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AN APPARENT INHIBITION BY "CIPC" OF RESPIRATION IN GERMINATING PEA SEEDS, SIMULATED BY A MORPHOGENETIC REACTION

by

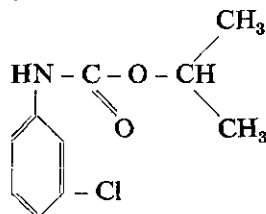
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1. INTRODUCTION

The observations reported in this paper have been collected in the course of a somewhat more extensive investigation by the second author, regarding the effect of the herbicide isopropyl N-(3 chlorophenyl) carbamate, more commonly referred to as "CIPC":



on the respiration of germinating pea seeds.

2. LITERATURE

The herbicide, CIPC, belongs to the carbamates, of which phenylurethane (ethyl-N-phenyl carbamate) has already been used by WARBURG (7) as an inhibitor of respiration. This author observed that inhibition by urethanes increased with the length of the alkyl chain (8), and refers to the same rule for inhibition of fermentation in yeast cells by alcohols, found by REGNARD back in 1889! (cf. [9]). Low concentrations of phenyl urethane, from 10 to 250 ppm were observed to produce stimulation of respiration, higher concentrations producing inhibition, in *Chlorella* (10).

AL-AISH and BROWN (1) in oat and rice seedlings observed inhibition of respiration by isopropyl-phenyl carbamate (IPC) in a concentration range from 0.5 ppm up to saturation.

SWANSON, SHAW and HUGHES (6), measuring respiration of cotton seedlings grown in contact with CIPC, found inhibition of respiration of decised root tips

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to increase with concentration while root elongation also was inhibited to a constant amount throughout the range of doses used in this case (1.6–16 lbs per acre).

Oxygen uptake after addition of the chemical from a side vessel to the untreated root tips after 30 minutes of measurement showed neither stimulation nor inhibition at the concentration of 31 ppm, and an inhibition down to 50% and 20% after 3 hours by 310 and 3100 ppm respectively.

Effects of carbamates on germination and growth have been variously reported in recent years. Investigators generally agree about inhibition of root (and shoot) growth, enlargement of cells, and prevention of mitosis (2, 3, 4).

IVENS and BLACKMAN (3) indicate that in the inhibition of root growth aside of an effect on the spindle in dividing cells some metabolic activity connected with mitosis may be involved.

3. MATERIAL AND METHODS

The oxygen uptake of peas (*Pisum sativum* L., var. Servo) was measured with a conventional WARBURG apparatus, using open manometers.

A large number of seeds was incubated in petridishes, containing a layer of sand sterilized by heating and soaked either with distilled water or with a solution of the herbicide, concentrations of 4 and 32 times 10^{-5} M (8.6 and 68.5 ppm, respectively) being applied.

The seeds were carefully laid out, so that they did not touch each other. Before, they were disinfected by a 7-minute exposure to strong sulphuric acid, and the seed coats taken off. In some of the earliest experiments the seed coats were not removed, it was noticed that they opened much earlier in the presence of the poison than in the blanks.

Each day, up to about 7 to 9 days, a sample of seedlings was selected from each of the 3 items, and the respiration rate studied in open WARBURG manometers (10 seeds or seedlings per vessel in 4 ccm distilled water or adequate herbicide solution, with 5 N potassium hydroxide in the center well) during about 1 hour. For each determination, a fresh sample was taken. During the respiration experiment, each sample of peas was under the same condition as during the previous germination period. A brief exposure to light was inevitable during preparation of the experiment.

A representative picture of the plantules, as obtained with different concentrations of CIPC after 115 hours is given in Plate 1.

We have comparatively studied the time course of respiration in inhibited and non-inhibited entire seedlings as well as in the plantules and the cotyledons separately.

In the present paper we will only discuss an experiment in which dry weight samples of entire seedlings as well as those of separately measured cotyledons and plantules were taken.

4. RESULTS AND DISCUSSION

a. Respiration in relation to germination and growth

Respiration of intact seedlings shows a picture as represented in figure 1a. The non-inhibited seedling shows a strong increase in respiration between about 50 and 200 hours of age. Such an increase is not observed in both series underlying inhibition, so that, at \sim 200 hours of age, a strong difference in respiration between inhibited and non-inhibited seedlings exists.

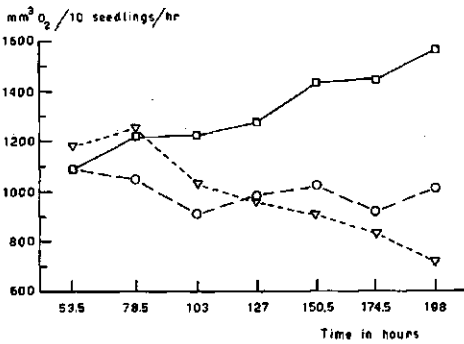


FIG. 1a. The respiration rate of inhibited and non-inhibited intact pea-seedlings, during germination.
 □ control (non-inhibited).
 ○ 4×10^{-5} Mol CIPC (= 8.6 ppm).
 ▽ 32×10^{-5} Mol CIPC (= 68.5 ppm).

FIG. 1b (top). The respiration rate of inhibited and non-inhibited plantules during germination (legend, see also fig. 1a).

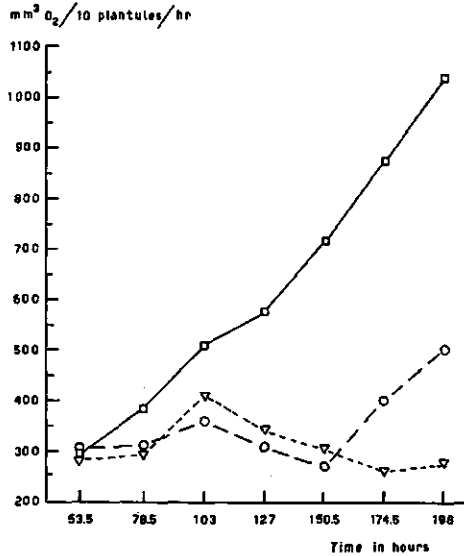


FIG. 1b (bottom). The respiration rate of inhibited and non-inhibited cotyledons during germination (legend, see also fig. 1a).

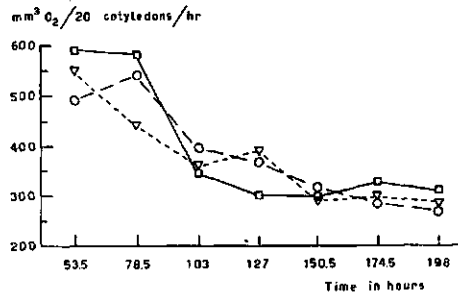
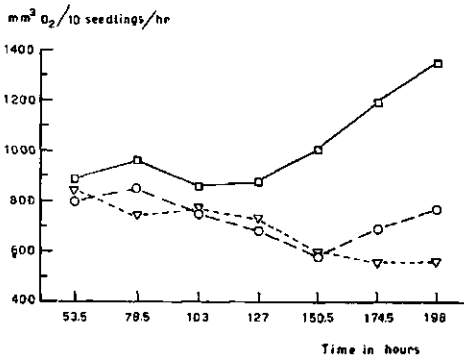


FIG. 1c. The combined respiration rates of the plantules and the cotyledons (inhibited and non-inhibited) during germination (legend, see also fig. 1a).



A comparison of the rate of respiration of intact seedlings (fig. 1a) with that of plantules and cotyledons taken separately (fig. 1b) shows that the combined respiration (fig. 1c) is about 20% reduced in the latter case (*cf.* figs 1a and 1c). In connection with data to be discussed below, this probably is due to reduction in plantule respiration by removal of its connection with the cotyledons.

Figure 1c moreover shows that the type of figure 1a is mainly due to the plantules.

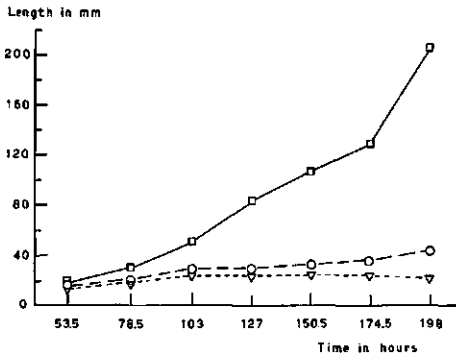


FIG. 2. Total length of the inhibited and the non-inhibited plantules during germination (taken after the respiration rate had been measured) (legend, see also fig. 1a).

At first sight, figure 1a seems relatively easy to understand, since the growth of the seedling is similarly inhibited (figure 2). It should, however, be observed that a real gain in dry weight is not to be expected in any of the cases, since germination proceeded in dark without external supply of energy in any form. Therefore, it is not very surprising that the rate of respiration per unit dry weight of the seedling (fig. 3) shows much the same picture as figure 1a: a strong increase (about twofold) with time in the non-inhibited sample, a slight decrease instead in both inhibited samples. There, thus, seems to be a real inhibition of intact seedling respiration per unit dry weight.

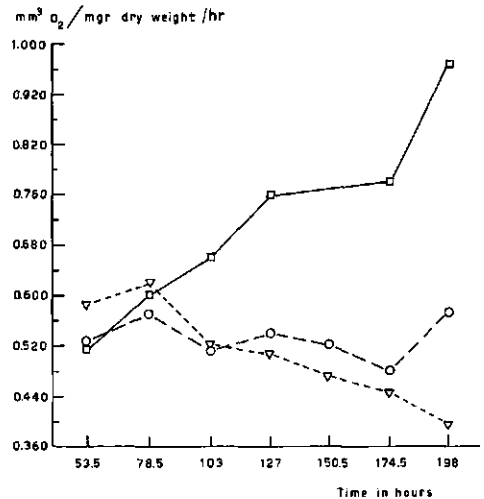


FIG. 3. The respiration rate per mg dry weight per hour of the entire seedlings (inhibited and non-inhibited) during germination (legend, see also fig. 1a).

The following analysis, however, indicates that it would be erroneous to conclude that CIPC in the concentrations applied, markedly affects the rate of cellular respiration.

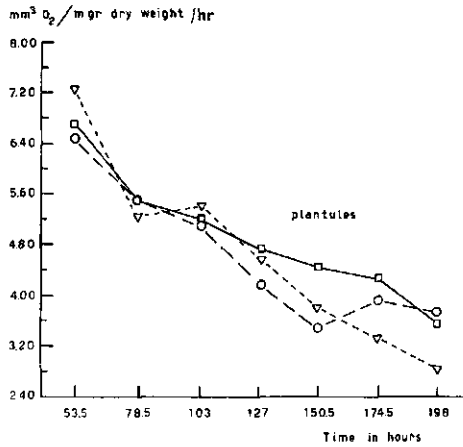


FIG. 4. The respiration rate per mg dry weight per hour of the plantules (inhibited and non-inhibited) during germination (legend, see also fig. 1a).

Figures 4 and 5 represent the time course of respiration per unit dry weight in isolated plantules and cotyledons, respectively. In the period under observation, plantule respiration rate decreases about 50% while no obvious effect of the inhibition treatments is manifest. Cotyledon respiration is about 20 times smaller, and also decreases somewhat with time. In this case no obvious effect of the inhibition is manifest either. These observations show that neither in the plantules nor in the cotyledons, CIPC in the concentrations used produces any marked effect on the rate of respiration per unit dry weight in any stage of development. Therefore, the effect observed per unit dry weight in seedlings of advanced development in figure 3 must be simulated. In figure 6, the time course of dry weight in plantules and cotyledons is represented. Cotyledon weight decreases about 20% in the period studied; there is no significant difference (owing to too large statistical errors) between inhibited and non-inhibited seedlings. Plantule dry weight increases in the same period, and manifestly more so in the non-inhibited seedlings than in the inhibited ones (*viz.*, 6 times against twice).

These observations, together with those of figures 4 and 5, contain the clue for understanding figure 3. According to figures 4 and 5, the respiration rate of the plantules – notwithstanding its decrease with time – at the end of the period still is about 20 times larger than that of the cotyledons. The amount of plantule

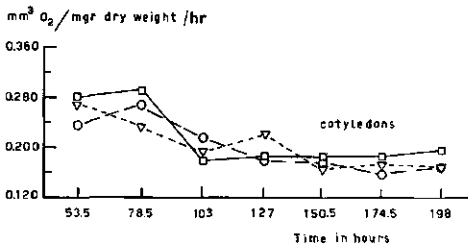


FIG. 5. The respiration rate per mg dry weight per hour of the cotyledons (inhibited and non-inhibited) during germination (legend, see also fig. 1a).

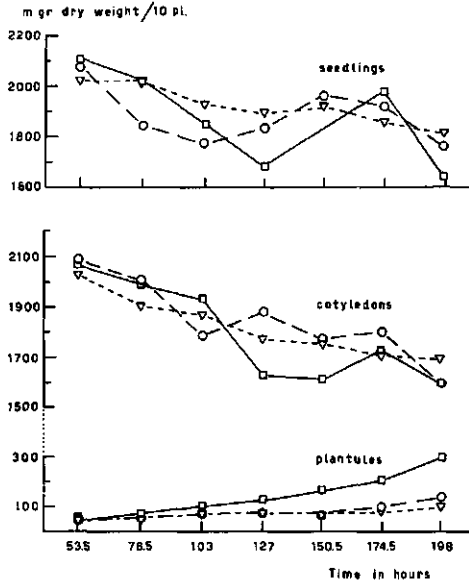


FIG. 6. The dry weight of entire seedlings (10), cotyledons (10 pairs), and plantules (10) (inhibited and non-inhibited) during germination (legend, see also fig. 1a).

tissue in the seedling, therefore, be it relatively small in relation to that of the cotyledons even at the end (see figure 6), gives an important contribution to total seedling respiration. It thus lays at hand to consider the important differences in dry weight development of the plantule, which are due to the inhibition (figure 6), as the real cause of the differences in seedling respiration rate per unit dry weight, observed towards the end of the period in figure 3. Inhibited and non-inhibited seedlings thus differ in that strongly different (but as such small) amounts of cotyledon material with a low respiration rate have served to build up plantule tissue with a high respiration rate. Whereas the differences in cotyledon material between inhibited and non-inhibited seedlings are negligible with respect to the rate of respiration, the differences in plantule material that have arisen by no means are. An estimation along this line on the basis of the data in figures 3 to 6 shows that the calculated differences in respiration rate of intact seedlings after 50 and 200 hours respectively, with and without inhibition, are in the right direction and of the right order of magnitude as compared with those in figure 3 if the difference of about 20% in intact seedlings as compared with separated plantules and cotyledons is kept in mind. In this estimation exactly the same figures for the separate respiration rates in inhibited and non-inhibited samples have been assumed.

Time	Plantule respiration (figs 6, 4)	Non inhibited		Seedling respiration per unit dry weight ($\text{cm}^3 \text{O}_2/\text{mg dry weight/hr}$)
		+	Cotyledon respiration (figs 6, 5)	
50 h.	50×6.80	+	2000×0.26	$= \frac{340 + 520}{2050} = \frac{860}{2050} = 0.42$
200 h.	300×3.60	+	1600×0.18	$= \frac{1080 + 290}{1900} = \frac{1370}{1900} = 0.72$

Inhibited

Time	Plantule respiration (figs 6, 4)	+	Cotyledon respiration (figs 6, 5)	=	Seedling respiration per unit dry weight (cmm O ₂ /mg dry weight/hr)
50 h.	50 × 6.80		2000 × 0.26		$\frac{340 + 520}{2050} = \frac{860}{2050} = 0.42$
200 h.	100 × 3.60		1600 × 0.18		$\frac{360 + 290}{1700} = \frac{650}{1700} = 0.38$
(Alternative):	100 × 3.60		1800 × 0.18		$\frac{360 + 325}{1900} = \frac{685}{1900} = 0.36$

The alternative seems more realistic with regard to the total seedling weight which should not be much less than that in the case of non-inhibition, but less realistic in view of the cotyledon data of figure 6. The ultimate difference in resulting seedling respiration, moreover, is negligible.

These calculations show that exactly the trend observed in figure 3 is reproduced: an about twofold increase over the period from 50 to 200 hours in the non-inhibited seedlings, and a slight decrease in the inhibited ones.

Thus, the marked apparent inhibition of respiration per unit dry weight in intact pea seedlings by CIPC developing during a germination period of 200 hours can be fully explained by differences in proportion of tissues with high and low respiration rates produced by the effect of the inhibitor on growth and development of the seedling.

Likewise, it is evident that the marked retardation of growth and development in the presence of the inhibitor is not due to lack of energy production by inhibition of respiration, since respiration is not inhibited. In this connection it is interesting to observe that WARBURG, already 1910, definitely states that (in sea urchin eggs) division of cells and nuclei was strongly inhibited at phenyl urethane concentrations which hardly affected respiration. It seems, therefore, that mainly its utilization in germination is inhibited. It seems likely to assume that this "uncoupling" is due to inhibition of some link apt to transfer energy from dissimilatory to assimilatory processes, probably the formation of energy-rich phosphate bonds. This might well be what IVENS and BLACKMAN (5) indicate as "some metabolic activity connected with mitosis".

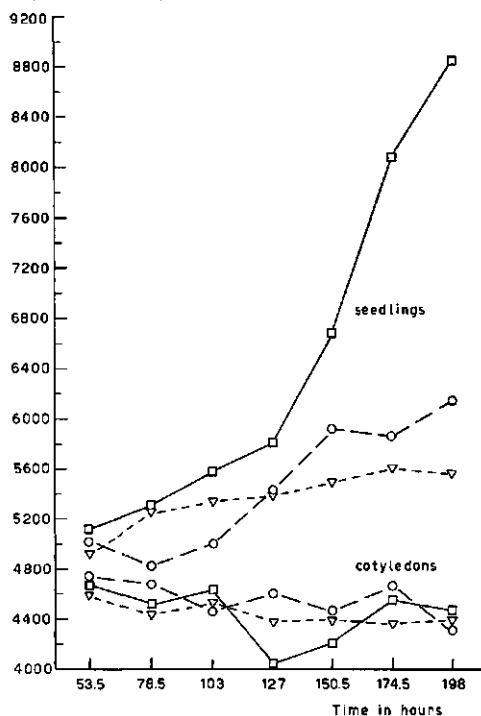
Additionally, it may be asked whether marked differences in root and shoot respiration rates exist. This has not been studied in any detail but some incidental observations tend to show that these differences are not very large.

b. Respiration and water content

It, furthermore, is of interest to consider the changes in water content taking place during germination, as far as this occurs during our experiments. The reason why this seems indicated, is apparent from figure 7 (a and b).

Figure 7b shows that the respiration of the plantules per mg fresh weight declines strongly during germination, in the same way for normal and poisoned plantules. Respiration per mg fresh weight in cotyledons shows a slight decline which, in percents, however, is quite important, and which is as well uninfluenced

m gr fresh weight / 10 pt.



m gr fresh weight / 10 pt.

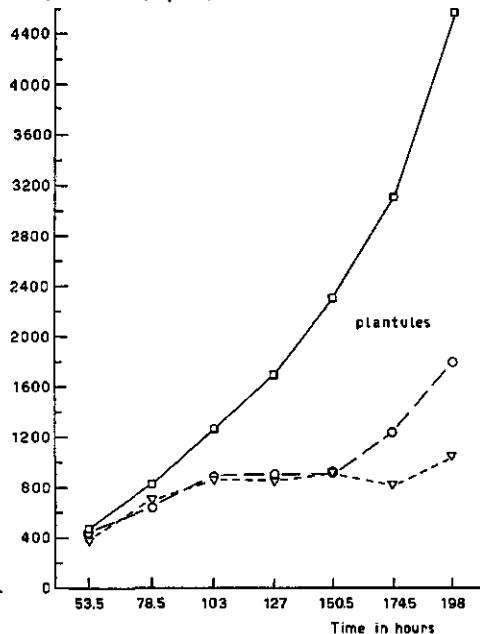


FIG. 7a. The fresh weight of entire seedlings, cotyledons (left), and plantules (right) (inhibited and non-inhibited) during germination (legend, see also fig. 1a).

$\text{mm}^3 \text{O}_2 / \text{m gr fresh weight / hr}$

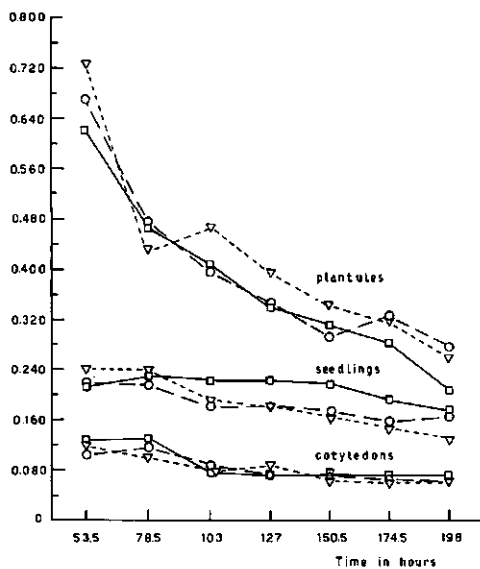


FIG. 7b. The respiration rate per mg fresh weight per hour of entire seedlings, plantules, and cotyledons (inhibited and non-inhibited) during germination (legend, see also fig. 1a).

by the poison. Respiration per mg. fresh weight of the entire seedling does not show great variation in time, and little – if some – reaction to the poison, while its values – as they should – are in between those of the plantules and the cotyledons.

Comparing figure 7b with figures 3, 4, 5, showing respiration per unit dry weight, it is obvious that the sets of curves for plantules and cotyledons are of the same type while that for the entire seedling is very different in that the respiration per mg dry matter of the non-inhibited seedling increases strongly with time. The latter observation can be understood – as has been explained in Section 4a – by the fact that, in the course of the growth period of the non-inhibited seedling, to which we will restrict ourselves for the moment, a steadily increasing amount of weakly respiring matter, of the cotyledons, becomes converted into strongly respiring matter of the plantules. This increase of the respiration per unit weight with time in the non-inhibited seedling is not – or only faintly – manifest with respect to fresh weight, as pointed out above. This fact suggests that the rate of respiration of the complete seedling is strongly coupled to the water content.

A simple overall calculation may convince of the reality of this suggestion, comparing the following data*)

1 mg fresh weight at beginning and end of experiment consumes ≈ 0.2 cmm O_2 /hr (fig. 7)

1 mg dry weight at beginning of experiment consumes ≈ 0.5 cmm O_2 /hr (fig. 3)

1 mg dry weight at end of experiment consumes ≈ 1.0 cmm O_2 /hr (fig. 3)

Thus, at the beginning:

1 mg dry weight consumes ≈ 0.5 cmm O_2 /hr

and, furthermore:

x mg dry weight + $(1-x)$ mg water consume 0.2 cmm O_2 /hr

Assuming that respiration is essentially coupled to the dry matter, *i.e.* the cell constituents, we obtain:

$$x = \frac{0.2}{0.5} = 0.4, \quad \text{and: } 1 - x = 0.6$$

Similarly, at the end:

1 mg dry weight consumes ≈ 1.0 cmm O_2 /hr

and

y mg dry weight + $(1-y)$ mg water consume 0.2 cmm O_2 /hr

which gives

$$y = \frac{0.2}{1.0} = 0.2, \quad \text{and: } 1 - y = 0.8$$

It thus appears that (beginning of the experiment):

0.4 mg dry matter + 0.6 mg water consume 0.2 cmm O_2 /hr

and (end of the experiment):

0.2 mg dry matter + 0.8 mg water consume 0.2 cmm O_2 /hr

From which it follows that:

1 mg dry matter + 1.5 mg water consume 0.5 cmm O_2 /hr

1 mg dry matter + 4.0 mg water consume 1.0 cmm O_2 /hr

Thus, in the mentioned region, increase of water content of the seedling from

*) This data is not exactly the experimental figures, yet near to these, and only serves to outline the principle.

~ 0.6 to ~ 0.8 enables the dry matter concerned to double its respiration rate. At the doubled rate, in our example, the average unit dry matter appears to be accompanied by nearly three times as much water as when showing the initial rate.

Certainly, also prior to our first observation (after 53½ h. of germination), water content of the seed, and germination rate will have increased considerably already, and a similar reasoning as given above, will obtain. This state, however, did not concern us in the present experiment.

The most obvious reason why increased water content is apt to stimulate respiration seems to be that particles, amply surrounded by water have better access to respiration substrates and oxygen than they have when they are in bigger "clusters" together.

We will now look into the actual values of the water contents recorded in the various parts during the experiment. Fig. 8 shows that, in cotyledons, plantules, and entire seedlings there is an increase in water content during germination, which is more pronounced for the non-inhibited seedlings, especially if the entire seedling is considered. Looking at the data after ~ 200 hours, those for the cotyledons vary from 61 to 65 per cent from the strongest inhibition to the normal ones, those for the plantules from 91 to 94 per cent, those for the entire seedlings from 67 to 82 per cent.

The reason why the increase in water content of the entire seedling is much more sensitive to the inhibitor than is the change in water content in both cotyledons and plantules, obviously is analogous to that in respiration. As much as respiration of the entire seedling is governed by the amount of weakly respiring material of the cotyledons converted into strongly respiring material of the plantules, so the water content of the entire seedling is governed by the amount of material of the cotyledons, with low water content, which is converted into

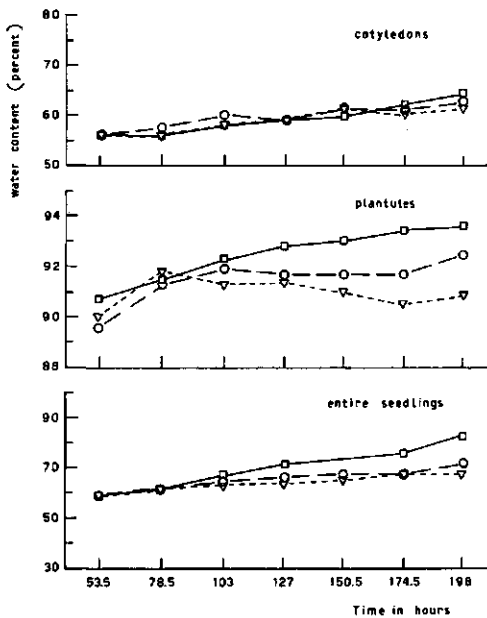


FIG. 8. The water content in percent $(1 - \frac{\text{dry weight}}{\text{fresh weight}}) \times 100$, of cotyledons, plantules, and entire seedlings (inhibited and non-inhibited) during germination (legend, see also fig. 1a). Note the different scales of the ordinate!

water-rich material of the plantules. The complete formulation of the difference between non-inhibited seedlings and inhibited ones thus appears to be: the difference in conversion during germination in the amount of weakly respiring, water-poor matter of the cotyledons into strongly respiring, water-rich material of the plantules. The mentioned conversion, as such, is the reason for the rise in respiration during germination.

It seems useful to list some of the data actually observed (non-inhibited seedlings only):

Object	Age	O ₂ -uptake/mg fresh weight/hr	O ₂ -uptake/mg dry weight/hr	Water content (%)
Plantules	53½ hrs	0.62 (fig. 7b)	6.8 (fig. 4)	91 (fig. 8)
"	198 hrs	0.20	3.6	94
Cotyledons	53½ hrs	0.12 (fig. 7b)	0.28 (fig. 5)	56 (fig. 8)
"	198 hrs	0.06	0.20	64
Total seedlings	53½ hrs	0.22 (fig. 7b)	0.52 (fig. 3)	58 (fig. 8)
"	198 hrs	0.18	0.96	82

It is evident that, in the intact seedling, the conversion of cotyledon material with about 60% water into plantule material with over 90% water is accompanied by about 20-fold initial increase in respiration rate. After 200 hrs this has resulted in an increase in water content of the total seedling to about 80%, accompanied by a twofold increase in overall respiration rate.

The picture, however, evidently is more complicated than has been assumed until now. In both plantules and cotyledons, respiration during the experiment shows a considerable decrease (per unit dry weight), accompanied by a slight, but regular increase in water content. This demonstrates the existence of conditions counteracting the enhancing effect of increase in water content upon the rate of respiration. In both cotyledons and plantules, this effect is even more obvious with respect to unit fresh weight than it is with respect to unit dry weight. It probably is difficult to understand this decrease in respiration accompanied by a slight increase in water content. The following points may be mentioned. In the plantules, further increase in weight may be increasingly due to tissues not actively contributing to respiration, e.g., cell walls, accompanied by the formation of cells with a central vacuole containing water not actively interfering with respiration. The former effect may be mainly responsible for the decrease in respiration rate per unit dry weight, the latter may further contribute to the understanding of the decrease in respiration rate per unit fresh weight. Besides this, it appears possible, that the respiratory "activity" per unit respiring material declines. For the cotyledons, the latter might be the most obvious reason for the decline in respiratory activity during the period of observation. This may be due to gradual exhaustion of respiratory substrates in the cotyledons and/or to preferent breakdown and translocation of "active" material. The obvious way for investigation of the problems referred to in this paragraph should be via correlation between the rate of respiration and some characteristic of the amount of "active" material, e.g. by way of the N-content. Unfortunately, we did not collect such data so far.

The above remarks, however, certainly do not invalidate the statement that the process of seedling growth, in its non-inhibited state, is characterized primarily by a strong increase in respiratory rate per unit dry weight, paralleled – or brought about? – by a concomittant water uptake.

This viewpoint leads to consider in some more detail the behaviour of the seedlings exposed to the herbicide.

Figure 3 has shown that the obvious increase in respiration rate per unit dry weight in the non-inhibited entire seedlings during the experiment is not manifest in the inhibited ones; there rather is a certain decline. This discrepancy is practically absent for plantules and cotyledons separately (figs. 4 and 5) as has been discussed in the previous section.

Remarkably, the above discrepancy between non-inhibited and inhibited intact seedlings is much smaller with respect to unit fresh weight (fig. 7b): The respiration rate per unit fresh weight of the entire seedlings drops from ~ 0.22 to ~ 0.18 in the intact seedlings, and from ~ 0.24 to ~ 0.13 in the highest concentration of the herbicide. This renders it obvious to look for effects of the herbicide on the changes in the overall water content during seedling development. This data is contained in fig. 8.

In the cotyledons, the water content for the highest poison concentration rises from 56 to 61%, for the non-inhibited seedlings from 56 to 64%. In the plantules this data is 90 to 91% against 91 to 94%, for the entire seedling the changes are 58 to 67%, against 58 to 82%. It is, therefore, obvious that the large deviation between inhibited and non-inhibited entire seedlings is mainly due to differences in proportion between water-poor and water-rich materials, similar to what was concluded for the differences in respiration in the preceding section. It seems, again, primarily this effect that is due to the herbicide.

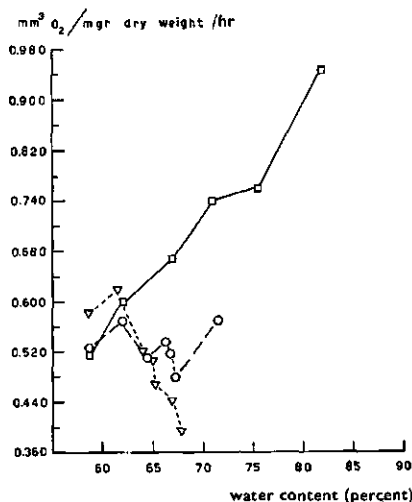


FIG. 9. The respiration rate per mg dry weight per hour of the entire seedlings (inhibited and non-inhibited) in relation to the water content in percent (legend, see also fig. 1a).

Fig. 9 shows the data of fig. 3, plotted against water content instead of time. The strong increase in respiration rate per unit dry weight with increasing water content in the intact seedling markedly deviates from the decrease in rate observed in the presence of the poison, especially at the highest concentration. (The deviation in slope beyond 150 hours, fig. 3, and beyond 65% water content, fig. 9, is due to the use of not quite comparable seedlings*). It is tempting to

*) At this time, among the seedlings selected were some which had germinated a few days later than the others, when some sterile water had been added anew to the Petri dish, so that they may in part have been in a somewhat lower average concentration of the herbicide.

explain, in analogy with the suggestion given earlier for the non-poisoned seedlings, the decrease of the respiration rate with increasing water content in the presence of the inhibitor (fig. 9) as due to increased accessibility of the tissue (for the poison) with increase in water content. This explanation requires the presence of some real inhibition of respiration. This indeed is apparent in the later stages in figs. 4 and 5, supporting the above suggestion. It should not be forgotten, however, that the main difference between non-inhibited intact seedlings and those in the presence of the poison consists in the difference in proportion between water-rich, strongly respiring material and water-poor, weakly respiring material. Consistent with the previous section, we may conclude that the main effect of the poison seems to be the uncoupling between the energy produced in respiration and the processes, inducing water uptake and promoting germination and seedling growth to proceed. These processes probably constitute a complicated biochemical chain between the initial action of the poison and its ultimate effect.

It appears tempting to interpret these results as evidence for the suggestion that the production of energy-rich compounds, like e.g., ATP, somehow is the driving force for water uptake upon which, in its turn, the progress of germination depends.

5. SUMMARY

The marked apparent inhibition of respiration per unit dry weight in intact pea seedlings by the herbicide CIPC, which develops during a germination period of 200 hours, is not primarily due to an effect of the inhibitor (in the concentrations applied) upon cellular respiration, but can be largely explained by a gradually developing difference in proportion between tissues with high respiration rate (plantule tissue) and those with low respiration rate (cotyledon tissue) produced by the effect of the inhibitor upon the rate of growth and development of the seedling.

The most likely action of the inhibitor, therefore, is uncoupling of the connection between the production of respiratory energy and its useful application in the process of germination.

Comparison of the effect of the herbicide with relation to unit fresh weight and unit dry weight reveals that increase in respiration activity per unit dry weight is closely correlated with increase in water content.

Thus, the conclusion that tissues with low respiration rate are replaced by tissues with high respiration rate during germination may be supplemented by the conclusion that the former, being low in water content, are replaced by tissues high in water content.

The most primary "useful application" of respiratory energy alluded to above which is inhibited by the herbicide may well be its promotion of water uptake or of some metabolic process which is a prerequisite for water uptake.

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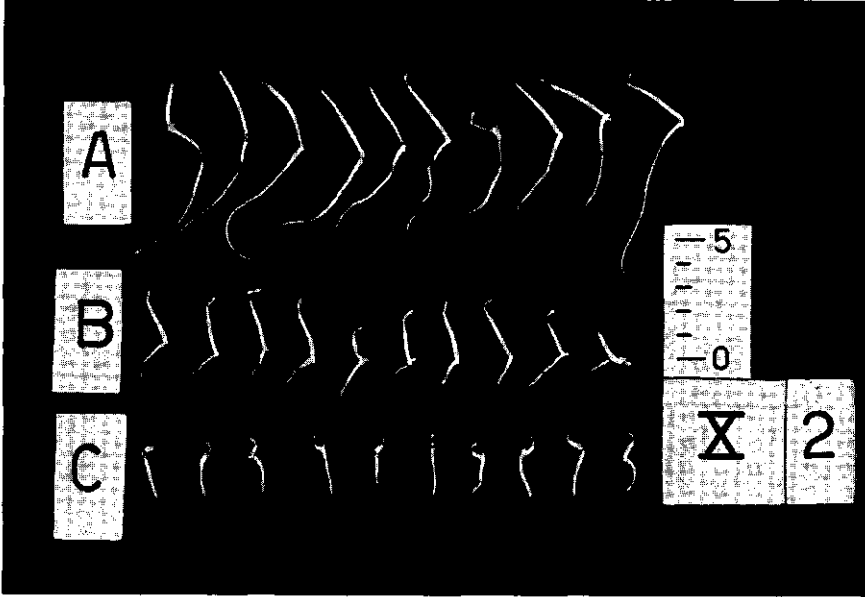


PLATE 1. Pea plantules germinated as intact seedlings (with cotyledons), without seedcoats, in the presence of 0 (A), 8.6 (B), and 68.5 (C) ppm CIPC respectively. Photograph taken after oxygen uptake of the plantules had been measured, 115 hours after the beginning of germination. Experiment X2, scale 0-5, in cm.