



Developmental and diurnal dynamics of transpiration and Hill reaction activity in *Rumex Maritimus* infected with smut fungus *Ustilago parletoreii* F.A Waldheim

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Abstract

This study examined the developmental and diurnal variation of transpiration and Hill reaction activity in the leaf lamina of *Rumex maritimus* infected with the smut fungus *Ustilago parletoreii*. Transpiration rates varied with developmental stage and time of day. The pre-sporulation stage showed the highest morning transpiration ($24.9 \pm 0.1 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 9:00 A.M., increasing to $27.0 \pm 0.1 \text{ mg cm}^{-2} \text{ hr}^{-1}$ at 11:00 A.M.), declining at noon ($14.0 \pm 0.2 \text{ mg cm}^{-2} \text{ hr}^{-1}$). Pre-flowering and flowering stages had moderate rates, while young- and mature-sporulation stages remained low throughout ($6.5\text{--}14.5 \text{ mg cm}^{-2} \text{ hr}^{-1}$). Hill reaction activity peaked during flowering ($0.88 \pm 0.02 \mu\text{g DCPIP hr}^{-1} \text{ g}^{-1}$), followed by pre-flowering (0.70 ± 0.03) and pre-sporulation (0.69 ± 0.02), with lower values in later sporulation stages ($0.54\text{--}0.40$). These results suggest that fungal infection influences both water loss and photosynthetic efficiency, with maximal physiological activity occurring before sporulation, reflecting interactions between developmental stage, pathogen, and diurnal environmental factors.

Keywords: *Rumex maritimus*, *Ustilago parletoreii*, transpiration, Hill reaction, leaf development, photosynthesis

Introduction

Plant physiological processes, including transpiration and photosynthesis, are strongly influenced by developmental stage, environmental conditions, and pathogen interactions (Taiz *et al.*, 2015; Jones, 2014) ^[8, 14]. Transpiration regulates water balance and nutrient transport, while photosynthetic efficiency, often assessed through the Hill reaction, determines energy availability for growth and reproduction (Farquhar & Sharkey, 1982; Good, 1963) ^[4, 6].

Pathogenic fungi, such as smut fungi (*Ustilago* spp.), can alter plant water relations and chloroplast function by disrupting stomatal regulation and photochemical activity (Smith *et al.*, 2011; Kranz, 2010). Infection may accelerate senescence, reduce stomatal conductance, and impair photosynthetic electron transport, leading to decreased growth and reproductive output (Fry, 1970; Davenport, 1959) ^[3, 5].

Rumex maritimus is a perennial herb inhabiting moist habitats and serves as a suitable model for studying host-pathogen interactions affecting physiological performance. Previous studies on *Rumex* species have characterized leaf water relations and photosynthesis during development (Pallas *et al.*, 1967; Lawlor, 2002) ^[10, 13], but little is known about the impact of smut fungus infection on these processes.

This study aims to investigate diurnal and developmental variation in transpiration and Hill reaction activity in *R. maritimus* infected with *Ustilago parletoreii*. We hypothesize that fungal infection will modify the normal developmental patterns of water loss and photochemical efficiency, with potential implications for plant productivity and stress tolerance. Therefore, the present study was undertaken to integrate measurements of transpiration and Hill reaction across different developmental stages and time intervals, in order to elucidate the physiological mechanisms underlying pathogen-induced changes in the host plant (Lv *et al.*, 2024; Tuba *et al.*, 1994^[11, 15]; Ye *et al.*, 2025).

Materials and Methods

Plant Material and Fungal Infection

Healthy and infected *Rumex maritimus* plants were collected from the research field. Leaves were categorized into five developmental stages: pre-flowering, flowering, pre-sporulation, young-sporulation, and mature-sporulation, following standard morphological criteria (Taiz *et al.*, 2015) ^[14]. Only leaves showing fungal infection symptoms were used to study pathogen effects.

Measurement of Transpiration Rate

Transpiration rate was determined using the cobalt chloride (CoCl₂) paper method, in which leaf lamina water loss is indicated by the color change of cobalt chloride from blue to pink (Jones, 2014; Larcher, 2003) ^[8, 9]. Fresh leaf laminae were excised, gently blotted to remove surface moisture, and weighed using an analytical balance. Leaves were exposed to natural ambient conditions, and weight loss was recorded at 9:00 A.M., 10:00 A.M., 11:00 A.M., and noon.

Transpiration rate was calculated as:

$$\text{Transpiration rate} = \frac{\text{Weight loss (mg)}}{\text{Leaf area (cm}^2\text{)} \times \text{Time (hr)}}$$

Leaf area was measured using graph paper tracing (Pandey & Sinha, 2006). Each measurement was performed in triplicate, and results were expressed as mean \pm standard error (SE).

Estimation of Hill Reaction Activity

Hill reaction activity was measured according to classical methods (Hill, 1939^[7]; Arnon, 1954), using 2,6-dichlorophenol-indophenol (DCPIP) as an artificial electron acceptor. Approximately 1 g of leaf lamina tissue from each developmental stage was homogenized in cold phosphate buffer (pH 6.5–7.0), filtered, and centrifuged to isolate chloroplasts.

Chloroplast suspensions were incubated with phosphate buffer and DCPIP, and reduction of DCPIP was monitored spectrophotometrically at 600 nm under illumination (Good, 1963; Fry, 1970) [5, 6]. Hill reaction activity was expressed as μg DCPIP reduced per hour per gram fresh weight (μg DCPIP hr^{-1} g^{-1} FW). All assays were performed in triplicate.

Results and Discussions

Transpiration Rate

The transpiration rate in the leaf lamina of *Rumex maritimus* infected with smut fungus *Ustilago parletoreii* exhibited clear diurnal and developmental variations (Table 1). Across all stages, transpiration increased from 9:00 A.M. to 11:00 A.M., followed by a sharp decline at noon.

At 9:00 A.M., the highest transpiration rate was observed in the pre-sporulation stage (24.9 ± 0.1 $\text{mg cm}^{-2} \text{hr}^{-1}$), while the mature-sporulation stage exhibited the lowest rate (10.50 ± 0.2 $\text{mg cm}^{-2} \text{hr}^{-1}$). This trend continued at 10:00 A.M. and 11:00 A.M., with pre-sporulation consistently recording the maximum values (26.3 ± 0.2 and 27.0 ± 0.1 $\text{mg cm}^{-2} \text{hr}^{-1}$, respectively). Pre-flowering and flowering stages showed intermediate transpiration rates, whereas young- and mature-sporulation stages maintained comparatively lower rates throughout the morning.

By noon, transpiration decreased markedly in all stages, ranging from 6.50 ± 0.3 $\text{mg cm}^{-2} \text{hr}^{-1}$ in mature-sporulation to 16.0 ± 0.2 $\text{mg cm}^{-2} \text{hr}^{-1}$ in pre-flowering and flowering stages. This diurnal pattern is consistent with stomatal regulation under varying light intensity and temperature, where maximum morning transpiration corresponds to higher irradiance and stomatal opening, while midday declines are likely due to partial stomatal closure to minimize water loss under stress conditions (Pallas *et al.*, 1967; Jones, 2014) [8, 13].

The elevated transpiration in the pre-sporulation stage may reflect peak physiological activity and pathogen-induced

alterations in stomatal function, while reduced rates in young- and mature-sporulation stages suggest declining stomatal efficiency and the onset of leaf senescence, which can be further influenced by smut infection (Taiz *et al.*, 2015) [14]. Overall, infection by *U. parletoreii* may exacerbate the decline in transpiration during later stages by affecting stomatal conductance and leaf water relations.

Hill Reaction Activity

The Hill reaction, representing the light-dependent phase of photosynthesis, also varied across developmental stages in infected leaves (Table 2). Maximum Hill reaction activity was recorded during the flowering stage (0.88 ± 0.02 μg DCPIP hr^{-1} g^{-1} FW), followed by pre-flowering (0.70 ± 0.03) and pre-sporulation (0.69 ± 0.02). A progressive decline was observed in the sporulation stages, with young-sporulation and mature-sporulation showing lower activities of 0.54 ± 0.03 and 0.40 ± 0.02 , respectively.

The high Hill reaction activity during flowering indicates optimal chloroplast function and photochemical efficiency to meet the energy demands of reproductive growth, whereas the decline in sporulation stages suggests senescence-associated degradation of chloroplast membranes and electron transport enzymes (Fry, 1970; Davenport, 1959) [3, 5]. Infection by *Ustilago parletoreii* may further impair chloroplast structure and electron transport, contributing to reduced photochemical activity in older leaf stages.

These findings highlight the coordinated decline of both transpiration and photosynthetic electron transport with leaf aging and pathogen infection. The alignment of reduced water loss and decreased Hill reaction activity suggests that the fungus may exacerbate the natural senescence process, potentially impacting overall plant productivity (Good, 1963; Jones, 2014) [6, 8].

Table 1. Rate of transpiration in the leaf lamina at different developmental stages of *Rumex maritimus*, infected with smut fungus *Ustilago parletoreii*, expressed in $\text{mg cm}^{-2} \text{hr}^{-1}$ at 9:00 A.M., 10:00 A.M., 11:00 A.M., and noon.

TIME	Pre-flowering	Flowering	Pre-sporulation	Young-sporulation	Mature-sporulation
09.00	20.4 ± 0.4	20.0 ± 0.6	24.9 ± 0.1	12.28 ± 0.1	10.50 ± 0.2
10.00	23.0 ± 0.5	22.6 ± 0.4	26.3 ± 0.2	12.85 ± 0.4	11.50 ± 0.2
11.00	25.0 ± 0.3	24.8 ± 0.3	27.0 ± 0.1	14.53 ± 0.3	12.05 ± 0.1
Noon	16.0 ± 0.2	16.0 ± 0.2	14.0 ± 0.2	9.0 ± 0.5	6.50 ± 0.3

*The data represent the means \pm SD of three replications

Table 2. Rate of Hill reactivity in the lamina tissues at different developmental stages of *Rumex maritimus*, infected with smut fungus *Ustilago parletoreii*, expressed as μg DCPIP hr^{-1} g^{-1} fresh weight.

Pre-flowering	Flowering	Pre-sporulation	Young-sporulation	Mature-sporulation
0.70 ± 0.03	0.88 ± 0.02	0.69 ± 0.02	0.54 ± 0.03	0.40 ± 0.02

*The data represent the means \pm SD of three replications

Conclusion

In *Rumex maritimus*, both transpiration and Hill reaction activity vary with developmental stage and time of day, and these patterns are significantly affected by infection with smut fungus *Ustilago parletoreii*. Peak transpiration occurred in the pre-sporulation stage, while maximum Hill reaction activity was observed during flowering. The decline in both parameters in young- and mature-sporulation stages indicates senescence and pathogen-induced impairment of stomatal and chloroplast function. These findings highlight the combined effects of developmental

progression and fungal infection on plant water relations and photosynthetic efficiency, providing insight into the physiological impact of smut infection on host plants.

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Competing interests

The authors have declared that no competing interests exist.

Authors' Contributions

All the authors have given equal contributions. All the authors read and approved the final manuscript.

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