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Morpho-quantitative analysis and severity assessment of *Uromycladium falcatarium* across elevation gradients and stand ages of falcata plantations in Mindanao, Philippines

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Abstract

The study was conducted to assess the teliospore morpho-quantitative variations and severity of *Uromycladium falcatarium* Doungsa-ard, McTaggart, Geering & R.G. Shivas causing gall rust disease across elevations and stand ages of falcata plantations in Mindanao. Two falcata plantations were identified across elevations from Low (0-400 m asl), Moderate (>400-800 m asl), and High (>800 m asl) and were classified as non-harvestable (<5yo) and harvestable (>5yo) for the stand ages. Likewise, matured galls were also collected from each plantation for microscopic analysis of the fungal teliospores. The result revealed a significant increase in disease severity *(P<0.01)* of gall rust in Falcata and teliospore length *(P<0.05)* of the fungus as influenced by an increasing elevation from 400 m asl and above. Meanwhile, the teliospore width *(P<0.05)* and wall thickness *(P<0.05)* significantly varied between stand ages which revealed a wider width in harvestable *(>5yo)* and thicker wall in non-harvestable *(<5yo)*, respectively. In addition, gall rust severity revealed a significant relationship between elevation and teliospore characteristics that correspond to the survival and prevalence of the fungus. This study provides substantial information and input in understanding the pathogen characteristics and survival under field conditions.

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Introduction

Falcata [*Falcataria falcata* (L.) Greuter & R. Rankin] is widely planted exotic species in the Philippines typically established in monoculture/intercropping systems which contribute approximately 70% of the country's log production, particularly in Mindanao (Santos *et al*., 2010; FMB-DENR, 2021). The species is native to Indonesia, Papua New Guinea, the Solomon Islands, and Australia and is known for its fast-growing characteristics and short cycle (Krisnawati *et al.,* 2011). It is utilized in the production of pulp, lightweight packaging materials, paper, veneer, plywood, and furniture including as a source of wood for light construction and as a species for soil conservation strategies because of its nitrogen-fixing potential (Doloriel, 2023; Alipon *et al.,* 2021; Marasigan *et al.,* 2023).

Gall rust caused by *Uromycladium falcatarium* Doungsa-ard, McTaggart, Geering & R.G. Shivas has been a major problem in Falcata plantations. The fungus produces teliospores that are dispersed by wind causing severe damage to all growth stages of Falcata, from seedlings to mature trees resulting in stunted growth and severe cases, death (Rahayu *et al.,* 2010; Widyastuti *et al.,* 2013). The fungus has been prevalent in the country with slight occurrence at low elevations and severe infection at higher elevations consequently resulting in susceptible Falcata plantations at increasing elevations favoring the disease development (Lacandula *et al.,* 2021; Rahayu *et al.,* 2020; Tulod *et al.,* 2023). However, the complex characteristics of the fungus remain a major concern in managing the disease in Falcata plantations.

The fungus *U. falcatarium* from *U. tepperianum* causing gall rust in Falcata was reclassified based on its teliospore wall morphology, host genus, and DNA sequence data (Doungsa-Ard *et al.,* 2015). Likewise, morphological characteristics of an organism are influenced by several factors including host specificity, environmental condition, genetic diversity, geographical isolation, and even human activities. Obligate parasites like rust fungus are known to infect plants where they interact and co-evolve (Chowdhury *et al.,* 2022), where the age of the host plant may influence the fungal infection. Several studies confirmed the influence of increasing elevations on the severity of gall rust disease in Falcata and the information on the morphological classification of the fungus was used only for taxonomic identification with limited information on the morpho-changes as influenced by elevations. Hence, this study aimed to assess the teliospore morphological-quantitative variations and severity of *U. falcatarium* across elevations and stand ages of falcata plantations.

Materials and methods

Study sites and classification

The study was conducted within selected Falcata plantations in Mindanao, Philippines (Fig. 1). Two falcata plantations were identified from Low (0-400 m asl), Moderate (>400-800 m asl), and High (>800 m asl) elevations and were classified as Non-harvestable (<5 years old) and Harvestable (>5 years old) stand ages (Table 1).

Table 1. Study sites and locations of collected gall rust samples of selected Falcata plantations in Mindanao

Elevations	Stand age	Location	GPS coordinates
Low (0-400 m asl)	Non-harvestable (<5yo)	Brgy. Mabuhay, Prosperidad, Agusan del Sur	8°41' 6" N, 125°57'55" E
	Harvestable (>5yo)	Brgy. Simbalan, Buenavista, Agusan del Norte	8°48'40" N, 125°26' 6" E
Moderate (>401-800 m asl)	Non-harvestable (<5yo)	Brgy. Sta. Ana, Malaybalay City, Bukidnon	8°7'30" N, 125°6'41" E
	Harvestable (>5yo)	Brgy. San Juan, Bayugan City, Agusan del Sur	8°52'4" N, 125°50'48" E
High (>800 m asl)	Non-harvestable (<5yo)	Brgy. Lunotan, Gingoog City, Misamis Oriental	8°43'17" N, 125°2'32" E
	Harvestable (>5yo)	Brgy. San Juan, Bayugan City, Agusan del Sur	8°51'41" N, 125°52'19" E

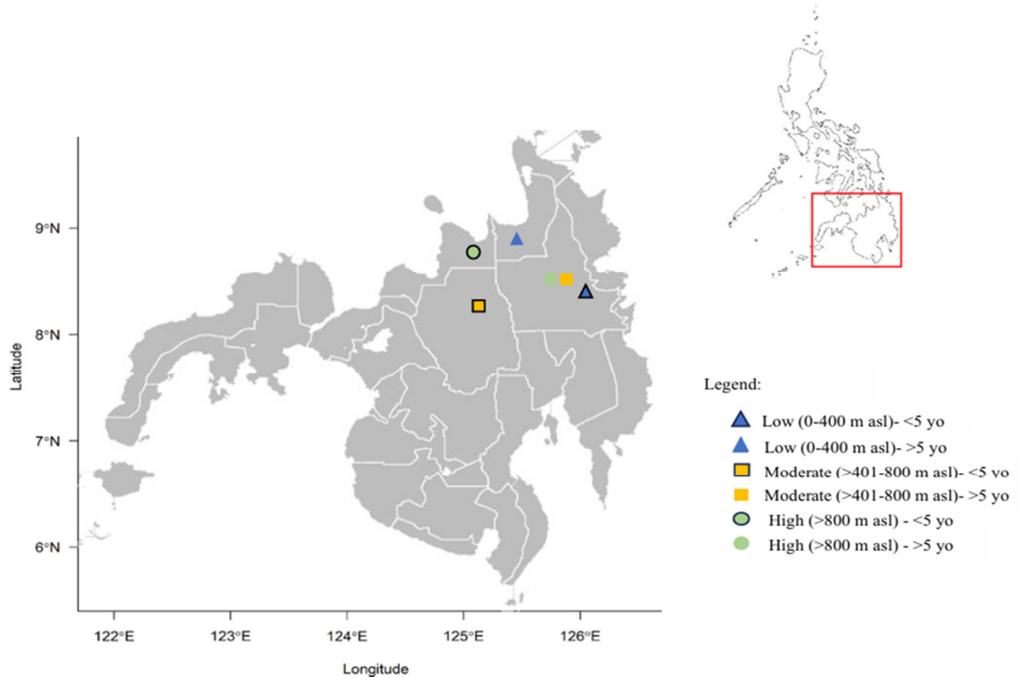

Fig. 1. Location map of the Falcata plantations in Mindanao, Philippines

Fig. 2. *Uromycladium falcatarium* in Falcata plantations. A -B galls on trunk (early stage and death stage) and twigs (matured stage); C. fungal teliospores (bar 10 µm)

Table 2. Gall rust severity assessment scale on Falcata trees

Severity scale	Description
0	No gall formation
1	1 to 10 % gall formation of the tree crown (Twigs and Branches) with no gall formation on the trunk
3	11 to 25 % gall formation of the tree crown with no gall formation on the tree trunk
5	25-50 % gall formation of the tree crown/ <25% gall formation on the trunk
7	51 to 75 % gall formation of the tree crown/ 26 to 50% gall formation on the trunk with active wood decay
9	More than 75% gall formation of the tree crown/ >50% gall formation on the trunk with active wood decay

Sampling procedure and severity assessment

A modified purposive sampling method with three randomly selected sampling blocks/plots (20×20m) consists of 60 falcata trees per plantation. A modified 9-point severity scale was used in the study based on the observed gall rust formation from the crown down to its trunk in Falcata trees (Table 2 & Fig. 2). The disease severity (DS) index scale for gall rust was the computed per plot using the formula below used by Rahayu *et al.*, 2020 with modification:

Disease Severity (DS) = [{(n0xz0) + (n1xz1) +...+ (n9xz9)}/ (NxZ)]×100

Where:
DS- Disease Severity
z0, z1, z3, z5, z7, z9- index score of gall rust presence 0, 1, 3, 5, 7 & 9
N- Total number of trees in one plot
Z- the highest score

Sample collection

Galls with brown/rusty color on the surface were collected from each plantation (Fig. 2B). The galls were brought to the Pest and Disease Laboratory of the ERDB-Forest and Wetland Research Development and Extension Center (FWRDEC) at Bislig City, Surigao del Sur.

Sample processing and morpho-quantitative measurement

The teliospores of the fungus were extracted carefully and placed in a glass slide with clear glycerin for preservation (Fig. 2C). The teliospores were examined using a light microscope (T720- AM) at 400x magnification with built-in tablet and camera application (S-EYE 1.10.7). A total of 150 teliospores per site and per classification were measured using the Image J application. The morpho-quantitative includes teliospore dimension (length and width), and wall thickness.

Data analysis

Using SPSS version 24, analysis of variance (ANOVA) was employed on the teliospore morpho-quantitative measurements across elevations and Tukey's HSD for the post-hoc test while the T-test was done between stand ages at $P \leq 0.05$ level of significance. Moreover, correlation analysis was performed between datasets (elevation, age, % severity, and teliospore characteristics) determining the level of significance at $P \leq 0.05$ level and interpreting the correlation coefficient values described by Turkbet *et al.* (2018).

Results

Disease severity

The severity of gall rust disease in Falcata plantations was presented across plantations (Table 3) and stand ages (Table 4). Statistical analysis revealed a highly significant variation in the severity of gall rust across elevations while no variation was recorded between stand ages of Falcata. The highest severity was recorded at High (>800 m asl) with 22.22%, which was comparable to Moderate elevation (>400-800 m asl) with 17.13%. However, it differs significantly from Low (0-400 m asl) with 4.68%, the lowest severity rating.

Table 3. Percent disease severity of gall rust (*U. falcatarium*) across elevations of Falcata plantations morpho-quantitative *of U. falcatarium*

Elevation levels	% Severity
Low (0-400 m asl)	4.68[b]
Moderate (>400-800 m asl)	17.13[a]
High (>800 m asl)	22.22[a]
F-test	**
%CV	55.69

Means of the same letter in a column are not significantly different at the 5% level using Tukey's HSD. **-highly significant

Table 4. Percent disease severity of gall rust (*U. falcatarium*) between stand ages of Falcata plantations

Stand ages	% Severity
Non-harvestable (<5yo)	14.29
Harvestable (>5yo)	18.47
F-test	ns

ns- non-significant

Table 5 shows the teliospore morpho-quantitative characteristics *of U. falcatarium* across elevations of Falcata plantations in Mindanao. Statistical analysis

revealed a significant variation in teliospore length while no significant variations were recorded in teliospore width, and wall thickness at different elevations. The highest teliospore length was recorded at High (>800 m asl) with 21.59 µm comparable to Moderate (>400-800 m asl) with 21.01 µm which differs significantly from Low elevation (0-400 m asl) with 19.61 µm which is the lowest.

Table 5. Teliospore morpho-quantitative *of U. falcatarium* across elevations of Falcata plantations

Elevation	Length (µm)		Width (µm)		Wall thickness (µm)	
	min-max	Ave.	min-max	Ave.	min-max	Ave.
Low (0-400 m asl)	16.08-24.62	19.61[b]	14.46-23.52	18.06	1.24-2.45	1.84
Moderate (>400-800 m asl)	15.90-25.57	21.01[a]	13.62-23.42	18.35	1.23-2.38	1.76
High (>800 m asl)	17-19-26.26	21.59[a]	13.67-23.18	18.82	1.25-2.67	1.76
F-test		*		ns		ns
%CV		4.59		2.45		2.97

Means of the same letter in a column are not significantly different at the 5% level using Tukey's HSD. *- significant, ns- non-significant

Table 6. Teliospore morpho-quantitative of *U. falcatarium* between stand ages of Falcata plantations

Stand ages	Length (µm)		Width (µm)		Wall thickness (µm)	
	min-max	Ave.	min-max	Ave.	min-max	Ave.
Non-harvestable (<5 yo)	15.90-26.26	20.64	13.87-23.10	18.33	1.22-2.67	1.81
Harvestable (>5 yo)	16.94-25.67	20.84	13.62-23.52	18.49	1.23-2.45	1.76
F-test		ns		*		*

*- significant, ns- non-significant

Table 7. Correlation coefficient (R) between gall rust disease severity and teliospore morpho-quantitative characteristics of *U. falcatarium* across elevation and stand age of Falcata plantation

	Elevation (m asl)	Stand age (yrs)	Disease severity (%)	Teliospore characteristics		
				Width (µm)	Length (µm)	Wall thickness (µm)
Elevation (m asl)	1.00					
Stand Age (yrs)	0.20	1.00				
Disease Severity (%)	0.94**	0.25	1.00			
Teliospore Width (µm)	0.58*	0.45	0.45	1.00		
Teliospore Length (µm)	0.83**	0.31	0.74**	0.59**	1.00	
Teliospore Wall Thickness(µm)	-0.42	-0.39	-0.48*	0.05	-0.39	1.00

*- significant, **- highly significant

On the Falcata stand ages (Table 6), the widest teliospore was observed in the harvestable (>5yo) stand age of Falcata with 18.49 µm compared to the non-harvestable (<5yo) with 18.33 µm. On teliospore wall thickness, the non-harvestable (<5yo) falcata reveals the thickest with 1.81 µm compared to the harvestable (>5yo) with 1.76 µm. Relationships gall rust disease severity, teliospore characteristics, and predictor variables

Table 7 confers, gall rust disease severity caused by *Uromycladium falcatarium* revealed a highly significant with a very strong correlation with elevation *(R=0.94)* and a strong correlation with teliospore length (*R=0.78*). However, gall rust disease severity observed a significant and negative moderate correlation with teliospore wall thickness (*R=-0.48*). Likewise, teliospore length revealed a highly significant very strong correlation between teliospore with elevation (*R=0.83*) and a moderate correlation with teliospore width (*R=0.59*). Also, teliospore width shows a highly significant and moderate correlation with elevation (*R=0.58*).

Discussion

The fungus, *U. falcatarium* causing gall rust disease in Falcata has been prevalent in the country since 1989 (Eusebio, 1998). The study

revealed that the severity of gall rust disease in falcata is more of an elevational influence than on the stand age. A consistent result was observed in Mindanao in the study conducted by Lacandula *et al.* (2021) and Tulod *et al.* (2023) in Falcata plantations, and Palma *et al.* (2021) in mixed plantations with increasing severity from 400 m asl and incidence of gall rust disease from 275 m asl with slight occurrence a lower elevation.

The teliospore produced by *U. falcatarium* was characterized in the study of Doungsa-Ard *et al.* (2015) and Doungsa-Ard *et al.* (2018) which are the isolates from the Philippines and Timor Leste, and Lelana *et al.* (2022) characterizing the isolates from Indonesia. With its described characteristics, the current study revealed that the teliospore morpho-quantitative dimensions of the fungus vary with a higher length at higher elevations with a wider width at harvestable stand age and thicker teliospore wall at non-harvestable stand age of Falcata that may influence its field survival. The teliospores produced by a rust fungus are thick-walled and resistant to cold or drying which is also its resting stage through the dormant state (Kolmer *et al.,* 2009). Further, the sedimentation rate of larger spores is higher compared to small spores affecting their survival in the field (Watkinson *et al.,* 2015; Golan and Pringle, 2017). The rust fungus is renowned for its intricate life cycle traits, especially its ability to disperse spores and survive in the field under a variety of environmental circumstances before reaching its ideal host or infection site (Helfer, 2014). According to Rahayu *et al.* (2020), a reduced gall rust disease incidence and severity were observed in conditions with more open sites, flat topography, absence of fog, greater age, and lower altitude while high relative humidity and low wind speed promoted gall rust disease development in Falcata plantation.

Conclusion

The result revealed a higher severity of gall rust disease in Falcata plantations as the elevation increases >400 m asl. The same with the teliospore length of the fungus that significantly increased at higher elevations >400 m asl while on the stand age revealed a wider teliospores width and thicker wall on harvestable (>5yo) and non-harvestable (<5yo) Falcata plantations, respectively. Moreover, gall rust severity revealed a significant relationship between elevation and teliospore characteristics that correspond to the survival and prevalence of the fungus.

Recommendations

The result of the study demonstrates the fungal characteristics across elevations and stand ages in Falcata plantations. Further studies on pathogen characterization at a higher magnification, classification, and survival must be done for a comprehensive understanding of the fungus for substantial input in the establishment of its management strategies.

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