in Kenya, a cuprous oxide fungicide (50% Cu) at a range of 0-5% (w/w) was applied by mist-blower five times a year at approximately 10 gal./acre. The results demonstrated: (i) a positive linear relationship between log₁₀ spray concentration and the amount of copper (expressed as log₁₀ mg. Cu/m.²+1) retained by the foliage; (ii) a significant negative linear relationship between spray concentration and the incidence of leaf rust (caused by *Hemileia vastatrix* Berk. & Br.); (iii) a positive linear relationship between concentration of fungicide applied and yield of coffee. An additional spray in May increased the mean annual yield by 2 cwt. coffee per acre and reduced a heavy rust attack. Trees receiving the highest spray concentration retained the most leaves; at lower concentrations there was considerable leaf fall but this was offset by new leaf production; unsprayed trees suffered severe leaf loss and were unable to produce as much new foliage.

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A new source of resistance against coffee leaf rust from New-Caledonian natural interspecific hybrids between *Coffea arabica* and *Coffea canephora*

Author L. Mahe

Author V. M. P. Varzea

Author D. Le Pierres

Author M.-C. Combes

Author P. Lashermes

Abstract The development of cultivars resistant to coffee leaf rust caused by the fungal pathogen *Hemileia vastatrix* is a priority in coffee breeding. However, only very few descendants of interspecific hybrids between *Coffea arabica* and diploid relative species have been used as resistance source. Identification of new sources of resistance appeared therefore particularly worthwhile. Hybrid plants derived from interspecific hybridization between *C. arabica* and *Coffea canephora* and found in neo-natural coffee tree populations of New Caledonia were therefore investigated. Amplified Fragment Length Polymorphism and microsatellites amplification were used to evaluate the genetic diversity of 14 hybrid plants, and rust resistance was evaluated by inoculation with a panel of rust races representing a large variability in virulence. An important genetic diversity was characterized in hybrid plants originating from introgressions into *C. arabica* from various *C. canephora* progenitors. On the 14 plants tested for leaf rust resistance, eight appeared resistant to all races investigated. Such plant material should represent a highly valuable resource for *C. arabica* breeding against coffee leaf rust.

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Designing pest-suppressive multistrata perennial crop systems: shade-grown coffee in Central America

Author C. Staver

Author F. Guharay

Author D. Monterroso

Author R. Muschler

Abstract During most of its cultivation in Central America, coffee (*Coffea arabica* L.) suffered few serious pest problems. However, over the past three decades, three factors contributed to significantly increase pest levels and losses: the recent introduction of new pests; more favorable conditions for existing pests, diseases, and weeds due to lower shade levels; and secondary pest problems caused by pesticide use. The strategy of

maximizing coffee production with pest control dominated by synthetic pesticides has not only increased yields substantially, but also production costs, pesticide resistance, and both human health and environmental risks. An analysis of the response of the food web in coffee plantations to varying levels of light and humidity associated with different shade levels provides the basis for identifying the optimum shade conditions which minimize the entire pest complex and maximize the effects of beneficial microflora and fauna acting against it. These optimum shade conditions for pest suppression differ with climate, altitude, and soils. The selection of tree species and associations, density and spatial arrangement, as well as shade management regimes are critical decisions for shade strata design. Site-specific knowledge of the seasonal food web dynamics permits growers to determine the appropriate seasonal shade management in order to further suppress pest levels. For example in a low-elevation dry coffee zone, 35 to 65% shade promotes leaf retention in the dry season and reduces *Cercospora coffeicola*, weeds, and *Planococcus citri*; at the same time, it increases the effectiveness of microbial and parasitic organisms without contributing to increased *Hemileia vastatrix* levels or reducing yields. In these conditions, shade should be at a maximum early in the dry season and at a minimum by the middle of the rainy season. Further research is needed on: the effects of individual tree species on the food web; the role of canopy architecture for coffee vigor, photosynthesis, leaf drying, pest susceptibility, and pruning regimes; and on simple observation methods and decision criteria for farmer management of tree-coffee-food web interactions.

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Coffee (*Coffea arabica* L.) genes early expressed during infection by the rust fungus (*Hemileia vastatrix*)

Author D. Fernandez
Author P. Santos
Author C. Agostini
Author M.-C. Bon
Author A.-S. Petitot
Author M. C. Silva
Author L. Guerra-Guimarães
Author A. Ribeiro
Author X. Argout
Author M. Nicole

Abstract The beverage cash crop coffee (*Coffea arabica* L.) is subject to severe losses caused by the rust fungus *Hemileia vastatrix*. In naturally resistant coffee plants, a specific hypersensitive reaction (HR) may be elicited early to stop fungal infection. To isolate host genes involved in HR, we undertook an expressed sequence tags (ESTs) analysis. Two cDNA libraries were constructed using suppression subtractive hybridization (SSH) and 527 non-redundant ESTs were generated from 784 randomly picked clones. Classification of the ESTs into several functional categories showed that more than one-quarter of the predicted proteins might encode disease resistance (R) proteins, stress- and defence-proteins, and components of signal transduction pathways. Twenty-eight differentially screened sequences (DSSs) were selected after differential hybridization of 1000 cDNA clones from each library. Investigation of the expression patterns of a subset of 13 DSSs showed higher levels of gene expression in inoculated plants compared with control plants. HR-up-regulation of transcript accumulation occurred for 9 out of the 13 genes 24 and 48 h after *H. vastatrix* challenge. Two genes encoded homologues of the Arabidopsis DND1 and NDR1 proteins, suggesting conservation of resistance signalling pathways in perennial plants. Other HR-regulated sequences matched receptor kinases, AP2 domain- and WRKY transcription factors, cytochromes P450, heat shock 70 proteins, glucosyltransferases and proteins of unknown function. The ESTs reported here provide a useful resource for studying coffee resistance responses and for improving *C. arabica* for durable disease resistance.

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Landscape context and scale differentially impact coffee leaf rust, coffee berry borer, and coffee root-knot nematodes

Author J. Avelino

Author A. Romero-Gurdián

Author H.F. Cruz-Cuellar

Author F.A. Declerck

Abstract Crop pest and disease incidences at plot scale vary as a result of landscape effects. Two main effects can be distinguished. First, landscape context provides habitats of variable quality for pests, pathogens, and beneficial and vector organisms. Second, the movements of these organisms are dependent on the connectivity status of the landscape. Most of the studies focus on indirect effects of landscape context on pest abundance through their predators and parasitoids, and only a few on direct effects on pests and pathogens. Here we studied three coffee pests and pathogens, with limited or no pressure from host-specific natural enemies, and with widely varying life histories, to test their

relationships with landscape context: a fungus, *Hemileia vastatrix*, causal agent of coffee leaf rust; an insect, the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae); and root-knot nematodes, *Meloidogyne* spp. Their incidence was assessed in 29 coffee plots from Turrialba, Costa Rica. In addition, we characterized the landscape context around these coffee plots in 12 nested circular sectors ranging from 50 to 1500 m in radius. We then performed correlation analysis between proportions of different land uses at different scales and coffee pest and disease incidences. We obtained significant positive correlations, peaking at the 150 m radius, between coffee berry borer abundance and proportion of coffee in the landscape. We also found significant positive correlations between coffee leaf rust incidence and proportion of pasture, peaking at the 200 m radius. Even after accounting for plot level predictors of coffee leaf rust and coffee berry borer through covariance analysis, the significance of landscape structure was maintained. We hypothesized that connected coffee plots favored coffee berry borer movements and improved its survival. We also hypothesized that wind turbulence, produced by low-wind-resistance land uses such as pasture, favored removal of coffee leaf rust spore clusters from host surfaces, resulting in increased epidemics. In contrast, root-knot nematode population density was not correlated to landscape context, possibly because nematodes are almost immobile in the soil. We propose fragmenting coffee plots with forest corridors to control coffee berry borer movements between coffee plots without favoring coffee leaf rust dispersal.

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Shade is conducive to coffee rust as compared to full sun exposure under standardized fruit load conditions

Author D.F. López-Bravo

Author E. de M. Virginio-Filho

Author J. Avelino

Abstract Shade effects on coffee rust are controversial, possibly because shade helps to prevent high fruit loads, which decreases leaf receptivity to the pathogen but, at the same time, might provide a better microclimate for germination and colonization. These two probable antagonistic pathways are combined under natural conditions. In order to clarify their individual effects, we dissociated the two factors by manually homogenising fruit loads under two light exposure situations, under shade and in full sunlight. The trial was set up in Turrialba, Costa Rica at 600 m of elevation, in a coffee plot initially under shade provided by the tree legume *Erythrina poeppigiana*. The plot was subdivided into two subplots: one was maintained under shade, whereas shade was eliminated in the

second subplot. In each subplot, we removed fruiting nodes from 40 coffee plants in order to obtain the following four levels: none, 150, 250, and 500 fruiting nodes per coffee plant. Coffee rust incidence and severity, along with plant growth and defoliation, were assessed on these coffee plants over a period of two years. Air and leaf temperatures, leaf wetness and relative humidity were also monitored. As expected, the intensity of the coffee rust epidemic increased in line with fruit load. We quantified a 28.9% increase in coffee rust incidence and a 129.2% increase in severity on plants with 500 fruiting nodes as compared to plants with no fruits. With the homogenised fruit load, the intensity of the coffee rust epidemic was greater in the shaded subplot, with a 21.5% increase in incidence and a 22.4% increase in severity. Two mechanisms were suggested. Firstly, we highlighted a dilution effect due to host growth which was 25.2% and 37.5% greater in full sunlight when considering new leaves or new leaf area respectively. Secondly, the microclimate was more conducive to coffee rust under shade, with lower intra-day temperature variations, due to lower maxima, and a higher leaf wetness frequency. We concluded that shade has antagonistic effects on coffee rust. Coffee rust is reduced by shade because shade reduces the fruit load. However, with an equivalent number of fruiting nodes, coffee rust incidence and, to a lesser extent, severity were greater under shade. The service provided by shade in controlling coffee rust is necessarily associated with a disservice that consists in reducing yield in the short term.

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The effect of coffee leaf rust on foliation and yield of coffee in Papua New Guinea

Author J. S. Brown

Author J. H. Whan

Author M. K. Kenny

Author P. R. Merriman

Abstract Coffee leaf rust epidemics of varying severity were created by application of fungicide treatments, of varying concentration, to experimental plots from 1989 to 1992. Detailed measurements of disease incidence and leaf fall showed that yield and foliation (number of leaves per lateral) in 1991 was affected by the disease incidence in the previous season. Analysis showed a significantly greater influence of disease on foliation, both within and between seasons, than on yield. This was confirmed by data from 1992. It was concluded that the effect of the disease on yield is indirect through induction of defoliation.

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Dispersal of uredospores of *Hemileia vastatrix* under field conditions

Author K.R. Bock

Abstract The number of uredospores of *Hemileia vastatrix* Berk. & Br. in the air at times which are critical for seasonal build-up of infection is excessively low; this, together with the effect of spore density on infection, suggests that wind plays no part in dispersal. Rain splash, on the other hand, disperses spores in quantity, the numbers being in direct proportion to the amount and intensity of individual showers. Showers in excess of approximately one-third of an inch are necessary for effective dispersal. A study of the distribution of spores on leaves within the coffee bush after rainfall of varying intensity confirms the close correlation between rainfall and spore movement, and shows that this movement is limited in range.

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Indirect biological control of the coffee leaf rust, *Hemileia vastatrix*, by the entomogenous fungus *Lecanicillium lecanii* in a complex coffee agroecosystem

Author D. Jackson

Author J. Skillman

Author J. Vandermeer

Abstract The entomopathogenic and mycoparasitic fungus *Lecanicillium lecanii* is known to attack both the green coffee scale, *Coccus viridis*, and coffee leaf rust, *Hemileia vastatrix*. Using multi-year surveys of *L. lecanii* and *H. vastatrix* prevalence, we demonstrate a previously unreported, one-year time lag between local epizootics (outbreaks) of *L. lecanii* and significant suppression of *H. vastatrix*. Epizootics of *L. lecanii* are associated with large populations of *C. viridis*, which are in turn associated

with colonies of their mutualistic partner, the arboreal-nesting ant *Azteca instabilis*. Therefore, these results suggest that effective conservation biological control of *H. vastatrix* using *L. lecanii* will be enhanced by an understanding of the self-organization process that gives rise to the emergent spatial distribution of the *A. instabilis*–*C. viridis* mutualism in this complex coffee agroecosystem.

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Additional physiological races of coffee leaf rust (*Hemileia vastatrix*) identified in Kenya

Author E. K. Gichuru

Author J. M. Ithiru

Author M. C. Silva

Author A. P. Pereira

Author V. M. P. Varzea

Abstract Coffee leaf rust (CLR), caused by the fungus *Hemileia vastatrix*, is among the most important diseases affecting coffee all over the world. In Kenya, it is currently the second most important disease, and breeding coffee to obtain new resistant cultivars has been a priority. Over time, new rust pathogenic races able to infect hitherto resistant coffee genotypes have been registered. To date, 49 races of the pathogen have been characterized all over the world. The most recent races to be characterized are able to infect derivatives of Timor Hybrid (HDT), which is a major source of resistance in breeding programs. This work aimed to identify new races of the pathogen in Kenya, emphasizing infected leaves sampled from CLR resistant varieties and breeding lines collected from two sites (Ruiru and Koru). Twenty-four samples were characterized, out of which 22 samples corresponded to new races of the pathogen. A total of six new races (III, XVII, XXIII, XXXVI, XLI and XLII) were characterized, revealing three new virulence genes (v1, v7, v8) and possibly a fourth virulence gene, the v9. This finding represents a serious threat to coffee production and also a challenge to coffee breeding programs that are in progress in Kenya.

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Cryptosexuality and the Genetic Diversity Paradox in Coffee Rust, *Hemileia vastatrix*

Author C. R. Carvalho
Author R. C. Fernandes
Author G. M. Carvalho
Author R. W. Barreto
Author H. C. Evans

Abstract Despite the fact that coffee rust was first investigated scientifically more than a century ago, and that the disease is one of the major constraints to coffee production - constantly changing the socio-economic and historical landscape of the crop - critical aspects of the life cycle of the pathogen, *Hemileia vastatrix*, remain unclear. The asexual urediniospores are regarded as the only functional propagule: theoretically, making *H. vastatrix* a clonal species. However, the well-documented emergence of new rust pathotypes and the breakdown in genetic resistance of coffee cultivars, present a paradox. **Methods and Results** Here, using computer-assisted DNA image cytometry, following a modified nuclear stoichiometric staining technique with Feulgen, we show that meiosis occurs within the urediniospores. Stages of spore development were categorised based on morphology, from the spore-mother cell through to the germinating spore, and the relative nuclear DNA content was quantified statistically at each stage. **Conclusions** Hidden sexual reproduction disguised within the asexual spore (cryptosexuality) could explain why new physiological races have arisen so often and so quickly in *Hemileia vastatrix*. This could have considerable implications for coffee breeding strategies and may be a common event in rust fungi, especially in related genera occupying the same basal phylogenetic lineages.

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Climate favourability to leaf rust in Conilon coffee

Author A. S. Capucho

Author L. Zambolim

Author P. G. C. Cabral

Author E. Maciel-Zambolim

Author E. T. Caixeta

Abstract The Brazil is the second largest producer of Conilon coffee (*Coffea canephora*), being the state of Espírito Santo responsible for producing 9.7 million bags of Conilon coffee. The biology of *Hemileia vastatrix*, etiologic agent of leaf rust, the main disease of this culture, is not well understood under the environmental conditions of Espírito Santo, Brazil. This study determined the ideal temperature and leaf wetness ranges for in vitro germination and infection of leaf discs in this pathosystem. For this, regression analyses with different temperature and leaf wetness ranges were performed. The results showed that a climate characterized by mild mean temperatures (between 21.6 °C and 23.6 °C) with foliar wetness associated with high relative humidity (>80 %) is the most favorable condition for the infection of Conilon coffee by *H. vastatrix*. The knowledge of the optimal temperature and moisture conditions for the infection can also be useful for developing systems to predict the occurrence of the disease in *C. canephora* from Brazil.

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Field screening of selected *Coffea arabica* L. genotypes against coffee leaf rust

Author B. M. Gichimu

Abstract Coffee Leaf Rust (CLR) is a fungal disease caused by *Hemileia vastatrix* Berk et Br. and is one of the major diseases of coffee. It causes premature leaf fall, yield loss and even death of the tree in severe cases. Coffee genotypes respond differently to biotic factors. This study was aimed at identifying potential sources of resistance genes to the disease. Forty five *Coffea arabica* L accessions were evaluated for their response to CLR under field conditions. CLR infection was assessed from the 45 genotypes subjected to similar field conditions in June 2010 when disease pressure was at peak. The experimental plot was laid out in a Randomized Complete Block Design with three replications. Each of the genotypes was represented by fifteen trees consisting of five trees per replication. Significant variation in tolerance to CLR was observed among the genotypes and some tolerant genotypes identified. HDT, Rumesudan, Barbuk Sudan, Ennareta, Geisha12, Babbaka Ghimira, Boma plateau and Tafari Kela were the most tolerant to CLR (recording a score of 0) while Drought Resistant II [DRII] was the most susceptible recording a score of 8. Most of the accessions that demonstrated high phenotypic resistance have not been utilized as sources of resistance to CLR in coffee breeding

programmes except HDT. Such genotypes could represent a highly valuable resource for *C. arabica* breeding against CLR if their reaction is confirmed.

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Extra Coffee Leaf Rust (CLR) is a fungal disease caused by *Hemileia vastatrix* Berk et Br. and is one of the major diseases of coffee. It causes premature leaf fall, yield loss and even death of the tree in severe cases. Coffee genotypes respond differently to biotic factors. This study was aimed at identifying potential sources of resistance genes to the disease. Forty five *Coffea arabica* L accessions were evaluated for their response to CLR under field conditions. CLR infection was assessed from the 45 genotypes subjected to similar field conditions in June 2010 when disease pressure was at peak. The experimental plot was laid out in a Randomized Complete Block Design with three replications. Each of the genotypes was represented by fifteen trees consisting of five trees per replication. Significant variation in tolerance to CLR was observed among the genotypes and some tolerant genotypes identified. HDT, Rumesudan, Barbuk Sudan, Ennareta, Geisha12, Babbaka Ghimira, Boma plateau and Tafari Kela were the most tolerant to CLR (recording a score of 0) while Drought Resistant II [DRII]) was the most susceptible recording a score of 8. Most of the accessions that demonstrated high phenotypic resistance have not been utilized as sources of resistance to CLR in coffee breeding programmes except HDT . Such genotypes could represent a highly valuable resource for *C. arabica* breeding against CLR if their reaction is confirmed.

Essential oils for rust control on coffee plants

Author R. B. Pereira

Author G. C. Lucas

Author F. J. Perina

Author E. Alves

Abstract Rust is considered the most important disease in coffee because it causes severe defoliation in plants and, consequently, reduction in productivity. This study evaluated the in vitro effect of essential oils of cinnamon, citronella, lemongrass, clove, tea tree, thyme, neem and eucalyptus on the germination of urediniospores of *Hemileia vastatrix*; the effectiveness of these oils to control rust on seedlings of coffee cultivars Catucaí 2SL, Catucaí IAC 62 and Mundo Novo 379/19 in the greenhouse; and the effect of more promising oils on urediniospores of *H. vastatrix* by transmission electron microscopy (TEM). All the essential oils inhibited the germination of urediniospores with increasing concentrations. All oils promoted partial control of the disease in the greenhouse. However, the oils of thyme, clove and citronella, at a concentration of 1000 µL L⁻¹, were most effective in controlling the disease on cultivars Catucaí 2SL, Catucaí IAC 62

and Mundo Novo 379/19, respectively. The images generated in TEM showed that urediniospores exposed to oils of clove, citronella and thyme promoted cellular disorganization and cytoplasmic vacuolization, which was more pronounced in urediniospores exposed to citronella oil. The oils of thyme, clove and citronella are promising for the control of rust in coffee.

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Endophytic microorganisms from coffee tissues as plant growth promoters and biocontrol agents of coffee leaf rust

Author H. S.A. Silva
Author J.P.L. Tozzi
Author C.R.F. Terrasan
Author W. Bettiol

Abstract A total of 234 strains of endophytic bacteria (217) and fungi (17) from coffee tissues were evaluated for their potential to control coffee leaf rust (*Hemileia vastatrix*) and to promote the growth of coffee seedlings. None of the fungal strains induced plant growth or reduced disease severity. Bacterial strains 85G (*Escherichia fergusonii*), 161G, 163G, 160G, 150G (*Acinetobacter calcoaceticus*) and 109G (*Salmonella enterica*) increased plant growth; the maximum increase was induced by strain 85G. This strain in vitro produced phosphatase and indol acetic acid. In an exploratory assay to control rust on coffee leaf discs, nine bacterial strains: 64R, 137G, 3F (*Brevibacillus choshinensis*), 14F (*S. enterica*), 36F (*Pectobacterium carotovorum*), 109G (*Bacillus megaterium*), 115G (*Microbacterium testaceum*), 116G and 119G (*Cedecea davisae*) significantly reduced disease severity when applied 72 or 24 h before challenging with the pathogen. Strains 3F, 14F, 109G, 115G, 119G, and 137G significantly reduced the severity of coffee leaf rust when compared to the diseased control in the seedling assay, when applied 72 h before challenging with the pathogen. Strain 109G was the most effective in this assay. Urediniospore germination was reduced 66% by strain 3F. There was no correspondence between the organisms that promoted seedling growth and those that reduced coffee leaf rust severity on seedlings or leaf discs.

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Occurrence of fungal diseases of *Coffea arabica* L. in montane rainforests of Ethiopia

Author A. Zeru

Author F. Assefa

Author G. Adugna

Author H. Hindorf

Abstract Coffee Berry Disease (CBD), *Colletotrichum kahawae*, Coffee Wilt Disease (CWD), *Gibberella xylarioides* and Coffee Leaf Rust (CLR), *Hemileia vastatrix* are the three major diseases reducing production and consumption of coffee in Ethiopia. A survey was conducted from July to September 2005 for CBD and CWD and from 2003 until 2007 for CLR in montane rainforest coffee areas of Ethiopia to estimate the occurrence and distribution of these diseases. Diseases were prevalent in all the surveyed forest coffee areas of Ethiopia: Harena, Bonga, Berhane-Kontir and Yayu. Depending on the forest coffee area the mean percent incidence of CBD ranged from 2 to 40 % in general and from 2 to 17.9 % at Berhane-Kontir and Bonga, respectively. The mean incidence of CWD varied from 2.4 % at Berhane-Kontir to 16.9 % at Yayu forest coffee areas. The mean incidence of CLR also varied for instance in 2005 from 32.2 % at Berhane-Kontir to 96 % at Harena forest coffee areas. The detection of the diseases during our surveys requires an integrated management of major coffee diseases for a sustainable conservation and wise use of coffee in montane rainforests of Ethiopia.

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Extra Coffee Berry Disease (CBD), *Colletotrichum kahawae* , Coffee Wilt Disease (CWD), *Gibberella xylarioides* and Coffee Leaf Rust (CLR), *Hemileia vastatrix* are the three major diseases reducing production and consumption of coffee in Ethiopia. A survey was conducted from July to September 2005 for CBD and CWD and from 2003 until 2007 for CLR in montane rainforest coffee areas of Ethiopia to estimate the occurrence and distribution of these diseases. Diseases were prevalent in all the surveyed forest coffee areas of Ethiopia: Harena, Bonga, Berhane-Kontir and Yayu. Depending on the forest coffee area the mean percent incidence of CBD ranged from 2 to 40 % in general and from 2 to 17.9 % at Berhane-Kontir and Bonga, respectively. The mean incidence of

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