

RESEARCH ARTICLE



Flexible drought deciduousness in a neotropical understory herb

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Abstract

Premise: Adaptive divergence across environmental gradients is a key driver of speciation. Precipitation seasonality gradients are common in the tropics, yet drought adaptation is nearly unexplored in neotropical understory herbs. Here, we examined two recently diverged neotropical spiral gingers, one adapted to seasonal drought and one reliant on perennial water, to uncover the basis of drought adaptation.

Methods: We combined ecophysiological trait measurements in the field and greenhouse with experimental and observational assessments of real-time drought response to determine how *Costus villosissimus* (Costaceae) differs from *C. allenii* to achieve drought adaptation.

Results: We found that drought-adapted *C. villosissimus* has several characteristics indicating flexible dehydration avoidance via semi-drought-deciduousness and a fast economic strategy. Although the two species do not differ in water-use efficiency, *C. villosissimus* has a more rapid growth rate, lower leaf mass per area, lower stem density, higher leaf nitrogen, and a strong trend of greater light-saturated photosynthetic rates. These fast economic strategy traits align with both field-based observations and experimental dry-down results. During drought, *C. villosissimus* displays facultative drought-deciduousness, losing lower leaves during the dry season and rapidly growing new leaves in the wet season.

Conclusions: We revealed a drought adaptation strategy that has not, to our knowledge, previously been documented in tropical herbs. This divergent drought adaptation evolved recently and is an important component of reproductive isolation between *C. villosissimus* and *C. allenii*, indicating that adaptive shifts to survive seasonal drought may be an underappreciated axis of neotropical understory plant diversification.

Resumen en Español

Premisa del estudio: La divergencia adaptativa a lo largo de gradientes ambientales es un factor clave de la especiación. Los gradientes de estacionalidad de la precipitación son comunes en los trópicos, sin embargo, la adaptación a la sequía es casi inexplorada en las hierbas neotropicales del sotobosque. Examinamos dos especies de caña agria neotropicales que divergieron recientemente, uno adaptado a la sequía estacional y otro que depende del agua perenne, para descubrir la base de la adaptación a la sequía.

Métodos: Combinamos mediciones ecofisiológicas en el campo y el invernadero con una evaluación experimental y observacional de la respuesta a la sequía en tiempo real para determinar cómo *Costus villosissimus* (Costaceae) difiere de *C. allenii* para lograr la adaptación a la sequía.

Resultados clave: Encontramos que *C. villosissimus*, que está adaptado a la sequía, tiene varias características que indican que evita la deshidratación a través de la caducidad y una estrategia de vida rápida. Aunque las dos especies no difieren en la eficiencia del uso del agua, *C. villosissimus* tiene una tasa de crecimiento más rápida,

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menor masa foliar por área, menor densidad del tallo, mayor nitrógeno foliar y una fuerte tendencia de mayores tasas fotosintéticas saturadas de luz. Estos atributos de la estrategia de vida rápida se alinean tanto con las observaciones basadas en el campo como con los resultados experimentales de sequía. Para sobrevivir a la sequía, *C. villosissimus* es caducifolia facultativa, perdiendo hojas inferiores durante la estación seca y creciendo rápidamente hojas nuevas en la estación húmeda.

Conclusiones: Revelamos una estrategia de adaptación a la sequía que, hasta donde sabemos, no ha sido documentada previamente en hierbas tropicales. Esta adaptación divergente a la sequía evolucionó recientemente y es un componente importante del aislamiento reproductivo entre *C. villosissimus* y *C. allenii*, lo que indica que los cambios adaptativos para sobrevivir a la sequía estacional pueden ser un eje subestimado de la diversificación de las plantas del sotobosque neotropical.

KEYWORDS

Costaceae, *Costus*, dehydration avoidance, drought adaptation, drought avoidance, drought tolerance, habitat isolation, monocots, recently diverged, tropical

A key driver of plant speciation is adaptive divergence across environmental gradients, such as gradients in water availability (Abbott, 2017; Ramírez-Valiente et al., 2018). Tropical plants undergo rapid rates of diversification (Kay et al., 2005; Madriñán et al., 2013; Lagomarsino et al., 2016) and frequently span precipitation and seasonality gradients, with many tropical herbs experiencing periods of drought (Richards, 1952). Nevertheless, tropical herb drought adaptation remains almost entirely unexamined (but see Mulkey et al., 1991). To understand adaptive divergence in species-rich tropical herbs, we must uncover their drought adaptation strategies.

Plants can adapt to aridity by tolerating or avoiding dehydration during drought (Lambers et al., 2008). Drought tolerance involves conserving water and is exemplified by evergreen plants with relatively long-lived, slow-growing, small, dense leaves (Lambers et al., 2008). In contrast, drought avoidance (here used as shorthand for dehydration avoidance during drought) is achieved either by completing a rapid lifecycle during times of seasonal water availability (drought escape) or by dropping their leaves for the duration of a dry season (drought deciduousness). Drought avoiding plants often grow (or regrow) rapidly when water is available and thus have relatively thin leaves with high water consumption and fast growth rates, aligned with a fast leaf/plant economic strategy (Sobrado, 1991; Reich et al., 1992; Wright et al., 2004; Reich, 2014). Plants can use a mix of tolerance and avoidance to deal with drought (Welles and Funk, 2021), and understanding the conditions under which certain water-use strategies are likely to evolve is a major goal in plant ecology (Robichaux et al., 1984; Ludlow, 1989; Wright, 1991; Tissue and Wright, 1995; Engelbrecht et al., 2000; Wright and Westoby, 2002).

Drought adaptation strategies are likely distinct in tropical herbs compared to temperate or tropical woody species. Woody tropical plants are sometimes drought deciduous, as in tropical dry forests (e.g., Markesteijn et al., 2011; Méndez-Alonzo et al., 2013) and other times are evergreen and employ leaf and stem level water

conservation traits and deep roots to survive in seasonal moist tropical forests (e.g., Wright et al., 1992; Engelbrecht et al., 2000). However, woody plants have greater resistance to hydraulic failure and access to deeper groundwater via deeper roots compared to herbaceous plants (Dória et al., 2018). Drought adaptation of tropical herbs is also likely to differ from that of temperate herbs, especially in tropical forests where the vapor pressure deficit is generally lower than in temperate systems (Cunningham, 2005). Such differences are underexplored because drought adaptation has received less attention in the tropics than in temperate systems, despite being a key component of tropical species distributions (Engelbrecht et al., 2007; Chen and Schemske, 2019).

Tropical tree distributions, and likely tropical herb distributions, are largely determined by water availability (Engelbrecht et al., 2007). Geographic gradients of precipitation and seasonality are common in the tropics, ranging from tropical dry forests with a 6-month dry season to perennially wet rainforests (Holdridge, 1967; Condit et al., 2011). Between these forests lie tropical seasonal moist forests, which typically have a 3–4-month dry season that is variable and often severe (Holdridge, 1967; Robichaux et al., 1984; Becker et al., 1988; Wright, 1991; Condit et al., 2011). Water stress on tropical herbs is therefore common but variable across the landscape, making species distributions across rainfall gradients dependent on drought adaptation. The unexplored potential for differential drought adaptation to contribute to rapid tropical herb diversification across rainfall gradients highlights the need for research on tropical herbaceous drought adaptation.

We studied ecophysiological and life-history traits that could facilitate divergent drought adaptation in two recently diverged parapatric understory herb species in Costaceae. *Costus villosissimus* (Maas) and *C. allenii* (Jacq.) are closely related species that show divergent adaptation along a steep gradient in rainfall seasonality across the Panamanian isthmus (Chen and Schemske, 2015, 2019). By comparing a species that occurs in seasonal moist forests (*C. villosissimus*) to a close

relative restricted to perennially wet forests (*C. allenii*), we can assess what traits are essential to drought adaptation. In particular, we asked how *C. villosissimus* achieves drought adaptation. If it employs drought avoidance via drought deciduousness, we expect a fast economic strategy compared to *C. allenii*, with traits facilitating rapid growth, water movement, and photosynthesis. Alternatively, if *C. villosissimus* employs drought tolerance, we expect a slow economic strategy involving more water conservative traits like slower growth and higher leaf mass per area (LMA) than *C. allenii*. We combined field and greenhouse trait data with responses to a greenhouse dry-down experiment and wild plant observations. Field data demonstrate trait differences between species in their natural habitats, whereas the standardized environment of the greenhouse allows us to establish whether differences are genetically based and to observe responses to water limitation in real-time while minimizing other environmental changes. Our multipronged comparison of close relatives enables a holistic understanding of divergent drought adaptation in these two species that we can interpret in the context of evolutionary diversification.

MATERIALS AND METHODS

Study system

Neotropical spiral gingers (*Costus*) are large, perennial understory monocots with a distinctive spiraling leaf arrangement that grow in Central and South American tropical forests. The genus has rapidly diversified within the last 3 million years (My), and close relatives commonly display climatic niche divergence (Vargas et al., 2020). *Costus villosissimus* and *C. allenii* diverged approximately 1.4 My ago (Mya) (95% CI: 0.4–1.82 My; Vargas et al., 2020) and are primarily reproductively isolated by differential habitat adaptation (Chen, 2011; Chen and Schemske, 2015).

In Central Panama, the habitat of *C. villosissimus* experiences a pronounced dry season of roughly 3–4 months in January–April, when there is little precipitation and lower soil water content than in perennially wet *C. allenii* habitat (mean dry season precipitation with 95% CI: *C. allenii* habitat = 92.6 ± 33.7 mm, *C. villosissimus* habitat = 69.2 ± 26.2 mm; mean year-round soil gravimetric water content with 95% CI [the difference is greater during the dry season]: *C. allenii* habitat = $49.4\% \pm 3.5\%$, *C. villosissimus* habitat = $32.0\% \pm 2.6\%$; Chen and Schemske, 2015). Furthermore, *C. villosissimus* grows in forest edges with higher light availability than *C. allenii*, which grows in shady forest understory (mean light availability as % photosynthetically active radiation [PAR] of full sun with 95% CI: *C. villosissimus* = $26.1\% \pm 4.9\%$, *C. allenii* = $11.3\% \pm 5.9\%$; Chen and Schemske, 2015). Higher light availability in the *C. villosissimus* habitat compounds drought stress because the air around the plant is likely to be drier and hotter than in the shadier *C. allenii* habitat. These differential habitat affinities are due to divergent habitat

adaptation as uncovered with reciprocal transplant experiments.

Reciprocal transplant experiments demonstrated that, despite partially overlapping ranges and shared pollinators, divergent habitat adaptation results in strong reproductive isolation (Chen, 2011; Chen and Schemske, 2015). When reciprocally transplanted, *C. villosissimus* has extremely low survival in the *C. allenii* habitat, and *C. allenii* cannot survive in the *C. villosissimus* habitat (Chen and Schemske, 2015). When transplanted into the *C. villosissimus* habitat, *C. allenii* has the greatest die-off in the first dry season and generally more die-off in dry seasons than wet seasons. This level of die-off contrasts with that of *C. villosissimus*, for which comparatively little die-off occurs during the same dry seasons (Chen and Schemske, 2015). Chen and Schemske (2019) further explored the role of drought adaptation in habitat isolation and found through reciprocal seed transplants in the wild that seed dormancy in *C. villosissimus* enables drought escape, whereas *C. allenii* seeds germinate immediately and then die during the wet season. The same study also showed clear differential survival of adults during a greenhouse dry down, which is consistent with greater adult *C. allenii* die-off during dry seasons in natural *C. villosissimus* habitat (Chen and Schemske, 2015, 2019). These studies demonstrate that drought adaptation is a key component of differential habitat adaptation and therefore reproductive isolation between *C. allenii* and *C. villosissimus*. Here, we expand on this work to understand the traits responsible for drought adaptation of mature plants. (Chen and Schemske, 2015).

Field observations

We sampled wild plants along Pipeline Road in Parque Nacional Soberanía, Panama (~9.15°N, -79.73°W), which transects the rainfall gradient from the Atlantic to the Pacific and includes both species' habitats. We collected leaf, stem, and rhizome samples from six individuals per species in 2019. For leaf collection, we standardized leaf age by collecting the 4th–6th fully expanded leaves from the top of the plant. We used these samples to determine leaf carbon isotope ratio ($\delta^{13}\text{C}$), leaf nitrogen content (leaf N), stomatal pore area index (SPI), stem density, theoretical hydraulic conductivity, and rhizome water content.

The stable isotope ratio of leaf carbon ($\delta^{13}\text{C}$) is related to the ratio of intracellular to ambient CO_2 (c_i/c_a) and is commonly used to infer long-term integrated water-use efficiency (WUE; Farquhar et al., 1982; Farquhar and Richards, 1984). The WUE is expected to be higher for drought-adapted plants but lower in fast economic strategy plants (Wright et al., 2004; Reich, 2014; Kooyers, 2015; Welles and Funk, 2021). Similarly, leaf N content is associated with photosynthetic capacity, and greater values align with a fast economic strategy (Field and Mooney, 1986; Reich, 2014). For leaf isotope and nitrogen content analysis, we ground dried leaf samples into a fine powder using a mill (3383L10 mini-mill, Thomas Scientific, Swedesboro, NJ,

USA). Leaf $\delta^{13}\text{C}$ and N content were measured via coupled EA-IRMS at the UC Davis Stable Isotope Facility (Davis, CA, USA) and are reported per mille (‰) relative to VPDB (Vienna Pee Dee Belmnite).

Stomatal pore area index (SPI) is a proxy for leaf gas exchange capacity and thus water lost to transpiration (Sack et al., 2003; Bucher et al., 2016). To measure SPI, we made epidermal prints of the abaxial side of fresh leaves with dental putty and clear nail polish (these species only have stomata on the abaxial leaf surface). One epidermal print failed, leaving us with six *C. villosissimus* and five *C. allenii* for stomata measurements. We counted and measured the guard cell length of all stomata in three fields of view per leaf (number of stomata ranged from 8 to 23) and standardized by field of view area to estimate stomatal density (SD, mm^{-2}) and stomatal guard cell length (SL, mm). We calculated SPI (unitless) as the product of stomatal density and the square of pore length. We also measured guard cell width and calculated theoretical anatomical maximum stomata conductance (g_{max}) using Equation 2 of Sack and Buckley (2016).

Stem density in *Costus* (herbaceous monocots) approximates investment in structural carbon (cell walls) and is negatively correlated with stomatal conductance (Ávila-Lovera et al., 2022). Lower stem density is therefore associated with lower investment in cell walls and higher stomatal conductance, which both align with a fast economic strategy. To determine stem density, we sectioned a 2-cm-long piece and measured fresh volume using mass displacement (Pérez-Harguindeguy et al., 2013). We then dried the stem to constant mass in an oven at 60°C for 72 h to obtain stem dry mass. We calculated stem density as the ratio between stem dry mass and fresh volume ($\text{g}\cdot\text{cm}^{-3}$).

Hydraulic conductivity is a measure of the ease of water movement through the xylem and has been found to differ between differentially drought-adapted tropical woody species (Engelbrecht et al., 2000). To calculate theoretical hydraulic conductivity, we fixed ~5 cm of stem in 100% ethanol and prepared sections as described by Ruzin (1999). In brief, we dehydrated 1-cm-long sections and embedded them in paraffin before making 10 μm thin sections using a rotary microtome (Leica RM2135, Leica Microsystems, Buffalo Grove, IL, USA). We mounted sections on glass slides and stained them with 0.05% v/v aqueous toluidine blue. We took photos at 50 $\times$ magnification using a camera attached to a compound light microscope (Zeiss Axio Imager, Zeiss, Oberkochen, Germany) and analyzed pictures in ImageJ (example cross section from each species: Appendix S1; ImageJ v. 1.53e; Rasband, 1997; Schneider et al., 2012). We could not obtain thin sections for two of six *C. allenii* stems, leaving us with data for four *C. allenii* and six *C. villosissimus*. We calculated average theoretical hydraulic conductivity ($k_{\text{h,theo}}$, $\text{kg}\cdot\text{m}\cdot\text{MPa}^{-1}\cdot\text{s}^{-1}$) from measurements of all the vessels (number ranged from 7 to 20) in each of three images of 2.414 mm^2 area per individual as

$$k_{\text{h,theo}} = \frac{\frac{\pi\rho}{128\eta}\sum_{i=1}^n(d_i^4)}{2.414 \times 10^{-6}}, \quad (1)$$

where ρ is the density of water at 20°C (998.2 $\text{kg}\cdot\text{m}^{-3}$), η is the viscosity of water at 20°C (1.002 $\times 10^{-9}\text{MPa}\cdot\text{s}$), and d_i is the diameter of the i th vessel element (Tyree and Ewers, 1991).

To assess potential differences in rhizome water storage as a drought adaptation, we measured rhizome water content. We collected a ~3 cm rhizome section to determine fresh and dry mass (dried to constant mass; 60°C for 72 h) and calculated water content as (Fresh mass – Dry mass)/Fresh mass ($\times 100$, %). We could not compare total rhizome investment because clonal reproduction via rhizomes makes isolating the belowground rhizomes from one individual challenging to do consistently.

To qualitatively assess leaf growth and death over the change of seasons, we took photos of three *C. villosissimus* individuals in Gamboa, Panama in the mid dry season (21 February 2021), the late dry season (7 March 2021), the early wet season (18 April 2021; approximately 2 weeks after the start of the wet season), and the middle of the wet season (15 July 2021).

Greenhouse observations

We further characterized leaf traits of individuals grown in pots in greenhouses at Michigan State University (MSU) and the University of California, Santa Cruz (UCSC). Plants were grown from seeds collected along Pipeline and Omar Torrijos roads in Parque Nacional Soberanía, Panama.

High LMA is associated with slow growth, long leaf longevity, and maintenance of leaf water content, whereas low LMA is associated with rapid growth and the ability to readily lose and regrow leaves (Wright et al., 2004; Reich, 2014). To obtain LMA, we collected four leaf disks of known area per plant between the midribs and the edges of the 3rd–5th leaves from the stem apex using a round squeeze punch, dried them at 60°C for 7 days, and measured dry mass (g). We collected data from 35 individuals of each species and calculated LMA as the ratio between mean dry mass and leaf disk area ($\text{g}\cdot\text{m}^{-2}$).

To estimate potential differences in leaf WUE, leaves from 10 individuals of each species were analyzed for $\delta^{13}\text{C}$ at the UC Davis Stable Isotope Facility (Davis, California, USA).

To understand differences in photosynthetic capacity, we calculated light saturated photosynthetic rate (A_{sat} ; see the section Analysis below for details) from light response curves. To generate light response curves, we measured photosynthetic rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) as a function of PAR for 10 individuals of each species with a portable infrared gas analyzer equipped with a fluorometer (LI-6400 and 6400-40; LI-COR Biosciences, Lincoln, NE, USA). All plants of both species were acclimated to the same common garden greenhouse conditions at MSU, with light levels similar to those of *C. villosissimus* habitat (~100–300 PAR). We measured photosynthetic rate at eight

different PAR levels ranging from 0 to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, representing the range of light levels experienced by both species in the field (Chen and Schemske, 2015). Leaves were measured at 30°C, ambient relative humidity, 400 ppm CO_2 , and high fan speed.

For growth rate calculations, we grew 20 *C. allenii* and 34 *C. villosissimus* from seed in growth chambers set at 25°C with a 9 h photoperiod of ~300 PAR light levels. The growth chamber was set up as a common garden with favorable conditions to understand genetic differences in growth rate. The short photoperiod approximates the window of light availability in dense tropical forests. We took initial height measurements when plants had ~3–5 true leaves and final measurements after 2–4 weeks in the growth chamber. We calculated stem absolute growth rate ($\text{cm}\cdot\text{day}^{-1}$) from these data.

Dry-down experiment

To assess whole-plant drought responses, we conducted a dry-down experiment in the MSU greenhouses on 45 individuals of each species. Stem data from this experiment (previously published by Chen and Schemske, 2019) revealed that *C. villosissimus* retained living stems for up to 9 months longer than *C. allenii* under common garden drought conditions and that once individuals lost all stems, they were dead and unable to resprout with rewatering. Here, we monitored leaf loss during the dry-down to determine whether *C. villosissimus* followed leaf retention patterns typical of drought tolerance or drought avoidance. We documented leaf loss as the loss of functioning (green) leaves, even though dried brown leaves were retained on the stem for up to a few months after death. We germinated plants for the dry-down in growth chambers, then potted them in 4-L pots and consistently watered them in the

greenhouse for 4–7 months to obtain similarly sized individuals before initiation of the drought treatment. To imitate the onset of a natural dry season, we watered plants weekly with progressively reduced volumes for 4 weeks, after which watering ceased. We recorded the number of green leaves before the drought treatment, every other week in the first 4 months of drought, and weekly for the remainder of the experiment. To assess resprout ability after 6 and 9 months, we fully rewatered 15 plants of each species, then recorded leaf growth on regular census dates for two months. For more detailed dry-down methods, see supplementary material for Chen and Schemske (2019).

Analysis

To generate values of A_{sat} , we fit nonrectangular hyperbolic functions to the photosynthetic light response curves using the `fit_aq_curve` function from the package `photosynthesis` (Stinziano et al., 2021) in R v4.0.3 (R Core Team, 2021). We conducted all other analyses in R v4.0.2 (R Core Team, 2020).

To assess differences between species in both field-collected trait data ($\delta^{13}\text{C}$, leaf N, SPI, stem density, theoretical hydraulic conductivity, and rhizome water content) and greenhouse-collected trait data ($\delta^{13}\text{C}$, LMA, A_{sat} , and growth rate), we conducted Welch's two-sample *t*-tests. We assessed normality both visually with normal Q-Q plots and with Shapiro–Wilk normality tests on within-species data and conducted a nonparametric Wilcoxon rank-sum test where data deviated from normal. In all such cases, significance levels did not differ between the *t*-test and the nonparametric test; thus, only *t*-test results are reported here. We did not apply multiple testing corrections given clear pre-testing reasoning for the importance of each trait to drought adaptation or to a slow vs fast economic strategy and follow the best practice reporting guidelines of

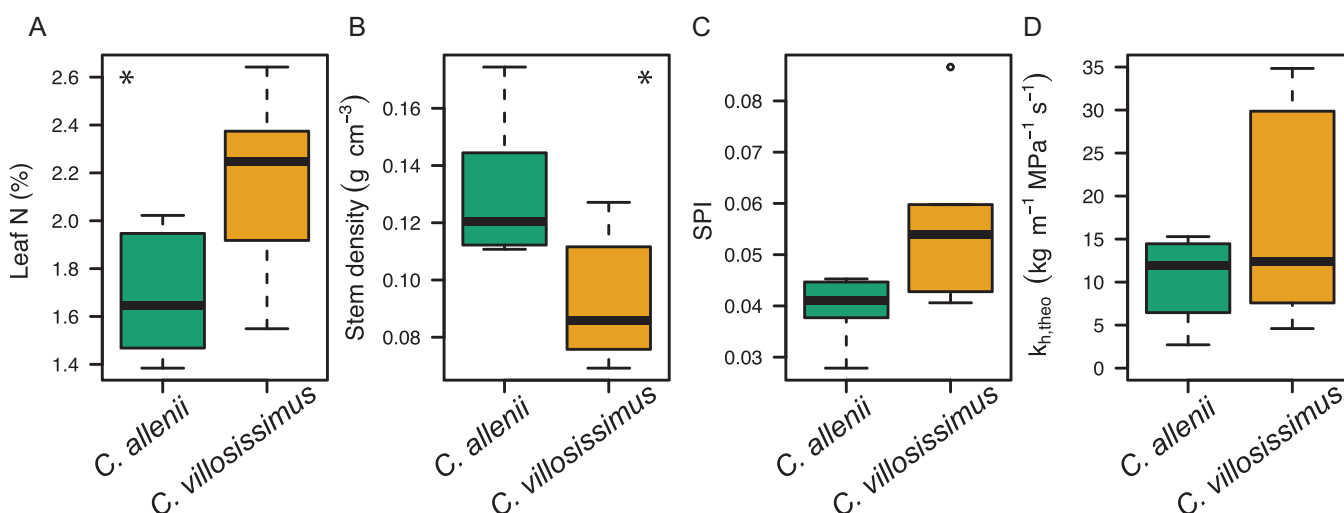


FIGURE 1 Trait data from wild *Costus villosissimus* and *C. allenii* collected from the field. From left to right: leaf nitrogen as a percentage of dry mass, stem density, stomatal pore index (SPI: Stomatal density $\times$ Pore length²), and average theoretical hydraulic conductivity ($k_{h,\text{theo}}$). Boxplots display the trait median (black line), interquartile range (box), 1.5 $\times$ interquartile range (dashed lines), and outliers (open circles). * $P \leq 0.05$.

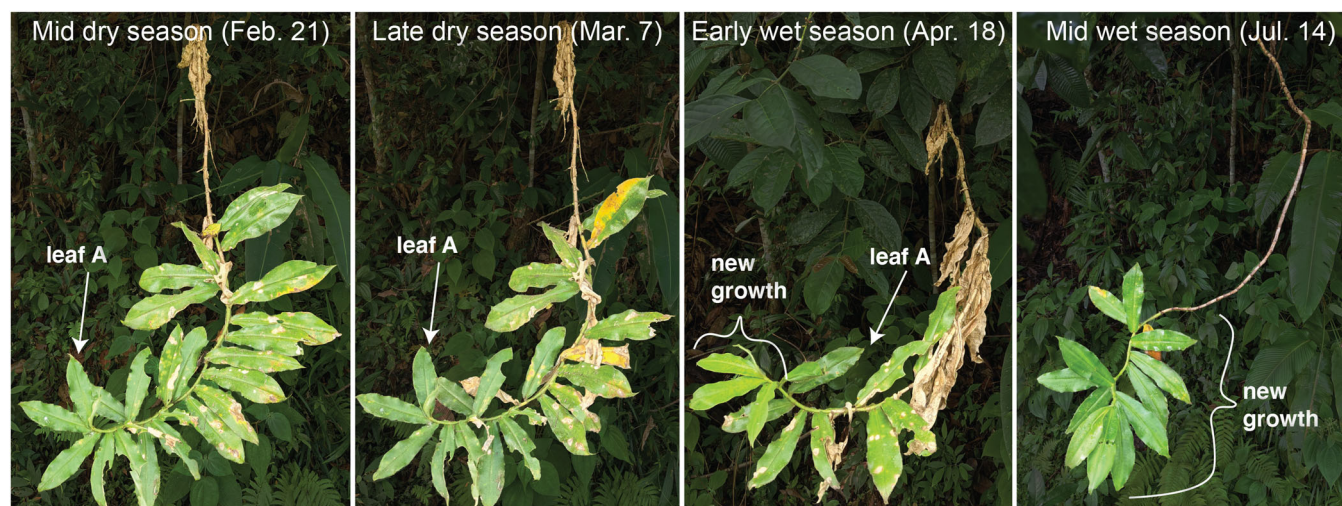


FIGURE 2 Growth of a *Costus villosissimus* individual in the field spanning the transition from dry to wet season in Gamboa, Panama, in 2021. The individual demonstrates progressive leaf loss from the middle of the dry season to the early wet season and rapid new growth in the wet season. Photographs from April were taken about 1 week after the initiation of regular precipitation. Image credit: E. Ávila-Lovera.

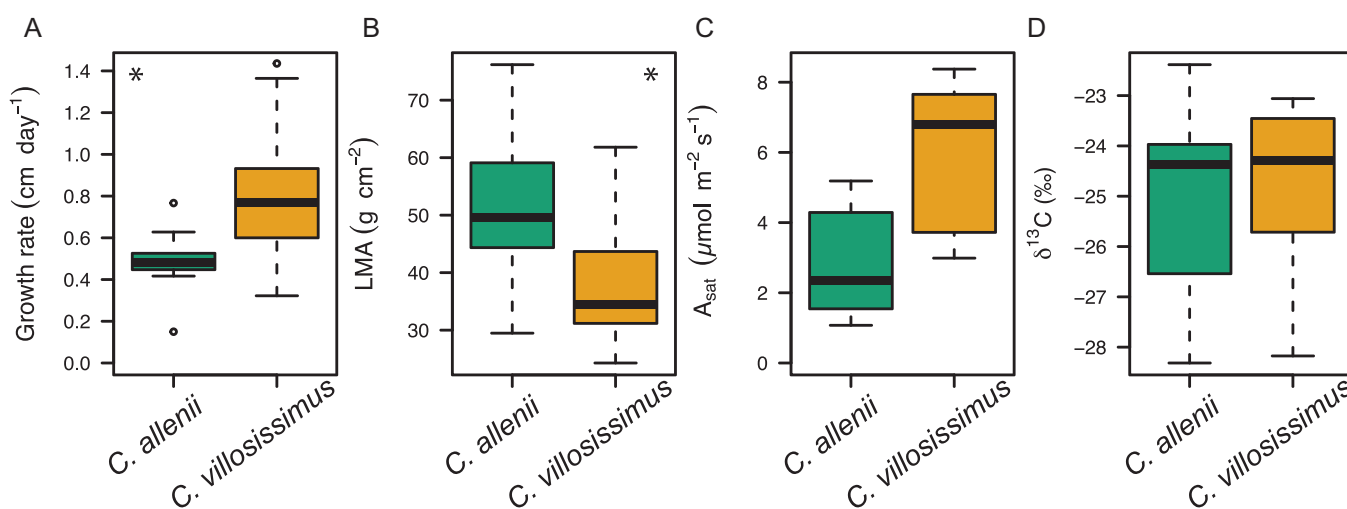


FIGURE 3 Trait data from greenhouse grown *Costus villosissimus* and *C. allenii*. Traits from left to right: growth rate, leaf mass per area (LMA), light saturated photosynthetic rate (A_{sat}), leaf carbon isotope ratio ($\delta^{13}\text{C}$). Boxplots display the trait median (black line), interquartile range (box), 1.5 $\times$ interquartile range (dashed lines), and outliers (open circles). * $P \leq 0.05$.

Moran, 2003). We also assessed correlations between trait pairs that were not already found to be uncorrelated for *Costus* by Ávila-Lovera et al. (2022). For trait pairs with Pearson correlation coefficient values above $|0.5|$, we evaluated the significance of the correlation with the rcorr function from the R package Hmisc (Harrell, 2022).

RESULTS

Field observations

We found several trait differences consistent with faster growth and higher photosynthetic rates in *C. villosissimus*

as compared to *C. allenii*, including higher leaf N and lower stem density. Leaf N content is $\sim 30\%$ higher in *C. villosissimus* than *C. allenii* (Figure 1A; $t = -2.555$, $\text{df} = 8.717$, $P = 0.032$). The difference in average leaf N content between species was greater than the average within site standard deviation of other *Costus* species' leaf N (ave. $\text{SD} = 0.239$, ave. diff = 0.478). Stem density is $\sim 40\%$ lower in *C. villosissimus* than in *C. allenii*, indicating lower resource requirements for growth (Figure 1B; $t = 2.761$, $\text{df} = 9.921$, $P = 0.020$). The difference in average stem density between our focal species was almost exactly equal to the average within site standard deviation of other *Costus* (ave. $\text{SD} = 0.037$, ave. diff = 0.038). Mean SPI is more than 40% higher for *C. villosissimus* than for *C. allenii*, although this

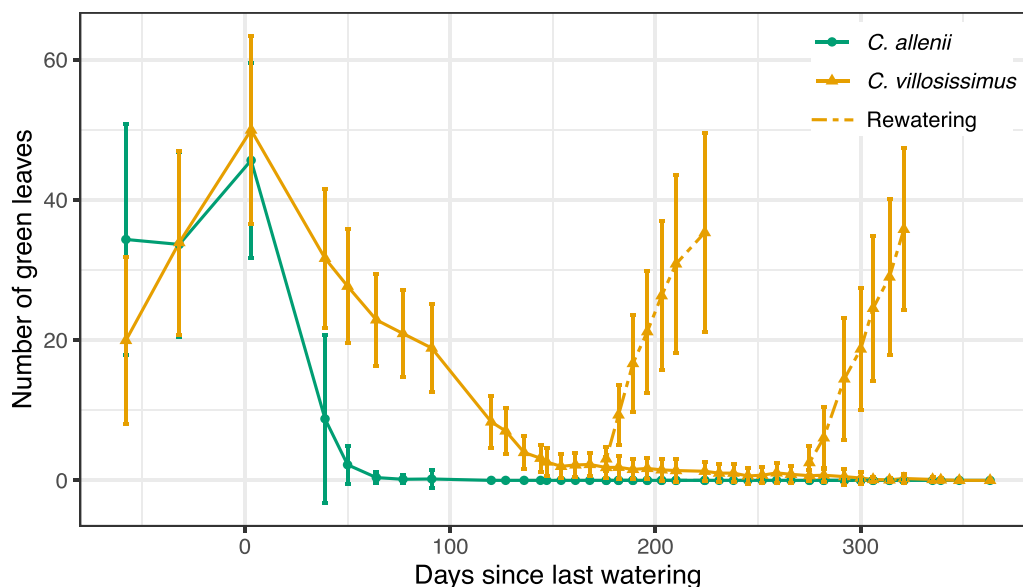


FIGURE 4 Leaf response of potted *Costus allenii* and *C. villosissimus* individuals during a dry-down experiment ($N = 45$ plants per species) and following two separate rewetting treatments ($N = 15$ plants per rewetting). No data exist for *C. allenii* rewetting because no individuals of that species regrew after rewetting. Data represent means $\pm$ SD.

difference is marginally insignificant (Figure 1C; $t = -2.268$, $df = 7.015$, $P = 0.058$). This trend is driven by differences in guard cell length ($t = -2.140$, $df = 8.953$, $P = 0.061$) rather than stomatal density ($t = -0.057$, $df = 7.279$, $P = 0.956$). When stomatal conductance is calculated as $g_{\max}$, we find no significant difference between species (Appendix S2; $t = -0.681$, $df = 7.2512$, $P = 0.517$). In both methods (SPI and $g_{\max}$), the range of values is greater for *C. villosissimus* than *C. allenii*. Mean theoretical hydraulic conductivity is higher on average in *C. villosissimus* as compared to *C. allenii*, although the difference is not statistically significant (Figure 1D; $t = -1.107$, $df = 7.315$, $P = 0.303$). Neither WUE, as approximated by $\delta^{13}\text{C}$ ($t = -1.871$, $df = 8.202$, $P = 0.097$), nor rhizome water content ($t = 0.260$, $df = 9.205$, $P = 0.800$) differed significantly between species. Only three trait pairs were significantly correlated: hydraulic conductivity and SPI (Pearson = 0.784, $P = 0.007$), hydraulic conductivity and $\delta^{13}\text{C}$ (Pearson = 0.75, $P = 0.012$), and, marginally, SPI and leaf nitrogen (Pearson = 0.61, $P = 0.047$).

Photos of wild *C. villosissimus* plants qualitatively demonstrate a pattern of gradual lower leaf death and growth cessation during the dry season, then rapid leaf regrowth at the onset of the wet season (Figure 2; Appendix S3).

Greenhouse observations

Traits measured in the greenhouse aligned with field-measured traits. Stem absolute growth rate under watered conditions is more than 50% faster in *C. villosissimus* than *C. allenii* (Figure 3A; $t = -5.559$, $df = 51.981$, $P < 0.0001$). *Costus villosissimus* also has smaller, thinner leaves than *C. allenii*,

captured by markedly lower LMA (Figure 3B; $t = 5.476$, $df = 67.205$, $P < 0.0001$). The difference in average LMA between our focal species was also greater than the standard deviation in LMA between other co-occurring *Costus* species (ave. SD = 11.520, ave. diff = 13.960). Light saturated photosynthetic rate (A_{sat}) in *C. villosissimus* was on average twice as high compared to *C. allenii*, although this was marginally insignificant (Figure 3C; $t = -2.254$, $df = 7.355$, $P = 0.057$). As in wild plants, $\delta^{13}\text{C}$ did not differ between species for greenhouse plants (Figure 3D; $t = -0.189$, $df = 17.909$, $P = 0.852$). No trait pairs were significantly correlated.

Dry-down experiment

Differential patterns of leaf loss are evident between species from the dry-down experiment (Figure 4). Rapid leaf die-off occurred for *C. allenii* in the first 50 days of the dry-down, with all leaves dead by the 100th day. All *C. allenii* were dead by the first rewetting and did not resprout. Conversely, leaf death for *C. villosissimus* was much more gradual, with some of the youngest green leaves present even after all *C. allenii* individuals had died. Further, *C. villosissimus* retained stems and therefore remained alive beyond total leaf loss, rapidly regrowing new leaves upon rewetting (Figure 4).

DISCUSSION

Tropical understory species commonly face seasonal drought (Richards, 1952), but we have little understanding of how they cope with it. Our research reveals a dehydration

avoidance strategy that is, to our knowledge, previously undocumented in tropical herbs. We found that a neotropical perennial monocot, *C. villosissimus*, adapts to drought primarily through facultative dry-season leaf death and rapid wet-season regrowth. This drought adaptation contrasts with its wet-forest-adapted close relative, *C. allenii*, which quickly loses all leaves under drought conditions and cannot recover. Conversely, *C. villosissimus* gradually loses green leaves, typically losing the oldest leaves first (Figures 2, 4; Appendix S3). Even under prolonged drought, *C. villosissimus* maintains leafless shoots and the ability to rapidly resprout, in some cases for over a year without water (Chen and Schemske, 2019). The dry season in the tropical moist forest of Panama is variable but typically lasts only about 3–4 months. Throughout this dry season, *C. villosissimus* will usually maintain multiple green leaves that facilitate rapid wet-season regrowth (Figures 2, 4; Appendix S3). Growth rate is more than 50% faster in *C. villosissimus* than in its drought-susceptible relative (Figure 3A). This more rapid growth than *C. allenii* is facilitated by traits associated with a fast economic strategy such as lower LMA and stem density and higher photosynthetic rates as indicated by greater leaf nitrogen and light-saturated photosynthetic rates (Figure 1; Gray and Schlesinger, 1983; Sobrado, 1991; Reich et al., 1992; Eamus, 1999; Wright and Westoby, 2002; Markesteijn et al., 2011; Reich, 2014). These traits likely resulted from adaptation to both the higher light environment and dry season by *C. villosissimus*. *Costus villosissimus* survives the dry season through a fast economic strategy that enables leaf loss and rapid regrowth characteristic of flexible drought-deciduousness.

The various lines of evidence we combine complement and corroborate one another in demonstrating differential drought adaptation enabled by a fast economic strategy in *C. villosissimus*. The field-measured traits and drought-induced leaf death in *Costus villosissimus* are consistent with controlled greenhouse measures of photosynthesis, growth, and whole-plant drought response, suggesting this drought adaptation is primarily genetic. Comparing *C. villosissimus* to its drought susceptible close relative allows us to understand which drought-associated traits are critical to divergent drought adaptation. Most trait differences between our focal pair were greater than the average site-level standard deviation for all *Costus* species at a variety of Central American sites (Appendix S4). Further, the suite of traits that function together in the semi-deciduous drought response of *C. villosissimus* has not previously been described and provides a stark contrast with the ecophysiology of the closely related *C. allenii*. Although we have low statistical confidence in some individual trait differences, all data trends for both greenhouse and wild plants are consistent with the drought avoidance via a fast economic strategy readily observed in real-time plant responses to experimental dry down and the natural dry season.

The drought adaptation strategy of *C. villosissimus* contrasts with the limited existing work on drought adaptation of tropical

herbs. In comparisons of two large-leaved, light-gap-affiliated, understory monocots in a tropical moist forest, WUE and leaf longevity were greater in the more drought-resistant species (Mulkey et al., 1991). The authors hypothesized that larger and deeper roots enabled this drought resistance. Notably, the more drought-susceptible species had traits in line with our drought-adapted species: shorter leaf longevity, higher leaf N, and higher photosynthetic capacity. These contrasting findings support a slow economic strategy for their drought-resistant species rather than a fast strategy as in *C. villosissimus*. The traits of *C. villosissimus* also contrast with the higher LMA and stem density in light-demanding tropical, herbaceous monocots as compared with shade-tolerant species (Rundel et al., 1998, 2020; Cooley et al., 2004; Swenson, 2009), highlighting the novelty of our finding of a fast economic strategy of drought-deciduousness in light-demanding *C. villosissimus*.

Drought adaptation has also been investigated in woody understory species in tropical moist forests. These woody species also contrast with our system and display traits indicative of drought adaptation through greater leaf dehydration resistance and more water conservative, slow economic strategies. For example, a drought-adapted *Piper* species has lower hydraulic conductivity than its wet-habitat-restricted relative (Engelbrecht et al., 2000), whereas we found the opposite trend (not statistically significant) in the hydraulic conductivity of *C. allenii* and *C. villosissimus*. Like *C. villosissimus*, some woody, moist-forest species demonstrate greater leaf production in the wet season than in the dry season. However, this phenology is decidedly less plastic than that of *C. villosissimus*, given that dry season irrigation did not impact total leaf production in those plants (Wright et al., 1992; Tissue and Wright, 1995). In contrast, *C. villosissimus* responds rapidly and dramatically with new leaf growth upon rewatering (Figures 2, 4; Appendix S3).

Our results are consistent with previous work indicating that drought adaptation in evergreen species is often facilitated by deep roots, whereas drought deciduousness is associated with shallower roots and, therefore, less access to water in the dry season (Sobrado, 1991; Hasselquist et al., 2010). *Costus*, as monocots, do not have taproots and are generally shallow-rooted. Although rhizomes likely provide some water reserves, the lack of differentiation in rhizome water content between *C. allenii* and *C. villosissimus* indicates that this may not be a critical part of differential drought adaptation.

Drought-deciduousness is common in tropical dry forest trees and Mediterranean climate shrubs (Mooney et al., 1970; Lambers et al., 2008; Huechacona-Ruiz et al., 2020). Drought-deciduous species in these ecosystems display similar fast economic strategy traits to *C. villosissimus*, including low LMA, low stem density, high leaf N, high maximum photosynthetic rates, and rapid growth rates (Gray and Schlesinger, 1983; Sobrado, 1991; Reich et al., 1992; Eamus, 1999; Markesteijn et al., 2011). Mediterranean climate woody shrubs are often drought-deciduous or semi-drought-deciduous (Mooney et al., 1970; Lambers et al., 2008). Semi-drought-deciduousness fits our findings in *C. villosissimus*,

whereby plants maintain some of the newest leaves during drought. For example, facultatively drought-deciduous species can drop all leaves or retain some of the youngest, depending on dry season intensity, just like *C. villosissimus* (e.g., *Lepichinia calycina*; Field and Mooney, 1983). Further, drought-deciduous Mediterranean climate shrubs tend to have shallower roots than their evergreen (but still drought-adapted) counterparts (Mooney and Dunn, 1970). There is also a trend toward increasing deciduousness with decreasing moisture in tropical dry forests (Méndez-Alonzo et al., 2013; Chaturvedi et al., 2021). Both trends align with semi-drought-deciduousness in *C. villosissimus* enabling facultative avoidance of dehydration during the unpredictable dry season, despite lacking taproots.

Whereas woody tropical species may be capable of drought resistance through deep roots and other mechanisms, shallow-rooted, herbaceous, tropical monocots are unlikely to adapt to drought in the same manner. Drought adaptation via facultative semi-drought-deciduousness, as seen in *C. villosissimus*, may represent an alternative pathway for rapid tropical drought adaptation. The evolution of drought adaptation in *C. villosissimus* is recent (~1 Mya) and has potential parallels in other *Costus* speciation events; divergence across gradients of precipitation and temperature seasonality has occurred multiple times in *Costus* (Vargas et al., 2020). Beyond *Costus*, drought adaptation via a highly flexible, whole-plant strategy may be mirrored in other similar clades of tropical understory herbaceous monocots. Monocots are widespread and diverse in a range of dry to wet tropical forest understories, such that adapting to seasonal precipitation via flexible drought-deciduousness may be an underappreciated avenue for tropical understory herb habitat differentiation.

Habitat isolation is often a key component of species divergence and maintenance (reviewed by Sobel et al., 2010) and is the primary reproductive isolating barrier between *C. allenii* and *C. villosissimus* (Chen, 2011; Chen and Schemske, 2015). Little to no survival in each other's habitats implies trade-offs. High resource investment in leaves and shoots is maladaptive in seasonally dry habitats when those leaves will die in the dry season and need to be regrown at the onset of the wet season. Conversely, wet habitats are hypothesized to have higher herbivore pressure (Janzen, 1970; Connell, 1971; Coley and Barone, 1996), and rapidly produced leaves may be more susceptible to herbivory and therefore maladapted to wetter habitats (Coley et al., 1985; Simms and Rausher, 1987; Herms and Mattson, 1992). Trade-offs like these form the basis of habitat isolation (Antonovics, 1976). Habitat isolation through differential drought adaptation could be an important but poorly understood axis of tropical understory herb diversification.

CONCLUSIONS

Here we described how a neotropical understory herb achieves drought adaptation that enables habitat isolation from its recently diverged relative. The drought-adapted

species, *C. villosissimus*, displays semi-drought-deciduousness and a fast economic strategy that enables rapid regrowth in the wet season after dry-season leaf die-off. Drought avoidance (dehydration avoidance) via semi-drought-deciduousness has not, to our knowledge, previously been documented in tropical understory herbs. Facultative semi-drought-deciduousness is especially adaptive for the highly variable and potentially severe dry seasons of tropical moist forests. Further, it is a strategy that enables drought adaptation in shallow-rooted species, such that it may be an important form of drought adaptation for other shallow-rooted tropical herbaceous monocots. Habitat isolation across precipitation seasonality gradients via such flexible drought adaptation may therefore contribute to tropical understory herb diversification.

AUTHOR CONTRIBUTIONS

All authors contributed to methodology and investigation. J.G.H. and E.A.L. did all data curation. J.G.H. did all formal analyses other than generating light response curves, which E.A.L. created. Aside from the graphical abstract by Erin Aiello, J.G.H. created all visualizations. K.M.K. was responsible for project funding acquisition and supervision. J.G.H. wrote the manuscript with reviews and edits from all authors.

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DATA AVAILABILITY STATEMENT

Data and analysis code are available online via Dryad Digital Repository at <https://doi.org/10.7291/D18H4S> (Harenčár et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Example stem cross sections for each species under 50× magnification.

Appendix S2. Boxplot of theoretical maximum stomatal conductance ($g_{\max}$) calculated from leaf prints of field-collected leaves. Boxplots display the trait median (black line), interquartile range (box), 1.5× interquartile range (dashed lines), and outliers (open circles).

Appendix S3. Growth of two additional *Costus villosissimus* individuals in the field spanning the transition from dry to wet season in Gamboa, Panama, in 2021.

Appendix S4. Analysis comparing trait differentiation between *Costus allenii* and *C. villosissimus* to broader variation in the genus.

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