59

4. **B. superba** Rich. Schomb., Reisen Br. Guian. 3: 1082. 1848; Progel in Martius, Fl. Bras. 6(1): 268, t. 72. 1868; Solereder in Engler & Prantl, Nat. Pflanzenf. 4(2): 36, f. 12. E-F. 1892. **Fig. 4.** S

Type: (British) Guiana: Mt. Roraima, Schomburgk 614 (939) (K, lectotype; isotypes: BM, F, G, GH, K, P).

Small tree, 7–10 m high. *Branchlets* pubescent or with a longitudinal line of pubescence below the stipular line. *Leaves*: petiole sparsely pubescent or glabrous, 3–5 mm long; blade narrowly ovate to elliptic, 1.5–2.5 × as long as wide, 4–10 × 2–5 cm, obtusely acute to acuminate at the apex, cuneate to rounded at the base, glabrous on both sides or pubescent on the costa beneath; 5–7 pairs of secondary veins; tertiary venation prominent beneath, less so above. *Inflorescence* rather lax, 3.5 × 3–6 × 6 cm. Peduncle, branches, and pedicels glabrous, with a longitudinal line of pubescence, or pubescent. Calyx glabrous or with a few hairs outside; tube 2 × 2–3 × 2.5 mm; lobes very unequal, more so than in the other species, triangular to narrowly oblong (the latter only in Schomburgk's collections), 1–7 × as long as wide, 0.5–5 × 0.3–1 mm. Corolla in the mature bud 2–4 × as long as the calyx, 12–15 mm long; tube 7 mm long; lobes 7–11 × 1 mm. Ovary 1.2 × 1.2 mm; style 3.5 mm long. Capsule 2 × 0.5–3 × 0.7 mm. Seed narrowly oblong, 13 × 2.5 mm; grain 2.5 × 1.5 mm. Colleters in the axils of the leaves.

Distribution: Venezuela (Bolivar) and (British) Guiana.
Ecology: Savannas (?). Alt. 650–1250 m.

VENEZUELA: Bolívar: Cerro Arepuchi, Río Caroní (Nov.) *Cardona* 1946 (US, VEN); Mt. Roraima District, near Arabupu (fr. Jan.) *Pinkus* 270 (F, G, GH, NY, S, US).
(BRITISH) GUIANA: Mt. Roraima., *Schomburgk* 613 (BM, paratype), 614 (939) (BM, F, G, GH, K, P, lectotype), s.n. (BR, paratype).

TIME, SPACE, LIGHT AND DARKNESS IN THE METABOLIC FLARE-UP OF THE SAUROMATUM APPENDIX*

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SUMMARY

In order to arrive at a better understanding of the flowering events in certain arum lilies, an effort has been made to integrate the relevant information provided by plant physiologists, naturalists, and plant biochemists with our own recent experiments. In *Sauromatum guttatum* Schott, the metabolic flare-up which, on the first day of flowering, occurs in the so called appendix of the inflorescence, is triggered by an agent (Van Herk's calorigen) that arises in the male flower primordia when these are exposed to the proper régime of light and darkness. A single, 5 to 6 hour exposure to darkness of an inflorescence kept in constant light suffices to induce the flare-up, a peak in heat- and CO_2-production occurring about 40 to 45 hours after the beginning of the "dark shot". Repeated exposures to darkness, at 24-hour intervals, shorten both the 40 to 45-hour lag-time and the critical length of each dark period, so that good responses occur after about 36 hrs. with a series of dark shots of 2 to 3 hours duration. The available evidence indicates that the site for perception of the second effective dark shot is in the appendix. After a single 6-hour dark shot, amputated appendices still develop heat and smell, provided the amputation is carried out at least 8 to 9 hours after the end of the dark shot. This indicates that the active principle formed in the male flower primordia as the result of the dark shot is not immediately released into the appendix. However, distribution in the appendix must be fast, since the various parts of the latter (base, middle and tip) heat up almost simultaneously, even though they do not display demonstrable differences in true lag-time, *i.e.*, in the period between the release of the triggering compound and the peak in metabolic activity; injection of extracted calorigen into properly treated appendix-sections always leads to a peak in metabolic activity about 30 hours later. In intact inflorescences, amputation or wounding of the spathe leads to a metabolic flare-up 36 hours later. The implications of the uncovered facts are briefly discussed.

1. INTRODUCTION

The following article, dealing with the events that characterize the flowering-sequence of certain arum lilies (mostly in the *Aroideae*, *Colocasioideae* and *Lasioideae sensu* A. Engler) can be seen as a first attempt to synthesise in a somewhat meaningful way the contributions made by naturalists (DORMER 1960; E. HEIMANS 1901; J. HEIMANS 1914; M. KNOLL 1926, 1956; KULLENBERG 1953; LAMARCK 1778; MEEUSE 1959 a and b, 1961, 1968 a and b; MEEUSE & HATCH 1960; VAN DER PIJL 1933, 1937; PRIME 1960; VOGEL 1963), plant physiologists (MATILE 1958; SCHMUCKER 1925, 1930) and plant biochemists (VAN HERK 1937 a, b and c; JAMES & BEEVERS 1950; HESS 1961, 1964; HESS & MEEUSE 1967;

* Dedicated to Professor Dr. G. van Iterson Jun.

OLASON 1967; SIMON 1962; SIMON & CHAPMAN 1961; SMITH & MEEUSE 1966).

The famous LAMARCK, in 1778, was the first to report on the development of considerable heat in the inflorescence of certain arum lilies. This phenomenon, based on a fierce respiration process, can lead to a difference in temperature with the environment which is about 15 °C in *Arum maculatum* and *A. italicum* (BUGGELN & MEEUSE, unpublished data; MATILE 1958) and even more in *Alocasia pubera* (VAN DER PIJL 1933). At the same time, there is production of a pronounced odor, fragrant in the case of *Alocasia* but more often carrion- or dung-like, and characteristic for each species. Its biological significance has been demonstrated elegantly by E. HEIMANS (1901), J. HEIMANS (1914) and F. KNOLL (1926). For 3 European *Arum*-species, it could be shown that the odor serves the purpose of attracting large numbers of small insects (mostly Psychodid flies in *A. maculatum* and *A. italicum*, small beetles in *A. nigrum*) which are thereupon trapped in the floral chamber where they are held prisoner for about a day. The pistillate or female flowers at the base of the floral column are the first ones to reach maturity; and, since some of the visitors may have come from other *Arum* inflorescences that were in the process of shedding their pollen, the likelihood of cross-pollination is great. The staminate or male flowers, grouped together in the region just above the female flowers, do not shower the captive insects with their pollen until many hours later, when certain wilting-phenomena (acting on the smooth and slippery wall of the floral chamber as well as on the bristles that bar its exit) begin to make escape from the trap possible. Powdered with pollen, some of the escapees may allow themselves to be caught again by an inflorescence that is still in the smelly (female) stage; here, they again ensure cross pollination.

Mixed fly- and beetle pollination has later been described for *Arum dioscoridis* by KULLENBERG (1953), for *Sauromatum guttatum* by MEEUSE (1959) and MEEUSE & HATCH (1960). Predominant beetle pollination occurs in *Dracunculus vulgaris* (SCHMUCKER 1930; MEEUSE 1959; MEEUSE & HATCH 1960) and in *Amorphophallus* (VAN DER PIJL 1937). Although details may vary widely, the pollination syndrome is *in principle* the same in all these cases. Chemical analysis of the odoriferous mixture emanating from aroid inflorescences (SMITH 1964; SMITH & MEEUSE 1966) has revealed amines and ammonia; these are known to be potential fly-attractants and can also be isolated from the exhalations of certain *Stapelia* flowers that are pollinated exclusively by large flies (Meeuse, unpublished data). The enzyme that decarboxylates valine to isobutylamine, one of the main compounds in the odor produced by *Arum maculatum*, can be shown to reach a peak when the inflorescence heats up (SIMON 1962). Thus, there is little doubt about the biological function of the smell, and the survival value it has for the plant. On the other hand, it has never been possible to demonstrate persuasively a direct insect-attraction by the heat (postulated, for no good reason, in the older British literature; see DORMER 1960). The sole role of the heat may thus well be the volatilization of the odoriferous compounds. However, this role can obviously be vital, and in this connection a case can be made for the idea that respiration in the aroid appendix, on the first day of

flowering, is of the uncoupled type (HESS 1964; HESS & MEEUSE 1967). The evidence shows that the level of ATP drops significantly on that day, so that there cannot be much trapping of energy in the form of high energy phosphate. The presence of a strong ATPase in the appendix (BUGGELN & MEEUSE 1967) and the cyanide-insensitivity of appendix slice respiration which seems to indicate an unusual type of electron transport chain (VAN HERK 1937; JAMES & BEEVERS 1950; MEEUSE 1966) may be significant.

One of the most impressive aspects of the aroid pollination syndrome is the wellnigh perfect timing of the events that follow each other in quick succession in the flowering process. In *Arum maculatum*, *A. italicum* and *A. orientale*, the peak in heat- and stench-production is normally reached late in the afternoon or early in the evening, undoubtedly in consonance with the life habits of the natural pollinators. In *Dracunculus vulgaris* and *Sauromatum guttatum*, the peak occurs in the morning – in the latter species between 7 and 11 a.m. (VAN HERK 1937). Under natural conditions, the unfolding-sequence of *Sauromatum* is always the same. The first signs are noticeable a short time after midnight, when the escalloped margins of the spathe just above the male flowers (see *fig. 1*) begin to move apart. Heat and smell begin to manifest themselves gradually in the early morning hours, while the female flowers reach maturity; the male flowers follow much later, as already described for *Arum*. The strictness and rigidity in the sequence convinced Van Herk that the events were governed by one special center in the inflorescence, a "site" that might produce a triggering-substance or hormone. Information on the exact position of the triggering-center was obtained by extirpation experiments. Provided these amputations were not carried out too soon, heating and smell production were, according to Van Herk, still possible after severing the spadix from the corm that produced the inflorescence; after elimination of the female flowers; of the yellow organs (modified flowers) above them; of sterile parts of the inflorescence; of the protective bract or spathe; and, finally, of the better part of the appendix itself. The only operation that could not be carried out with impunity was the scraping-off of the male flower primordia, which prevented heat- and smell-development, provided the scraping was done at least a day (22 hours) before the expected heating-process. Amputation of the male flowers at a later moment still permitted heat- and smell-production, indicating that the triggering principle had already begun to leave the primordia and was now present in the appendix; the metabolic explosion or avalanche could no longer be stopped. Experiments in which the appendix was cut off confirmed the conclusion. Of the 3 appendices that exhibited reduced heat- and smell-production after being cut off from the inflorescence and put with their base in water, the one that showed the closest-to-normal temperature-rise (1.3 °C) turned out to be cut off 20 hours before the heating began; for the other two, where the rises were 0.7 °C and 0.5 °C, the cut-off times were (resp.) 21 hours and 20 hours before heating. This shows that the active principle or trigger is present in the appendix about 22 hours before the heating starts, *i.e.*, 25–30 hours before peak-time. Van Herk also succeeded in extracting the triggering-principle from male flower primordia first ground

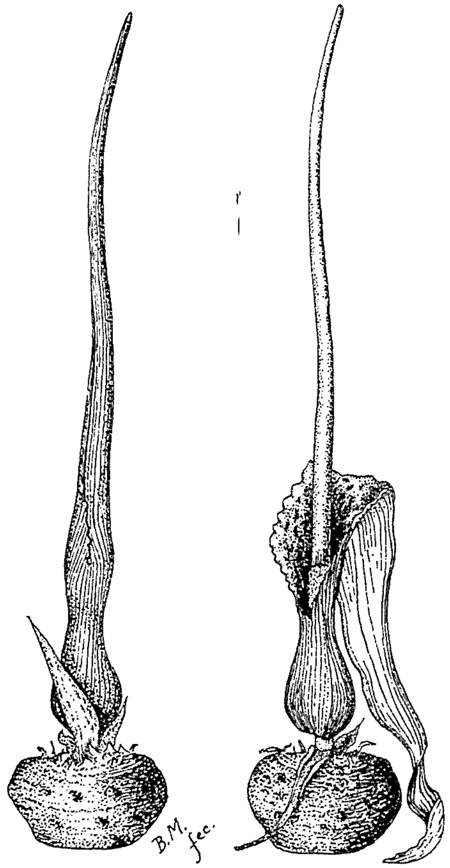


Fig. 1. *Sauromatum* inflorescences.
Left: D-day minus one.
Right: D-day.

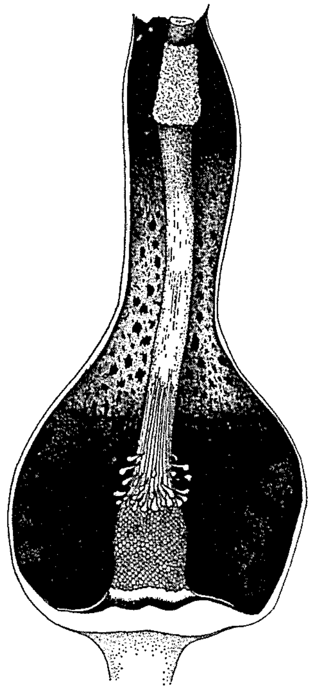


Fig. 2. Floral chamber of *Sauromatum guttatum* Schott, showing female flowers (base), male flowers (top) and clubshaped organs.

up with water with icecold acetone; after elimination of the organic solvent, injection of the principle in the form of an aqueous solution into appendices that had been amputated two days previously (but had not "fired") resulted in heating, after the expected lag-time of about a day. Various amino acids, vitamins, sugar phosphate and inorganic phosphate could not replace the triggering-substance in this action. In conversation, Van Herk later referred to his newly discovered hormone as *calorigen*, a name which we will adopt here for the sake of brevity, even though we share Van Herk's view that active extracts such as he prepared may also affect other flowering-phenomena, such as the unfolding of the spathe; calorigen may have multiple effects, or (alternatively) more than one hormone may be present in the extract.

2. STATEMENT OF THE PROBLEM

Research of pioneer quality always leads to new questions, and this laudatory dictum certainly applies to Van Herk's work. The rest of our present article should be seen in that light; our aim has not been to criticize, but to build on the solid foundation which Van Herk has laid. With this in mind, we shall raise and discuss the following (interconnected) points.

1. The question can be asked: "What triggers the trigger"? Is calorigen always found in male flower primordia of the proper developmental stage, or do its appearance and presence depend strongly on external circumstances? Surprisingly, Van Herk (although mentioning the activity-peak between 7 and 11 a.m.) has made no attempt to establish a connection with the normal day/night régime, or to study the effects of light and darkness; he routinely kept the developing inflorescences in fairly strong, constant light. In our Seattle laboratory, such treatment always resulted in very poor, delayed unfolding of the inflorescence, with an unspectacular rise in temperature. On the other hand, we can confirm Van Herk's report that anthesis in constant light is non-synchronized; the production of heat and smell may occur at any time of the day or night.
2. It follows that Van Herk, at least initially, was at the mercy of his material; with the exception of his calorigen experiments, where he could start the heating-process at will by injecting the hormone, his work shows a "retro-active", *post mortem* quality that must have led to much waste. Because in constant light it is impossible to judge with accuracy the readiness of *Sauromatum's* inflorescence to open and heat up, there must have been many appendices that were lost to experimentation because (in retrospect!) they were cut off too early and did not heat up at all. Synchronization would also have been very helpful in the extraction of calorigen, a process which, according to Van Herk, requires at least ten inflorescences blooming simultaneously.
3. Conceptually, it is possible to take issue with the extirpation experiments, because Van Herk's reasoning was based solely on the idea that elimination of certain floral parts would still *permit* anthesis; no attention was paid to the possibility that amputations of this type may *induce* (or at least influence) the metabolic flareup, and no control experiments were run to cover this contingency. Below, we shall see that amputation of the spathe does indeed have a triggering effect!
4. In the extirpation experiments, the presence of calorigen in the male primordia was deduced from its presence in the appendix – into which the hormone was obviously *released*. However, the experiments do not permit a conclusion as to the possibility that calorigen resides in the male flower primordia (perhaps in the form of a precursor?) for an extended period without being released, and do not allow an estimate of the length of that period.

3. EXPERIMENTAL PART

3.1. Influence of the light/dark regime

The first experiments indicating an effect of light & darkness on the anthesis of arum lilies were performed by SCHMUCKER (1925) with *Arum maculatum*. With very few exceptions, inflorescences transferred to darkness a few days before expected opening-time, and then kept in darkness, never unfolded. The reverse experiment, with constant light, resulted in normal anthesis except that the natural synchronization was lost; of the 51 inflorescences still healthy at the end of the series, 22 opened between midnight and noon, 29 in the second half of the (24 hour) day. Reversal of day and night resulted in anthesis that was normal and synchronized, except for the moment of "peaking" which in 35 out of 42 cases occurred in the small hours of the (light) night.

With the Javanese species *Amorphophallus variabilis*, Van der Pijl was able to demonstrate that for a given inflorescence the moment of daybreak is decisive; the onset of stench-production, which normally falls at around 4:30 p.m., could be shifted forward and backward by changing the moment of dawn artificially.

The influence of an exposure to light, preceded and followed by a period of constant darkness, was demonstrated elegantly by MATILE (1958) with *Arum italicum*. Illumination by light from a small incandescent bulb for about two hours turned out to be essential. Shorter exposures gave increasingly weaker responses, 15 minutes being the threshold value for any measurable response to occur. The temperature maximum always manifested itself about 24 hours after the start of the illumination. With a view to what is going to follow, we prefer to paraphrase this Matile conclusion by stating that the temperature maximum follows about 22 hours after the beginning of the final dark period. Adoption of this 22-hour lag-time enables us to correlate the natural moment of peaking, late in the afternoon, with the time of sundown which in April and May (when *Arum italicum* in Central and Western Europe is in bloom) falls at about 7–8 p.m. Furthermore, the likelihood that the dark period is decisive and the exposure to light only a prerequisite is suggested by numerous literature data on short-day plants, especially cocklebur where the induction of flowering (although admittedly a different phenomenon) requires "long nights" of at least 8½ hours.

In our own light & dark experiments, concerned mostly with *Sauromatum guttatum* and aimed at finding the minimum effective length of the dark period, we have followed two approaches. In one series, inflorescences kept in constant light were subjected to a daily "dark shot" for several days, in such a way that the beginnings of the successive dark shots were separated by a time-span of 24 hours. For any given inflorescence, the length of the successive dark shots was kept constant, but among different inflorescences it was varied between 1 hour and 6 hours. In the other approach, a developing inflorescence was kept in constant light until judged "ripe" (on the basis of experience and growth curves); it was then exposed to a *single* dark shot, varying in length over a wide range from one inflorescence to another, and returned to constant light. (In one case, constant light was simply followed by constant darkness). Two criteria were used to assess the response to the light & dark treatment: the temperature rise of the appendix, measured with the aid of thermistors, and the hourly and total CO_2-production, determined with a special conductivity-method designed by

Mr. John Klima of this department. Simultaneous application of the two criteria to one and the same appendix was practiced in only a few cases, but correlation between the two was found to be excellent (see *fig. 3*) so that the single-criterion approach can safely be accepted as satisfactory.

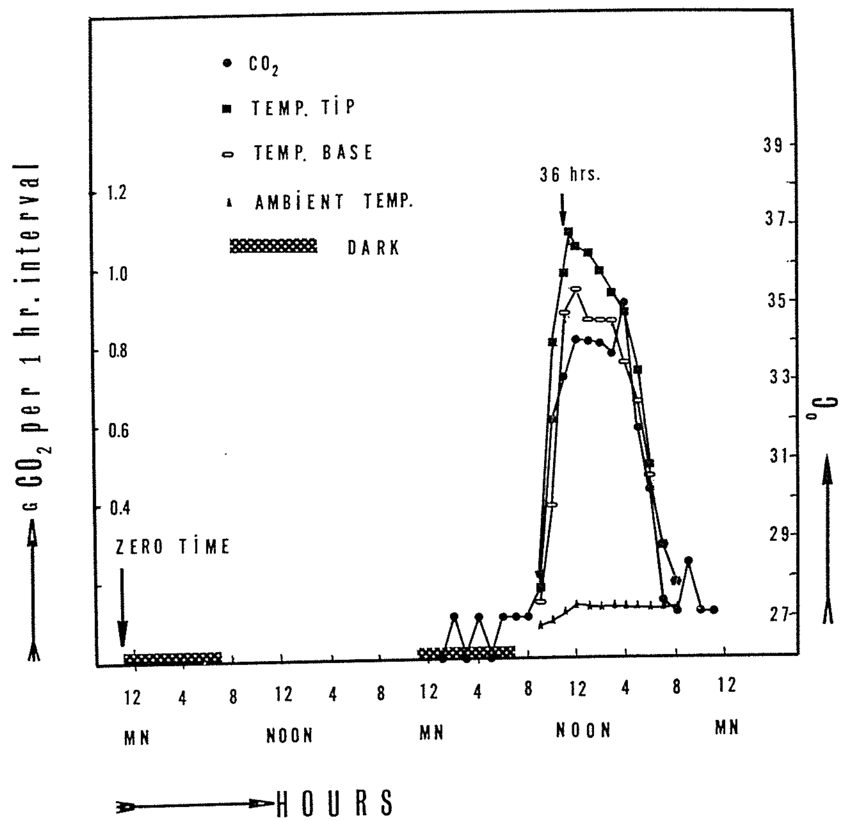


Fig. 3. Correlation between heat- and CO_2-production in the appendix of *Sauromatum* during the metabolic flare-up.

Our *fig. 4* illustrates some of our results. In the case of single dark shots, a 5-hour "night" represents the borderline case (BUGGELN 1969a). The lag-time between the beginning of the dark shot and the moment of peaking is about 48 hours. For single 6-hour dark shots, the lag-time is about 45 hours (average of 14 experiments; range 40–48 hours). For dark shots repeated at one-day intervals for 2 or 3 days, the length of the lag-period is reduced to about 36 hours and the critical length of the dark period to 2 hours.

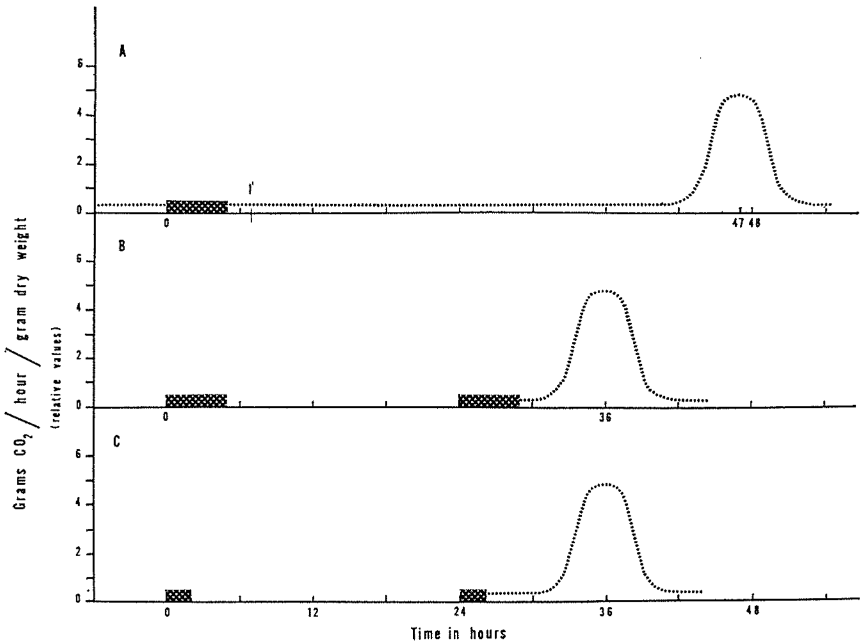


Fig. 4. Variation in the lag-periods of the metabolic flare-ups in appendices of *Sauromatum* inflorescences exposed to different light/darkness regimes.

3.2. Influence of light-quality and intensity, under a 12 hr./ 12 hour light & darkness régime

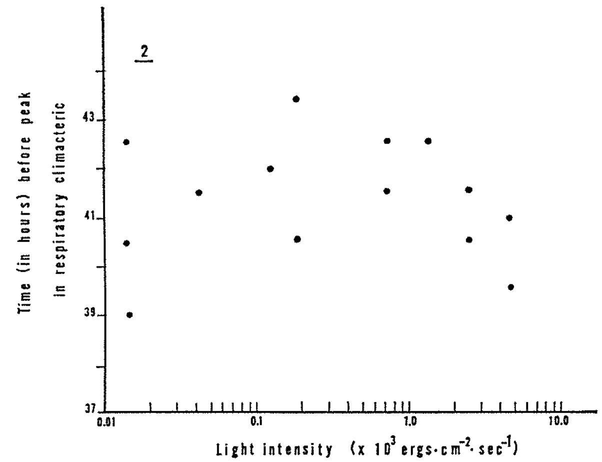
For this series, filters of sufficient monochromaticity were constructed on the basis of an article by Zalik & Miller (1960). The wave length regions chosen were blue (with a peak at 4,480 Ångström), green (5,200 Ångström), red (6,880 Ångstrom) and far red (7,000 Ångström). With the aid of fine screens, light intensities were varied for all four colors, and also for white light, between 0.008 $\times 10^3$ ergs/cm²/ sec. and 7.0 $\times 10^3$ ergs/cm²/sec. These intensities were measured directly with a radiometer. For each individual treatment and inflorescence, we established the length of the lag-period until peak-time, and the total CO_2 production per g of wet weight and per g of dry weight, these weights being determined one day after the day of the flare-up.

At least three conclusions emerge from the results:

a. In the set-up described, there does not seem to be a simple, straight-line relationship between light-intensity and response in terms of CO_2-production; a full response can be displayed at amazingly low light-intensities (fig. *5* and *6*).

b. Most surprisingly, there are no striking differences between the responses to different wavelength regions. Far red, when replacing white light, is not "seen as" darkness.

Fig. 5. Relationship between light intensity and lag-period in the metabolic flare-up of *Sauromatum* appendices. Light-regime: 12 hr./12 hr., light/darkness.



c. The average lag-time, for all intensities and colors (including white light) is 40.3 hours (number of experiments 99, range 37,5–44,5 hours). There is no simple functional relationship between length of lag-time and light-intensity. If the intensity of the response is gauged by the length of the lag-period (a long period indicating a weak response, a short one a violent reaction), it can be said that a good response can be obtained at very low light-intensities; this confirms the conclusion reached under a). Nevertheless, it must be clear that the average lag-time established here, under very artificial conditions, represents a ceiling-value, a maximum that exceeds the normal, natural lag-time. It is not far-fetched to assume, for the latter, a period of about 36 hours – close to the one found in our experiments with repeated dark shots of 2 or 3 hours, and useful if one wishes to correlate natural peak-time and sundown.

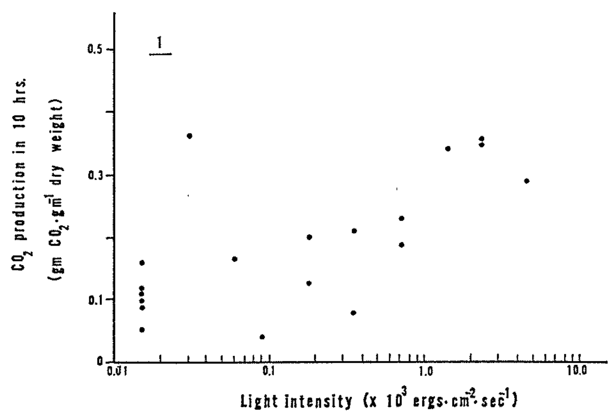


Fig. 6. Relationship between light-intensity and total CO_2 produced in the metabolic flare-up of *Sauromatum* appendices. Light-regime: 12 hr./12 hr., light/darkness.

3.3. Site of perception of the single dark shot

This question has been investigated carefully by BUGGELN (1969b). In order to preclude possible effects of the spathe-amputation (an operation that cannot be avoided if one wishes to expose *only* the male flower zone), the amputation was carried out several days before flowering; the naked spadices were then employed when they had reached the proper developmental stage. For details we refer to Buggeln's thesis. The results show that it is indeed the region of the male flower primordia that perceives the dark shot stimulus. Controls involving exposure to darkness of other floral parts showed no response. It is worthy of note that Matile (who, like Buggeln, used naked spadices in the crucial experiments) obtained a full-fledged respiratory response in *Arum italicum* when only the male flower primordia received a light-shot interrupting a prolonged period of constant darkness.

3.4. Release of calorigen from the male flower primordia

BUGGELN (1969b) obtained information on the presence of calorigen in the appendix administering one effective 6-hour dark shot to inflorescences kept in constant light, and then cutting the appendices off after various time intervals, *e.g.* 2, 4, 6, 8, 10 hours etc. after the end of the dark shot. Appendices isolated in this fashion and kept with their base in water did not heat up unless the cutting-off took place 8–9 hours after termination of the dark period, indicating that calorigen (or its precursor) is held in the male flower primordia for a considerable length of time.

3.5. Site of perception of the second effective dark shot

BUGGELN (1969b) found that appendices cut off at least 9 to 10 hours (see above) after an effective 6-hour dark shot (given to the whole inflorescence) and then exposed to a second dark shot 24 hours after the first one, display a metabolic flare-up with a lag-time that is definitely shortened when compared with the lag-period for the single shot. The average lag for single shots is 44.7 hours (14 observations), for double shots as described here 34.8 hours (10 observations).

The second dark shot must therefore be perceived by the appendix.

3.6. Speed of distribution of calorigen in the appendix

VAN HERK (1937b, c) has already recorded, and we can confirm, that upper and lower half of an activated appendix heat up almost simultaneously. In some cases, the appendix tip heats up middle or base while in other cases base or middle react first. One of the most elegant ways to illustrate this situation is to use a mixture of certain cholesterol esters (cholesteryl propionate, -decanoate and -oleate) which, due to the presence of liquid crystals, indicates a change in temperature by a change in color. The mixture we used the most frequently had a composition guaranteeing a color change from red through yellow and green to blue-violet over the temperature-range 26–29.4 °C. It was applied directly, in a very thin layer, to a *Sauromatum* appendix that was in the process of heating up while kept in an incubator at 25 °C. If there were a very slow acropetal trans-

port of calorigen in the appendix, one could expect the base always to heat up before middle and tip, unless there were a compensating mechanism manifesting itself in the form of a progressive decrease in lag-time from base to tip. The absence of such a compensating mechanism must, however, be deduced from experiments in which relatively small pieces of appendix were treated with calorigen (see below). Thus, the transport of calorigen after its release from the male flower primordia must be relatively fast, compared with the long lag-time. At this moment, the distribution-mechanism is entirely obscure.

3.7. Time-budget for the appendix

If a 6-hour dark-shot, given only once, leads to a total lag-time (until peaking) of about 45 hours, and calorigen-release comes 8–9 hours after the end of the dark-shot, the remaining "true" lag-time is 28–29 hours. The latter period should agree, at least roughly, with the lag-time after calorigen-injection observed by Van Herk and ourselves. BUGGELN (1969b), using dilute calorigen preparations obtained, in part, by Van Herk's method, observed a lag-time of 28 hours (average of 14 determinations). VAN HERK (1937c) mentions 22 hours, but in evaluating this figure, two facts should be kept in mind. First, Van Herk usually used much more concentrated preparations, and it follows unmistakably from his fig. 3a that dilution of the calorigen leads to an increase in the lag-time (in spite of Van Herk's different interpretation). Second, the actual heating-up period is not included in the 22-hour figure; the lag-period until peaktime can thus safely be assumed to be 25–27 hours. In summary, we can state that the claims we are making as to the lengths of dark-shot, release-time and "true" lag-period are realistic.

3.8. Influence of spathe-amputation

For inflorescences kept in constant light until "ripe", it can be shown that amputation of the spathe consistently leads to a metabolic flare-up, with a peak 36 hours after the amputation. Since the lag-time agrees closely with the one found for experiments where repeated dark-shots are given, it is likely that amputation and the anthesis-inducing light/dark régime affect the same process. In principle, amputation can be seen as the elimination of an inhibitor provided by the spathe. In order to check this hypothesis, experiments were run in which a number of 5 cm vertical slits (about 15) were made in (and through) the wall of the floral chamber with the aid of a razor blade. It is unlikely that such treatment could have much effect on the transport (export) of substances produced by the spathe. Nevertheless, the slashing resulted in a flare-up with a lag-time of approximately 36 hours. Another possibility (appealing, because it might provide a bridge to the light & dark experiments) is that slashing or amputation will bring about a change in the gaseous atmosphere within the floral chamber. This hypothesis was checked by cutting two square windows of approximately 2 cm^2 in the wall of the floral chamber of a young and still growing inflorescence, on opposite sides; to further promote gas circulation through the floral chamber, the upper half of the spathe was cut away. When such inflorescences were raised in constant light until "ripe", and the spathes were then cut away, a metabolic flare-up still followed, with a lag-time of about 35 hours. The conclusion must be that wounding does *not* work through an influence on the internal gas atmosphere of the floral chamber; the wounding is effective *per se*. At present, it is hard to suggest a mechanism for this effect. It would be interesting to investigate the relationship between the number of vertical slashes made and the magnitude of the response.

3.9. Nature of calorigen

BUGGELN (1969b) has developed a convenient, economical calorigen test by placing small (5–6 cm) pieces of appendix have their base capped by the cut-off fingertip of an "examination" glove. This arrangement acts as a well – the appendix sections being hollow – into which test substances may be added. The plant tissue is kept in place with a rubber band encircling the glove-tip, and the temperature-rise (if any) is registered with the aid of thermistors. Positive reactions were obtained with calorigen extracts prepared by Van Herk's method, and also with fractions isolated from such extracts with the aid of Sephadex columns, other molecular sieves, and thin layer chromatography. These methods reveal that calorigen must have a molecular weight of less than 1,000. On a thin layer plate (Silicagel-G), with a mixture of acetone and water (85:15, v/v) as the running-fluid, the activity moves to a position with an R. F. value of approximately 0.97–0.99. In the lyophilized state, purified calorigen preparations could be kept for at least 2½ months without complete loss of activity. The hormone could not be replaced by gibberellic acid (GA_3) or by cyclic (3′,5′) AMP administered at pH 6.1 and 5.3, respectively. The threshold value for the development of a considerable "aroma" seems to be much lower than that for inducing a measurable temperature rise. In one experiment, where smell was the sole criterion, the inhibitor cycloheximide administered together with the calorigen seemed to prevent the latter's action.

3.10. Discussion and prognosis

As a routine matter, calorigen is extracted from male flower primordia about 4 hours before the appendix peaks. As already reported, a certain amount of the hormone then has already moved into the appendix, so that extraction at this juncture cannot be considered to be economical. Efforts should be made to extract calorigen (or its precursor) from male flower primordia immediately after a single effective dark-shot, and also (in a series of parallel experiments) at regular intervals afterwards.

Cyclic (3′,5′) AMP, the so-called "second messenger" produced in many different cell types under the influence of hormones (representing the "first messengers") has thus far been found in such disparate places as *E. coli* cells, human urine, cellular slime molds (where it probably represents the natural acrasin) and many different mammalian cells. This compound thus seems to be well-nigh ubiquitous in biological systems. In spite of its failure to substitute for calorigen in our tests (a failure that may well be due to penetration difficulties),

determinations of the compound in appendix tissue should be made at regular intervals, after a single effective dark shot, in order to find out whether or not it plays the role of a second messenger in this higher plant material also.

The long lag-time in the appendix, after release of the calorigen and before peak-time, can easily be reconciled with the assumption of a *de novo* synthesis of enzymatic protein. It is known that gibberellin, acting on the aleurone cells of barley grains, induces a *de novo* synthesis of α-amylase. The metabolic level at which gibberellin, in this case, works may therefore be that of messenger-RNA formation (or action?); de-repression of certain genes may well be involved. Seen against this background, it seems logical to initiate calorigen studies where emphasis is laid on the use of agents that interfere with protein synthesis or with purine- and pyrimidine metabolism (puromycin, cycloheximide; in general, purine- and pyrimidine analogs, ethionine etc.). It is interesting and gratifying to note that the study of aroid appendices has, in quite a logical fashion, moved from the realm of natural history (pollination studies) into that of molecular biology. As already explained elsewhere (MEEUSE 1968a, 1968b), our experiments have also opened the door to investigations concerning the possible involvement of the phytochrome system in flower anthesis.

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THE WOOD STRUCTURE OF DICRANOSTYLES (CONVOLVULACEAE)*

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SUMMARY

The anatomy of the mature wood of three species of the South American genus of woody climbers *Dicranostyles* Bth. is described and compared with that of the secondary wood of other genera of the *Convolvulaceae*. The stems are characterized by the occurrence of concentric rings of included phloem tissue. In most characters the three species are quite similar, except for the absence of rays over two cells wide in *D. guianensis*. Instead of these, aggregate rays occur at intervals between consecutive rings of included phloem. *D. mildbraediana* posses-ses fibre tracheids with next to the normal large bordered pits numerous very large irregular slits giving the impression of a helical sculpturing of the walls. From a comparison with other Convolvulaceous genera like *Bonamia*, *Ipomoea*, *Maripa*, *Neuropeltis*, and *Prevostea*[1] it appears that nearest to *Dicranostyles* in general appearance as well as in anatomical structure is the genus *Maripa*. The resemblance to *Ipomoea* seems only superficial. The relation between anatomical conformities of genera and their position in the various systems of subdivision of the family is discussed.

1. INTRODUCTION

In the course of a study of a collection of woody climbers gathered in Suriname by Dr. J. van Donselaar some difficulties arose over two stems with concentric bands of included phloem. Though the wood structure as seen with a hand lens was reminiscent of *Moutabea* and *Securidaca*, the sterile or, respectively, fruiting herbarium vouchers excluded Polygalaceous relationship. As described elsewhere (MENNEGA 1968) these herbarium specimens were eventually identified as species of *Dicranostyles*, a genus of *Convolvulaceae* hitherto unknown from Suriname. It turned out that the wood structure is in agreement with some other genera of this family.

2. LITERATURE

In his treatment of lianas with abnormal secondary growth PFEIFFER (1926) mentions 12 genera of *Convolvulaceae* with the concentric type of included phloem, in his terminology "corpus lignosum circumvallatum". *Dicranostyles* is not among these. Earlier SCHENK (1893) studied mature wood of lianas, chiefly from South Brazil. *Argyreia*, *Convolvulus*, *Ipomoea* and *Porana*, an

* Dedicated to Professor Dr. C. E. B. Bremekamp.
[1] Though *Prevostea* is a synonym of *Calycobolus* (HEINE 1963) the name *Prevostea* will still be used in the present paper to follow the current anatomical literature.