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Climate change and Uredinales (rust fungi) on Pleurothallid orchids in Monteverde

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Lindsey Parsons
Department of Biology, Berea College

ABSTRACT

The purpose of this experiment was to determine if the decrease in mist frequency observed in Monteverde, has an effect on Uredinales (rust fungi) infections in *Pleurothallis aristata* and *Pleurothallis segoviensis* orchids and to determine if the infection in *P. segoviensis* contributed to the rate of herbivory and number of flowers the orchid produced. In mist experiments infected samples of both species of orchids were placed in either a misted and control condition and observing the change in infection rates over a 20-day period. Under natural conditions, number of leaves, leaves with infection, leaves with herbivory, and number of flowers were counted on samples of *P. segoviensis* to determine if there were any significant interactions among these factors. Results reveal that additional mist does have an effect on rust fungus infection rates; with a decrease in mist, rust fungus infection rates increased. Infection rates also cause higher rates of herbivory and lower rates of flower production. Thirty-eight species of Pleurothallid orchids were found in the short twenty-day study in the forest near the Estación Biológica de Monteverde and in the Jardín de Orquídeas. Monteverde, famous for Pleurothallid orchids is experiencing a decrease in mist frequency, which puts the orchids here in a dangerous situation when dealing with rusts.

RESUMEN

El propósito de este experimento fue determinar si el cambio del clima, a saber, la disminución de la frecuencia de la niebla, tenía un efecto en las infecciones de Uredinales (Hongos de herrumbre) en las orquídeas de la familia Pleurothallid *Pleurothallis aristata* y *Pleurothallis segoviensis*. Tambiín se determinó si la infección en *P. segoviensis*contribuyó al índice de herbivoría y al número de flores que la orquídea produjo. Los métodos para las muestras incluyeron experimentos de la niebla en ambas especies de orquídeas que fueron colocadas en condiciones de niebla y de control y observaciones del cambios en índices de infección. El número de hojas, hojas infectadas, hojas con herbivoría, y el número de flores fue contado en muestras de *P. segoviensis.* para determinar si habían interacciones significativas entre estos factores. Los resultados revelaron que la frecuencia de la niebla tieneun efecto en los índices de infección del hongo de herrumbre cuaudo la frecuencia de al niebla disminuye, los índices de infección del hongo de herrumbre aumentan. Los índices de infección también causaron índices más altos de herbivoría y índices de producción floral menores. Monteverde, famoso por las orquídeas de la familia Pleurothallid, está experimentando una disminución en la frecuencia de niebla que ponea las orquídeas esta zona en una situación peligrosa en referencia a la infección del hongo de herrumbre.

INTRODUCTION

Uredinales are one of the largest groups of plant parasitic fungi with an estimated 5,000 species (Alexopoulous *et al.* 1996). They usually display high host specificity and use two unrelated host plants during their very complex life cycle (Heath 1997). Rust fungi appear to be obligate biotrophs, meaning they only live on living plants (Wingfield 2003). They gain entry to the host via either growing into stomatal openings or by direct penetration of host epidermal cells (Alexopoulous *et. al.* 1996). Heath (1997) points out that although these parasites usually do not kill their host plants they can cause damage. Intracellular invasion of the rust hyphae and haustoria has profound effects on the cellular structure and activity of the host plant (Heath 1997).

I have observed rust fungus of the genus *Trentopohlia* on *Pleurothallis aristata* and *Pleurothallis segoviensis* at the Estación Biológica de Monteverde. This relationship between Pleurothallid orchids and Uredinales is concerning in Monteverde and unfortunately virtually nothing is known about the interaction. This area is abundant with epiphytic plants, including Pleurothallids. The potential threat of rust fungus infections on these organisms could cause much damage to the plants.

Along with this threat, Pleurothallid orchids in Monteverde are also experiencing climate changes that are compromising their health. The nighttime temperature is increasing and the amount of precipitation is decreasing, including mist (Pounds *et al.* 1999). This change has had a significant effect on the birds, anurans, and lizards in the region. Anoline lizard and anuran populations, both sensitive to rainfall variability, have declined with the decrease in mist frequency and increased nighttime temperatures (Pounds *et al.* 1999). It is very possible that this decline is also affecting the flora in Monteverde, specifically Pleurothallids. Arboreal flora is exceptionally sensitive to climate; they are dependent on the atmosphere for moisture and required ions (Benzing 1998).

These climate changes, particularly the decrease in mist frequency, may have an effect on Pleurothallid's rust infection rates, possibly making it harder for orchids to cope with rust fungus. Additionally, these climate changes may affect the impact rust fungi are having on the overall health of the orchids, specifically the rate of herbivory and number of flowers produced. Finally, not knowing what orchids have what rust fungus infections puts scientist at a disadvantage when trying to take action to change the patterns of degradation.

I hypothesize that Pleurothallid orchids experiencing decreasing mist frequency climate changes will have a harder time dealing with rust fungi, as shown by pre- verses post-climate change rates of infection. I also hypothesize that the percentage of leaves with herbivory will increase with higher rust fungus infections. Likewise, the number of flowers produced will decrease with a higher rate of rust infection.

METHODS

The study consisted of three parts that will be referred to as the (1) mist experiment, (2) rust, herbivory and flower health observation and (3) Uredinales on Pleurothallids survey.

Mist Experiment

The study site for the mist experiment was a small orchid garden close to the Estación Biológica de Monteverde. Forty samples of *Pleurothallis aristata* and 32 samples of *Pleurothallis segoviensis* (nomenclature as in Hammel *et al.* 2003) were obtained and tied with nylon string to long branches for easy observation. All of *P. aristata* samples had leaves infected with an unidentified rust fungus and about half of *P. segoviensis* had leaves infected with what was thought to be the same fungus. Each *P. aristata* was cut back to 40 leaves with a varying number of infected leaves (3 - 20 with average of about 10). In the same way, each *P. segoviensis* was cut back to 5 leaves with a varying number of infected leaves (0 - 3). After the orchids were set up, pruned and counted, I spread the rust fungus spores with a paintbrush to the other leaves on the orchids. Twenty of *P. aristata* were in a misted group (approximately 6ml twice daily) and the other 20 were in a control group, receiving no added mist. Likewise, 17 *P. segoviensis* were treated with mist and 15 as a control. At five-day intervals I counted the number of infected leaves and non-infected leaves for each plant of each treatment. In order to track the change of infection rate on each *P. segoviensis* leaf, each leaf was placed into one of five categories describing the amount of infection it was experiencing (1 : none, 2 : small spot, 3 : <50% infected, 4 : >50% infected and 5 : dead).

A 2-Way ANOVA was conducted to determine if the number of infected leaves at the end of the experiment depended on the treatment and/or time. Several analyses of Co-Variance (ANCOVA) were conducted as well to determine if the number or proportion of infected leaves at the end of the experiment depended on the treatment and/or the number or proportion of infected leaves infected at the beginning of the experiment. For *P. segoviensis* frequency distributions were created to see if there was a change in infection rates on the individual leaves.

Rust, Herbivory, and Flower Health Observation

Observations of the relationships between rust, herbivory, and flower health were made for *P. segoviensis* in the forest and regenerating pasture around the Estación Biológica de Monteverde and at a garden in Cañitas. Samples of *P. segoviensis* were found and the number of leaves, leaves infected with rust fungi, leaves with herbivory, and flowers were recorded. A simple linear regression analysis was conducted to see if the percent of infected leaves had an effect on the percent of leaves with herbivory and the percent of flowers.

Uredinales on Pleurothallids Survey

Finally, the Uredinales on Pleurothallids survey was conducted in the forest and regenerating pastures around the Estación Biológica de Monteverde and the Jardín de Orquídeas. The orchid flora was inspected for rust fungi. Leaves that were infected with rust were collected for identification of the orchids.

RESULTS

Mist Experiment

During the study period there were two days of precipitation and twice everyday the misted groups were misted. Results for the ANCOVA revealed that in both *P. aristata* and *S, segoviensis* the number of infected leaves at interval four significantly depended on the treatment (*P. aristata*: $p = 0.0049$; *P. segoviensis*: $p = 0.0010$) and number of infected leaves at interval zero (*P. aristata* and *P. segoviensis* $p = <.0001$). Similarly, the proportion of infected leaves at interval four significantly depended on the treatment (*P. aristata*: $p = 0.0037$; *P. segoviensis*: $p = 0.0041$) and proportion of infected leaves at interval zero (*P. aristata*: $p=<.0001$; *P. segoviensis*: $p = 0.0008$). Finally, the increase of infected leaves from intervals zero to four significantly depended on the treatment (*P. aristata:* $p = 0.0049$; *P. segoviensis*: $p = 0.0010$) but did not significantly depend on the number of leaves infected at interval zero (*P. aristata*: $p = 0.4421$; *P. segoviensis*: $p = 0.1324$).

The 2-Way ANOVA showed that the number of infected leaves for both species significantly depended on the treatment (*P. aristata* and *P. segoviensis* $p = <.0001$) (Fig. 1) and time (*P. aristata:* $p = <.0001$; *P. segoviensis*: $p = 0.0039$). The p values for the effect of time on number of infected leaves was typically only significant at a ten-day interval (Table 1).

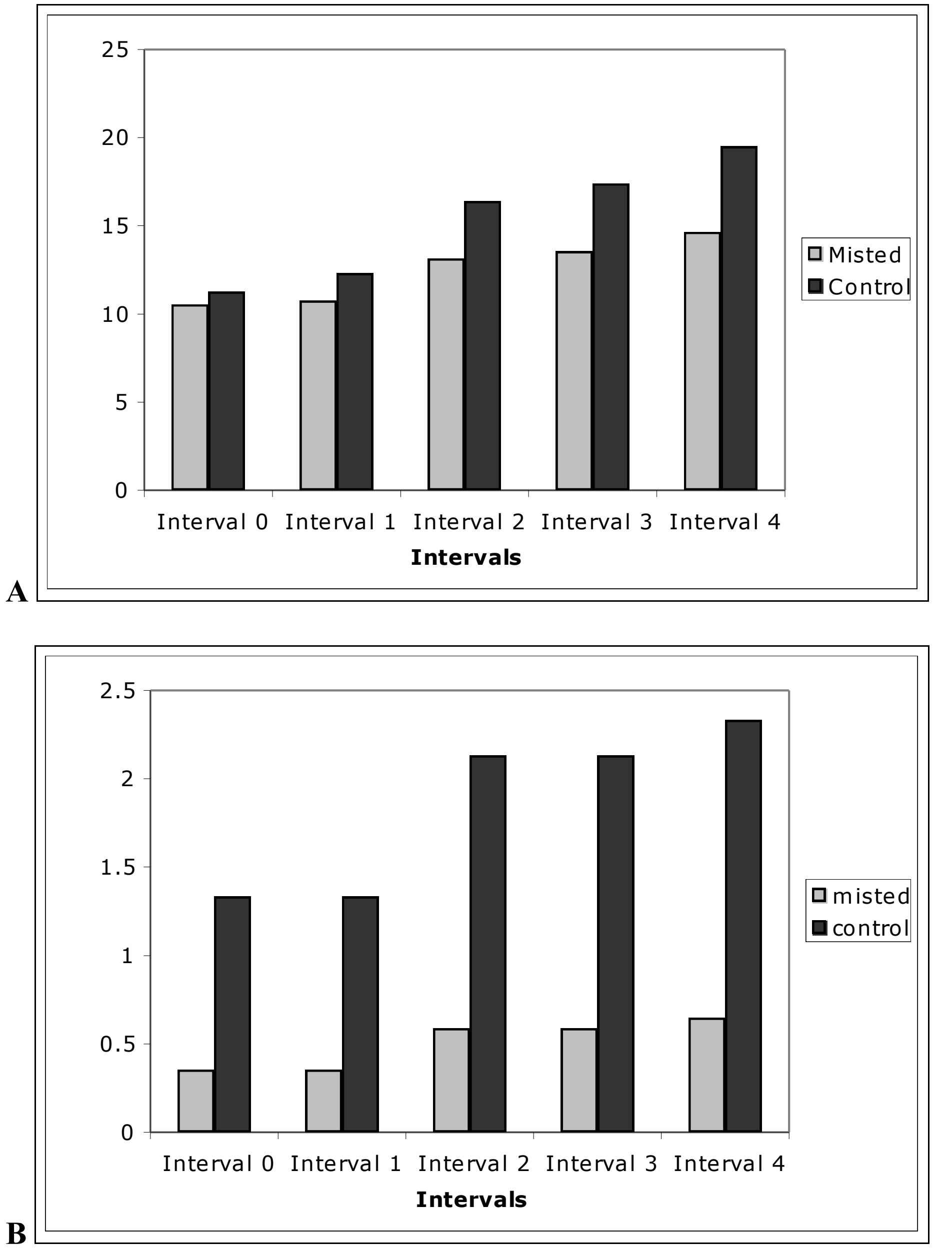


FIGURE 1. Rate of Uredinales (rust fungus) infection in (A) *Pleurothallis aristata* and (B) *Pleurothallis segoviensis* over five day periods in two different treatments misted and control. Number of infected leaves significantly depends on treatment [(A) n = 40 (20 misted; 20 control), p = <.0001] [(B) n = 32 (17 misted; 15 control), p = <.0001] and time [(A) n = 40 (20 misted; 20 control), p = <.0001] [(B) n = 32 (17 misted; 15 control), p = 0.0039].

TABLE 1. Fisher's PSLD for time as an effect on the number of leaves infected with Uredinales (rust fungi) in Pleurothallid orchids (A) *Pleurothallis aristata* and (B) *Pleurothallis segoviensis.* Intervals zero to one and all other intervals are five-day periods. S notes significances. Notice that in most cases it takes ten or more days to see a significant effect of time on the infection rates.

Week	Mean Difference	P-Value		Week	Mean Difference	P-Value	
0-1	-.625	.5014		0-1	0.000	---	
0-2	-3.875	<.0001	S	0-2	-.542	.0311	S
0-3	-4.575	<.0001	S	0-3	-.542	.0311	S
0-4	-6.175	<.0001	S	0-4	-.671	.0079	S
1-2	-3.250	.0006	S	1-2	-.542	.0311	S
1-3	-3.950	<.0001	S	1-3	-.542	.0311	S
1-4	-5.550	<.0001	S	1-4	-.671	.0079	S
2-3	-.700	.4515		2-3	0.000	---	
2-4	-2.300	.0141	S	2-4	-.129	.6082	
3-4	-1.600	.0863		3-4	-.129	.6082	

B

The change in individual leaf rust infection for *P. segoviensis* was recorded by placing each leaf into a category of infection at intervals 2, 3, and 4. The frequency distributions showed that the frequency of leaves per category did not change over 15 days (Fig. 2).

Rust, Herbivory, and Flower Health Observation

The regression analysis for the 54 samples of *P. segoviensis* showed a significant positive relationship between the percent of leaves per plant infected with rust fungus and the percent of leaves per plant with herbivory (R^2 = 0.222, p = 0.0003) (Fig. 3). There was also a significant negative relationship between the percent of infected leaves per plant and percent flowers produced per plant (R^2 = 0.221, p = 0.0003) (Fig. 3).

Uredinales on Pleurothallids Survey

The results for the Uredinales on Pleurothallids survey are summarized in Table 2.

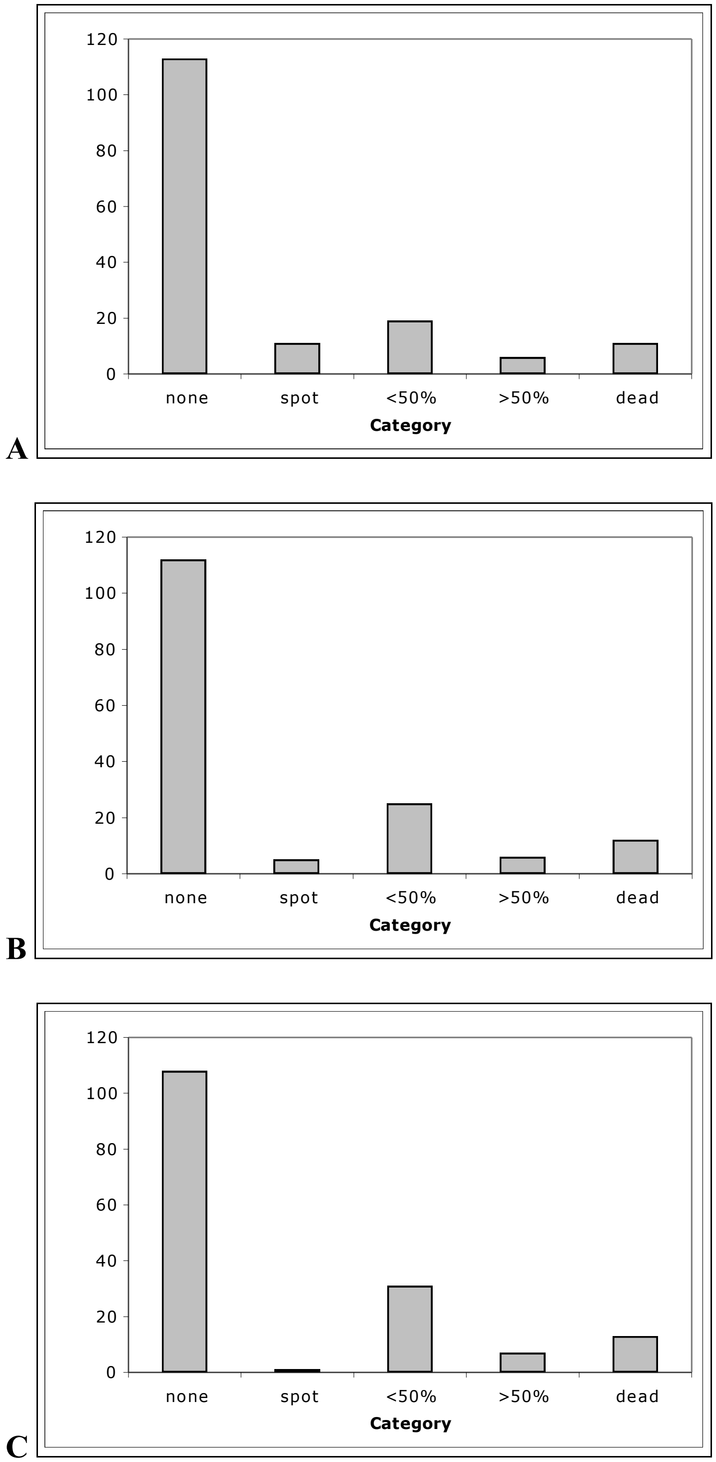
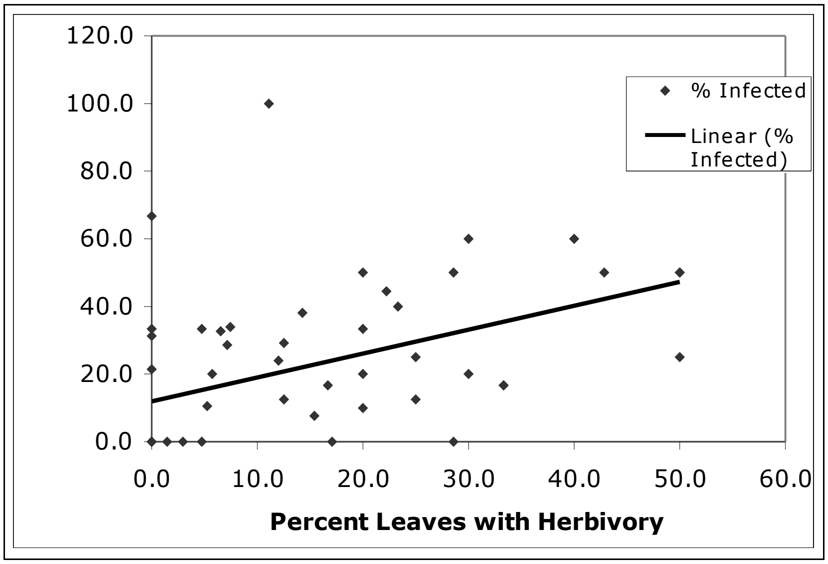
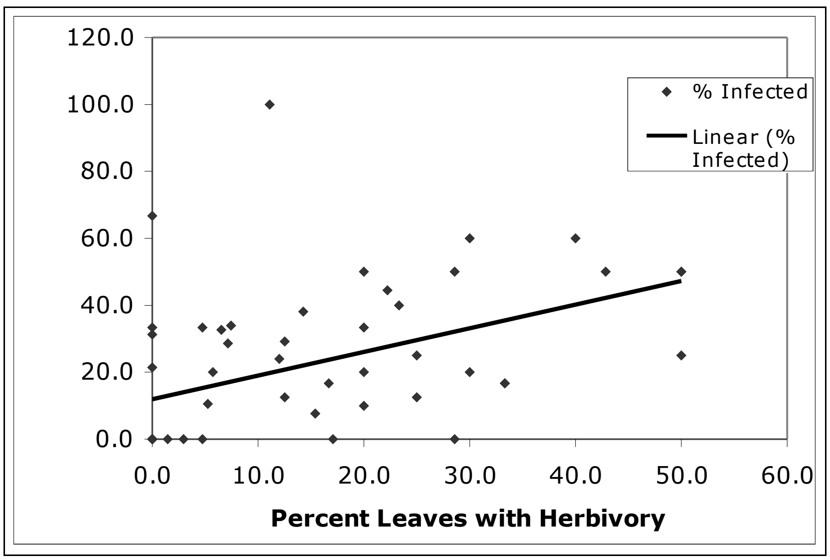


FIGURE 2. Frequency distribution of Uredinales (rust fungi) infection category in *Pleurothallis segoviensis* by number of leaves. Categories were (1) no infection, (2) small spot, (3) <50% infected, (4) >50% infected, and (5) dead. Graph A is 10 days into the experiment, B 15 days, and C 20 days. The frequency of leaves per category did not change over 15 day period.



A



B

FIGURE 3. Regression plots for *P. segoviensis.* (A) As percent of leaves infected with Uredinales (rust fungi) increases the percent of leaves with herbivory also increases (R^2 = 0.222, p = 0.0003). (B) As the percent of infected leaves increases the number of flowers produced decreases (R^2 = 0.221, p = 0.0003).

TABLE 2. Pleurothallid orchid species found in the wild around the Estación Biológica de Monteverde and at Jardín de Orquídeas with rust fungus on leaves

Genus	Specific Epithet	Wild	Jardín
Epidendrum	*spp.*	X	
Scaphyglottis	*spp.*	X	
Maxillaria	*spp*	X	
Dracula	*astute*		X
Dracula	*carlueri*		X
Dracula	*erthrochaete*		X
Dracula	*pusilla*		X
Lepanthes	*barbosae*		X
Lepanthes	*confusa*	X	
Lepanthes	*gracillima*		X
Lepanthes	*jimenezii*		X
Lepanthes	*lindleyana*	X	
Lepanthes	*monteverdensis*		X
Madevalia	*calura*	X	
Pleurothallis	*amparonana*		X
Pleurothallis	*barbulata*		X
Pleurothallis	*bothros*		X
Pleurothallis	*calyptrostele*		X
Pleurothallis	*cardiothallis*		X
Pleurothallis	*cogniauziana*		X
Pleurothallis	*colossus*		X
Pleurothallis	*crescentilabia*		X
Pleurothallis	*dolichopus*		X
Pleurothallis	*immersa*		X
Pleurothallis	*leucantha*	X	
Pleurothallis	*pantasmi*		X
Pleurothallis	*pilostoma*		X
Pleurothallis	*pompalis*		X
Pleurothallis	*quadrifida*		X
Pleurothallis	*segoviensis*	X	X
Pleurothallis	*segregatifolia*		X
Restrepia	*muscifera*	X	X
Restrepia	*trichoglossa*	X	X
Restrepiopsis	*tubulosa*		X
Stelis	*microchila*	X	X
Trichosalpinx	*memor*	X	X
Zootrophion	*endresianum*		X

DISCUSSION

The hypotheses that I set forth were supported by my data. Decreased mist does have an effect on Uredinales infections on the Pleurothallid orchids *P. aristata* and *P. segoviensis.* During the study period there were only two days of precipitation and the misted groups were misted twice everyday. With decreased rates of mist, the orchids are more vulnerable to rust fungus infections. Additionally, *P. segoviensis* with rust infections are more susceptible to herbivory and produce fewer flowers.

Over my 15-day experiment period for individual leaves in *P. segoviensis*, the frequency of leaves per category did not change. It would be interesting to run this experiment for a longer period to see how the leaves changes over time with rust fungus infections.

The effect of time on infection rates was one of interest. The fact that it took approximately ten days for an increase of infection to be significant gives insight to the time frame that Pleurothallids are dealing with. This is a relatively short period of time, indicating that Uredinales have the capacity to spread quickly on their host.

In conclusion, the health of Pleurothallid orchids in the Monteverde area is in jeopardy. This threat could also be extrapolated to other orchid species. The effects of climate change are having a negative effect on many organisms in the area (Pounds *et al.* 1999) and these orchids are not excluded. Pleurothallids, as part of the epiphyte flora, promote biodiversity and provide abundant and diverse resources for fauna (e.g., food, shelter, breeding sites, etc.) (Benzing 1998). They are essential for nutrient cycling in the Monteverde cloud forest and scientist do not know exactly what impact there absence would have on the ecosystem.

ACKNOWLEDGMENTS

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