ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVIII — nr 2
1969

Observations on the development of plants. XV

Effect of CCC, gibberellin and vernalization on the development of *Salvia pratensis* sown at various dates

A. LISTOWSKI

As shown by previous experiments *Salvia pratensis* passes to the generative phase and develops normally its inflorescence under various external conditions (Listowski 1965; 1966). Longer vernalization followed by exposure to long day (or continuous light) seems to induce most uniform and normal florescence. Moreover, the plants flower without vernalization under short day or in varying light conditions along the lines: long—short—long or short—long. Under these conditions, however, only a part of the plants flowers. Under long day with low light intensity florescence of part of the plants is also observed. It would seem that the experimental population, thus also natural populations, consist of plants in which the inhibitory barriers for florescence lie at various levels.

Continuous daylight, irrespective of the light intensity, as well as long day with high intensity are systems which inhibit flowering. If even in a part of these plants the stem does elongate and some buds are set, they degenerate at a very early phase of development. Vernalization gives no effect when the plants are grown under short day after this treatment.

Under different day length but only by very low intensity sometimes shortened stems are formed, or normal ones but strongly foliated and with rudimentary generative organs. This problem was analysed in more detail in the experiments described below by comparing the development of plants seeded in the period of waning light on 13th Aug. with those under rising light conditions on 14th March. Simultaneously the effect of CCC and gibberellin was investigated, and a part of the plants sown on 13th August were subjected to natural vernalization for 3, 6 and 9 weeks.

DESCRIPTION OF DEVELOPMENTAL TYPES OF SHOOT

At the beginning of development the plant forms a rosette of some dozen leaves (photo 1). Flowering is terminal, the primordium of the flower first appearing in the form of a cone or pyramid in the middle of the leaf rosette. The growth of the stem starts only somewhat later. The normal flowering shoot is not branched or has few branches. The flowers are arranged as if in whorls, two to eleven in each.

Photo 1. Plant in leaf rosette phase

The stem leaves are few, the lowest pair is smaller and longer than the rosette ones, the following pair is already greatly reduced (photos 2 and 3).

Under conditions deviating in some degree from optimal a number of modifications is observed in the normal development of the shoot.

a) The first deformation consists in a declining activity of the growth apex its dying as early as the rosette phase. This causes the neighbouring resting (most frequently two) axillary buds, to start growing. In this way lateral rosettes develop which in turn elongate into shoots.

b) A symptom of inhibition of elongation growth is often formation of shortened shoots. Elongation growth is slow and of short duration, owing to this a short shoot develops with one or two internodes. At its top two small deformed leaves appear differing in shape from the rosette ones, then the activity of the growth apex subsides (photos 4 and 5).

A shortened shoot may also arise from axillary buds. Photo 6 shows a plant with growth apex which has died and a shortened shoot has developed from an axillary bud.

c) Well developed shortened shoots with a larger number of leaves are shown in photos 7 and 8. In these cases the plants are vegetatively well developed with large leaves and profuse foliage.

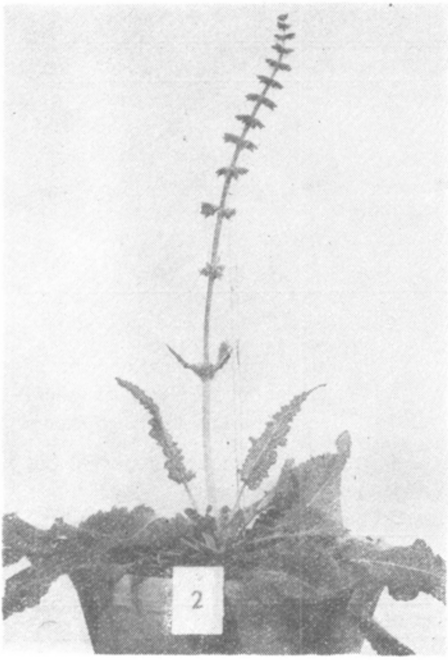

Photo 2

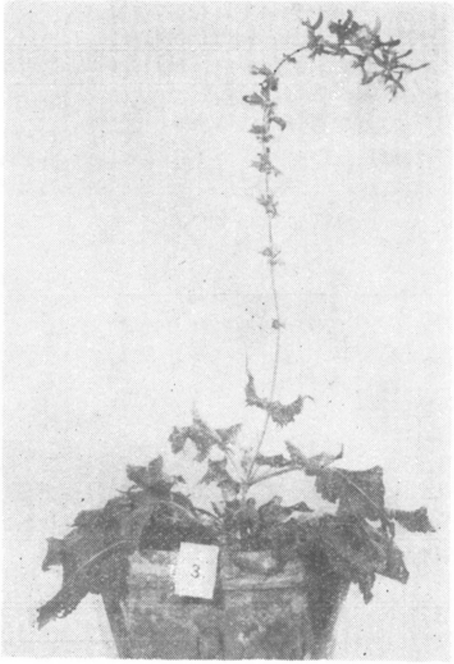

Photo 3

Photo 2. Plant with normally developed shoot

Photo 3. Plant with normally developed flowering shoot but with a distinctly vegetative lower part of stem

d) If elongation growth is prolonged as it often happens under the influence of gibberellin, long shoots form, but with shorter internodes than in normally developing plants and with a varying intensity of the vegetative tendency (photo 9). Frequently a secondary apical rosette develops here (photo 10). In other cases the generative part of the stem is rudimentary (photo 11, 12).

e) Formation of stems is observed, abundantly foliated in the lower and middle parts whereas in the upper part relatively few flower whorls develop.

In all the "deviations" from normal development described, the developmental rhythm is changed. When the rhythm is normal the inflorescence differentiates in advance of the period of elongation activity of the subapical meristem (see below "cone phase"). In the "vegetative" type of development, elongation growth starts by formation of the stem, the differentiation and leaf growth processes are intensified, finally, a greatly shortened under-develop generative part develops as a rule.

Autumn-winter combination

The plants were seeded on Aug. 13th in a glasshouse under natural waning daylight. Up to the beginning of October 12 percent of the plants reached the "cone" phase, that is exhibited a normal type of generative development. These plants

Table 1

Combination	n	Number of plants (March 20): in rosette phase	shortened shoot	vegetative shoot	with rudimentary generative part	with normal flowering stem	with dead main growth apex	Development of axillary buds
control	7	2	4	0	0	1	5	—
CCC I	7	3	3	0	0	1	4	distinct strong development of axillary buds. Part of them flowers, part as shortened shoots
CCC II	7	1	5	1	0	0	6	
CCC III	7	3	3	0	0	1	4	
GA_3I	7	3	0	2	2	0	4	lateral shoots less numerous but flower frequently
GA_3II	7	1	0	3	2	1	1	
GA_3III	7	1	2	2	0	2	3	

Table 2

Combination		n	Number of plants: in rosette phase	shortened shoot	vegetative shoot	with rudimentary generative part	with normal flowering stem	with dead main growth apex	Development of axillary buds (lateral shoots)
V_3	K	7	1	4	1	1	0	5	Relatively weak, only in 2 plants lateral shoots with rudimentary part
	CCC	7	1	5	0	1	0	4	Intensive development of lateral shoots, short., veget., to normally flowering
	GA_3	7	1	1	3	2	0	2	Less numerous than under CCC, mostly with rudiment. generat. parts, less frequently flowering
V_6	K	7	0	4	0	3	0	4	Lateral shoots after drying up of main growth apex flower frequently
	CCC	7	3	2	2	0	0	2	Numerous later. shoots, short. or elong., flowering
	GA_3	7	0	0	5*)	1	0	1	Relat. numerous, usual. veget. or rudimentary gener. part *) secondary rosette in 4 plants
V_9	K	6	0	5	0	0	1	3	Frequently reach flower. phase
	CCC	8	0	0	1	1	6	0	Generally veget. in lower part, flower in upper part
	GA_3	8	0	0	2*)	4	2	0	Not numerous *) secondary rosette in 1 plant

Photo 4. Shortened shoots

were discarded. At three dates: Nov. 20th (I), Dec. 12th (II) and Dec. 21st (III) part of the plants were treated with CCC (50 mg) or gibberellic acid GA_3 (10 mg), moreover, the effect of vernalization for 3, 6 and 9 weeks on the development of the plants, in interaction with CCC and gibberellin was tested (Tables 1 and 2, observation date March 20th).

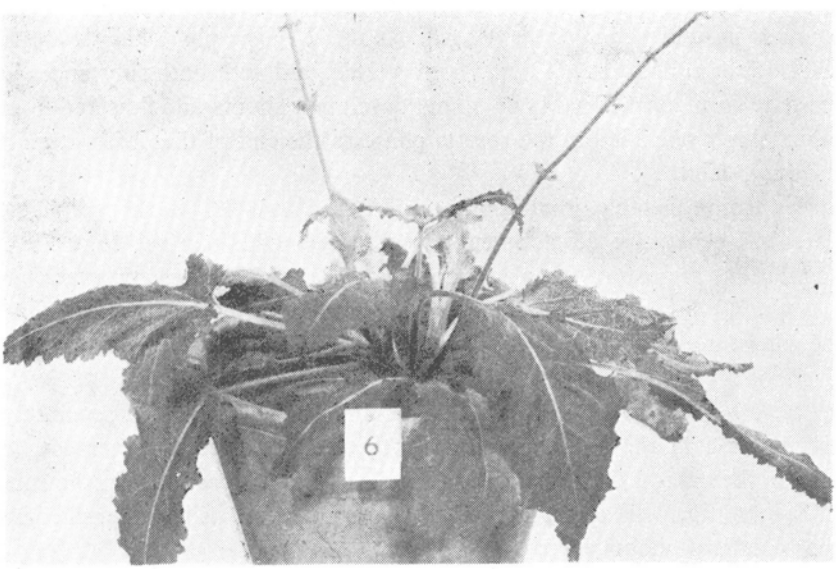

Photo 5. Shortened shoot, lateral one normal

Photo 6. Shortened lateral shoot

Spring-summer combination

The seeding date was March 14th, and the plants grew the whole time in a glasshouse under natural changing daylength. At the 2-leaves phase they were treated with CCC (50 mg) and GA_3 (10 mg.), respectively and with both substances jointly. After rosette formation part of the plants developed shoots and flowered normally. The other plants remained in the rosette phase to the end of the observation period (end of July, table 3).

As seen from this table, joint application of CCC and GA_3 in rising light systems stimulates the generative development as compared to that in the controls. Under these conditions almost all the plants come into flower. The growth apex elongates into a normally blooming stem, at the same time the development of axillary buds becomes more intensive, this finding expression in a distinctly increased number of lateral shoots.

On the other hand, under the action of GA_3 or CCC applied separately only a slight increase in the florescence frequently was observed together with earlier flowering in the case of CCC treatment. Thus, the growth processes in the subapical part of the main growth apex and in the axillary buds are visibly intensified and the number of lateral shoots increases.

The mean length of the stem, however, remains the same in all the combinations:

Table 3

Combination	Observ. date / Phase / n	Number of plants: 1st June			15th June			1 st July			flowering plants, %	lateral shoots
		rosette	veget. shoot	flower. shoot	rosette	veget. shoot	flower shoot	rosette	veget. shoot	flower. shoot		
K	19	16	1	2	14	2	3	12	0	7	40	weak development of axillary buds lateral shoots not numerous
GA_3	10	5	3	2	5	0	5	5	0	5	50	few lateral shoots
CCC	10	4	5	1	3	2	5	2	2 (short)	6	60	2—3 lateral shoots per plant
CCC+GA_3	10	1	8	1	1	1	8	1	0	9	90	no differences in degree of branching of shoots
GA_3+CCC	10	3	4	3	1	3	6	1	0	9	90	

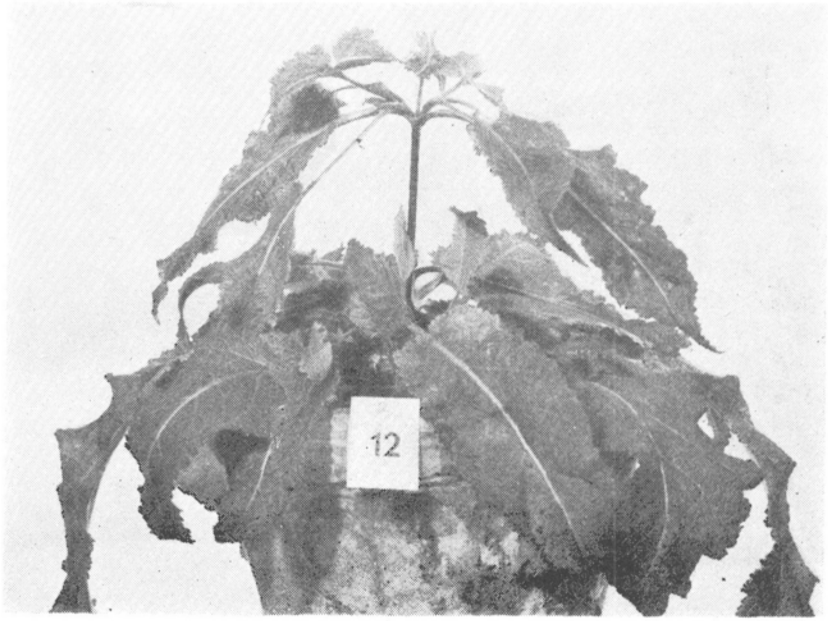

Photo 7. Vegetative shoot

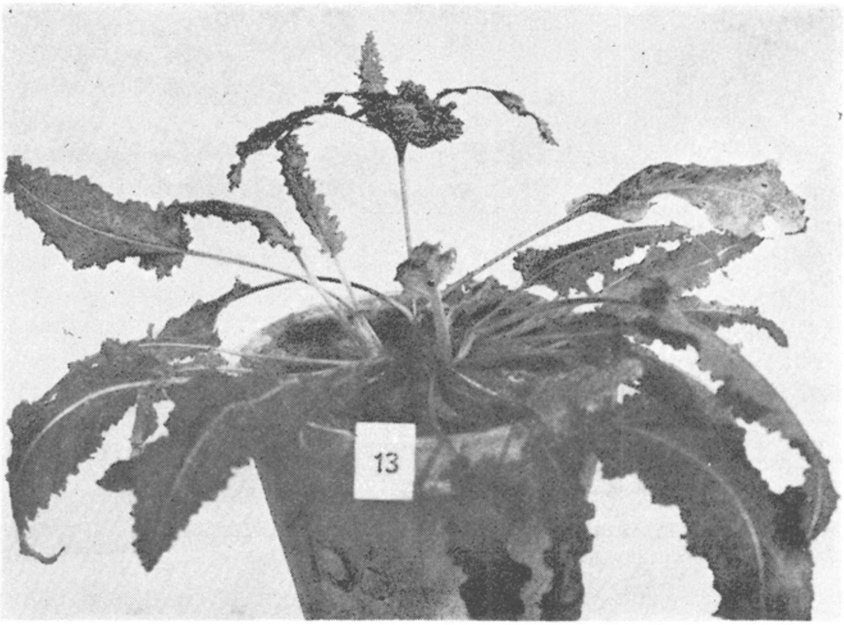

Photo 8. Vegetative shoots shortened

control, CCC- and GA_3-treated. The shoots are somewhat less branched in the combination with GA_3.

In the combination sown in late summer the effect on development is only analogous, in part of the plants, more frequently it is different. This difference consists above all in the frequent occurrence of various types of vegetative development of the stem, and on the other hand, in dying back of the growth apex.

The autumn and winter development of nonvernalized plants sown in late summer is the same in the controls and the CCC-treated combination. Mostly a shortened shoot develops, and then growth ceases and the growth apex dies. Plants subjected to the action of CCC differ from the control ones only in a distinctly more intensive development of the axillary buds.

Gibberellin, on the contrary, intensifies markedly elongation growth of the stem, this resulting in long, profusely foliated stems with a rudimentary inflorescence or a secondary rosette.

The effect of shorter vernalization, and in the control combination also 9-week vernalization, is limited almost exclusively to an intensification of growth processes. The rate of leaf differentiation and leaf growth increases, therefore the leaves are distinctly larger. A more intensive development of axillary buds is also observed. Nevertheless, even after 9 weeks of vernalization, in the control combination plants in which only shortened shoots have developed prevail. However, a distinct effect of six-, and still more of nine-week vernalization could be observed in plants treated with CCC and gibberellic acid. The vegetative character of their development, stimulated so strongly by GA_3 in the control or shorter vernalized plants, becomes less pronounced in conditions of longer lasting vernalization. This is manifested by a decrease in the number of plants with a secondary rosette and the prevalence of plants with a rudimentary or even more or less normal inflorescence. It may, therefore, be claimed that a shift occurs towards a stronger generative tendency in development. This shift is very pronounced in plants subjected to CCC-treatment and vernalized for 9 weeks. As a result, in a large majority of the plants, normal inflorescences develop with simultaneous intensified growth of lateral shoots.

It does not seem possible on the basis of the present and earlier experiments to present a full model of *Salvia pratensis* development. Nevertheless the reaction of this plant seems very interesting in several respects:

1. *Salvia* seems to be a plant in which generative induction and initiation occur in various light conditions: under short day, and short- long-day, and in general, as the result of prolonged vernalization. Circumstances decisive for blooming may also occur after initiation. The barriers inhibiting further development of the inflorescence may occur at various phases of the differentiating generative process. Inhibition may also come into play at the bud stage. The buds set may die and be shed as it is frequently observed under continuous illumination and in the case of insufficient light intensity.

2. *Salvia* may be considered a good example of a plant in which the difference between the activity of the eumeristems and that of the subapical meristem is pronounced. The rhythm of stem development, i.e. of elongation growth of its

Photo 9

Photo 10

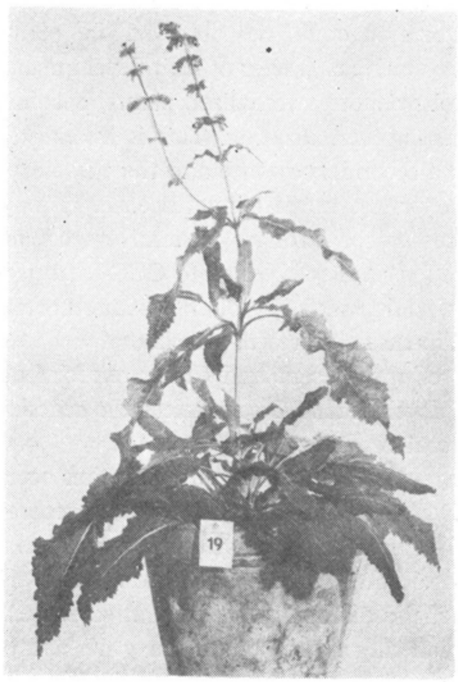

Photo 11

Photo 12

Photo 9. Vegetative shoot
Photo 10. Vegetative shoot with secondary rosette
Photo 11. Lower part of stem vegetative, higher normal whorls of flowers
Photos 12. Shoots with rudimentary inflorescence

vegetative part, on the one hand, and the development of the inflorescences on the other, changes in dependence on the light and thermic conditions prevailing.

3. In conditions nonoptimal for the development of generative organs the intensity and character of stem growth and leaf differentiation vary in dependence on the conditions. Generally speaking, two lines of development may be distinguished, one leading to a strong development of stem and leaves which sometimes end in the formation of rudimentary generative organs, and the other, occurring when the deviations from the optimum for meristematic activity are large, is manifested by the short-shoot pattern, this in turn being associated with the dying back of the growth apex. Before this death formation of new leaves ceases, however, the plant as a whole does not die: one, two, or less frequently, more axillary buds emerge from the state of growth and differentiation stagnation. The death of the growth apex and cessation of elongation growth may be considered as the expression of disturbances in the metabolism of the plants under nonoptimal light conditions.

To sum up the effect of gibberellic acid, it may be said that it is analogous to that observed in other plants (Chouard 1960). It may be interpreted on the basis of the hypothesis assuming that at various development stages different gibberellins come into play, or it may be considered from the viewpoint of the supposition that these differences are related to the gibberellins level, or the interactions between gibberellins and other growth substances (Baldev and Lang 1965, Michniewicz 1963). In the present experiments the influence of gibberellin on elongation processes, and the resulting activation of the subapical meristem were clearly demonstrated. The effect of exogenous gibberellin is most pronounced in Salvia growing under nonoptimal light conditions, that means when the level of endogenous gibberellin synthesis is low, this resulting in a strong stimulation of the vegetative development of the stem.

The interaction of vernalization with GA_3 leads to differentiation of the generative organs, although they often degenerate subsequently. Thus, the vegetativ development processes are prolonged here beyond the strictly vegetative stage. This may be due to the declining role and direct influence of gibberellin or to a change in the character of its action owing to the abolition by vernalization of the barriers inhibiting generative development.

As far as the effect of CCC is concerned, this substance activates in the first place the development of the axillary buds. In interaction with vernalization it influences flowering positively. A particularly favourable effect, but only in the period of summer high light intensity conditions, was observed when CCC and GA_3 were applied jointly.

The data pointing to the stimulating effect of CCC on generative and axillary bud development are so far not abundant, nevertheless they are available. In cereals the effect of CCC on the crop was more frequently negative than positive, however, an elongation of the head and uppermost internode (Michniewicz et al. 1967) and an increase in the number of grains in the head (Michniewicz et al. 1967; Mayer et al. 1963; Listowski et al. 1967) were noted, and what is still more interesting in this connection, an increased contribution of lateral shoots to the yield, indicating more intensive tillering (Primost 1964; Listowski et al. 1967; Barbier et al. 1966). For the broad bean, Sójka (1967) reports an increased number of racemes and pods. In *Azalea* speeded up efflorescence with intensified bud setting was observed (MacDowell 1966). More intensive shoot development and a tendency to branching occurred in alfalfa (A. Jelinowska, private communication). Sebanek (1966) demonstrated that axillary bud development is stimulated in pea upon treatment with CCC and gibberellin. He believes that CCC weakens the apical domination as do high gibberellin doses, giving in effect an increased development of side shoots. On the other hand, this author sees a positive influence of gibberellin of moderate and low concentration manifested in the development of lateral buds. In the present experiments the intensity of development of axillary buds in the combination subjected to CCC+GA treatment was not higher than in plants to which CCC alone was applied, thus one cannot speak here of any summation of the effects of both these substances.

The above quoted results can only be treated as observations and on their basis one could hardly advance hypotheses as to the mechanism of CCC action, and still less in the case of *Salvia* where a distinctly positive effect of CCC applied jointly with gibberellic acid on the process of flowering was observed. The interaction between CCC and GA_3 has been studied by various authors who generally stress the antagonistic relation between the retardant and gibberellin, consisting, maybe, in that CCC reduces the biosynthesis of endogenous gibberellins (Harada et al. 1965; Michniewicz 1965; Ninnemann 1964; Kochler 1965). This antagonism involves only the process of elongation growth (Sachs and Kofranek 1963) and it is not a general observation. In plants or in conditions in which endogenous gibberellin synthesis does not exceed the level optimal for growth, the growth-inhibiting effect of CCC would be pronounced. At an "excessive" endogenous gibberellin level this influence may not be noticeable, the same may be true in the case when gibberellin does not play so essential a role in the process of growth of the given organ (Knypl 1966). It should be added that in *Salvia* no difference in the length of shoots was noted in the plants of the combination treated with CCC and with gibberellic acid, in the controls and in the combination in which these two substances were applied jointly.

As regards the initiation of florescence, the influence of retardants has only been analyzed in long-day *Samolus parviflorus* (Baldev and Lang 1965). Amo 1618 as well as CCC had an effect opposite to that of gibberellin, inhibiting the process of initiation. These results, however, as also shown by the present observations, cannot be generalized.

The stimulating effect of CCC in plants developing in autumn and winter appeared only in interaction with vernalization of 9 weeks duration. Investigations on the effect of CCC on the vernalization course were performed only on wheat grain subjected to vernalization (Michniewicz 1965; Günther 1966). In the light of these experiments, CCC inhibits the vernalization of winter cereals when applied before this treatment, whereas it has no effect after vernalization. Neither was this inhibitory effect noted in cereals sown in spring. Günther (1966) believes that this may be not only the result of the interaction with GA, but that it may be connected with the presence of an inhibitor which decomposes under the influence of vernalization as observed by Kentzer (1966).

In *Salvia* vernalization abolishes in all cases the barriers inhibiting generative development, that is both when the plants grow under continuous illumination and long day, or in autumn and winter under very poor illumination. This leads to the supposition that we are dealing here in the first place with an anti-inhibitor effect of vernalization. Only when the given inhibitor is inactivated or decomposed, other influences may become manifest such as the effect of CCC, positive in this case as far as flowering is concerned.

SUMMARY

Observations were undertaken on the development of *Salvia pratensis* in two periods: under waning (seeding Aug. 13) and rising (seeding March 14) light conditions. The effect of CCC, of gibberellic acid and of both these substances applied jointly was investigated. Part of the plants of the autumn combination were subjected to vernalization for 3, 6 and 9 weeks under natural conditions. In the plants developing in autumn various courses of development were noted, ranging from remaining in the rosette phase through various types of shoot development: shortened, normally vegetative with double rosette, vegetative with rudimentary inflorescence, up to normally flowering specimens (see successive photos). The results of the observations are compiled in tables 1, 2 and 3. The proportions of plants with various developmental types of shoots varies in the particular combinations sown at both dates. In the plants seeded in spring (table 3) a stimulation of generative development was observed under joint application of CCC and gibberellic acid, CCC alone mainly activated axillary bud development, whereas the number of normally flowering plants was not much higher than in the controls. Plants developing in the autumn—winter period without vernalization were not affected by CCC. Both in this combination and in the controls, generally a shortened stem develops or the plant remains in the rosette phase, the growth apex dying back in both cases. Gibberellin stimulates elongation growth leading to the formation of a distinctly vegetative shoot, frequently with a secondary rosette. The influence of vernalization of short duration is limited to a certain enhancement of vegetative growth (e.g. larger leaves). Under 9 week vernalization a distinct intensification of flowering in the combination with CCC, and decreasing vegetative tendencies in the combination with gibberellic acid (manifested as the domination of plants with rudimentary inflorescence but without double rosette) were observed.

The effect of gibberellin did not differ in character from that in the cases reported and described in the literature. On the other hand, the effect of CCC and of this substance applied jointly with GA_3 cannot be interpreted on the basis of the present results nor of the scarce literature data.

The action of the retardants alone as well as in interaction with gibberellic acid is no doubt a process more complicated than it was until recently believed. One might assume that this influence may vary, to quote as example: the „anti-resting" effect on axillary buds, thus a decrease of apical domination under the action of CCC, and on the other hand, few, but actually observed cases in which CCC stimulates definite steps of generative development, finally, and most frequently, cases of elongation growth inhibition which, however, does not occur in all plants.

(Entered: December 9, 1968 r.)

REFERENCES

Baldev B., Lang A., 1965, Americ. J. of Bot. 52: 4.
Barbier S., Mayer H., 1966, Plant a. Soil 34: 167.
Chouard P., 1960, Ann. Rev. of Pl. Phys. 11: 191.
Günther C., 1966, Naturwissenschift. 53 (10): 256.
Harada H., Lang A., 1965, Plant Phys. 40: 176.
Kentzer T., 1967, Acta Soc. Bot. Pol. 36: 8.
Knypl J. S., 1966, Acta Soc. Bot. Pol. 35: 611.
Köhler D., 1965, Planta 67: 1.
Listowski A., Pawłowska J., Żebrowski Z., 1967, Sprawozd. z dośw. z CCC za lata 1965—66, IUNG R (9) 92.
McDowell T. C., Larsen R. A., 1966, Proc. of the Americ. Soc. of Horticult. Sc. 88: 600.
Mayer H. H., Primost E., Rittmeyer G., 1962, Bodenkultur 13: 27.
Michniewicz M., 1963, Wiadom. Botan. 7: 117.
Michniewicz M., 1965, Naturwissensch. 52: 88.
Michniewicz M., Chromiński A., Belt H., 1967, Roczn. N. Roln. 93-A-1, 131.
Ninnemann H., Zeevart J. A., Kende H., Lang A., 1964, Planta 61: 229.
Primost E., 1963, Bodenkultur 14: 209.
Primost E. 1964 (mimeograph) copy Arbeitstsgem. d. Saatzuchtleiter, 41.
Sachs P. M., Kofranek A. M. 1963. Amer. J. of Bot. 50: 772.
Sebanek J. 1966. Flora 156 (6): 573.
Sójka E. 1967. Sprawozd. z dośw. z CCC za lata 1965—66, JUNG R (9) 163.
Stoddart J. L., 1963, Nature 199, 1270.
Stoddart J. L., 1965, Ann. of Bot. NS 29, 116.

Obserwacje nad rozwojem roślin

XV. Wpływ CCC, gibereliny i jaryzacji na rozwój *Salvia pratensis* wysianej w różnych terminach

Streszczenie

W niniejszych doświadczeniach przeprowadzono obserwacje nad rozwojem *Salvia pratensis* w dwóch okresach: w okresie malejących (siew 13.VIII) i rosnących (siew 14.III) układów świetlnych, analizowano przy tym wpływ CCC, kwasu giberelinowego oraz łączny obu tych substancji. Prócz tego część roślin serii jesiennej jaryzowano w ciągu 3, 6, i 9 tygodni w warunkach jaryzacji naturalnej.

U roślin rozpoczynających życie jesienią zaobserwowano różny przebieg rozwoju, od trwania w fazie rozety poprzez różne typy rozwoju pędów skróconych, wegetatywnych z podwójną rozetą wegetatywnych z uwstecznioną częścią kwiatostanową aż do normalnie kwitnących (patrz kolejne fot.). Wyniki obserwacji podane są w tabelach 1, 2 i 3. Udział różnych typów rozwojowych łodygi w poszczególnych kombinacjach w obu terminach siewu jest różny.

Przy siewie wiosennym (tab. 3) obserwuje się stymulację rozwoju generatywnego przy łącznym stosowaniu CCC i kwasu giberelinowego. Natomiast samo CCC wpływa przede wszystkim na uaktywnianie się rozwoju pączków kątowych, natomiast liczba roślin normalnie kwitnących jest niewiele tylko większa od kontroli.

Przy siewie jesienno-zimowym bez jaryzacji wpływu CCC nie zaobserwowano. W tej kombinacji, jak i w kontrolnej, najczęściej dochodzi do wytworzenia się pędu skróconego albo roślina

pozostaje w fazie rozety, z tym że w obu tych wypadkach obserwuje się obumieranie głównego wierzchołka wzrostu. Giberelina stymuluje wzrost elongacyjny, w związku z czym dochodzi do, wytworzenia się wyraźnie wegetatywnej łodygi często z wtórną rozetą. Wpływ krótkiej jaryzacji ogranicza się jedynie do pewnego wzmocnienia wzrostu wegetatywnego, między innymi liście są większe. Przy 9-tygodniowej, a więc dłuższej jaryzacji, obserwuje się wyraźne uintensywnienie zakwitania w kombinacji z CCC, a malejące tendencje wegetatywne w kombinacji z kwasem giberelinowym (co objawia się jako dominacja roślin z uwstecznionym kwiatostanem, ale bez podwójnej rozety).

Charakter działania gibereliny nie odbiega od innych poznanych i opisanych w literaturze wypadków. Co się tyczy natomiast wpływu CCC, jak i łącznego oddziaływania CCC i GA_3 niniejsze wyniki, jak i inne bardzo zresztą nieliczne dane, które można znaleźć w literaturze, nie pozwalają jeszcze na szerszą interpretację obserwowanych faktów.

Charakter działania samych retardantów, jak i retardantów w interakcji z kwasem giberelinowym, jest napewno procesem bardziej złożonym niż to do niedawna sądzono. Można by założyć, iż oddziaływania te mogą być różne — przykładem: „antyspoczynkowy" wpływ na pączki kątowe, a więc zmniejszanie się pod wpływem CCC dominacji apikalnej, z drugiej strony nieliczne, ale jednak obserwowane wypadki, w których CCC stymuluje określone etapy generatywnego rozwoju, wreszcie najczęstsze wypadki hamowania wzrostu na długość, efekt który znów nie występuje u wszystkich roślin.