THE EFFECT OF VARIOUS UNCOUPLERS ON THE RESPIRATION OF APPENDIX TISSUE SLICES OF SAUROMATUM GUTTATUM SCHOTT (ARACEAE) AT VARIOUS STAGES OF ANTHESIS[1]

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SUMMARY

In the presence of glucose, the respiration of appendix tissue slices of the arum lily *Sauromatum guttatum* Schott is markedly stimulated by the uncouplers 2,4-dinitrophenol, Na-azide and pentachlorophenol *after* the first day of flowering. There is no appreciable effect on the first flowering-day itself, and only a minor stimulation (if any) at younger stages. These results are interpreted on the basis of the hypothesis that, on the first day of flowering, respiration is of the "uncoupled" type. The relative lack of response to the uncouplers in the young flowering-stages is probably due to a rapid turnover of ATP in connection with the spectacular growth of the inflorescence.

1. INTRODUCTION

Biochemically speaking, it can be maintained that in plant cells heat is just an "undesirable" waste product in the process of biological oxidation; the main direct function of the latter process can be seen as the creation of high energy compounds (e.g., ATP). Ecologically, to be sure, the development of heat by plants may confer certain advantages upon the generating individuals, e.g. when young plants have to push their way up through snow banks in early spring. Perhaps the most fascinating case of ecological advantage of heat-production is provided by certain arum lilies, such as *Arum*- and *Sauromatum* species. As explained elsewhere (MEEUSE 1966; BUGGELN & MEEUSE 1967; SMITH & MEEUSE 1966), the heat developed in their inflorescences during anthesis may well have the specific biological function of evaporating the odoriferous substances that attract the insect pollinators. The survival value of the very spectacular heat production is thus immediately obvious. For evolutionary purposes, it should certainly be worthwhile to look into the biochemical mechanisms underlying it, the more so since the heat production (being geared to the life habits of the pollinators) manifests itself within a very short, well-defined, time span, a circumstance which leads to the strong suspicion that respiration here is of the "uncoupled" type. HESS (1964) has indeed provided experimental evidence indicating that during the period of heat- and smell production the level of ATP

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in the so-called *appendix* (the heat- and smell-generating organ, VOGEL's (1963) "osmophore") drops significantly.

The present series of experiments was designed to test the effect, on appendix slices of various physiological ages, of the wellknown artificial uncouplers 2,4-dinitrophenol (DNP), pentachlorophenol and sodium azide. If respiration in the appendix during the "odoriferous" stage is indeed "uncoupled", in the sense that ATP either is not formed on any significant scale or is decomposed immediately, then no respiration-stimulating effects of the uncouplers should be found for that stage. On the other hand, the addition of appropriate concentrations of DNP, azide or pentachlorophenol to appendix slices cut at a later stage of flowering, when respiration has gone back to a much lower, "normal", level, should have a very marked stimulatory effect. As regards the stages preceding anthesis, we did not have a clear-cut *a priori* opinion. Respiration here is fairly low (although not quite as low as it is in resting storages organs such as potatoes and other tubers). On the other hand, however, it should be kept in mind that the appendix, in the week preceding anthesis, is one of the fastest-growing plant organs on record (SMITH 1964; MEEUSE 1966), with a spectacular buildup of cell wall materials, starch and protein; in such situations, there is a very rapid turnover of *ATP*, and *a priori* little effect of uncouplers can be expected. For documentation of this viewpoint, we refer to GAUR's (1957) and BEEVER's (1961) data concerning DNP effects on carrot tissue of increasing physiological age.

2. MATERIALS AND METHODS

Plants of *Sauromatum guttatum* Schott were grown in the New Botany Greenhouse on the campus of the University of Washington, Seattle, Wash. The corms were harvested in the fall, and the inflorescences were allowed to develop from these in the laboratory or greenhouse at room temperature.

Since we were primarily interested in metabolic processes whose development and level of activity could be measured as a function of the flowering sequence in *Sauromatum*, it was necessary to define an arbitrary timescale which would describe the morphological development of the inflorescence. In this paper the following designations will be used to identify the chronological age of the tissue being investigated:

(a) D-DAY (female flower anthesis stage) refers to the day of the unfolding of the spathe and the closely following production of heat and stench in the appendix.

(b) D-DAY+1 or D+1 (male flower anthesis stage) refer to one day after the peak of heat and smell.

(c) D-DAY+2 or D+2, D-DAY+3 or D+3, etc., refer to the number of days after D-DAY that the tissue was harvested.

(d) D-DAY–1 or D–1, D-DAY–2 or D–2, etc., refer to the number of days before D-DAY that the tissue was investigated.

In order to determine (retroactively!) the age of the various preflowering or "D minus" stages, a stump of the appendix was always left intact and in place on

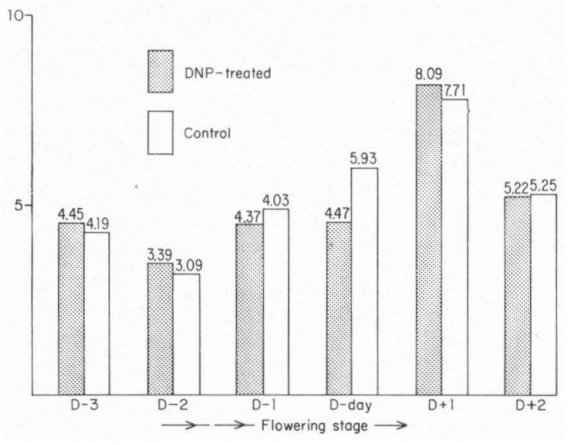

Fig. 1. Q'_{0_2}-values for appendix tissue slices of *Sauromatum* treated with 10^{-4} M DNP.

the inflorescence. By the warming and stench production in these stumps it is possible to accurately determine the occurrence of D-day. Elimination of the upper part of the appendix does not interfere with the normal flowering sequence (VAN HERK 1937). In each case where "D minus" material was investigated, the cut surface of the stump was coated with lanolin paste to prevent the remaining tissue from drying out.

The experiments which involved the use of D-day tissue were carried out in the morning, when the spadix was warm and was producing the carrionlike odor.

In all experiments, the appendix tissue used was consistently obtained from the middle third of the appendix. This material was hand-sectioned into a large aliquot of buffer, the slices being about 0.3 mm in thickness. Warburg exper-

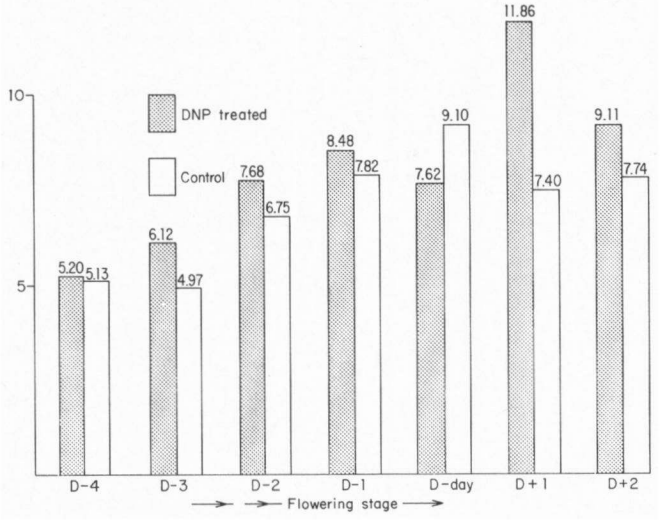

Fig. 2. Q'_{0_2}-values for appendix tissue slices of *Sauromatum* treated with 5×10^{-4} M DNP.

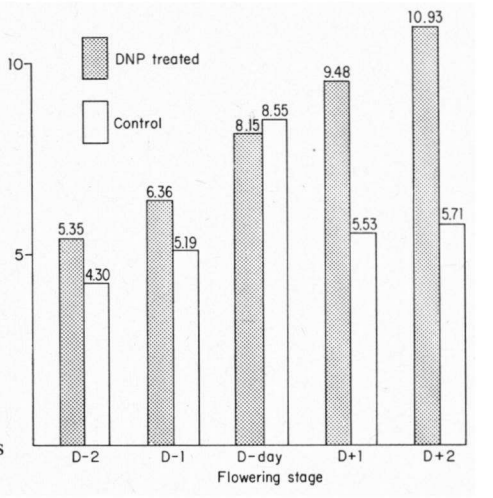

Fig. 3. Q'_{O_2}-values for appendix tissue slices of *Sauromatum* treated with 10^{-4} M NDP.

iments were run in the conventional way; CO_2 outputs were determined by using the two flasks method with paired samples. Tissue samples were added to the flasks containing DNP or pentachlorophenol or azide in the required concentration, and measurements were begun after a 45-minute equilibration period. The results of these experiments are recorced in *tables 1–5* and *figures 1–5*.

In evaluating the raw data, a difficulty was presented by the fact that the pre-D-day appendix is very rich in starch, whereas the post-D-day stages are almost starch-free. To express the magnitude of respiration (gas exchange) on a simple

Table 1. Effect of 10^{-4}M DNP on the respiration of *Sauromatum* appendix tissue slices. Warburg experiments, temperature 19°–23°C. (a) Flasks measuring O_2 uptake, main compartment: 8–10 slices *Sauromatum* spadix tissue, 2.7 ml 0.03M phosphate buffer pH 5.0 containing 2% glucose, 0.3 ml DNP pH 5.0, final concentration as above, or 0.3 ml buffer. Center well: 0.2 ml 30% KOH, filter paper wick. (b) Flasks measuring excess CO_2 production: legend as for (a) except that 0.2 ml buffer was substituted for the KOH.

Tissue stage	DNP R.Q.	Control R.Q.	DNP Q'_{O_2}		Control Q'_{O_2}	
D-day−3	0.995	0.735	4.45		4.19	
D-day−2	1.100	0.979	3.86	av. 3.39	3.33	av. 3.09
D-day−2	0.932	0.898	2.91		2.85	
D-day−1	1.020	1.040	4.37		4.83	
D-day	1.040	1.060	2.89	av. 4.47	4.38	av. 5.93
D-day	1.260	1.200	6.04		7.47	
D-day+1	0.998	0.761	6.98	av. 8.09	5.58	av. 7.71
D-day+1			9.20		9.83	
D-day+2			6.45	av. 5.22	5.08	av. 5.25
D-day+2			3.98		5.41	

In this and all subsequent tables, Q'_{O_2} is defined as O_2 uptake per mg starchless dry weight per hour.

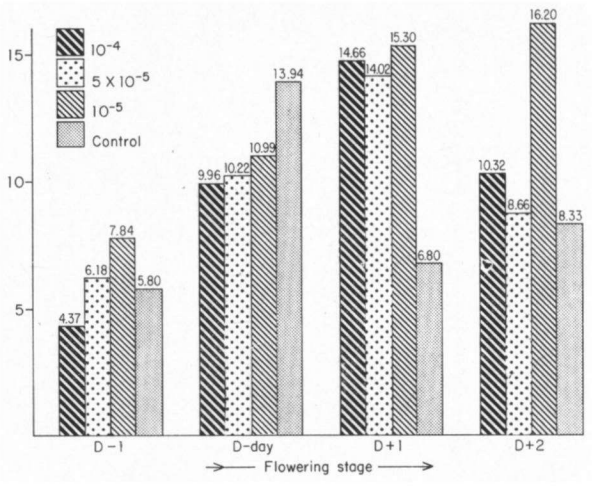

Fig. 4. Q'_{O_2}-values for appendix tissue slices of *Sauromatum* treated with pentachlorophenol.

dry weight basis would clearly tend to underrate the activity of the pre-D-day tissues. For this reason, all results have been expressed on a "dry weight minus starch" basis. OLASON (1967) has determined the starch-content of the *Sauromatum* appendix at various stages by first gelatinizing the starch with hot water, then converting it to glucose with the aid of *Cryptochiton* digestive enzymes, and finally assaying the free hexose with fungal glucose-oxidase (notatin), which is highly specific. He thus arrived at the following figures for the starch-content on successive days:

Flowering stage	D–3	D–2	D–1	D-day (early)	D+1	D+2	D+3
Starch, as % of dr. wt.	46.5	50.5	52.0	34.5	6.5	4.5	3.3

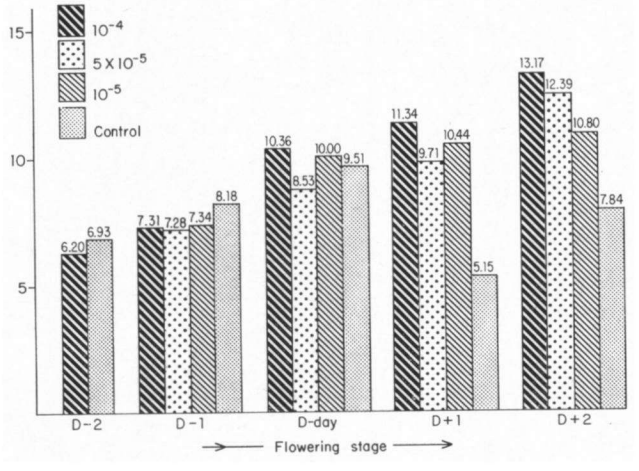

Fig. 5. O'_{O_2}-values for appendix tissue slices of *Sauromatum* treated with azide.

Table 2. Effect of 5.10^{-5}M DNP on the respiration of *Sauromatum* appendix tissue slices Legend the same as for Table 1 except for final DNP concentration.

Tissue stage	DNP R.Q.	Control R.Q.	DNP Q'_{O_2}		Control Q'_{O_2}	
D-day−4	1.070	1.030	5.20		5.13	
D-day−3			6.12		4.97	
D-day−2			7.68		6.75	
D-day−1			6.93		7.28	
D-day−1			9.15	av. 8.48	8.74	av. 7.82
D-day−1			9.82		7.84	
D-day−1	0.837	0.937	8.03		7.41	
D-day	0.964	0.961	5.83		6.89	
D-day	0.912	0.941	9.58	av. 7.62	12.30	av. 9.10
D-day			7.44		8.12	
D-day+1	0.989	0.897	9.75	av. 11.86	8.14	av. 7.40
D-day+1	0.899	0.880	13.96		6.66	
D-day+2	0.860	0.904	11.07	av. 9.11	9.84	av. 7.74
D-day+2			7.15		5.64	

Table 3. Effect of 10^{-1}M DNP on the respiration of *Sauromatum* appendix tissue slices. Legend the same as for table 1 except for final DNP concentration.

Tissue stage	DNP R.Q.	Control R.Q.	DNP Q'_{O_2}		Control Q'_{O_2}	
D-day−2	1.050	1.180	5.35		4.30	
D-day−1	1.110	1.250	3.22	av. 6.36	3.43	av. 5.19
D-day−1			9.49		6.95	
D-day	1.220	1.440	3.00	av. 8.15	2.43	av. 8.55
D-day			14.38		15.45	
D-day			7.08		7.77	
D-day+1	1.090	1.200	4.97	av. 9.48	4.10	av. 5.53
D-day+1			13.99		6.96	
D-day+2			11.87	av. 10.93	5.47	av. 5.71
D-day+2			9.98		5.94	

The Q'_{O_2} data were submitted to standard analytical statistical methods. This analysis shows that there is no statistically significant effect by DNP on the O_2 uptake of appendix tissue slices from *Sauromatum* in the pre-D-DAY stages or at D-DAY. However, at stages D+1 and D+2 DNP elicits a significant (95% level of significance) stimulation in O_2 uptake in these tissues. Even though DNP at 10^{-5}M seems to lower the R. Q. in all stages tested, this same consistency of effect was not shown with the other two levels of DNP. For this reason the R. Q. data were not treated with statistics.

Admittedly, the figure for early D-day is still somewhat arbitrary, since the starch-content changes so rapidly on that day.

3. RESULTS

The results are presented in *tables 1–5* and *figs. 1–5*.

The Q_{O_2} data were submitted to standard analytical statistical methods. This

Table 4. Effect of pentachlorophenol on the respiration of *Sauromatum* appendix tissue slices. Warburg experiments, temperature 20 to 21 °C. Main compartment: 8–10 slices *Sauromatum* spadix tissue, 2.7 ml 0.03M phosphate buffer pH 7.8, 0.3 ml pentachlorophenol, final concentrations indicated below, or 0.3 ml buffer. Center well: 0.2 ml 30% KOH, filter paper wick.

Tissue stage	Q'_{O_2}							
	Control		10^{-4}M		5×10^{-5}M		10^{-5}M	
D-day−1	5.80		4.37		6.18		7.84	
D-day	14.23	av. 13.94	9.75	av. 9.96	10.11	av. 10.22	12.91	av. 10.99
D-day	13.65		10.16		10.33		9.06	
D-day+1	5.80		12.50		14.77		16.40	
D-day+1	6.40	av. 6.80	15.15	av. 14.66	12.13	av. 14.02	13.05	av. 15.30
D-day+1	8.19		16.33		15.17		16.45	
D-day+2	4.95		4.55		8.59		13.78	
D-day+2	11.03	av. 8.33	12.82	av. 10.32	15.09	av. 8.66	17.00	av. 16.20
D-day+2	9.00		13.58		2.30		17.82	

analysis shows that there is no statistically significant effect by DNP on the O_2 uptake of appendix tissue slices from *Sauromatum* in the pre-D-DAY stages or at D-DAY. However, at stages D+1 and D+2 DNP elicits a significant (95% level of significance) stimulation in O_2 uptake in these tissues. Even though DNP at 10^{-5}M seems to lower the R.Q. in all stages tested, this same consistency of effect was not shown with the other two levels of DNP. For this reason the R.Q. data were not treated with statistics.

The blank spaces which occur in the preceding tables under the "R.Q." headings are deliberate omissions. The Q_{O_2} data indicated that the DNP was affecting respiration of the plant material, but in no case was a high "apparent R.Q." manifested. This led to the conclusion that although the DNP was penetrating the tissue successfully, aerobic CO_2 production due to alcoholic fermentation was not enhanced under the existing experimental conditions. Determinations of R.Q.'s were subsequently discontinued.

Table 5. Effect of azide on the respiration of *Sauromatum* appendix tissue slices. Warburg experiments, temperature 21 to 22 °C. Main compartment: 8–10 slices *Sauromatum* spadix tissue, 2.7 ml 0.03M phosphate buffer pH 5.0, 0.3 ml azide, final concentrations indicated below, or 0.3 ml buffer. Center well: p.2 ml 30% KOH, filter paper wick.

Tissue stage	QO_2							
	Control		10^{-4}M		5.10^{-5}M		10^{-5}M	
D-day−2	6.93		6.20		–		–	
D-day−1	7.16		6.30		6.84		6.82	
D-day−1	8.38	av. 8.18	7.86	av. 7.31	7.72	av. 7.28	7.86	av. 7.34
D-day−1	8.99		7.78		–		–	
D-day	11.14		11.74		9.52		11.18	
D-day	7.88	av. 9.51	8.98	av. 10.36	7.54	av. 8.53	8.81	av. 10.00
D-day+1	5.60		10.70		–		–	
D-day+1	4.70	av. 5.15	11.98	av. 11.34	9.71		10.44	
D-day+2	7.84		13.17		12.39		10.80	

4. DISCUSSION

The results of the present investigation can be summarized by stating that the uncouplers DNP, Na-azide and pentachlorophenol, applied in the appropriate concentrations, exert a pronounced stimulatory effect upon the respiration of *Sauromatum* appendix slices when administered at post-D-day stages. (In evaluating *fig. 4*, it should be kept in mind that the low average value for 5×10^{-5} M DNP on D+2 may have been caused by the one unusually low figure of 2.30 (*table 4*), which has the earmarks of being due to simple experimental error. Without it, the graph would be even more convincing).

In consonance with our expectations, there is no stimulatory effect of the uncouplers on D-day. The fact that before D-day the stimulatory influence is only slight (or even lacking) may perhaps be attributed to the rapid turnover of ATP in the fast-growing appendix. Final judgment on this hypothesis must await the result of experiments carried out on an appendix in which D-day has artificially been delayed, although growth has already ceased. We now know that such a delay can be brought about by administering an appropriate light-regime to the inflorescence.

The stimulation of respiration by uncouplers after D-day leads to values for the oxygen consumption which compare favorably with those that are found naturally on D-day. This fact is important for a variety of reasons. In the first place, it seems permissible to conclude that a good proportion of the respiratory apparatus (composed of enzymatic proteins and cofactors) is still intact. VAN HERK, who considered flavoproteins to be important agents in the terminal respiration of the *Sauromatum* appendix, has reported that before D-day most of the flavines were present in protein-bound form, so that they could not be dialyzed out; after D-day, a considerable proportion of the flavines (46%) was present in free form, a fact that would lead to the suspicion that the flavoproteins, after D-day, are progressively destroyed. A combination of Van Herk's observations and those made by us would seem to permit two possible conclusions. *Either*, the flavoprotein is originally present in abundance, so that a certain amount of inactivation can be "absorbed" with impunity, i.e., without doing serious harm to the capacity of the respiration system; *or*, the flavoprotein systems in the appendix are no longer very important in respiration after D-day. In this context, it is worthy of note that the cyanide-insensitivity of *Sauromatum* appendix slices (usually thought of as indicating a major role of flavoproteins in respiration) does not change appreciably during the flowering-sequence (Meeuse, unpublished).

In the presence of the uncouplers and glucose, the respiration of post-D-day appendix slices does not seem to be severely limited by a dearth of respiratory substrate. This finding agrees with an observation by Van Herk, who noticed that the respiration of post-D-day appendix tissue could be boosted by the addition of glucose, in contrast to the respiration of earlier stages. A severe qualitative change in the nature of the respiratory substrate does not seem likely from an inspection of the R.Q. values.

Adopting the now classical viewpoint that the main effect of uncouplers such

as DNP lies in their influence on the levels of ATP, ADP and inorganic phosphate (Pi), it is tempting to postulate that respiration in the post-D-day stages is controlled by the availability of the phosphate acceptor ADP and/or the level of Pi. From BUGGELN & MEEUSE's data (1967) on the mitochondrial ATP-ase of the appendix, we can conclude that its activity in the post-D-day stages is higher, not lower, than before. This makes it difficult or impossible to interpret the changing response to uncouplers of the appendix tissue during the flowering process on the simple basis of a change in ATP-ase activity. After D-day, respiration seems to be tightly coupled to oxidative phosphorylation. Why this is the case at this age and apparently not at earlier stages (in spite of the latters' lower ATP-ase activity) remains a mystery.

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