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BOTANY

THE INFLUENCE OF PHOTOPERIOD, DORMANCY BREAKING AND GROWTH HORMONE TREATMENT ON POPLAR CUTTINGS

BY

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§ 1. *Introduction*

The first author was confronted with the problem of stimulating cuttings from the Southern hemisphere at the end of the growing season of the parent trees into immediate growth on their arrival in The Netherlands. By the end of the dutch spring, on the longest day of the year, the cuttings should already have rooted and expanded buds. At the same time the parent trees in South Africa would be in a condition of deep rest.

It was obvious that some preliminary research would be necessary for such results to be secured [5], part of which is shortly forthcoming [6], part of which will be reported on here.

The part that is forthcoming will deal with the winterrest problem and the stimulation of the opening of the buds. The problems dealt with here are: (i) further research on the shooting ability of the leaf buds, and (ii) the stimulation of root formation. The experiments have been carried out in the early spring, February and March 1952, with cuttings from poplars, grown in The Netherlands. The cuttings used in the present paper differed from the cuttings, that were to be expected two months later from South Africa in that they were in the state of afterrest. The South African cuttings would be in a state of preliminary rest or perhaps of beginning middle rest.

Due to her situation between 24° and 34° southern latitude day length in South Africa nowhere is very long. As the South African cuttings would come from rather short-day parent trees the present investigations have been partly carried out with cuttings from *Populus robusta* SCHNEIDER trees that from May 1951 until autumn were grown in an artificially shortened day. These trees henceforth are called short-day parent trees. Experiments were also run with cuttings from the same species grown in the normal day length of The Netherlands; they are called long-day parent tree cuttings. Supplementary work has been performed with long-day parent tree material of *Populus serotina* HARTIG. For details with regard to the treatment of parent trees cf. REINDERS-GOUWENTAK [6].

§ 2. *Methods*

Two-year-old branches were used for cuttings. The cuttings were 12 cm in length. About half of the cuttings in each group had no outwardly visible buds: they are called budless cuttings. Each group held 18–25 cuttings, one half of which was destined to be taken out for root control after 14 days.

It had appeared from work already cited (6) that our *Populus robusta* strain as regards vegetative growth is essentially a long-day plant. At the time the experiments described here were carried out, i.e. in February and March, the natural day length was not yet 12 hours. So with regard to light, it seemed appropriate to lengthen the day with artificial light at any case in some of the groups. This was essential as the South African cuttings later in May would have to be exposed to the long natural day of The Netherlands even if in their own country they had been grown in short-day conditions. The 4 different day lengths chosen are: extremely-short, a short-day and two kinds of 16 hour-days.

Extremely-short (group III) was given for 14 consecutive days as complete darkness and for the rest of the experimental duration substituted by the same long day as in group IV. For a short-day the cuttings were exposed to the normal winterday light (group II). The long-day of 16 hours was supplied by exposing sets to normal winterdaylight and lengthening the day from 6.00h–8.30h and from 17.30h (or 18h from 20th February on)–22.00h with incandescent light of 25 watt lamps (group I) or with TL tubes (group IV) at a height of 40 cm over the top of the cuttings.

Further, in three of the groups (I, II, III) cuttings have been treated in 4 different ways: (*a*) exposed to a hot water treatment for dormancy breaking and afterwards treated for root stimulation with growth hormone, (*b*) hot water treatment, but no growth hormone application, (*c*) growth hormone application without rest breaking treatment, (*d*) control (no rest breaking, no growth hormone). In group IV because of shortage of material only treatments (*a*) and (*c*) could be given.

As the budless cuttings within the duration of the experiments did not shoot from sheathed or regenerated buds, they clearly show that rapid shooting can not be obtained without the presence of lateral buds. These cuttings therefore have been cancelled in the experiments on shooting. In the rooting experiments they have been included.

For rest breaking purposes we used MOLISCH's hot water method and immersed the cuttings for 1 minute in water of 50° C (subgroups *a* and *b*).

Those cuttings which received the hormone treatment (subgroups *a* and *c*) have been soaked at the basal ends for 12 hours in a water solution of 100 p.p.m. sodium salt of heteroauxin, each subgroup of 12 or 13 cuttings in a glass that contained 75 cm^3 of the solution.

The experiments started on February 5th with the planting of the cuttings, in a rooting medium of one part of peat and two parts of sand, those which got the extremely-short-day in a darkened space in the greenhouse at a relative humidity of 100 % or in a darkroom with a temperature of 20–22° C and a relative humidity of 75 %, the other ones in the ordinary greenhouse at a temperature of about 18° C and a relative humidity, which of course daily varied between 90–75 % and 50 %.

§ 3. *Experiments with cuttings from Populus robusta short-day* (9 *hour-day*) *parent trees*

The results set out in Table I clearly show the beneficial effect of 14 days of darkness (group III) for shooting compared with any of the kinds of light or day lengths tried (group I, II, IV). Group III had grown in complete darkness in the greenhouse (100 % relative humidity) except for a short time daily when the box was opened for care and control purposes. In group III 24 of the 42 cuttings shooted, i.e. 57 % as against not yet 10 % in the light-groups.

TABLE I

Number of cuttings of Populus robusta from short-day parent trees that shooted within 14 days after treatment. Figures between brackets indicate the total number of cuttings with buds present

Rest breaking treatment Growth hormone treatment	yes yes } *a*	yes no } *b*	no yes } *c*	no no } *d*
III "darkness"	9 (11)	8 (13)	3 (9)	4 (9)
I long day (with incand. light)	0 (11)	0 (12)	0 (9)	0 (10)
II short day	1 (7)	1 (10)	0 (10)	0 (10)
IV long day (with TL light)	2 (9)	—	0 (10)	—

Further, it appears from Table I that the rest breaking treatment is very important. This is shown by the subgroups *a* and *b* of group III which as compared with the corresponding subgroups *c* and *d* shooted much better. It is also shown in the groups I, II and IV since the sole cuttings which opened buds are found in the subgroups where dormancy has been broken.

Group III after those first 14 dark days has been placed in natural day the length of which has been brought up to 16 hours with TL lamps. The small shoots, etiolated before, within a few days became green and developed into normal shoots.

Virtually all cuttings after the second 14 days of growth had opened the buds or bore shoots. Yet, subgroups III *a* and III *b* in their further development had retained the advantage they had secured in the 14 days of darkness as was shown by the greater length of the shoots.

The root formation in the various groups is shown in Table II. The figures set out are average numbers of roots per cutting of groups dug up after 14 days and of other groups after 28 days. The cuttings that had been dug up after the first period of 14 days have been replanted and checked again at the end of the second period but as the root number in some of these cuttings was less than at the end of the first period, these figures have not been recorded. Root numbers for cuttings with buds opened and those for budless cuttings or with buds not yet opened are given separately.

From Table II it appears that there also is a beneficial effect of 14 days of darkness on root formation as rooting at the end of that period in group III with 2,9, 2,8, 2,5 and 1,6 in the cuttings with buds opened is superior over the corresponding subgroups of I, II or IV and the same is the case with the budless cuttings.

The beneficial effect of 14 days of darkness (in the greenhouse, relative humidity 100 %) is still present after 28 days when for the second period

TABLE II

Average number of roots of Populus robusta cuttings from short-day parent trees with buds and without buds. For legends of roman figures consult Table I

	After 14 days							
Hot water treatment. .	yes } a		yes } b		no } c		no } d	
Growth hormone ,,	yes		no		yes		no	
	buds		buds		buds		buds	
	+	—	+	—	+	—	+	—
III	2,9	2,8	2,8	4,3	2,5	3,4	1,6	2,3
I	1,2	0,4	0,5	0,5	0,2	1,4	0,0	1,1
II	0,3	0,6	1,2	0,7	0,0	0,3	0,6	1,0
IV	0,3	0,9	—	—	1,2	1,1	—	—
	After 28 days							
Hot water treatment. .	yes } a_1		yes } b_1		no } c_1		no } d_1	
Growth hormone ,,	yes		no		yes		no	
	buds		buds		buds		buds	
	+	—	+	—	+	—	+	—
III	5,2	4,1	3,9	4,0	9,6	4,4	2,6	4,7
I	2,0	1,5	1,8	2,7	2,3	2,3	1,2	0,6
II	2,3	1,8	0,5	2,1	1,8	1,4	0,0	1,2
IV	4,8	2,0	—	—	1,6	1,1	—	—

of 14 days the cuttings have been grown in a 16-hour day composed of 10½ hours of winter daylight and 5½ hours of TL light. Subgroup IV *a* with 4,8 in the series with buds seems to be an exception, but the figure is such high because of one cutting with 13 roots whereas the other ones shew only 2–4 roots.

From the foregoing it is evident that for stimulation of root formation group III is also the best one, i.e. the group which got 14 days of darkness followed by a period of 14 days in a 16-hour daylight condition. For this long day the natural day was lengthened with TL light, no other light sources were tried nor shorter day lengths applied. This is because of the results of our previous, shortly forthcoming work [6]. Besides, the South African cuttings this research was undertaken for would have to grow in the long summerdays of our country and so the sole question

TABLE III

Group	19 February 1952				4 March 1952			
	a	b	c	d	a_1	b_1	c_1	d_1
III	10 of 10	11 of 11	12 of 12	10 of 12	12 of 12	11 of 12	11 of 12	12 of 12
I	4 of 13	5 of 12	6 of 13	3 of 12	9 of 12	10 of 12	10 of 12	6 of 12
II	6 of 13	6 of 12	2 of 13	5 of 12	10 of 12	8 of 12	11 of 12	7 of 12
IV	6 of 12	—	7 of 12	—	11 of 12	—	9 of 12	—

with regard to daylight presented here was whether good growth in long day conditions could be expected.

How much ahead rooting is in group III is also shown if the numbers of rooted cuttings are compared, see Table III.

In group III 80 %–100 % of the cuttings had already rooted on February 19th, whereas in the other groups only 25 %–50 % had formed roots. At the end of the experiment (March 4th) the rooting percentage still is better in group III, but the difference has become less as the rooting response in III now varies from 90 %–100 % and in the other groups from 50 %–90 %.

As to the results of the various treatments *a*, *b* etc., it may suffice to consider only the results within group III. Since there is no significant difference between the subgroups at the end of the first 14 days, the results after 28 days will be the only ones discussed.

The budless cuttings of group III (Table II) after 28 days with 4,1 and 4,4 or 4,0 and 4,7 roots per cutting in the various subgroups do not show any effect of the rest breaking treatment. This can not be explained by a supposition that budless shoots do not show dormancy since this hypothesis has been refuted by work on cambial activity in budless shoots [1, 2]. It might be due to the rather large variation in rooting response of the cuttings.

In the cuttings-with-buds a rest breaking effect seems to be present at least between subgroup *c* and *a* with 9,6 and 5,2, but in a negative sense. If the difference 4,4 $\pm$ 2,5 between the two is a statistically reliable one, which for the moment we shall assume, there may be concluded that an artificial rest breaking treatment although beneficial for dormancy breaking in buds is not beneficial for root formation. Further there may be concluded that it is not the rest breaking process itself but the *artificial* rest breaking which impedes root formation. For, as is proved by the spontaneous opening of the buds, dormancy in subgroup *c*-with-buds has been broken by natural means. Yet, this subgroup shows the high root number 9,6.

As to the influence of growth hormone treatment (Table II, group III), there is a difference between treatment and no-treatment but again only in those cuttings the buds of which had shooted in subgroup *c* and *d* and perhaps also in subgroup *a* and *b*.

That such a difference has not been found with the budless cuttings might be due to various causes. One of these is the variation question already referred to. Other possibilities will not be discussed as being fruitless until more research has brought further elucidation of the rooting problem in Populus.

The experiments reported on in this paragraph show that for cuttings from short-day parent trees in the period of afterrest shooting and rooting response are best after a first period of growth in darkness, followed by a long day of 16 hours (of natural winter daylight supplemented with

TL light in the early morning and after sunset). If root formation and bud development are considered apart from each other, breaking of dormancy would be favourable for the latter process only. However, since root formation in subgroup a_1 is satisfactory and bud development with no rest broken subgroup c_1 is bad, for the promotion of shooting and rooting breaking of dormancy and growth hormone treatment can be recommended.

§ 4. *Rooting response of Populus serotina at relative humidity* 100 % *and* 75 %

It has already been mentioned that group III for the first 14 days had grown in a blacked out part of the greenhouse where relative humidity was 100 %, whereas the relative humidity in the light parts of the greenhouse was kept as good as possible at 75 %. So the better response of group III could have been due to relative humidity (e) rather than to darkness. This, however, was not the case as was seen with cuttings of *Populus serotina*. Cuttings of this species, used because robusta material was no more available, have been grown in 75 % e in a darkroom and in 100 % e in the darkened space of the greenhouse. Dormancy has been broken and growth hormone treatment applied. The experiment lasted 14 days.

In 75 % e an average number of 7,1 roots was counted, in 100 % e there were not only subterranean roots formed but besides these there arose some roots in the air 5,2 and 2,0 respectively which brought the total number at 7,2. So, no difference in total rooting response was found and the superiority of group III over the light-groups can be attributed to the factor darkness.

It may be noted that the number of roots which is 7 in this experiment is higher than the number of roots found with the short-day parent tree cuttings of *P. robusta*. This is not due to a species difference, but to the fact that the *P. serotina* parent tree had grown in natural day length and thus is a long-day parent tree. This is proved by the experiments with *P. robusta* cuttings of long-day parent trees which will be discussed in the next paragraph.

§ 5. *Experiments with Populus robusta cuttings from long-day parent trees*

The data in Table IV to the left show the shooting response after 11 days of growth with the various photoperiods, after a treatment for dormancy breaking and root stimulation. The figures between brackets indicate the total number of cuttings present with-a-bud. To the right are inserted the data for the corresponding subgroup from the short-day parent trees of Table I.

From Table IV it appears that there is no difference in shooting response between cuttings from short-day parent trees and those from long-day parent trees, shooting in both being favoured by 11 days of darkness (group III).

TABLE IV

For legends see the text

Subgroup a	Number of shooted cuttings from	
	long-day parent trees	short-day parent trees
III "darkness"	9 (12)	9 (11)
I long day (with incand. light) . . .	2 (8)	0 (11)
II short day	1 (9)	1 (7)
IV long day (with TL light)	2 (10)	2 (9)

As in the experiments with the short-day parent tree cuttings group III after the period of nearly a fortnight of darkness has been afterwards brought in the long-day-conditions-with-TL-light. Its cuttings developed normally.

The rooting ability of these cuttings in number of roots and in percentage of rooting is set out in Table V together with the rooting response after 27 days. The cuttings have been treated for dormancy breaking and for root stimulation on February 21st and allowed to grow for 27 days thus corresponding with the a_1 subgroups of Table II. Of group III a set of cuttings could also be grown for 11 days, this set thus being comparable to III a of Table II. There was no material available to check the separate effect of dormancy breaking and growth hormone on the long-day parent tree cuttings.

TABLE V

Average number of roots per cutting and percentage of cuttings rooted, after 11 and after 27 days, of cuttings taken from long-day parent trees of Populus robusta. Averages of 8–12 cuttings

Time of checking	11 days		27 days		11 days	27 days
Hot water treatment . . . . . . Growth hormone treatment . . .	yes yes } a		yes yes } a_1		Percentage of cuttings rooted	
	buds		buds		buds	buds
	+	–	+	–	+ and –	+ and –
Group III	7,0	6,2	16,7	8,6	100	100
Group I	–	–	15,6	7,1	–	100
Group II	–	–	12,9	7,5	–	100
Group IV	–	–	19,0	9,5	–	100

There are several differences between the rooting response of cuttings from the short-day parent trees of Table II and III, and those from the long-day parent trees in Table V. Of the latter ones 100 % of the cuttings of all subgroups a_1 had rooted after 27 days whereas in the former ones this was only so in group III. Furthermore, rooting in all of the groups and subgroups is much stronger than in the corresponding ones in Table II.

The main difference, however, is that now rooting is best in group IV, whereas in the short-day parent tree cuttings the best rooting response was in group III.

So in cuttings of long-day parent trees the best shooting response is shown after a first period of growth in darkness, whereas the best rooting response is in the long day of 16-hours composed of natural daylight with a supplementing illumination of TL light in the early morning and after sunset. Since, however, shooting in this long day is insufficient, the photoperiodic treatment of group III is recommended for vegetative propagation. As no work with various combinations of growth hormone application and rest breaking treatment (subgroup *b, c, d*) has been done information is not as complete as for short-day parent tree cuttings.

§ 6. *Discussion*

Populus robusta, essentially a long-day plant in The Netherlands, when grown in different day length conditions does not only show the well known photoperiodic response with regard to growth in length of the shoots, termination of seasonal growth and leaf fall, but she also shows a photoperiodic response with regard to root formation and bud development of the cuttings.

The long-day parent tree cuttings in our experiments, in all kinds of light conditions tried, inclusive of darkness, in the period of afterrest rooted better — in all of them for 100 % — than did the cuttings from short-day parent trees. The latter, however, rooted for 100 % in some of the light conditions only, in others for 90 % or less.

MOSHKOV and KOCHERZHENKO [4] already realized the importance of the photoperiodic treatment of the parent plants but found the relation between the rooting ability of the cuttings and the photoperiodic treatment of the parent plants essentially determined by the transition to dormancy. For the short-day parent tree cuttings or the long-day ones the dormant period was found at different times of the year and the rooting percentage, then, was greatly reduced. After that period the rooting percentage in both kinds of cuttings if they were grown in continuous light again amounted to 100 % and so the authors failed to observe a different photoperiodic behaviour of the cuttings.

We in our experiments got the best results for both types of cuttings in different light conditions, long days for cuttings from long-day parent trees and a period of darkness for those from short-day parent trees.

The different results may be due to the fact that MOSHKOV only reports on the percentage of rooted cuttings but does not mention the number of roots per cutting. These data, as follows from our work, are not correlated. The rooting percentage (Table III) may be 100 %, while the number of roots per cuttings is small (Table II).

Since the cuttings of the long-day parent trees rooted nearly as well in a 16 hour photoperiod of winter day-light supplemented with incandescent light of low intensity as in the short photoperiod of the normal winterday, *Populus robusta* cuttings of long-day parent trees with regard to rooting response may be called day neutral cuttings but as regards number of roots there is a preference for long day.

The cuttings of short-day parent trees which are rooting badly in long days or in the short winterday of about 10 hours and prefer a period of darkness before being exposed to light are day neutral but as to number of roots they are extremely short-day plants.

The long-day parent tree cuttings, in our experiments, rooted best in long day conditions (Table V) but the difference with the "darkness" group was only small. So, from a practical point of view, it can be recommended to try first of all the effect of — the inexpensive — darkness (see our group III) for vegetative propagation of *Populus* species.

The influence of rest breaking and of growth hormone application in the 4 combinations possible has been examined for the short-day parent tree cuttings. It is shown that in our experiments the rest breaking treatment — a hot bath of short duration — and subsequent growth hormone treatment did not give such satisfactory results as the growth hormone treatment alone if the number of roots in the darkness-group of Table II is considered. This same fact has been found by GUMPELMAYER [3] for cuttings of long-day parent trees.

However, in vegetative propagation problems, there is not only the rooting response to be considered but also the opening of the buds and their development into shoots.

In both long-day and short-day parent tree cuttings shooting was strongly promoted in group III i.e. the group in which cuttings for the first 14 days have been grown in complete darkness prior to the long day conditions. Within group III there always was a definite beneficial effect of the rest breaking treatment. GUMPELMAYER in the same state of afterrest reports an inhibiting effect on bud development. This author, however, grew the cuttings in the short day of late winter and as we have been able to show (Table IV) the effect of the short late winter day is poor. The inhibiting effect of the rest breaking treatment stated by GUMPELMAYER presumably is due to unfavourable photoperiodic conditions.

If, as in propagation studies, rooting and shooting have to be considered simultaneously, from a practical point of view the best procedure will be a compromise between the results obtained for the two processes. Cuttings of *Populus robusta* in the period of afterrest may be best grown by treatment for dormancy breaking and growth hormone application followed by 14 days of darkness prior to planting in the propagation frame and, if days are still too short, appropriate lengthening of the day with light of rather high intensity.

Summary

In cuttings from parent trees of *Populus robusta* which trees were grown

in artificial short-days, dormancy breaking in the period of afterrest is beneficial for bud development. Root formation is strongly promoted by soaking in a growth hormone solution and slightly impeded by rest breaking treatment. As in cuttings the two processes have to be promoted both dormancy breaking and growth hormone treatment are advisable. The best photoperiodic treatment of the cuttings is growth in darkness for about two weeks and subsequent growth in long days, as this treatment promoted both root formation and bud development more than any of the light conditions.

The same procedure can be advised for cuttings from the long-day parent trees in this species but here the largest number of roots was found in the long day.

Root formation in number of roots per cutting in the long-day parent trees was always better than in the short-day trees in the same conditions.

Populus robusta cuttings from long-day parent trees with regard to rooting response have been found to be day neutral but with preference for long day, as in this light condition the largest number of roots is formed. For the same reason those from short-day parent trees are day neutral with preference for the extremely short day.

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LITERATURE

1. Gouwentak, C. A., Proc. Ned. Akad. v. Wet., Amsterdam **44**, 654–663 (1941).
2. ——— and A. L. Maas, Med. Landbouwhogeschool Wageningen **44**, Verh. 1, 3–16 (1940).
3. Gumpelmayer, E., Phyton 1, 154–169 (1949).
4. Moshkov, B. S. and I. E. Kocherzhenko, Comptes Rend. Acad. Sc. URSS **24**, 392–395 (1939).
5. Reinders–Gouwentak, C. A., Proc. Kon. Ned. Akad. v. Wet., Amsterdam **C 56**, 66–70 (1953).
6. ———, shortly to be issued.