Temporal dynamics and biocontrol potential of a hyperparasite on coffee leaf rust across a landscape in Arabica coffee’s native range

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ABSTRACT

Agroforestry systems can provide habitats for a rich biodiversity including multitrophic interactions, which presents opportunities to develop natural pest control. Shade coffee systems in several coffee growing areas of the world host such unique habitats where pests and their natural enemies interact. One of the major global challenges for coffee production, coffee leaf rust caused by the fungal pathogen Hemileia vastatrix is attacked by the fungal hyperparasite, Lecanicillium lecanii. However, we lack insights in the dynamics and biocontrol potential of the hyperparasite on coffee leaf rust from landscapes in Arabica coffee’s native range. To understand the temporal dynamics across landscapes and environmental drivers of the rust and hyperparasite, and the potential for biocontrol of the rust by the hyperparasite, we studied the rust and hyperparasite during the dry and wet seasons for three consecutive years at 60 sites across a gradient of coffee management in southwestern Ethiopia. We found that coffee leaf rust was more severe during the dry season, whereas the hyperparasite was more severe during the wet season in two out of three years. The rust growth rate from the wet to the dry season transition was negatively related to the hyperparasite index during the wet season, implying a potential top-down control. Coffee leaf rust was generally more severe at lower altitudes in the dry season, whereas the hyperparasite was more severe at high altitude. The rust incidence increased with management intensity, while the hyperparasite was more common under less intensive management. This study could be interesting in that it represents a landscape where Arabica coffee originated and the rust and hyperparasite might have a long co-evolutionary history. Our findings highlight the potential of the hyperparasite to suppress the rust’s growth rate from the wet to dry season transition when the rust severity could otherwise be at its peak. We show that less intensively managed landscapes with dense shade levels are likely to increase hyperparasite abundance and result in an improved top-down control of the rust. However, more detailed knowledge is needed on the interaction of these species to assess its importance for reducing rust induced yield losses or the risk of rust outbreaks.

1. Introduction

The spatial and temporal variation in abundance of a given species can be caused by variation in its resources, often referred to as bottom-up control (Blüthgen et al., 2004; Hunter and Price, 1992). However, top-down interactions that can strongly modify such relationships are well-documented from aquatic systems (Carpenter et al., 1985). Trophic cascades are also common in terrestrial systems. For example, herbivorous insects are often regulated by predators (Vidal and Murphy, 2018) or parasitoids (Anderson et al., 2019). Such knowledge is also widely utilized in biological control of insect pests in agriculture (Meyling and Eilenberg, 2007). However, the diversity, commonness and distribution of natural enemies of plant diseases and whether these natural enemies have the potential to reduce disease levels is less known both for natural and agricultural systems.

Hyperparasitism is a condition in which a parasite is infected by another parasite (Holt and Hochberg, 1998), and could potentially play a similar role as other top-down interactions (Gleason et al., 2014;
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Parratt and Laine, 2016). So far, one of the best studied examples of hyperparasitism on fungi is the infection of chestnut blight causal agent, the fungus Cryphonectria parasitica, by the hypovirus Cryptophycomyceta hypovirus-1 (Gov), in which the hyperparasite antagonizes fungal growth and reduces its devastating effect on chestnut populations (Milgroom and Cortesi, 2004). Another example is the effect of the fungal hyperparasite Ampelomyces quisqualis on the powdery mildew fungus Podosphaera plantaginis attacking the ribwort plantain Plantago lanceolata, where infection by the hyperparasite is shown to reduce pathogen overwintering (Parratt and Laine, 2018; Tollenaere et al., 2014). Likewise, belowground hyperparasites could play a key role in the top-down control of plant diseases in agroforestry systems (Mulaw et al., 2010; Muleta et al., 2007). Nevertheless, we know little concerning the coupling between disease and hyperparasite dynamics within agroforestry systems (Avelino et al., 2011).

One interesting system is the attack of coffee leaf rust Hemileia vastatrix by the hyperparasite Lecanillicium lecanii (Vandermeer et al., 2009). This is relatively well-studied in Mexico were the highest hyperparasitism levels were reported from traditionally managed coffee systems (Jackson et al., 2012a; Vandermeer et al., 2014). While coffee leaf rust has had devastating outbreaks in most parts of the coffee growing areas (Avelino et al., 2015), in some areas no such outbreaks have been reported (Hindorf and Omondi, 2011; McCook, 2006). Several authors have suggested that the hyperparasite might play a role in the lower epidemics of the disease in those areas (Hindorf and Omondi, 2011; Vandermeer et al., 2009). In Ethiopia, which represents the main centre of origin of Arabica coffee Coffea arabica (Anthony et al., 2002; Tesfaye et al., 2014) and perhaps also the rust and the hyperparasite (McCook, 2006), coffee leaf rust can be found in all management systems (Samnegard et al., 2014; Zewdie et al., 2020). However, little is known about the distribution of the hyperparasite, its habitat requirements and its potential top-down control of the rust across this landscape.

Our general objective here is to assess the spatial and temporal dynamics of the coffee leaf rust and the hyperparasite and the potential biocontrol of the rust by the hyperparasite across an environmental and management gradient in southwestern Ethiopia. We followed the rust and the hyperparasite on Arabica coffee at 60 sites during three years in both the wet and dry season. For the temporal dynamics across the six surveying periods we ask: 1) How do the incidence of coffee leaf rust and the hyperparasite vary between seasons and years? 2) Do the incidence within sites correlate over time? and 3) Does the hyperparasite index in one season affect the rust growth rate towards the subsequent season? For the spatial variation within surveying periods we ask: 1) What is the relationship between the coffee leaf rust incidence and the hyperparasite incidence across the landscape? 2) Which environmental and management variables explain variation in the incidence of coffee leaf rust across the landscape? and 3) Which environmental and management factors affect the incidence of the hyperparasite?

2. Materials and methods

2.1. Study system

Southwestern Ethiopia is characterized by a mosaic landscape with large contiguous forest areas mixed with small forest patches, open agricultural fields and home gardens (Lemessa et al., 2013). Coffee is the major cash crop for the community and there is a long tradition of coffee cultivation, hence the crop has both economic, cultural and social values for the society (Manlosa et al., 2019). Across the landscape, coffee grows in a broad management gradient ranging from forests with little or no management and a dense shade canopy to more intensively managed coffee in plantations with a more open canopy (Hundera et al., 2013). Hemileia vastatrix Berk. & Br. is an obligate parasite of coffee leaves (Kushalappa and Esdes, 1989) that causes yellow or orange powdery pustules or lesions on the underside of the leaves (Fig. 1). The rust spores, which are called urediniospores, are adapted for wind-dispersal, but short-distance dispersal also happens through rain splash and coffee laborers and rain can act to release the spores to the air as well (Boudrot et al., 2016). The white fungal hyperparasite Lecanillicium lecanii (Zimm.) Zare & W. Gams (Zare and Gams, 2001) grows and feeds on the coffee leaf rust (Hemileia vastatrix), in which the hyperparasite antagonizes fungal growth and reduces its devastating effect on chestnut populations (Milgroom and Cortesi, 2004). Another example is the effect of the fungal hyperparasite Ampelomyces quisqualis on the powdery mildew fungus Podosphaera plantaginis attacking the ribwort plantain Plantago lanceolata, where infection by the hyperparasite is shown to reduce pathogen overwintering (Parratt and Laine, 2018; Tollenaere et al., 2014). Likewise, belowground hyperparasites could play a key role in the top-down control of plant diseases in agroforestry systems (Mulaw et al., 2010; Muleta et al., 2007). Nevertheless, we know little concerning the coupling between disease and hyperparasite dynamics within agroforestry systems (Avelino et al., 2011).

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2.2. Site selection and environmental variables

The study was conducted in Gera and Gomma districts of Jimma zone in southwestern Ethiopia between 7°37′–7°56′N and 36°13′–36°39′E (Fig. 2a, b). To assess the temporal dynamics and drivers of the rust and hyperparasite, we selected 60 sites across the landscape. The focus during site selection was to obtain a data set representing a broad gradient in coffee management with as little spatial autocorrelation as possible (Fig. 2b). The final set of sites varied in many aspects such as canopy cover, management intensity, and altitude (1506–2159 m a.s.l., which is basically in the known altitudinal range of coffee in Ethiopia). At each of the 60 sites, we established a 50 × 50 m plot (Fig. 2c), where we recorded the abiotic and biotic environmental variables. In the central 30 × 30 m, we identified and tagged 16 coffee shrubs close to the intersection of each 10 × 10 m quadrant (Fig. 2c).

Within each site, we recorded a set of environmental variables: i) Altitude was recorded with Garmin GPSMAP 64 s; ii) Canopy cover was calculated using ImageJ (version 1.50i) (Schneider et al., 2012) based on vertical photos taken above the coffee shrubs at five points using a pocket Nikon Coolpix S2800 camera; iii) Shade trees with DBH > 20 cm were identified and counted in the 50 × 50 m plot; iv) Coffee density is the number of coffee shrubs larger than 1.5 m in the 30 × 30 m quadrant; and v) Coffee structure index as a measure of the management intensity. In short, this index captures the typical growth habit of the coffee shrubs

Fig. 1. The coffee leaf rust, caused by the fungal pathogen Hemileia vastatrix, can be recognized by the orange powdery spores. On top of the rust fungus grows the hyperparasite Lecanillicium lecanii, which is characterized by the white spores.
subjected to a gradient in management with smaller values referring to the less intensively managed forest sites, and higher values referring to the more intensively managed sites (Zewdie et al., 2020). We also estimated coffee yield based on berry counts from the 16 selected coffee shrubs at each site during the three wet seasons 2017, 2018 and 2019.

2.3. Survey of coffee leaf rust and the hyperparasite

We surveyed the coffee leaf rust and the hyperparasite during the dry (January–April) and wet (July–August) season for three consecutive years (2017–2019). On each of the 16 coffee shrubs per site, we recorded data on coffee leaf rust and the hyperparasite on at least 20 leaves at each branch position (i.e., at the lower, middle and upper part) of the coffee shrub (see Zewdie et al., 2020). Counts from the three branch positions of the shrub were pooled together prior to the analysis. More specifically, for the coffee leaf rust we recorded incidence (i.e. the proportion of infected leaves out of the total number of surveyed leaves) and average severity (i.e., percentage leaf area covered by rust on the subset of rust-infected leaves, visually estimated at the shrub level after the survey of the >60 leaves). For the hyperparasite, we recorded incidence (i.e., the proportion of leaves with the hyperparasite out of the total number of rust-infected leaves) and average severity (i.e., percentage of the area of rust covered by the hyperparasite on leaves with the hyperparasite, visually estimated at the shrub level after inspection of all rust-infected leaves). For the hyperparasite we recorded incidence (i.e., the proportion of leaves with the hyperparasite out of the total number of rust-infected leaves) and average severity (i.e., percentage of the area of rust covered by the hyperparasite on leaves with the hyperparasite, visually estimated at the shrub level after inspection of all rust-infected leaves). From these measures, we further calculated total severity (henceforth denoted only "rust severity") of the rust by multiplying rust incidence by average severity. For the hyperparasite we calculated an index called "hyperparasite index" by multiplying the hyperparasite incidence by average hyperparasite severity. We assume that there is no (strong) correlation between rusted area on a leaf and the proportion of the rusted area being infected by the hyperparasite. Thus, this index is related to the parasitism rate and we believe that it is a relevant metric for the potential capacity of the hyperparasite to control the rust. During the first dry season, we recorded only coffee leaf rust incidence and hyperparasite incidence. For details of the metrics and the response variables used in this study, see Table S1, and definitions in Madden et al. (2017).

2.4. Statistical analyses

For the analyses, we used the framework of generalized linear (mixed) effects models. Generalized linear mixed models (GLMMs) were fitted using the glmer function (Bates et al., 2015) and linear mixed effects models (LMEs) were fitted using the lmer function, both from the R package lme4. The function Anova in the car package and rand in the lmerTest package were used to extract the significance of the fixed and random effects, respectively. All the analyses were performed in R version 3.5.1 (R Core Team, 2018). For a detailed overview of questions, response variables, model structures, transformations, model distributions and link functions, see Table S2, but see below for a brief verbal description of the statistical approach for each research question.

2.4.1. Temporal dynamics

To explore variation in the incidence of the rust and hyperparasite between seasons and years, we modelled rust and hyperparasite incidence as a function of ‘season’ (wet or dry), ‘year’ (2017, 2018 or 2019) and their interaction. We included ‘site’ as a random effect to both the rust and hyperparasite models. We fitted linear mixed effects model for the severity of coffee leaf rust and hyperparasite index with ‘season’, ‘year’ and their interaction as explanatory variables and ‘site’ as a random variable.

To investigate whether the rust and hyperparasite were consistently...
higher in some sites than in others, we first ran a linear mixed effects (LME) model with log-transformed incidence of coffee leaf rust or incidence the hyperparasite as the response variable and ‘site’ as a random effect, in separate models. From this, we calculated the intra-class correlation coefficients (ICC) by dividing the site level variance by the total variance (site level variance + residual variance). A high ICC value indicates that each site has a consistent level of incidence (either high or low) of the rust or the hyperparasite in the different surveying seasons.

To understand whether the hyperparasite abundance in one season affected the coffee leaf rust growth rate during the subsequent season, we ran a linear regression for the logarithm of the rust growth rate as a function of the hyperparasite incidence (1st season) or index during the previous season. The rust growth rate \( \lambda \) was calculated as:

\[
\lambda = \frac{\text{CLR}_t + 1}{\text{CLR}_{t+1}}
\]

Where \( \lambda \) refers to the rust growth rate, \( \text{CLR}_t \) refers to the rust incidence during season \( t \) and \( \text{CLR}_{t+1} \) refers to the rust incidence during the subsequent season \( t + 1 \). We also included the coffee leaf rust incidence (1st season) or severity in season \( t \) as a covariate to account for the possibility that growth rates might be higher if the initial rust incidence or severity is low than high.

2.4.2. Spatial variation

To assess the general relationship between coffee leaf rust and the hyperparasite across sites during the same time period for the six survey seasons, we modelled the coffee leaf rust incidence as a function of hyperparasite incidence or hyperparasite index using a generalized linear mixed model (GLMM) with ‘site’ as a random effect. We ran separate models for each survey period.

To identify the environmental and management drivers of coffee leaf rust incidence, we modelled coffee leaf rust incidence as a function of altitude, coffee density, shade canopy cover, shade trees with DBH > 20 cm, and coffee structure index. We also included fruit load from the same season (wet season models) or the preceding season (the dry season models) since that can affect rust levels (Avelino et al., 2004; López-Bravo et al., 2012). To account for surveying date, we included the fixed continuous variable ‘surveying date’. To account for over-dispersion, we included ‘site’ as a random factor. Before the analysis, all predictor variables were scaled to zero mean and unit variance to obtain standardized coefficients. Models were simplified by dropping the least significant term from the model, until all predictor variables had a \( p \)-value below 0.1 (Crawley, 2012). To identify whether the hyperparasite incidence or index could explain additional variation in the rust incidence, beyond what was already explained by the abiotic and biotic environment, we re-ran the models described above with the additional explanatory variable hyperparasite index. As we lacked data on hyperparasite index for the first year, we used hyperparasite incidence in that analysis.

To understand variation in the hyperparasite incidence (1st season) or index among sites, we ran a linear model for each of the six seasons with hyperparasite incidence or index as response variable and the same...
predictor variables as in the rust models. Since the occurrence of the hyperparasite depends on the presence of the rust (obligate host of the hyperparasite in this landscape to our knowledge), rust incidence was always included as a covariate in these models. The same procedure as for the rust models was employed.

3. Results

3.1. Temporal dynamics

The incidence of both coffee leaf rust and the hyperparasite varied strongly between seasons and years (Table S3, Figs. 3 and S1). Rust incidence was generally higher during the dry season, but the difference was less pronounced for the first year (significant interaction between year and season, Fig. 3a, b). The seasonal variation of the hyperparasite

Fig. 4. The relationship between coffee leaf rust growth rate (incidence in season $t+1$ / incidence in season $t$) and hyperparasite index during the preceding season (in panel (a) the x-axis denote hyperparasite incidence since we lack data on the index for this transition). Black dots represent the log-transformed growth rates of coffee leaf rust for each of the 60 sites. Red solid regression lines are superimposed on the graphs when $p < 0.05$ and the grey shaded region shows the 95% confidence interval around the fitted line. In panel (d) the rust growth rate vs. hyperparasite index is shown for low and high rust severities, respectively, to illustrate a significant interaction effect. The dashed horizontal line represents zero growth. DS and WS refer to the dry and wet season, respectively. Panels (a–e) refer to the five successive rust transitions during the six surveying seasons (see Table S5 for statistics). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
incidence was less clear with a strong interaction effect between year and season (Fig. 3). The largest difference between seasons was that the first wet season had higher incidence compared to both the preceding and subsequent dry seasons (Fig. 3c, d). Severity of coffee leaf rust, as well as hyperparasite index varied in a similar way between the different seasons and years as the data on incidence (Table S3, Fig. S2).

There was only a weak evidence for a consistency in the incidences of the rust (ICC = 0.11; p = 0.004) and the hyperparasite (ICC = 0.07; p = 0.069) within the sites based on the different survey periods (see also Fig. 3b, d; Table S4).

For the two transitions from the wet to dry season, hyperparasite index was negatively related to the rust growth rate (Fig. 4b, d, Table S5). In other words, at sites with high hyperparasite index the subsequent season rust growth rate was lower implying a potential top-
down control function. However, note that the rust growth rate was still positive between the seasons in most sites in the first transition (most points above the 0-line in Fig. 4b). In the second transition between wet and dry seasons (Fig. 4d), the top-down effect was stronger for sites with lower rust severities as shown by the significant interaction between hyperparasite index and rust severity in season t (Table S5). However, for the transitions from dry to wet season, when there was a general decline in rust incidence through defoliation of heavily rusted leaves (most point under the 0-line in Fig. 4a, c, e), the pattern was weak and inconsistent (Fig. 4, Table S5).

3.2. Spatial patterns

The incidence of coffee leaf rust varied much between the sites across the landscape during all surveys, with a minimum of 0–8.3% affected leaves to a maximum of 35–62%, depending on surveying period (Table S3). The hyperparasite co-occurred with the rust less frequently but also showed higher variation across the landscape with maximum 11–66% of the rusted leaves colonized, depending on surveying period (Table S3). There was a positive relationship between coffee leaf rust incidence and hyperparasite index across the sites for three out of six surveying periods (Fig. 5, Table S6).

The coffee leaf rust incidence varied in relation to different drivers and in different directions during the different survey periods (Table 1). For example, the rust incidence decreased with altitude during the 2017 and 2019 dry season, whereas the reverse was true during the wet season of the same years. High management intensity (i.e. high coffee structure index) had a negative effect on rust incidence in three of the six surveying periods (Table 1). The hyperparasite contributed positively to the rust model during the 2017 and 2018 surveying periods, but not during the 2019 (Table 1, last column and values in parentheses).

The hyperparasite index varied much in relation to several drivers during the different surveying periods (Table 2). The hyperparasite was absent from the majority of the sites during the 2017 dry season and was only prevalent towards the last surveying dates, hence, ‘surveying date’ significantly contributed to the model (Table 2). In the two wet seasons 2017 and 2019, when the hyperparasite was most abundant, the hyperparasite index was higher in sites with higher shade tree cover (Table 2). In three out of the six surveying periods, the rust incidence was positively related to the hyperparasite index (Table 2).

4. Discussion

We investigated the temporal dynamics of the coffee leaf rust – fungal hyperparasite interaction on Arabica coffee across a landscape in its native range, as well as the potential for the hyperparasite to control outbreaks of the coffee leaf rust. Both the leaf rust and the hyperparasite showed strong seasonal variation, with rust infection generally more severe, but hyperparasite infection less severe, during the dry season. We found a negative relationship between the hyperparasitism rate (i.e. hyperparasite index) in the wet season and the growth rate of the leaf rust from the wet season to the subsequent dry season, suggesting a possible top-down control of the leaf rust during its peak season. The rust and its hyperparasite seem to have slightly different climatic niches since they differ in their relationship with altitude, suggesting an opportunity to promote the hyperparasite by managing for a colder and more humid microclimate.

4.1. Temporal dynamics of the rust and hyperparasite

Our study showed that coffee leaf rust was generally more common during the dry season, but that the intensity also varied between years. This finding is in line with other studies from Ethiopia but from different landscapes than ours (Chala et al., 2010; Daba et al., 2019; Garedew et al., 2019) and also from other tropical countries (Bock, 1962; Martins et al., 2015). The seasonal variation in rust incidence and severity could be attributed to the development of the rust spores, as spore germination and leaf infection takes place during the rainy season when there is enough moisture on the leaves (Chala et al., 2010). It then takes several months for new infections to develop visible rust pustules (Avelino et al.,

### Table 1

| Coffee leaf rust incidence | Estimate | Altitude | Shade trees > 20 cm DBH | Canopy cover | Coffee density | Coffee structure index | Fruit load | Surveying date | Hyperparasite index |
|---------------------------|----------|----------|-------------------------|-------------|----------------|------------------------|-----------|-----------------|-------------------|
| 2017 Dry season           | SC       | −0.64 (−0.64) | 0.15 (0.14)            | −           | −              | Na                     | −0.45 (−0.54) | (0.21)          |
|                           | p        | <0.001    | −0.009 (0.12)          | <0.001      | −              | Na                     | <0.001    | (0.027)         |
| 2017 Wet season           | SC       | 0.83 (0.49) | −0.94 (0.85)           | 0.71 (0.74) | −              | Na                     | (0.77)    |                 |
|                           | p        | <0.001 (0.019) | <0.001 (<0.001)       | 0.001       | −              | (<0.001)               | (0.001)   |                 |
| 2018 Dry season           | SC       | −0.28 (−0.36) | −0.28 (−0.36)        | −0.036 (−0.04) | −              | (0.40)                 | (0.002)   |                 |
| 2018 Wet season           | SC       | 4.42 (8.25) | −0.69 (0.33)           | 1.12 (0.62) | 0.70 (0.33)    | (0.80)                 | (10.07)   |                 |
|                           | p        | >0.026 (0.006) | >0.001 (0.004)       | 0.017 (0.227) | <0.001 (0.026) | <0.001 (0.132)         | <0.001    |                 |
| 2019 Dry season           | SC       | −0.26 (−0.25) | 5.67 (1.46)           | 16.92 (4.94) | 10.48 (2.26)  | Na                     | (13.26)   |                 |
|                           | p        | <0.001 (−0.001) | 3.25 (3.04)           | 4.96 (5.00) | 7.19 (7.19)   | (0.08)                 | (0.783)   |                 |
| 2019 Wet season           | SC       | −0.45 (−0.45) | 0.072 (0.081)         | 0.026 (0.025) | 0.008 (0.007) | (0.15)                 | (0.42)    |                 |
|                           | p        | 0.021 (0.023) | 5.29 (5.14)           | 4.84 (2.53) | 3.15 (3.22)   | (0.517)                | (0.073)   |                 |

Sequential model selection was performed after accounting for environmental and management variables that significantly contributed to the model and afterwards by adding the hyperparasite index to understand how it can affect the variation of the coffee leaf rust. Values in brackets are outputs after the hyperparasite index was added to the minimum model.

* Log-transformed. Na – we do not have yield for the 1st dry season.
The infections intensify during the wet season and reach peak levels at the start of the dry season (Daba et al., 2019; Hindorf and Omondi, 2011). As the dry season advances, the rust spores could face shortage of moisture, which limits further infections and heavily infected leaves drop off the coffee shrub, which then leads to a lower rust incidence and severity in the late dry season (Daba et al., 2019; Garedew et al., 2019), which is a likely explanation for the negative relationship with surveying date in two of the surveyed dry seasons. Coffee leaf rust is a biotrophic pathogen, i.e. only grows on living leaves and large leaf drop can lead to less infection the subsequent year, sometimes causing a biennial pattern of rust intensity (Avelino et al., 2004; Waller, 1982); a pattern difficult to evaluate in our data that only covers three years. However, it is worth noting that for both the rust and the hyperparasite there is a correlation between the incidence in 2017 and 2019.

In contrast to the rust intensity, the hyperparasite intensity was generally higher during the wet season. Moreover, the hyperparasite showed more variation between years and seasons than the rust. Our finding that the hyperparasite was more common during the wet season coincides with the previous suggestions that it prefers humid conditions (Eskes et al., 1991; Staver et al., 2001; but see Martins et al., 2015). However, as the hyperparasite totally depends on the rust, all seasonal environmental factors that limit the rust will also affect the hyperparasite (Jackson et al., 2012a).

The extent to which hyperparasites feeding on fungal plant pathogens regulate the epidemics of their host pathogens is little studied compared to similar top-down effects on insect herbivores by parasitoids or birds (Cuevas-Reyes et al., 2007; Hassell, 2000; Vidal and Murphy, 2018). We found a negative relationship between the rust growth rate and the hyperparasite index during the wet to dry season transition, when the rust otherwise peaks. However, in the first such transition the absolute growth of the rust between the wet and dry season was still positive in all sites (the negative regression slope was still above 0; see Fig. 4b). Importantly, the negative relationship between rust growth and hyperparasite index was not due to variation in initial rust levels. However, in one year the reduction in growth by the hyperparasite was larger in sites with lower rust levels, indicating that the pattern might be variable irrespective of the rust levels. In our study area, and unlike in Mexico where the hyperparasite has an association with scale insects (Vandermeer et al., 2009), the rust is to our knowledge the only resource for the hyperparasite. Thus, hyperparasite germination would be expected to take place on the already developed rust lesions. Hence, the hyperparasite most likely needs some time to develop before it attains the potential to suppress the rust level. Jackson et al. (2012a) found a one-year time lag for a negative effect of the hyperparasite on coffee leaf rust intensity. However, such longer time lags might perhaps also be related to biennial fluctuations in fruit load and rust (Merle et al., 2020) especially in areas where the hyperparasite does not have an alternative host. On the other hand, Vandermeer et al. (2009) found a negative rust-hyperparasite relationship even during the same season, which could be due to the presence of an alternative host. Through feeding on the spores and reducing severity of the rust, the hyperparasite can delay leaf-fall and thus extend their co-occurrence (Staver et al., 2001). In this case, the hyperparasite could start germination before the rust becomes severe and thus provide suppression of the rust growth as observed in our study during the wet to dry season transitions. Alternatively, rusted leaves with the hyperparasite that fall down to the ground can contribute as an initial inoculum source for the hyperparasite through rain splash since soil has been shown to serve as a reservoir for a viable propagules of the hyperparasite (Jackson et al., 2012b).

### 4.2. Spatial patterns of the rust and hyperparasite

Coffee leaf rust and the hyperparasite had a positive relationship across sites in several of the survey periods, which is logical given that the rust is the resource on which the hyperparasite depends. In line with this finding, Martins et al. (2015) reported a positive correlation between rust severity and hyperparasite incidence irrespective of season from their studies in coffee plantation in Brazil. Similarly, Tollenaere et al. (2014) found a strong positive relationship between the prevalence of the hyperparasite Ampelomyces spp. and powdery mildew fungi *P. plantaginis* naturally infecting the wild plant host *P. lanceolata* in Åland Archipelago, Finland.

Various environmental and management factors affected coffee leaf rust incidence with varying strength and direction of effects (see also Zewdie et al., 2020). The rust was generally more common at lower altitudes in the dry season which is the peak of the rust intensity. At higher altitudes, relatively low night temperatures could increase the latent period of the rust and limit its epidemic development (Chala et al., 2010; Waller, 1982). The pattern seemed to be stronger in the dry season surveys followed by a drop in the incidence and severity during wet season. This is likely due to the fact that at lower altitudes severe rust infection leads to leaf-fall and subsequent reduction in the disease inoculum so that the remaining leaves will have lower rust severity towards the end of dry season and early wet season. It is clear that other factors by themselves or in interaction with the abiotic environment also are involved in explaining the variability in rust intensity across the landscape. For example, the tendency for a biennial fruit set in coffee (DaMatta et al., 2008; Merle et al., 2020) can contribute to the

| Hyperparasite index | Estimate Altitude Canopy Shade trees >20 cm DBH | Coffee structure index | Coffee density | Surveying date | Coffee leaf rust incidence |
|---------------------|-----------------------------------------------|------------------------|----------------|-----------------|----------------------------|
| 2017 Dry season     | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - |
| 2017 Wet season     | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - |
| 2018 Dry season     | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - |
| 2018 Wet season     | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - |
| 2019 Dry season     | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - |
| 2019 Wet season     | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - | SC  - -  - - |
variability between sites in rust intensity (Avelino et al., 2004; López-Bravo et al., 2012). The genetic variability of both the coffee, the rust and the hyperparasite across the landscape most likely also contributes to the variability across sites, although we did not have the opportunity to examine that.

Our results suggest that the hyperparasite is more responsive to the moist environment that results from the buffering effect of the shade trees that maintain a humid microclimate within the coffee canopy as also suggested by (Staver et al., 2001). In line with this, it seems to be more sensitive to UV radiation (Galvão and Bettiol, 2014). Thus, there seem to be a slight difference in niche requirements between the rust and the hyperparasite along a moisture gradient, where low humidity on the leaf surface could be more limiting for the hyperparasite than the rust (Shinde et al., 2010), even if also the rust responded positively to higher canopy cover in one of our models. In a greenhouse experiment, Drummond et al. (1987) show that a high humidity (>95 %) is essential for the germination and infection process of the hyperparasite, Verticillium lecanii (currently referred to as Lecanicillium lecanii) on whitely Trialeurodes vaporariorum.

4.3. Management implications

Intensified management to improve yields often trades-off against other goals such as biodiversity conservation (Geeraert et al., 2019). One aspect in this nexus is the question of natural pest control. Much would be gained if it would be possible to develop management options with less need of herbicide and pesticide use (Lewis et al., 1997; Vandermeer et al., 2014), while still enabling smallholder farmers to improve their revenues. Nature based sustainable management approaches are also of urgency now more than ever to combat probabilities of sudden outbreaks of diseases in response to for example climate change (Davis et al., 2012). Interestingly, complex landscapes in agricultural areas and agroforestry systems by virtue of their nature enable multitrophic interactions, which presents opportunities to develop natural pest control (Perfecto et al., 2014; Rusch et al., 2013). However, to date, top-down controls of plant diseases are not widely implemented in agroforestry systems, despite the promising biocontrol options in both coffee and cocoa plantations (Avelino et al., 2011; Bailey et al., 2008; Mulaw et al., 2010; Muleta et al., 2007). There is also evidence from other systems suggesting that hyperparasites can play a key role as biological control agents (Gleason et al., 2014; Milgroom and Cortesi, 2004; Parratt and Laine, 2016) both in managed (Goett et al., 2008; Romero et al., 2007) and natural systems (Parratt and Laine, 2018; Tollenaere et al., 2014).

In the Ethiopian context, it seems like a moist microclimate is conducive for the hyperparasite on coffee leaf rust. This suggests that it is important to maintain relatively high shade levels to support the top-down regulatory function of the hyperparasite. Yet, too much shade will reduced the yield, and we still do not know how important the suppression of the rust by the hyperparasite is in terms of gains in yields or reduced risks for larger outbreaks. To develop sustainable management systems that take advantage of biotic interactions there is a need for a better ecological understanding of the system (Pell et al., 2010). Thus, studies that focus on obtaining a detailed knowledge of drivers of the hyperparasite dynamics, as well as its life-history, would be advised for a better understanding of the between and within season dynamics of the rust and hyperparasite for the possibility of top-down effects. One starting point could be to follow the dynamics of both the rust and the hyperparasite at the level of individual coffee leaves in different habitats at shorter time intervals but for several consecutive seasons.

Authors’ contributions

B.Z., A.J.M.T. and K.H. conceived and designed the experiment. B.Z. and B.A. conducted the field work. B.Z., A.J.M.T. and K.H. analyzed the data and B.Z. wrote the first draft, and all authors contributed to the final manuscript.

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.n5b2zhb1>

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2021.107297.

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