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APPENDIX 1. Complete data set for individual classes in Mango, Slope, and Waterhole troops in Palo Verde National Park, Costa Rica (Troop Size: large = 27, medium = 12, small = 4). Each bout was classified as including one or two behaviors.

				Percent of bouts with activity			
Troop	Time	Age/Sex	Total observation bouts	Rest	Alert	Move	Feed
Mango (Large)	Morn	F	10	20	20	50	70
		FWI	8	25	63	63	38
		J	5	20	40	40	80
		M	11	27	45	55	36
	Mid	F	21	86	10	5	10
		FWI	6	67	33	17	0
		J	8	75	38	25	13
		M	15	27	80	13	7
	Aft	F	9	22	33	22	56
		FWI	5	0	40	60	80
		J	3	33	0	67	100
		M	5	40	40	20	40
Waterhole (Medium)	Morn	F	13	46	23	38	38
		FWI	12	42	58	42	17
		J	14	50	7	50	43
		M	18	44	50	22	17
	Mid	F	18	89	11	6	11
		FWI	17	76	24	12	0
		J	18	72	28	0	17
		M	20	85	15	5	5
	Aft	F	9	44	0	22	56
		FWI	8	25	38	63	25
		J	7	14	43	43	71
		M	9	22	89	67	0
Slope (Small)	Morn	F	5	100	20	0	0
		FWI	6	83	50	0	17
		J	6	67	0	33	50
		M	12	67	58	8	8
	Mid	F	2	100	0	0	50
		FWI	1	0	100	0	0
		M	5	40	40	0	40
	Aft	F	2	100	50	0	0
		FWI	1	100	0	0	0
		J	1	100	0	100	0
		M	5	40	20	20	40

THE EFFECT OF LIGHT AVAILABILITY AND TEMPERATURE ON THE CLOSURE RESPONSE TO TACTILE STIMULUS IN *NEPTUNIA PLENA*

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Abstract. *Neptunia plena* is an aquatic mimosoid plant that closes its leaves in response to tactile stimulus. This response may be an adaptation to reduce (1) insect herbivory, (2) potential damage from aquatic birds who walk on top of this floating plant, or (3) desiccation in ancestral terrestrial forms. Because ATP and enzymes regulate the closing mechanism of *N. plena*, sunlight availability and temperature will affect leaf closing and reopening time following disturbance. Leaf response times were slower in the shade, supporting the prediction that ATP availability limits leaf responses. Response times in sun and shade were quicker in the afternoon, which may be a result of higher temperatures increasing enzymatic activity. Our results indicate that irradiance and temperature can impact tactile responses in leaves, but more exploration is needed to understand the evolutionary and adaptive roles of the closing response mechanism in *N. plena*.

Key Words: Fabaceae, *irradiance*, Mimosoideae, *Na^+/K^+ ATPase pump*

INTRODUCTION

Neptunia plena, an aquatic Mimosaceae plant, responds to tactile stimulus by closing its leaves. Hypotheses to explain why the leaves close in response to disturbance include reducing herbivory by insects such as bruchid beetles (Ricklefs 1973: 197) or reducing damage from water birds walking over the mat of vegetation while feeding. In terrestrial species with the same response to stimuli, leaves close to avoid wind desiccation (Raven 1986: 509). Because aquatic plants are not water-limited, it is possible that the response in aquatic mimosoids is an anachronism from its terrestrial ancestors.

The mechanism that causes leaf closure response involves the establishment of an electrical and/or diffusional gradient through the active transport of Na^+ and K^+ ions (Johnson 1983: 430). Because the mechanism is energy demanding (involves an ATP pump), we hypothesized that leaf closure could be affected by the availability of photosynthetically active radiation. Because the mechanism is also enzyme regulated, reaction rates could vary with environmental temperature. We predicted that closing and reopening times would generally be slower in the morning because of cooler temperatures, and be slower in the shade because of lower light intensity. We tested these predictions through a combination of diurnal observations and shading experiments.

METHODS

We conducted this study in the Laguna Palo Verde marsh in Palo Verde National Park, Costa Rica, on 13 - 14 January 1998. To reduce photosynthesis, we placed three 1 m^2 shade shelters over patches of *N. plena* in the marsh. Three equal-sized sun plots were established within 5 m of the shade shelters. The shade structures were black plastic sheets ≈ 30 cm above the vegetation mat, closed on both the top and two sides to exclude sun during the morning and afternoon, while still allowing wind movement.

Shade structures were erected at 11:00 on 13 January 1998. The next day, we measured *N. plena* response time in the morning (07:30 - 09:00) and again in the afternoon (12:45 - 13:45). At each site, we disturbed 15 randomly chosen leaves and recorded the time it took the leaf to (1) close completely and (2) initiate reopening. Air and water temperatures were measured both inside and outside each shade shelter and in each sun plot at all sites at both sampling times.

Closing and reopening times were tested for normality and homoscedasticity then analyzed with a two-way analysis of variance for effects of time of day, shade, and time x shade. We analyzed temperature data with a t-test.

RESULTS

Plants in the sun closed faster than shaded plants ($F_{1,8} = 27.77$, $P < 0.001$) and plants also closed faster in the afternoon than in the morning ($F_{1,8} = 30.09$, $P < 0.001$); there was no shade x time interaction ($F_{1,8} = 3.31$, $P = 0.11$; Fig. 1). Shade treatments increased reopening times in the morning but not in the afternoon (Fig. 2; $F_{1,8} = 14.35$, $P = 0.005$ for main effect of shade; $F_{1,8} = 17.70$, $P = 0.003$ for shade x time interaction).

Water temperature increased by ~ 2.5 °C from morning to afternoon. Air temperature increased by ~ 5 °C from morning to afternoon. Shade shelters had little or no effect on temperatures (± 0.7° C of ambient).

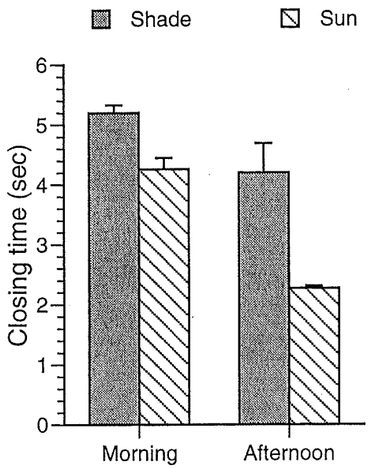

FIG. 1. Closing time of *Neptunia plena* leaves following disturbance (mean ± SE).

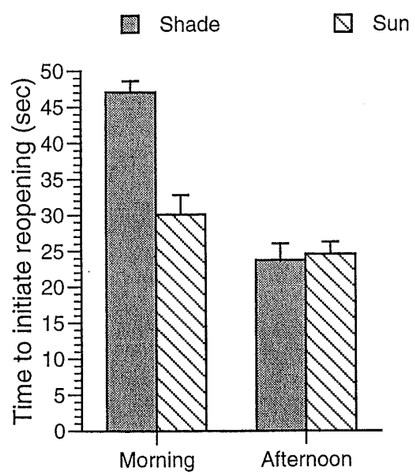

FIG. 2. Time until *Neptunia plena* leaves initiated reopening following disturbance (mean ± SE).

DISCUSSION

The response in *N. plena* leaves to disturbance was affected by both irradiance and time of day. The slower response in the shade versus the sun may be related to lower local energy stores. Leaf sugar concentrations are presumably increased by photosynthesis, and therefore, leaves in the shaded treatment may have had less ATP to support leaf responses to tactile stimuli. Warmer temperatures were the likely explanation for the accelerated response times in the afternoon given that the closing and reopening mechanisms are regulated by enzymes. It is possible that enzyme limitations are more important for reopening than closing mechanisms because reopening is believed to be driven by the dissipation of a previously established diffusion gradient more than it depends on the immediate release of ATP. Because there was no difference in reopening time between shade and sun treatments in the afternoon, it is possible that a temperature threshold on enzymatic reactions may have been reached for both treatments.

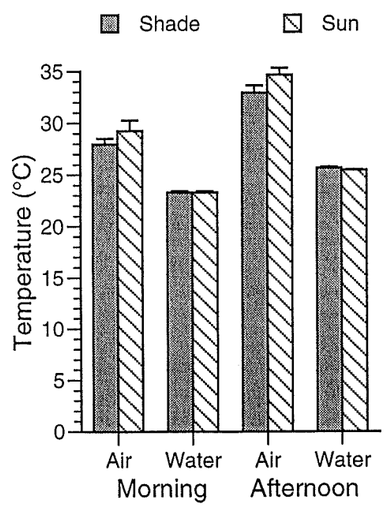

FIG. 3. Air and water temperature in morning and afternoon in sun and shade treatments (mean ± SE).

Presumably there are costs and benefits associated with the tactile responses of *N. plena* leaves. Closing of leaves represents an energetic cost to the plant, in both the act of closing the leaf and in the reduced potential for photosynthesis while shut. Leaves in the shade may reopen more slowly because they receive less light and thus have less photosynthetic potential to lose than leaves in the sun. However, there are also benefits associated with the closing of leaves. Closing response may reduce herbivory by startling insects or making foliage less appealing to large grazers (Raven 1986: 509). Patterns of temperature and irradiance could influence both the leaf response rates and the cost - benefit relationships. Future studies of herbivory pressure and reopening mechanisms in *N. plena* would aid our understanding of the ecological relevance of leaf tactile responses.

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