Zeitschrift: Berichte der Schweizerischen Botanischen Gesellschaft = Bulletin de la Société Botanique Suisse

Herausgeber: Schweizerische Botanische Gesellschaft

Band: 74 (1964)

Artikel: Experiments with Helianthus annuus hypocotyls on IAA-C14 transport in relation with temperature

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DOI: https://doi.org/10.5169/seals-52029

Experiments with Helianthus annuus hypocotyls on IAA-C^{14} transport in relation with temperature

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Manuscript received on July 30, 1964

The first observations on auxin transport in relation to temperature were reported by van der Weij (1932). His conclusion, still reported in textbooks, can be summarized as "the rate is independent of temperature and the amount of auxin transported for unit time does depend in temperature". Van der Weij based his results on the determination of the amount of auxin from the donor agar block, passing through coleoptile sections of fixed lengths to a receptor agar block, in fixed time intervals. Went and White (1939) published further supporting observations.

Recently, Gregory and Hancock (1955), using the same technique, studied the transport of auxin in relation to temperature in woody plants, completing their experiments with apple shoots. These authors stated that "the conclusion of van der Weij that temperature is without effect in the rate of auxin transport is not well founded". Their experiments showed that "temperature markedly affects both rate of transport and amount transported".

However, Pilet (1951) observed that increase in temperature had a stimulating effect on basipetal transport of auxin, though it had no such effect on either acropetal or lateral transport of auxin.

The contradictory results reported indicate that no satisfactory explanation of auxin transport in relation to temperature has yet been reached. We have therefore followed the transport of radio-active IAA applied to the cotyledons through *Helianthus* hypocotyls at various temperatures in order to observe transport in relation to temperature in the case of intact dicotyledoneous seedlings.

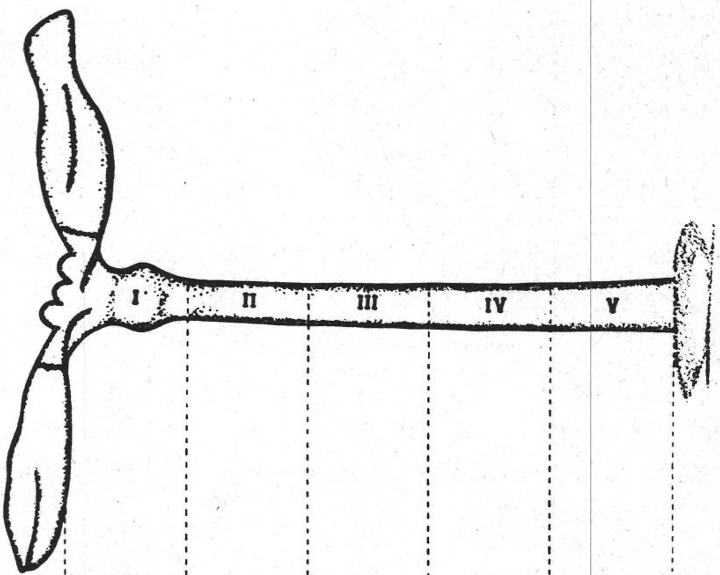
Materials and methods

Helianthus annuus hypocotyls were selected for the work, grown as previously described by Vardar (1959–1962). From the seedlings uniform plants were chosen and placed in a constant temperature chamber (EMI

Electronics Ltd. Type RA 607 modified by us) and allowed to equilibrate overnight. To the temperature stabilized hypocotyls, IAA-C^{14} was applied to the cotyledons as described by Vardar (1959–1962). After being kept for 1.5 or 3 h in vertical position, 1 cm sections were prepared and separately ground for determination of C^{14} content. Counting was done in a Nuclear Chicago M5 gas flow counter. The radioactive auxin (Indol-3-Acetic acid-α-C^{14}) was supplied by Waltham Tracer Laboratory; specific activity 1.1 mc/mM, concentration used 0.11 mg/ml; amount applied per plant 2.2 μg with an activity of 12,000 cpm as recorded under the standard counting conditions.

Experiments

Hypocotyls were grown for six days in a greenhouse under normal lighting conditions and those of 45 mm length selected for the obser-

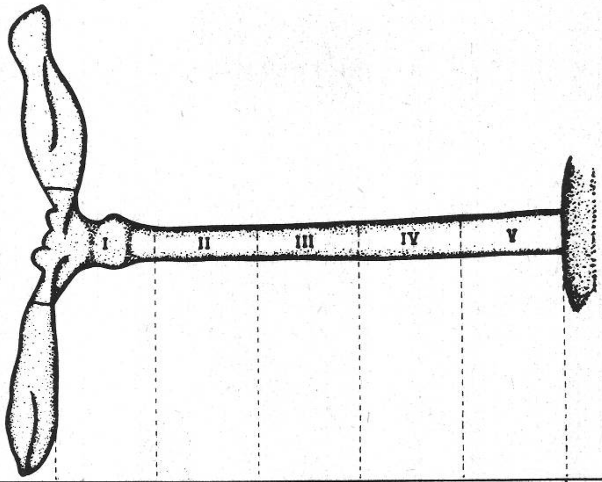


Fixed temperature	Exposure time	Radio activity measured in different segments, cpm / segment				
		Ist section	IInd section	IIIrd section	IVth section	Vth section
15 °C	1½ h	1105.22	21.61	11.67	7.09	4.47
	3 h	1292.73	84.60	40.20	38.00	36.57
25 °C	1½ h	1155.45	66.86	45.55	25.44	18.14
	3 h	1253.39	133.67	55.53	46.57	37.09
35 °C	1½ h	1404.89	94.05	60.93	35.34	20.37
	3 h	1024.13	272.15	69.11	44.79	31.63

Table 1

Comparison of radio activity measured in different segments of *Helianthus annuus* hypocotyls. (In each case the experiments were repeated 20 times and each planchette counted for 25 minutes) vations. The selected plants were placed in a constant temperature chamber in the vertical position and allowed to equilibrate overnight; three fixed experimental temperatures were used, 15 °C, 25 °C and 35 °C. On each, 100 μl IAA-C^{14} solution were applied (50 μl to each cotyledon) the following morning as a droplet along a line border made about 0.5 cm above the base end of each cotyledon, as previously described by Vardar (1959–1962).

After periods of 1.5 or 3 h at constant temperature the portion of each cotyledon above the line of treatment with IAA-C^{14} was excised and the remaining portion of stem sectioned into 5 lengths each 1 cm long. Each section was ground and mounted on a planchette for counting. Counting was performed on a Nuclear Chicago gas flow windowless counter. Results are shown in Table 1.



Fixed temperature	Exposure time	Relative activity in different segments of hypocotyl					Total activity found in hypocotyl
		Ist section	IInd section	IIIrd section	IVth section	Vth section	
15 °C	1 ½ h	92.1	2.0	1.0	0.6	0.4	96.1
	3 h	107.7	7.0	3.0	3.0	3.0	123.7
25 °C	1 ½ h	96.0	5.5	3.8	2.0	1.5	108.8
	3 h	104.4	11.1	4.6	3.9	3.0	127.0
35 °C	1 ½ h	117.0	7.8	5.0	2.9	1.7	134.4
	3 h	85.3	22.6	5.7	3.7	2.6	119.9

Table 2

Relative amounts of the transported IAA-C^{14} found in the different levels of *Helianthus* hypocotyl at three fixed temperatures and two different exposure times. (In each case the experiments were repeated 20 times and each planchette counted for 25 minutes)

Note: All figures are adjusted to a calculated amount of 1000 units applied to the plant, individual figures per section being fractions of this total

Thus we have made a comparison of the transport of IAA-C^{14} applied on the cotyledons in hypocotyls kept under different temperature conditions for various time intervals. Assuming the amount of radioactivity applied to be 1000 units, regional calculations were made and the relative amount of activity for different levels of the hypocotyls were determined (Table 2).

Conclusion

Comparison of the relative amounts of radioactivity (Table 2) which correspond to transported IAA-C^{14} determined in succeeding layers of hypocotyls subject to three different temperature conditions and two different times, and especially comparison of the total radioactivity (Figure 1) found in the hypocotyls show that temperature affects the transport capacity of IAA-C^{14}.

It will be seen that with increasing temperature the transport of IAA-C^{14} increases at least to an optimum temperature of 25 °C. These

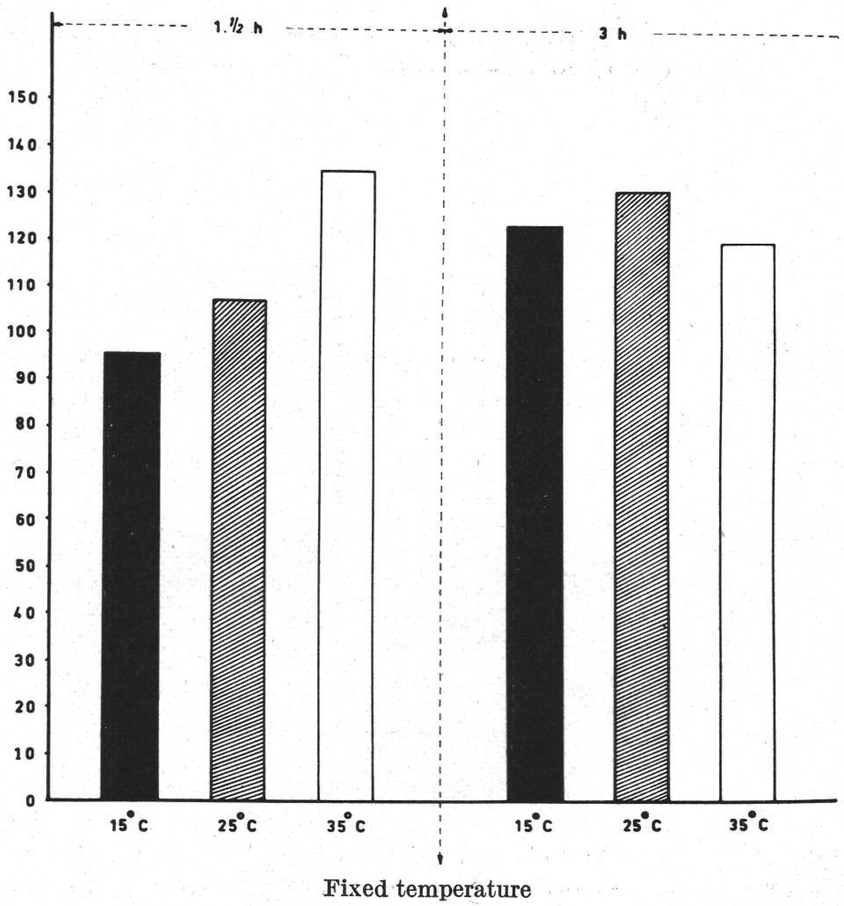


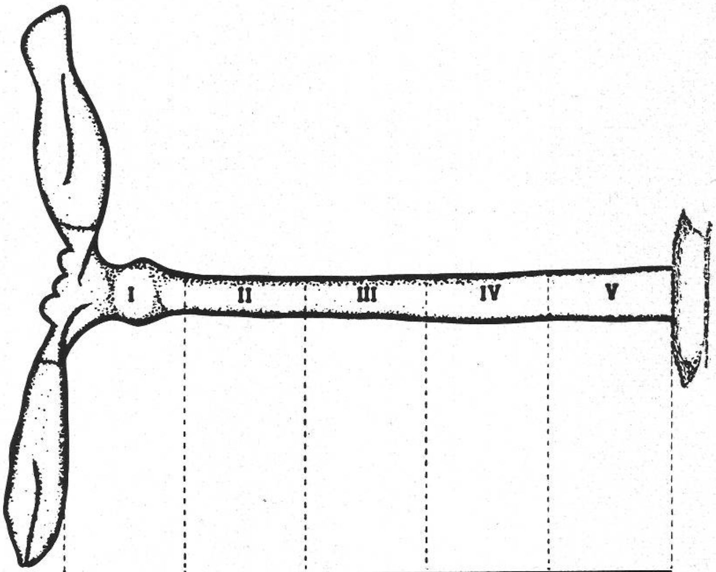
Figure 1

Histogram of radioactivity found in hypocotyls at different temperatures results are in agreement with the results of van der Weij (1932) on coleoptiles and Gregory and Hancock (1955) on woody plants.

In this case, the temperature dependence of auxin transport capacity has been shown for young intact dicotyledoneous seedlings by a simple and sensitive radioactive method.

In order to compare the effect of temperature on the rate of transport, Table 3 has been calculated on the basis of the percentage transported to different distances assuming total transport to be 100%.

These values show clearly that the rate of transport of IAA-C^{14} varies with variation in present temperature.



Fixed temperature	Exposure time	Percentage of activity in different segments of hypocotyl				
		I^st section	II^nd section	III^rd section	IV^th section	V^th section
15 °C	1½ h	95.8%	2.1%	1.0%	0.6%	0.4%
	3 h	87.1%	5.7%	2.4%	2.4%	2.4%
25 °C	1½ h	88.2%	5.0%	3.5%	1.8%	1.4%
	3 h	81.8%	8.7%	3.6%	3.1%	2.4%
35 °C	1½ h	87.0%	5.8%	3.7 %	2.1%	1.3%
	3 h	71.1%	18.8%	4.8%	3.1%	2.2%

Table 3

Comparison of activity found in the different segments expressed as percentage of the total activity found in hypocotyls

Note: In calculating the value for each case the total activity found in hypcotyl was accepted as 100 and the percentage of activity calculated for different sections

These results on intact young dicotyledoneous seedlings are in agreement with those of Gregory and Hancock (1955) obtained for woody plants and do not agree with the conclusion of van der Weij (1932). Thus 87.1 % of the activity was found in the first section of hypocotyl at 15 °C for three hours and only 5.7 % for the second section, while at 35 °C the values were 71.1 % and 18.8 % respectively. This shows that the transport rate is greater at 35 °C than at 15 °C. Kaldewey (1963) observed the same relation in the peduncles of ***Fritillaria.***

Inspection of the results for the other sections is consistent with the above conclusion. The results are plotted in Figure 2.

Here the interesting and the unexpected point is the fact that very low but significant radioactivity has been noted even in the fifth section of the hypocotyl in such a short period as 1.5 h. This cannot be explained as

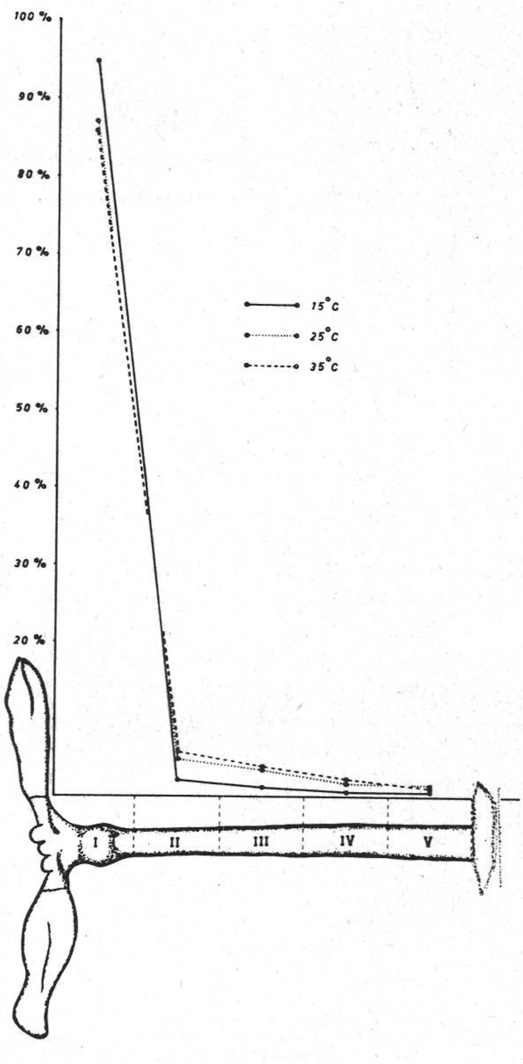


Figure 2

Graphic comparison of transported IAA-C^{14} in different segments a normal auxin transport. This case was recently discussed personally with Dr. Kaldewey. It is probable that the transport of even such a small quantity of IAA-C^{14} which moves very rapidly is different from the known normal auxin transport. I believe this problem deserves further investigation. On the other hand comparison of the results at 35 °C with those at the lower temperatures shows that an optimum temperature effect (Figure 1) is obtained at 25 °C in agreement with the results of Gregory and Hancock (1955).

Acknowledgement

The author wishes to express his appreciation to CENTO and to NATO for the technical assistance and for the equipment that made this research possible.

Summary

The contradictory results reported indicate that no satisfactory explanation of auxin transport in relation to temperature has yet been reached. We have therefore followed the transport of radio-active IAA applied to the cotyledons through *Helianthus* hypocotyls at various temperatures in order to observe transport in relation to temperature in the case of intact dicotyledoneous seedlings.

The results obtained indicated that both the rate and the amount of transport of IAA-C^{14} are dependent on the temperature in the case of intact dicotyledoneous seedlings.

Zusammenfassung

Der Transport C^{14}-markierter Indolylessigsäure in intakten Keimlingen von *Helianthus annuus* ist in Intensität und Geschwindigkeit von der Temperatur abhängig. Die Werte für 25 °C und 35 °C sind deutlich höher als diejenigen für 15 °C.

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