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Insights from excrement: invasive gastropods shift diet to consume the coffee leaf rust and its mycoparasite

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Agroecosystems are almost always by definition composed of novel assemblages of organisms from various parts of the world (Perfecto and Vandermeer 2015). As ecologists, we have little ability to predict *a priori* how interactions within these novel assemblages will organize themselves and what their impacts will be within and adjacent to agricultural production. While it may be possible to make coarse predictions about well-studied organisms, as with natural enemy release in non-native ranges, it is less often the case that we are able to predict the development of novel interactions which result from host shifts in new ecological contexts

(Agosta 2006; Nylin et al. 2018). This is an issue highlighted by the study of invasive species as well as the many disastrous attempts at classical biological control (Simberloff & Stiling 1996). Here we highlight this unpredictability of agroecosystems by reporting on a widely distributed invasive snail described as being an herbivore, apparently shifting its diet to consume a globally important fungal pathogen of coffee (McCook & Vandermeer 2015), the coffee leaf rust (CLR), *Hemliea vastatrix*. Both field observations and laboratory experiments show that the widespread invasive snail, *Bradybaena similaris*, along with other members of the gastropod community in Puerto Rico, are consuming CLR uredospores (here simply referred to as spores) (Fig. 1). Importantly, CLR lesions that produce these spores are characteristic of "mature" infections on leaves and are the transmissible stage of the pathogen (Talhinhas et al. 2017). Additionally, laboratory experiments show that *B. similaris* also consumes a known biological control agent of CLR, the mycoparasitic fungus, *Lecanicillium lecanii* (Vandermeer et al. 2009; Jackson et al. 2012).

Initial field observations in 2016 of brightly orange colored snail excrement on the undersurface of coffee leaves (Fig. 1, panels B and D) on various farms in the central mountainous region of Puerto Rico led to the insight that there may be a snail consuming spores of the coffee leaf rust. Later that summer, hundreds of the invasive *B. similaris* and a native Carribbean snail, *Bulimulus guadalupensis,* were found on the Estación Experimental Agrícola Adjuntas along with the characteristic orange excrement. To explore which of the snails was consuming CLR, both species were collected along with leaves containing CLR and preliminary experiments showed that after 24 hours *B. similaris* cleared the coffee leaves of CLR spores while *B. guadalupensi* failed to consume any CLR.

After the observations in 2016, we returned to collect *B. similaris* at the same location to conduct more extensive laboratory trials the following year. Given the high incidence of the mycoparasite *L. lecanii* growing on CLR lesions in the region, we sought to determine whether *B. similaris* consumes it in addition to CLR. Coffee leaves were collected from various farms in the region, and the percentage of a leaf covered in CLR lesions with spores was estimated along with the number of *L. lecanii* patches. A single coffee leaf and a single *B. similaris* were placed together in dark containers for 24 hours after which the percentage of CLR and number of *L. lecanii* patches were again quantified. After exposure to the snail for 24 hours there was an average reduction of 30 ± 4 percentage of CLR and 17.4 ± 3.8 in the number of *L. lecanii* patches (Fig. 2A). We also corroborated that the orange excrement we observed in the field is associated with the consumption of CLR spores (p-value =0.001, R^2=0.53, slope=-0.07 ± 0.017) and also its mycoparasite *L. lecanii* (p-value = 0.003, R^2=0.47, slope= -0.07 ± 0.02) (Fig. 2B). Additionally, laboratory results suggest density-independent consumption rates of the CLR by *B. similaris*. The linear regression is not significant when considering all the data (p-value =0.11, R^2=0.17, slope= -0.39 ± 0.23), but there is a clear trend in the data when removing the single point where *B. similaris* consumed no CLR at all (p-value = 0.01, R^2=0.40, slope= -0.52 ± 0.18) (Fig. 2C). Furthermore, our experiments suggest that *B. similaris* consumes more CLR when a given leaf has more *L. lecanii* (Fig. 2D). Although this relationship is only significant when we remove an outlying point of very high number of *L. lecanii* patches (all data: p-value = 0.37, R^2=0.06, slope= -0.16 ± 0.18; outlier removed: p-value = 0.014, R^2=0.38, slope= -0.59 ± 0.21), it suggests that there may be non-linearities in how *B. similaris* consumes CLR when *L. lecanii* is present on a leaf. The exact mechanism driving this pattern is not clear due to the strong relationship between the amount of CLR on a leaf and the number of *L. lecanii* patches (p-value = 0.01, R^2=0.37, slope= -0.83 ± 0.29).

Our experiments and field observations confirm that the invasive *B. similaris* is one of the spore predators of CLR in Puerto Rico. Interestingly, even though *B. similaris* has been described as one of the most widely distributed invasive land snails, it has never been described as consuming other than plant material. In fact, there appears to be only one case in the literature of mollusks specifically consuming rust fungi, which found that the black slug, *Arion ater,* preferentially grazed on leaves infected by a rust fungus (Ramsell & Paul 1990). This is distinct from what we are observing in this system, as the gastropods do not seem to be consuming any plant material, but only the rust fungus and its mycoparasite. The irony of *B. similaris* consuming CLR in Puerto Rico is that it has been described as a severe agricultural pest of many crops in various regions around the world (Idris and Abdullah 1997). In fact, *B. similaris* has been shown to be resistant to a number of control methods implemented in agricultural systems.

Following our experiments, our research team began to pay closer attention in surveys of CLR around the central mountainous region of Puerto Rico as part of ongoing research, and made note of other gastropods apparently consuming CLR spores (Fig. 1, panels C and D). It can be seen from these photos that they are on leaves that show spores having been cleared off portions of the leaves in addition to their guts being full of presumed bright orange CLR spores. Fig. 1C shows a snail in the process of defecating brightly colored orange excrement, and Fig. 1D shows orange excrement in the lower right hand portion of the photo. While these gastropods have not yet been identified, they do not bear resemblance to any of the known native gastropods.

These observations and experiments give rise to a number of interesting questions from both a scientific perspective and as having potentially important implications for the production of coffee. Further work is needed to understand the potential trade-offs *B. similaris* and other gastropods may provide to coffee agroecosystems given our understanding of other elements within the system. For example, *L. lecnaii* is a well-studied biological control agent of CLR (Vandermeer et al. 2009; Jackson et al. 2012; Hajian-Forooshani et al. 2016), and the effect of *B. similaris* (and potentially other gastropods) consuming it along with CLR needs to be understood especially in light of results suggestive of *B. similaris* consuming more CLR when *L. lecanii* is present. Related theoretical work suggests that when an herbivore is consumed by both a predator and a pathogen which exhibit intraguild predation, the intraguild predation (i.e. the predator eating prey infected with the pathogen) can be a stabilizing force that could prevent the outbreak of the herbivore (Ong and Vandermeer 2015). In short, there are non-obvious but potentially consequential implications which stem from these observations. The work summarized here provides evidence that the orange excrement observed in the field is indeed representative of consumption of CLR (Fig 2B).

CLR is the most economically significant pest in coffee around the world, and has been introduced in nearly every coffee producing country worldwide. Here we present what is, to our knowledge, the first case of gastropods feeding on CLR, thus shedding light on a potentially important element of autonomous biological control in coffee agroecosystems (Vandermeer et al. 2010). This work highlights how the ecological theater in which interactions play out turns an agricultural pest in one system to a biological control agent in another. The extent to which *B. similaris* consumes CLR in its native regions, or other introduced regions of the world where coffee is cultivated, is currently unknown. Undoubtedly part of the unpredictability of agroecosystems results from the particular combinations of native and introduced biodiversity, and we suggest that understanding the ecology of these systems will provide key insights in how to manage them. In many agroecosystems technocentric approaches are becoming the norm, where efforts to control supersede efforts to understand the basic ecology. It is our hope that more agronomists start making observations like the ones presented here and that more ecologists leverage their perspectives to help find solutions to issues confronting farmers in agroecosystems around the globe.

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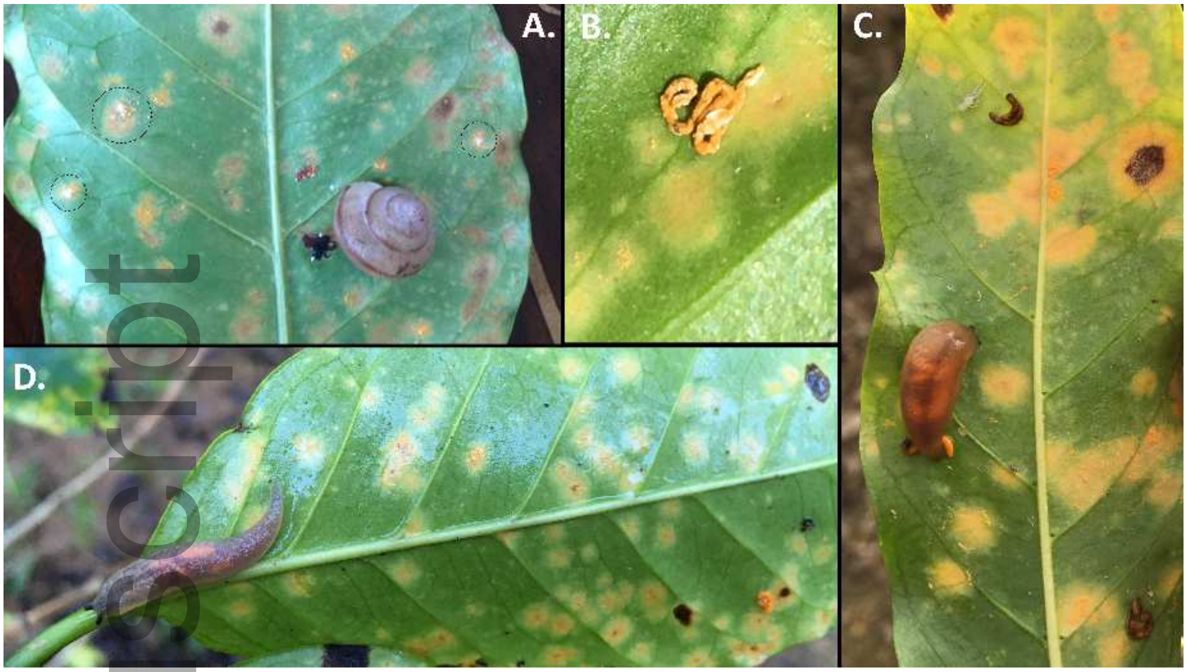
Figure captions

Figure 1. A.) *B. similaris* on coffee leaf with CLR and small white patches of *L.lecanii* circled in black. Note that some CLR lesions have spores (bright orange and textured) and others do not (drab orange with smooth texture), B.) the characteristic orange excrement that led to the idea that gastropods could be consuming CLR, and C.) & D.) two unidentified gastropods with their

guts full of what appear to be CLR spores in addition to the orange excrement on leaves from the field.

Figure 2. 2017 laboratory experiments exposing leaves with CLR and *L. lecanii* to *B. similaris* for 24 hours. A.) Percentage of CLR spores and the number of *L. lecanii* patches on a leaf pre and post exposure to *B. similaris*. B.) The number of snail excrement associated with the change in both the % CLR and the number of *L. lecanii* patches. C.) The change in CLR and the initial amount of CLR on a leaf. The dark red line shows the regression including all points and the orange line excludes the one outlying point where no CLR was consumed. D.) The change in CLR associated with the initial number of *L. lecanii* patches on a leaf. The black point indicates the particularly high density outlier. The grey regression line includes all points; the black regression line excludes the outlier.



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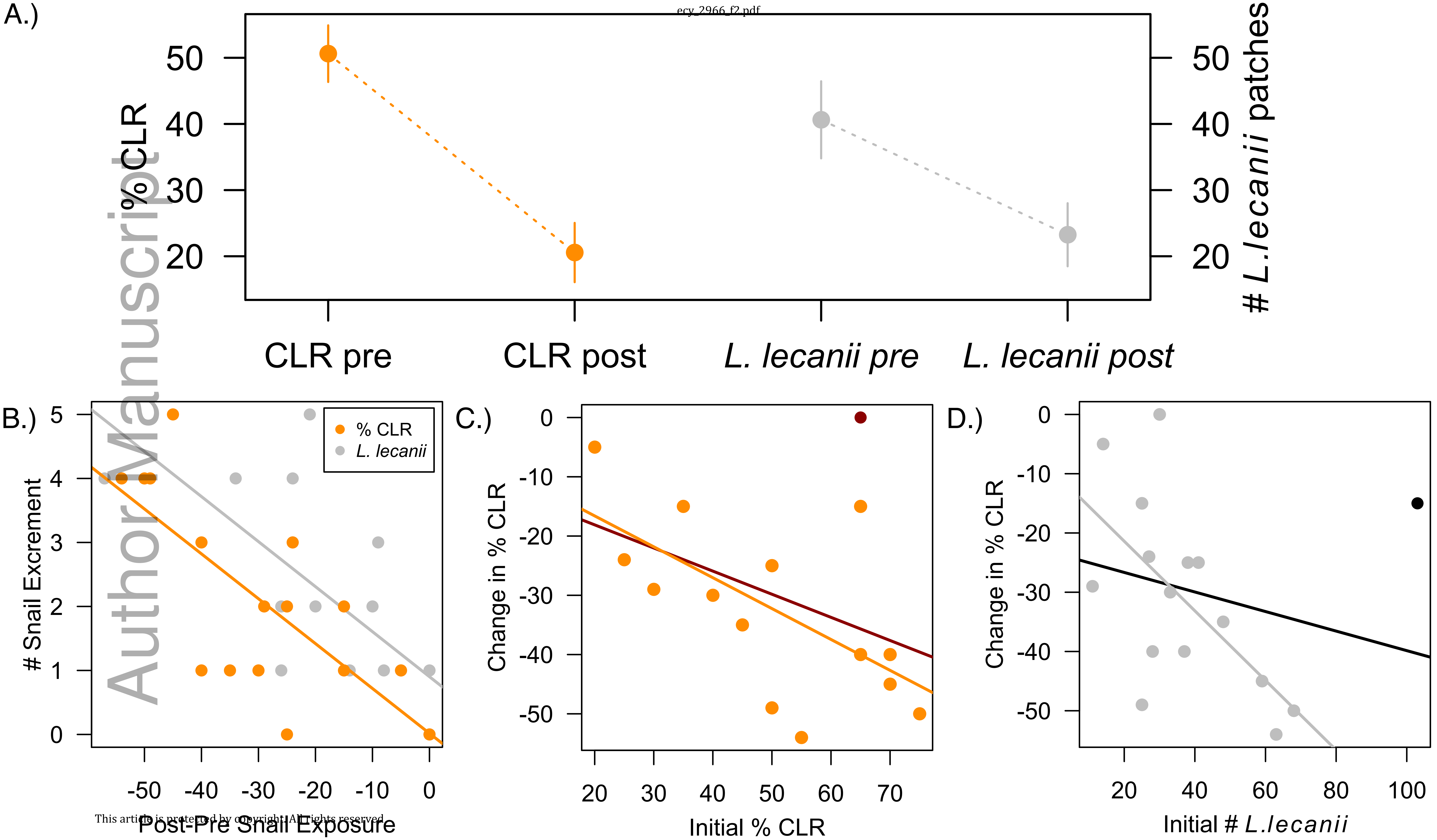
A.)
% CLR
50
40
30
20
L.lecanii patches
CLR pre
CLR post
L. lecanii pre
L. lecanii post
B.)
Snail Excrement
5
4
3
2
1
0
-50
-40
-30
-20
-10
0
Post-Pre Snail Exposure
% CLR
L. lecanii
C.)
Change in % CLR
0
-10
-20
-30
-40
-50
20
30
40
50
60
70
Initial % CLR
D.)
Change in % CLR
0
-10
-20
-30
-40
-50
20
40
60
80
100
Initial # L.lecanii