# Factors influencing flower bud formation on the pear tree cultivar 'Doyenne du Comice'. III. Saccharides, nitrogen compounds and some mineral elements contents in pear leaves and shoots

## FRANCISZKA JAUMIEŃ

Department of Pomology, Warsaw Agricultural University, ul. Nowoursynowska 166, 02-766 Warszawa, Poland

(Received: October 13, 1980)

#### Abstract

Long shoots inhibited in growth by treatment with chlormequate contained more reducing sugars in mid July than did the control ones growing vigorously. Storage starch accumulation was earlier in the former shoots than in he control ones, and they also contained more nitrogen compounds, especially protein, and significantly more calcium, magnesium, iron and zinc then the controls. Comparison of long shoots with growth partly inhibited by the action of chlormequate, on which flower buds form in the subapical part, and of spurs on which an apical flower bud forms, with the long shoots of control vigorously growing trees where flower buds do not form, indicates that initiation of flowering in the pear tree is associated with a high level of storage compounds, both organic and inorganic, in the stem.

#### INTRODUCTION

Attention has been called quite long ago to the significance of nutritive compounds and the interrelations between them, influencing the initiation of flowering of fruit trees. According to Klebs (1903), Kraus and Kraybill (1918) (quoted after Bielińska, 1957), prevalence of carbohydrates over nitrogen compounds favours flower bud formation in plants. At first the decisive role in this process in apple trees was ascribed to sugars, then to starch (Hooker, 1920; Harley et al., 1942). The experiments of Kraybill et al. (1925) (quoted after Bielińska, 1957) demonstrated that it is not only the ratio of starch to nitrogen, but also the absolute amounts of these compounds that play a significant role in the formation of flower buds on trees.

Numerous investigators called attention to the role of nitrogen compounds in the initiation of flowering of fruit trees (H e i n i c k i e, 1930; D e l a p, 1967). A doubtless relation was, however, only found between flowering initiation

and protein content (Potter and Phyllips, 1930; Ursulenko, 1955). A certain role in the process of blossoming is also attributed to mineral compounds such as phosphorus, calcium, potassium and others (Feucht and Arancibia, 1968; Hołubowicz, 1970; Bould and Parfitt, 1973).

The purpose of the present study was the comparison of saccharide, nitrogen compound and some mineral component levels in pear shoots inhibited in growth by treatment with chlormequate and in spurs before setting of flower buds with the levels of the same substances in long vigorously growing shoots which do not form flower buds.

#### **METHODS**

# Chemical investigations

Dry weight, reducing sugars and total nitrogen, soluble compounds and protein nitrogen contents in leaves and axes of long shoots and spurs of trees treated and untreated with chlormequate (see Part I) were determined in 1972, 1973 and 1974. In 1973 arginine, asparagine, aspartic acid, glutamine, serine with glutamic acid and threonine were additionally determined. The content of eight mineral elements: Fe, Mn, B, Zn, K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, Ca and Mg was determined in 1974. Samples for chemical analyses were taken each year at two dates: in mid June

Samples for chemical analyses were taken each year at two dates: in mid June  $(T_1)$  and mid July  $(T_2)$  from six trees sprayed with chlormequate and six control untreated ones. The long shoots were excised at their base and after measuring their length divided into two halves. For chemical determinations 50 g of leaves and long shoot axes were taken from the upper and lower halves. Two hundred spurs were collected from each of two control trees with their apical flower buds and 50-g samples of fresh leaves and spur axes were prepared.

The material was collected in the morning hours (8 - 11 a.m.). Air-dry material was used for chemical analyses.

Reducing sugars were determined by the method of Hagedorn-Jensen in the modification of Fujita and Iwatake (K a c z k o w s k i and T o c z k o, 1969). Total nitrogen was determined by the Kjeldahl method, protein nitrogen was calculated from the difference between total nitrogen and nitrogen of soluble compounds. For preparing extracts for soluble compounds nitrogen and amino acid nitrogen determinations alcohol-water extraction was applied. One gram of dry ground material was infused with 15 ml ethanol and, after about 15 h of extraction at room temperature centrifuged. The residue was extracted with 15 ml boiling 80 per cent ethanol for 0.5 h under reflux condenser and centrifuged. The

sediment was extracted with boiling water for 5 min on a water bath. Then 96 per cent ethanol was added to precipitate the proteins and after about 10 h the mixture was centrifuged. The combined extracts were evaporated to dryness at about 50°C. The residue was washed twice with a small amount of water to a final volume of 5 ml.

Amino acids were separated by thin-layer chromatography. Plates were covered with a 0.25 mm thick silica gel layer-G and 20  $\mu l$  of the extract was placed on them as well as standard solutions of the amino acids in a 0.1 mg/ml concentration. The plates were then developed twice in a phenol-water 4:1 system. The chromatograms were first air-dried, then for about 30 min at 60°C and colour was developed with an acid ninhydrin solution. The amino acids were visualized on the chromatograms by means of ninhydrin, eluted with methanol and their content was determined by means of spektol at wavelength 506 nm.

Potassium and calcium were determined by flame photometry, phosphorus by the molybdenum-vanadium method. Magnesium, iron, zinc and manganese contents were determined with the single-beam spectrometre for atomic absorption: Atom-Absorption-Spectrophotometre PEKIN-ELMER 300 with the use of an acetylene-air mixture. Boron was determined by the colorimetric method with curcumin.

The contents of all the determined compounds were calculated as per cent of dry weight and in grams or milligrams in reference to 50 g fresh weight. The results are given only in conversion to fresh weight.

Analysis of variance was applied for elaboration of the results, separately for the stems and leaves of the upper and lower parts of the long shoots and for the spurs. In each analysis two combinations were taken into account (chlormequate and control) and two dates in three replications. For evaluation of the significance of the difference Student's t test was applied. Two levels of significance were adopted:  $a_1 = 0.05$  and  $a_2 = 0.01$ . Moreover, a level close to significant with a probability of error within the limits 0.05-0.1 was considered. The calculated data are given in the tables.

# Microscopic investigations

The presence of starch in various tissues of the stem was revealed in material collected in mid June and mid July 1972, 1973 and 1975 by examining in a microscope cross sections of the subapical, middle and basal parts of long shoots sprayed with chlormequate and control ones and cross sections of spurs stained with alcohol solution of I in KI. The presence of starch grains in the colenchyma, cortical parenchyma, starch sheath, phloem, xylem and pith was quantitatively estimated according to a 5-grade score.

## **RESULTS**

# Chemical analysis of leaves and stems

## 1. Dry weight

Dry weight of leaves and stems of trees inhibited in growth was higher than in the vigorously growing ones. Wider differences were noted in the subapical than in the basal part of the shoots.

In mid June, in the initial phase of elongation growth, three to four weeks after spraying the trees with chlormequate the differences in dry weight were slight, whereas in mid July the shoots with weaker growth ending earlier showed a 14 to 26 per cent higher dry weight of leaves and 21 to 31 per cent higher of shoots in their upper part as compared with the control still elongating ones. Spurs always have the highest dry weight content (Table 1).

Table 1

Dry weight content (g/50 g fresh weight) in leaves and stems of trees treated (C) with chlormequate and untreated ones (K)

Dates of sample collection		Upper part of shoot		Lower part of shoot		Spurs	
Year	Date	K	С	K	С	K	С
			Leaves	3			
1972	17 VI	15.7	16.4	17.5	17.2	17.5	17.3
	11 VII	17.4**	19.9	19.1	19.7	19.2	18.8
1973	14 VI 18 VII	16.0 16.1**	15.9 20.1	18.2 18.9**	17.8 19.9	17.7 19.5	18.0
1974	19 VI	16.6	16.5	19.1	18.5	18.5	18.1
	15 VII	14.7**	18.5	18.6	19.0	17.0	18.1
			Stems				
1972	17 VI	13.6 <sup>0</sup>	15.2	16.2	16.4	17.8	18.0
	11 VII	14.9**	18.1	18.2*	20.3	20.6	19.9
1973	14 VI	15.3	15.6	15.8	15.5	16.7	16.7
	18 VII	14.6**	17.8	17.6**	19.0	19.6	-
1974	19 VI	13.9**	15.2	15.9*	16.4	18.6	18.8
	15 VII	12.4**	16.2	15.8**	17.7	18.9	18.7

Odifference close to significant (a = 0.1), \*significant difference ( $a_1 = 0.05$ ), \*\*difference highly significant ( $a_2 = 0.01$ ) between K and C.

## 2. Sugars

Reducing sugars content is higher in leaves than in the shoot axes (Tables 2, 3). In mid July the content of these compounds was significantly higher in the subapical part in the leaves and stems treated with chlormequate as compared with that in the controls. These differences amounted to 13 per cent in 1972, 22 per cent in 1973 and 42 per cent in 1974 for leaves and correspondingly 15, 28 and 140 per cent for the stems. In the basal part of stems treated with chlormequate in 1973 and 1974 the reducing sugar level is also significantly higher than in control shoots. The differences in 1972 were small and nonsignificant. The sugar content in spurs is closer to that in long shoots inhibited in growth by chlormequate than to that in controls.

Table 2

Reducing sugars content (g/50 g fresh weight) in leaves and stems of trees treated (C) with chlormequate and untreated ones (K) in June (T<sub>1</sub>) and July (T<sub>2</sub>)

Year	Date	Upper part of shoots		Lower part of shoots		Spurs	
		K	С	K	C	K	C
			Leave	s			
1972	$\mathbf{T}_1$	1.37	1.15	1.08	1.16	1.13*	0.86
	T <sub>2</sub>	1.19	1.29	1.18	1.17	1.18*	0.93
1973	T <sub>1</sub>	1.31	1.39	1.56 <sup>0</sup>	1.46	1.35	1.41
	T <sub>2</sub>	1.24**	1.53	1.47	1.41	1.39	_
1974	т <sub>1</sub>	1.67	1.70	1.46*	1.85	0.97°	1.25
	T <sub>2</sub>	0.97**	1.38	1.16	1.30	1.11	0.96
			Stem	s			
1972	T <sub>1</sub>	0.71	0.77	0.74	0.70	0.89	0.79
	т2	0.78	0.87	0.79	0.87	0.99	0.83
1973	Т1	1.61	1.66	0.77	0.72	0.70	0.72
	т2	0.61 <sup>0</sup>	0.77	0.80*	0.89	0.81	_
1974	$T_1$	1.12**	1.35	1.02**	1.44	0.87	0.84
	T <sub>2</sub>	0.43**	1.05	0.48**	0.93	0.69 <sup>0</sup>	0.57

Explanations as in Table 1.

Table 3

Reducing sugars content after hydrolysis (g/50 g fresh weight) in leaves and stems of trees sprayed (C) with chlormequate and unsprayed ones (K) in June (T<sub>1</sub>) and July (T<sub>2</sub>)

Year	Date	Upper part of shoots			Lower part of shoots		rs
	_	K	С	K	С	K	С
			Leaves				
1972	$T_1$	1.58	1.41	1.36	1.35	1.45°	1.18
	$T_2$	1.55	1.75	1.58	1.64	1.56*	1.25
1973	т <sub>1</sub>	1.45	1.59	1.69	1.67	1.53	1.62
	T <sub>2</sub>	1.48**	1.81	1.75	1.66	1.61	-
1974	$\mathbf{T}_1$	1.92	1.93	1.89	2.18	1.20°	1.51
	T <sub>2</sub>	1.16**	1.65	1.37	1.53	1.38	1.16
			Stems				
1972	<b>T</b> <sub>1</sub>	0.95	0.95	0.97	0.96	1.26	1.07
	T <sub>2</sub>	1.07	1.23	1.17°	1.32	1.36	1.13
1973	т <sub>1</sub>	0.73	0.80	0.91 <sup>0</sup>	0.85	0.83	0.83
	T <sub>2</sub>	0.79*	1.01	1.06*	1.14	1.12	_
1974	$T_1$	1.40 <sup>0</sup>	1.58	1.23**	1.65	1.08	1.09
	T <sub>2</sub>	0.52**	1.25	0.59**	1.11	0.81	0.71

## 3. Nitrogen compounds

Total nitrogen content is significantly higher in long shoots after chlormequate treatment than in the control ones. In the upper part of shoots the differences amount for leaves to 13 per cent in 1972, 24 per cent in 1973 and 34 per cent in 1974. For stems the differences were around 50 per cent in the years 1973 and 1974 and as low as 14 per cent in 1972. In the basal part of the shoots the differences in total nitrogen content were smaller amounting for the leaves to 15, 17 and 30 per cent, and for stems to 41, 23 and 21 per cent, respectively. In the spurs of trees exposed to chlormequate and untreated ones the total nitrogen content is similar and close to the content in the upper part of long shoots treated with chlormequate (Table 4).

Table 4

Total nitrogen content (g/50 g fresh weight) in leaves and stems of trees sprayed (C) with chlormequate and unsprayed ones (C)

Dates of sample collection		Upper part of shoots		Lower part of shoots		Spurs	
Year Date		K	С	K	С	K	С
			Leaves				
1972	17 VI 11 VII	0.333 0.349	0.327 0.396	0.368 0.377	0.379 0.432	0.408 0.415	0.414 0.429
1973	14 VI 18 VII	0.309 <sup>0</sup> 0.318**	0.289 0.421	0.329** 0.378**	0.379 0.435	0.374 0.424	0.388
1974	19 VI 15 VII	0.281 0.259*	0.281 0.322	0.313 <sup>0</sup> 0.306	0.343 0.397	0.355 0.326 <sup>0</sup>	0.370 0.369
			Stems				
1972	17 VI 11 VII	0.179 0.165	0.167 0.188	0.158** 0.118**	0.128 0.166	0.209 0.191	0.225 0.208
1973	14 VI 18 VII	0.221 0.136**	0.212 0.211	0.171 0.151**	0.170 0.181	0.233 0.210	0.232
1974	19 VI 15 VII	0.174 0.104**	0.174 0.156	0.134 0.114**	0.137 0.138	0.208 0.183	0.214 0.191

Soluble compounds nitrogen occurred in a significantly higher amount in shoots sprayed with chlormequate only in 1973 and 1974. In mid July these differences in the upper part of long shoots treated with chlormequate (Table 5,  $T_2$ ) amounted to 17 and 23 per cent, respectively, in leaves and 56 and 26 per cent in stems. The differences in the basal part of long shoots are usually non-significant.

There are wider differences in the protein nitrogen level than in soluble compounds nitrogen (Table 6). In the subapical part of chlormequate-treated long shoots the amount of protein nitrogen was in mid July by 15, 34 and 25 per cent higher in the leaves and by 18, 56 and 57 per cent higher in the stems as compared with the controls in the years 1972, 1973 and 1974, respectively. These differences were lower in the basal part of the shoots amounting to 16, 17 and 34 per cent in the leaves and 46, 23 and 21 per cent in the shoot axes, respectively.

Table 5

Soluble nitrogen content (g/50 g fresh weight) in leaves and stems of trees treated (C) with chlormequate and untreated ones (K) in June (T<sub>1</sub>) and July (T<sub>2</sub>)

Years	Date	Upper part of shoot		Lower part of shoot		Spurs	
		K	С	K	С	K	С
			Le	aves	-		
1972	T <sub>1</sub>	0.043°	0.032	0.029	0,037	0.048	0.044
	T <sub>2</sub>	0.037	0.036	0.038	0.037	0.040	0.045
1973	$T_1$	0.029 <sup>0</sup>	0.024	0.026	0.029	0.028	0.029
	T <sub>2</sub>	0.024*	0.028	0.029	0.029	0.028	_
1974	т <sub>1</sub>	0.037	0.034	0.035	0.037	0.048	0.050
	T <sub>2</sub>	0.035**	0.043	0.039	0.037	0.037	0.042
		_	St	ems			
1972	Т1	0.026	0.020	0.021	0.024	0.033	0.032
	$T_2$	0.027	0.024	0.024	0.029	0.032	0.031
1973	T <sub>1</sub>	0.039	0.040	0.035	0.037	0.072	0.072
	T <sub>2</sub>	0.034**	0.053	0.041	0.045	0.050	_
1974	$T_1$	0.026	0.029	0.024	0.026	0.029*	0.035
	T <sub>2</sub>	0.019*	0.024	0.022*	0.028	0.027	0.027

Additional determinations performed in 1973 demonstrated that in mid July in the upper part of chlormequate-treated stems the content of the amino acids in point and amides was significantly higher than in the control shoots, and very close to that in spurs (Fig. 1). Shoots with partly inhibited growth (spurs and long shoots sprayed with chlormequate) and ending elongation growth in June contained in their upper part in July 77 per cent more aspartic acid, 75 per cent glutamine, 65 per cent serine with glutamic acid, 62 per cent asparagine, 47 threonine, 34 per cent arginine than shoots which still continued intensive growth at this time. In the basal part of treated long shoots only the arginine and asparagine contents were significantly increased. The amounts of other compounds were also increased but nonsignificantly. The leaves of all shoots exhibited similar amounts of the pertinent compounds.

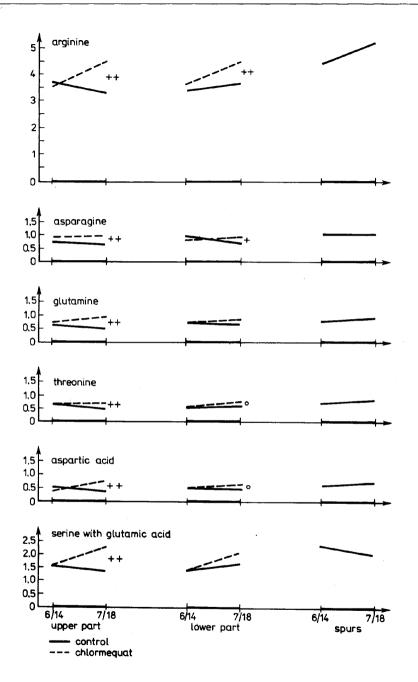


Fig. 1. Arginine, asparagine, aspartic acid, glutamine, serine with glutamic acid and threonine contents in mid June and mid July in 50 g of fresh weight of pear shoots sprayed and unsprayed with chlormequate

Table 6

Protein nitrogen content (g/50 g fresh weight) in leaves and stems of trees treated (C) with chlormequate and untreated ones (K) in June (T<sub>1</sub>) and July (T<sub>2</sub>)

Years	Date		Upper part of shoots		Lower part of shoots		Spurs	
		K	C	K	С	K	C	
			Leave	es				
1972	T <sub>1</sub>	0.289	0.296	0.339	0.342	0.359	0.370	
	T <sub>2</sub>	0.313*	0.361	0.339 <sup>0</sup>	0.395	0.375	0.383	
1973	T <sub>1</sub>	0.280°	0.265	0.303**	0.349	0.347	0.358	
	T <sub>2</sub>	0.294**	0.393	0.349**	0.407	0.396	_	
1974	T <sub>1</sub>	0.243	0.247	0.278 <sup>0</sup>	0.306	0.308	0.320	
	$T_2$	0.224**	0.279	0.267**	0.357	0.289	0.326	
			Stem	18				
1972	$T_1$	0.153	0.146	0.137	0.105	0.176	0.193	
	T <sub>2</sub>	0.139	0.164	0.094**	0.137	0.159	0.178	
1973	$T_1$	0.181	0.172	0.135	0.134	0.161	0.160	
	$T_2$	0.101**	0.158	0.110**	0.135	0.160	-	
1974	$T_1$	0.148	0.145	0.110	0.111	0.178	0.179	
	$T_2$	0.084**	0.132	0.091**	0.110	0.156	0.163	

#### 4. Mineral elements

Significantly more calcium, magnesium, iron and zinc was present in shoots which have completely finished their elongation growth in mid July, as compared with control shoots continuing to grow vigorously (Tables 7, 8). The upper part of long shoots sprayed with chlormequate contains 100 per cent more calcium than the control ones, 57 per cent more magnesium in leaves and 33 per cent more in stems. The difference in the iron content in stems in favour of those treated with chlormequate is 68 per cent and in zinc content 51 per cent. The differences in the leaves are slight, they also are much smaller in the basal part of long shoots than in the upper part. Iron and calcium contents are highest in the spurs.

There are no marked seasonal changes in phosphorus content in the upper part of treated long shoots and control ones. In the leaves and axes of the lower part of control long shoots, however, the content of phosphorus diminishes from

Table 7

K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, Ca, MgO contents (g/50 g fresh weight) in June (T<sub>1</sub>) and July (T<sub>2</sub>) of the year 1974 in leaves and stems of trees treated (C) with chlormequate and untreated ones (K)

Element	Upper p of shoo			Lower of sh		Spurs	
		K	С	K	С	K	С
		· · · · · · · · · · · · · · · · · · ·	Leave	S			
K <sub>2</sub> O	<b>T</b> <sub>1</sub>	0.33**	0.29	0.37**	0.29	0.35**	0.29
-	$T_2$	0.30°	0.28	0.39**	0.31	0.33**	0.26
$P_2O_5$	T <sub>1</sub>	0.09	0.10	0.07	0.08	0.07*	0.08
2 0	T <sub>2</sub>	0.09	0.09	0.06*	0.07	0.06*	0.07
Ca	$T_1$	0.14	0.16	0.19*	0.22	0.27	0.31
	$T_2$	0.13**	0.24	0.21**	0.25	0.33	0.37
MgO	$T_1$	0.08*	0.07	0.08	0.09	0.10	0.11
	т2	0.07**	0.11	0.08*	0.10	0.11	0.12
100			Stems				
к <sub>2</sub> о	T <sub>1</sub>	0.24°	0.22	0.21*	0.18	_	_
_	т2	0.21	0.22	0.21*	0.18	0.18	0.16
$P_2O_5$	<b>T</b> <sub>1</sub>	0.07	0.07	0.04	0.04	****	
- 0	T <sub>2</sub>	0.07	0.07	0.03**	0.05	0.08	0.08
Ca	т <sub>1</sub>	0.14*	0.17	0.15	0.18	_	
	$T_2$	0.12**	0.24	0.14*	0.19	0.57	0.56
MgO	$T_1$	0.07*	0.06	0.05*	0.06		_
	T 2	0.06**	0.08	0.04*	0.05	0.09*	0.11

mid June to mid July and increases in the axes of treated long shoots. Owing to this, the difference in phosphorus content between the latter shoots is significant in July. The phosphorus content in the upper part of the shoots is higher than in the lower, both in chlormequate-treated and control shoots. The phosphorus level is higher in the axes of spurs than in those of long shoots (Table 7).

In mid June boron content in the upper part of the stem is markedly higher in long shoots inhibited in growth and in spurs as compared with that in control long shoots. After one month these differences diminish and become nonsignifi-

Table 8

Fe. Mn. B and Zn contents (g/50 g fresh weight) in June  $(T_1)$  and July  $(T_2)$  of the year 1974 in leaves and stems of trees treated (C) with chlormequate and

untreated ones (K)

Element	Date	Upper part of shoots		Lower of she	-	Spurs	
		K	C	K	C	K	C
			Leave	s		-	
Fe	T <sub>1</sub>	3.47	3.55	3.00	3.48	4.43	4.33
	т2	2.26°	2.94	4.29	3.77	3.79	4.71
Mn	T <sub>1</sub>	1.04°	0.74	1.26	0.85	1.36	1.29
	т2	0.83*	1.23	1.19	1.31	1.15	1.27
В	Т1	0.48	0.64	0.40*	0.54	0.46	0.53
	т2	0.42	0.45	0.43 <sup>0</sup>	0.56	0.35*	0.53
Zn	T <sub>1</sub>	0.78	0.77	0.67 <sup>0</sup>	0.84	0.65	0.76
	T <sub>2</sub>	0.86	0.76	0.91	0.95	1.14	0.87
			Sten	18			
Fe	T <sub>1</sub>	1.47**	2.02	1.46	1.55	<del>-</del>	_
	т2	1.22**	2.05	1.52*	1.97	3.88	4.07
Mn	T <sub>1</sub>	0.36**	0.14	0.35*	0.12	_	_
	T <sub>2</sub>	0.30	0.39	0.31	0.37	0.29	0.31
В	т <sub>1</sub>	0.25**	0.43	0.26	0.32	0.50°	0.76
	T <sub>2</sub>	0.29	0.35	0.20*	0.31	0.41	0.46
Zn	T <sub>1</sub>	0.52	0.51	0.41	0.53		
	т2	0.45**	0.68	0.45 <sup>0</sup>	0.64	1.00	1.05

Explanations as in Table 1.

 $T_1 - June 19. T_2 - July 15.$ 

cant because the boron content in spurs and treated long shoots diminishes, while it increases in control long shoots. The boron level in the lower part of sprayed long shoots was at this time significantly higher (Table 8).

In shoots treated with chlormequate the potassium content is significantly lower, especially at the earlier date. In mid July these difference persist only in the leaves. They are much smaller and significant only in the basal part of long shoots (Table 7).

Manganese content both in leaves and shoots shows no noticeable tendency (Table 8).

# Starch presence in the stem

Observations over three years demonstrated that storage starch is present in very low amounts in elongating parts of the stem. In 1972 it was absent around mid June in the subapical part of vigorously growing shoots. It appeared only in the starch sheath in 1973 and 1975 and in some few parenchymatous cells of the forming protoxylem and in the pith (Fig. 2). In the subapical part of shoots sprayed with chlormequate, which ceased to elongate large and numerous starch

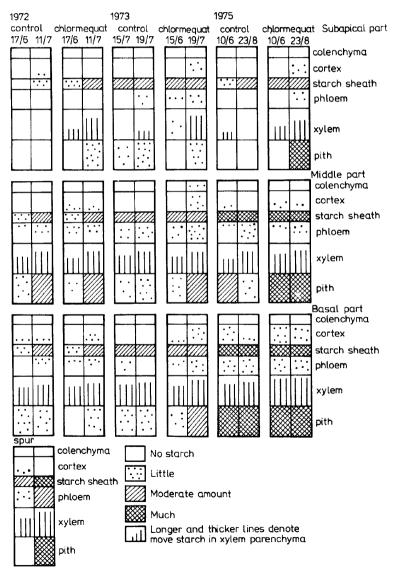


Fig. 2. Quantitative estimation of starch in the particular tissues on cross sections of the pear tree stem

grains can be seen at this time in the cells of the starch sheath and this substance begins to accumulate in the parenchyma of secondary xylem on the side facing the pith, which now forms a broad layer.

In the middle nonelongating parts of vigorously growing control shoots and those inhibited in growth by chlormequate treatment starch appears in similar amounts. It is found in the starch sheath, and in small amounts in the phloem parenchyma and in the pith.

Treated and untreated shoots show in their basal parts starch in the parenchymatous cells of all tissues. It is more abundant in the xylem rays and in the pith of this part of the shoot than in the middle part, both in treated and untreated trees (Fig. 2).

In mid July wide differences are noticeable in the amount of starch accumulated in te subapical part of the shoots, in dependence of the intensity of their growth. At this time in shoots still continuing elongation the beginning of starch accumulation is noted in the xylem, only scarce grains in the cortex parenchyma and mostly none in the pith. In the subapical part of shoots, however, which grew less intensively and finished elongation growth, the xylem is full of starch, there is some in the pith, phloem parenchyma and cortex parenchyma. Large starch grains appear in the cells of the starch sheath both in shoots treated with chlormequate and in the controls (Fig. 2). Somewhat more starch appears in the phloem and cortex parenchyma of sprayed shoots than in the controls (Fig. 2).

Spurs were investigated for the presence of starch only in 1972. In mid June numerous starch grains were found in the starch sheath and xylem parenchyma. Only scarce grains are visible at this time in the phloem parenchyma and cortex parenchyma cells. After the lapse of one month the starch accumulation in spurs is very high. All parenchymatous cells of the xylem and those of the pith are filled with starch grains of various size. The phloem parenchyma is also filled. Starch is also abundant in the cells of the cortex (Fig. 2).

## DISCUSSION

# Starch

The observations performed indicate that storage starch accumulation in pear stems is connected with their elongation growth. In the period of shoot elongation starch accumulates only in parenchymatous cells, outside the vascular cylinder, forming what is called the starch sheath. In the parenchyma of differentiating xylem, the layer of which occupies only 8 per cent of the stem cross section radius, there is very little starch. In other tissues it is absent. When growth is less intensive, and particularly when elongation growth ceases in treated

shoots, starch rapidly accumulates in the xylem parenchyma and pith cells and further in phloem and cortex parenchyma.

The differences in the quantity of accumulating storage starch in vigorously growing and inhibited in growth long shoots or in spurs which by nature grow weakly, results probably from the different intensity of elongation growth of these shoots. They appear mainly in the subapical part. Here in long shoots sprayed with chlormequate and in spurs storage starch accumulates in the xylem in large amounts in mid June. The xylem layer occupies at this time 15 - 23 per cent of the cross section radius.

Early ending of elongation growth of the treated shoots makes rapid starch accumulation possible in the tissues. In mid July all parenchymatous cells of the subapical part of treated shoots and of spurs are filled with starch. This is the period of the beginning of differentiation of flower buds on spurs and in the subapical part of chlormequate-treated shoots.

The here presented results of observation on the course of starch accumulation in pear shoots agree with those of investigations of other authors concerning apple trees. S w a r b r i c k (1928), in apple shoots above the ring, and Włodek and Bielińska-Czarnecka (1963) and Grochowska (1973), in apple spurs forming flower buds, noted starch accumulation in the course of June. According to their unanimous opinion, slowed down growth favours starch accumulation. As claimed by these authors starch appears in general more abundantly in xylem and in the pith than in the cortex parenchyma and phloem.

Filipovich and Rowe (1977) did not obtain, by inhibiting with SADH the growth of young apple trees of the 'Jonathan' variety, apparently a significant increase of starch content in the shoot apexes as compared with the controls. They calculated the starch content in relation to dry weight which was significantly lower in the shoot apexes of controls where the tissues are more hydrated than in those inhibited in growth; hence the high ratio of starch to dry mass in the apexes of stems not treated with SADH.

# Sugars

Three-year investigations demonstrated that stems inhibited in growth after spraying with chlormequate and the control ones as well as spurs exhibited in mid June very similar reducing sugars contents. In mid July, however, that is about 50 days after treatment, a significantly increased amount of these compounds was found in the upper and lower parts of shoots sprayed with chlormequate. The leaves from the lower part of these shoots which had grown before spraying contained more reducing sugars as early as the first part of June, and in the upper part later in mid July.

The higher reducing sugars content in leaves from shoots treated with chlormequate may result from the better assimilation conditions due to anatomical changes in the leaves (wide intercellular spaces in the spongy parenchyma and longer palisade parenchyma cells) owing to the influence of the retardant (H a l f a c r e and B a r d e n, 1968; E a t o n and L u i, 1970) or to a delay in their ageing (H a l f a c r e and B a r d e n, 1968). The high and rather uniform simple sugars and starch contents along the whole length of the stem in sprayed shoots is probably the consequence of an increase in the amount of living tissue in the stem, a better supply of assimilates by the leaves and a reduced efflux in acropetal direction (M o n s e l i s e and L u c k w i l l, 1974) because of inhibition of elongation growth, and perhaps also in basipetal direction as the result of declining root growth (S c h u m a c h e r et al., 1967).

# Nitrogen compounds

Shoots sprayed with chlormequate contained in mid July also much more nitrogen compounds than did unsprayed ones. The content of protein nitrogen was significantly higher in long shoots inhibited in growth than in vigorously growing ones. Wide differences, however, were noted in the upper part of the shoots and in leaves amounting to 15-34 per cent for leaves and 18-57 per cent for shoots in dependence on the year. The protein compounds content in the subapical part of treated shoots and spurs with an apical bud was similar.

Soluble nitrogen content in shoots was much lower than that of protein nitrogen, but its amount was also significantly higher in the upper part of long shoots treated with chlormequate and in spurs than in long shoots of control trees. In this nitrogen fraction arginine dominated in all shoots. Serine with glutamic acid was less abundant and so was aspartic acid, asparagine, glutamine and threonine. In shoots with slowed down growth there was more than 60 per cent more aspartic acid, glutamine, serine with glutamic acid and asparagine and 47 - 39 per cent more threonine and arginine than in the vigorously and longer growing shoots.

After the end of elongation growth nitrogen is utilized above all for secondary growth and tissue differentiation in the stem and for storage forms. Therefore, the higher nitrogen content, particularly in its storage form, that is in protein compounds in the shoots may be explained by the reduced utilization of nitrogen compounds for new growth as compared with that in vigorous and long growing shoots. B i e l i ń s k a et al. (1957, 1964) report that total nitrogen content in their experiments was always higher in apple spurs than in long shoots.

The present results over three years showed distinctly that nitrogen compounds accumulate mainly in the form of protein and, by analogy with apple trees, it may be presumed that the storage in pear trees also consists of protein nitrogen, although soluble nitrogen compounds including arginine may also play

some role. Protein nitrogen is the main nitrogen storage compound in apple trees and it accumulates in the cortex and xylem, but the role of storage substance is mainly played by protein in the cortex (Tromp, 1970; Tromp and Ovaa, 1971 a, b; 1973; Spencer and Titus, 1972). In Oland's (1959) opinion soluble compounds nitrogen, and particularly free amino acids serve as storage nitrogen. O'K e n n e d y et al. (1975) and O'K e n n e d y and T i t u s (1979) claim that the soluble form on nitrogen compounds may constitute the storage form, but protein nitrogen is most important in this respect and it is accumulated in the cortex and xylem. In the soluble fraction the participation of arginine in free amino acids is most important, particularly in the xylem and this compound is considered by the above named authors as the storage form. The fact that arginine is the main storage amino acid in apple trees has been earlier established by B i e l i ń s k a et al. (1957, 1964). A higher arginine per cent was found by Tromp and Ova a (1967, 1971 a, b) in xylem sap in the autumn-winter period, whereas during the spring-summer season aspartic acid and asparagine, glutamic acid, glutamine, threonine and serine dominated. B o 11 a r d (1953, 1956) reports that aspartic and glutamic acids and their amides form in apple-tree roots and those of other plants and are transported with the transpiration stream through the xylem to the above ground parts. Spencer and Titus (1971) ascertained by means of <sup>14</sup>C that glutamine is transported in equal amounts through the phloem and xylem, and asparagine probably only through the xylem. This might suggest that anatomical changes in the xylem, consisting of a reduction of the number and lumen of the vessels, and thus restricting the transpiration stream, may influence the level and the ratio of the nitrogen compounds to sugars and the direction of nitrogen metabolism. These suppositions still have to be confirmed by precise biochemical investigations. It may, however, be stated at present that in pear trees the main storage form is protein nitrogen, and among soluble compounds arginine. It would seem also that shoots with reduced growth dynamics contain markedly more storage nitrogen, the more the higher the level of saccharides and the more there is of storage tissue in the shoot, this being characteristic of stems with reduced elongation growth dynamics.

# Mineral elements

Determination of mineral elements demonstrated a high accumulation of calcium, magnesium, iron and zinc in shoots with weak growth dynamics. Spurs always exhibited the highest content of these mineral substances, and in the upper part of long shoots sprayed with chlormequate their content was also high.

Ystaas (1972) and Drake et al (1974) noted an increase in calcium content in leaves of apple and pear long shoots in the period between June and September, and Hołubowicz (1970, 1971) observed a similar fact in

the leaves and buds of spurs. In the experiments of Bielińska and Włodek (1958) and Barrera-Guerra and Słowik (1978) spurs always contained more calcium than did long shoots. Calcium transported through phloem and xylem and not taken up by young leaves after the end of elongation growth is retained in the stem. It is accumulated in one year and may be utilized in the next (Wieneke and Führ, quoted by Borys, 1979).

A higher iron, magnesium and manganese content, but somewhat lower potassium amount in per cents of dry weight were found by B a r r e a-G u e r r a and Słowik (1978) in spurs than in long shoots of the 'McIntosh' apple. They also noted that the contents of these substances differed in dependence on the position of the shoots in the tree crown. R o b i n s o n (1975) reports that chlormequate applied in the form of spray always increases the macroelements concentration in the above ground parts of plants. An exception is potassium the amount of which decreases. SADH, on the contrary, applied to apple trees causes a drop in the calcium level, but has no noticeable influence on P, K and Mg. According to V e l a r d e and S a l a m a n c a (1978), SADH applied at the moment of most intensive apple tree growth increased significantly the K and decreased Ca, Fe and Mn contents without any major effect on the amount of Mg, Zn and Cu.

Potassium content in the leaves and shoots sprayed with chlormequate was significantly lower when converted to per cent of dry weight (results not included), whereas when converted to grams of fresh weight the differences were smaller or even insignificant as compared to those noted in the upper part of long shoots (Table 7). Potassium probably plays a more important role in the growing parts than in those with depressed elongation growth dynamics or in parts which have ended growth. In this situation uptake and accumulation of potassium may be limited by antagonistic ions such as magnesium, the amount of which is significantly higher in weakly growing shoots.

Smaller differences than in the case of calcium or iron were observed in mid July in the phosphorus content of shoots with different growth dynamics. Younger leaves, that is those in the upper part of control long shoots and from shoots treated with chlormequate contained more  $P_2O_5$  in relation to fresh weight than did older leaves. The content of this compound was highest in the axes of spurs, but similar to that in the upper part of long shoots inhibited in growth on which flower buds formed and in the upper part of vigorously growing long shoots which remained vegetative. This situation may have changed later.

Phosphorus compounds play a central role in cell metabolism, therefore, phosphorus is present in larger quantities in young leaves than in older ones. Rubin (quoted by Bielińska and Włodek, 1958) found more phosphorus in young tissues than in older ones, but its content was highest in flowers and flower buds. Bielińska and Włodek (1958) found more phosphorus in spurs than in apple tree long shoots and Fritzsche et al.

(1964), Fe u c h t (1966, 1967), Fe u c h t and A r a n c i b i a (1968) and B a x t e r (1972) detected large phosphorus quantities in flower buds of numerous fruit trees in the period of their formation and before blossoming. B o u l d and P a r f i t t (1973) found a direct relationship between the phosphorus content in the leaves and the number of flower buds set on apple trees.

Uptake and accumulation of phosphorus compounds depends probably on the elongation growth dynamics and requirement of the plant for these compounds. Hołubowicz (1970) noted small variations in the period between June 21 and July 31 in leaves and buds of the apple cv. 'James Grieve', while Harley et al. (1958) demonstrated in apple tree sand cultures that the amount of <sup>32</sup>P transported from the roots to the leaves was very small, although it increased with time. The requirements of plants in the initial period of growth were satisfied from reserves. The phosphorus accumulated in pear and apple spurs is probably used by the flowers developing on them in the spring of the next year.

Chemical analyses demonstrated a high boron content in the period beginning with June and a marked depression after one month in spurs and in the upper part of long shoots inhibited in growth by treatment with chlormequate. Smaller periodical variations occurred in control long shoots, particularly in their lower part in both kinds of long shoots. The role of boron in plants is as yet not well understood. More data are available on the consequences of boron deficit than on its participation in cell metabolism. It is known, however, that variations in its content in the above ground parts of pear trees are very wide (Huguet and Woodbridge, quoted after B o r y s, 1979).

Interpretation of the differences in the content of mineral compounds in plants is difficult, above all in account of their diverse role in cell metabolism. It may, therefore, be supposed that the controversial results obtained by many investigators are only apparently contradictory. They depend, namely, in a high extent on the developmental phase of the plant and on the dynamics of their growth, whereas observance of calendar dates may lead to errors. Determination of mineral or organic compounds only in leaves and only from the central part of the stem does not represent their role. This is particularly true for storage compounds which have a decisive influence on the development of plants in the initial phase of the next vegetation period.

# SUMMING UP OF RESULTS FROM PARTS I, II AND III AND GENERAL DISCUSSION

Investigations of many years duration demonstrated that flower bud differentiation in the pear tree cv. 'Doyenne du Comice' occurs in the climate of Poland similarly as in the apple tree and starts in the second half of July. Flower buds form mostly on spurs.

In the trees of this cultivar sprayed with chlormequate elongation growth was weak and flower buds formed also in the subapical part of the long shoots. The mean length of these shoots was about 25 cm and they were by more than one half shorter than those of unsprayed trees.

The end of internode elongation in sprayed shoots occurred in mid June, as confirmed by the differentiation and lignification of the phloem fibers and secondary xylem elements in the subapical part. At the tip of these shoots the apical bud begins to form at this time. Elongation growth of the long shoots ends under the action of chlormequate about four weeks before the flower buds begin to differentiate on them. Long shoots on control trees grow vigorously in June and continue growth over July and even up to August. Flower buds form but seldom on these shoots.

Growth inhibition due to chlormequate has a distinct influence on the anatomical structure of the pear stem. Differentiation of the particular tissues in stems sprayed with chlormequate has a different course than in unsprayed ones. After ending of elongation growth the cortex in the subapical part of the sprayed shoots begins to extend and this leads to the formation of a characteristic thickening in this part of the shoot. There are no major changes in the phloem caused by chlormequate, but secondary xylem increment is weak. Multi-row rays appear in the xylem and the conducting elements are less numerous and have a markedly reduced lumen. As a consequence of these changes the proportion of parenchyma in the xylem of the weakly growing stems is larger than in the xylem of vigorously growing ones of control trees. The changes occurring in the anatomical structure of the subapical part of long shoots sprayed with chlormequate thus lead to a distinct prevalence of living elements capable of accumulating storage substances over dead elements playing a mechanical conducting role. The anatomical structure of the stem in the subapical part of long shoots inhibited in growth is similar to that of spurs in which growth of the internodes is still stronger inhibited. It is, therefore, possible that the changes in the anatomical structure of the stem under the influence of chlormequate are the consequence of elongation growth inhibition in the shoots.

After the end of elongation growth, accumulation of storage substances begins in the stem. Three-year studies demonstrated that during weakened growth, and particularly after the end of elongation growth caused by chlorme-quate treatment the content of reducing sugars is higher in these shoots than in the controls. Accumulation of storage starch also occurs earlier in long shoots of treated trees than in the control ones. In mid July there is very much starch in the subapical part of long shoots inhibited in growth in the xylem parenchyma, somewhat less in the pith, phloem perenchyma and cortex parenchyma. At this time the beginning of starch accumulation is observed in the corresponding control long shoots in the xylem and scarce grains in the cortex parenchyma. In the pith it is usually absent. In the middle and basal parts of long shoots sprayed

with chlormequate there is more starch than in the same parts of control long shoots. Accumulation of storage starch begins earliest, however, in spurs characterized by the lowest growth intensity. In mid July there is much starch in the xylem parenchyma and the pith cells of these shoots. There also is much in the cells of the cortex and the phloem parenchyma. These data suggest that storage starch accumulation in the pear stem in mid summer is connected with a depressed elongation growth.

Before the differentiation of flower buds, in mid July long shoots sprayed with chlormequate contain also much more nitrogen compounds, and especially protein than the control ones. Protein nitrogen is the main storage form of nitrogen in the pear tree. In the spurs and also in the treated long shoots the protein nitrogen level is similar and significantly higer than in untreated vigorously growing long shoots. These difference probably are the result of a lower utilization of nitrogen compounds for growth increments by the shoots with depressed growth dynamics. The seasonal fall of the protein nitrogen level from mid June to mid July in the treated shoots and spurs is much smaller than in the long shoots of control trees. The widest differences in the protein nitrogen level are noted in the upper part of unsprayed long shoots and those sprayed with chlormequate. In this part there also are wide differences in soluble compounds nitrogen, particularly arginine, which probably also is a storage form of nitrogen. It occurs in largest quantities in the upper part of sprayed long shoots and in spurs. It is an irrefutable fact that shoots with a weakened and early ending elongation growth, on which flower buds form contain much more nitrogen in protein and arginine form than the vegetative vigorously growing ones..

The results of annual studies suggest that long shoots sprayed with chlormequate contain significantly more calcium, magnesium, iron and zinc than the vigorously growing unsprayed long shoots. The levels of these mineral components are higher in spurs and in the upper part of weakly growing long shoots treated with chlormequate than in the upper part of control shoots. The differences in phosphorus and boron contents between these shoots are not so pronounced, but their amount in spurs is also highest.

Comparison of long shoots with depressed growth by the action of chlormequate, on which flower buds form in the subapical part, and of spurs forming an apical flower bud with long shoots of control trees characterized by vigorous growth on which no flower buds form indicates that flowering initiation in the pear trees is associated with a high level of both organic and inorganic storage compounds in the stem.

Many factors no doubt influence the processes leading to the accumulation of a definite level of storage compounds, indispensable for the induction of changes associated with the transition of the meristem of the apical bud from the vegetative to the generative phase. Many hypotheses have been advanced for elucidation of this transition. The here obtained results confirm some of them and

allow to explain some seemingly contradictory views concerning the role of certain factors favouring flower bud formation in fruit trees.

At the beginning of the present century attention was called to the role of sugars and further starch, and particularly the ratio of these substances to nitrogen compounds in flowering initiation (Klebs, 1903; Kraus and Kraybill, 1918; quoted after Bielińska, 1957; Hooker, 1920; Harley et al., 1942). The experiments of Kraybill (quoted by Bielińska, 1957) and later of Grochowska (1973) demonstrated the significance Of the saccharide level and particularly of starch in apple spurs in the period of flower bud initiation.

Heinickie (1930) and Delap (1967) pointed out the significance of nitrogen compounds and Potter and Phyllips (1930) and Ursulenko (1955) of protein nitrogen. The latter authors found a high protein nitrogen content in apple spurs forming flower buds. Bielińska (1956, 1957) also detected in apple spurs a total nitrogen content higher than in long shoots and prevalence of protein nitrogen over soluble compounds nitrogen. According to Tromp (1970), Tromp and Ovaa (1971a, b; 1973), Spencer and Titus (1972) protein nitrogen is a storage form of nitrogen in apple trees. On the basis of the author's own results it would seem that in the pear tree the main storage form is also protein nitrogen. Soluble compounds nitrogen including arginine may also be a storage form in the pear tree like in the apple (Oland, 1959; O'Kennedy et al., 1975; O'Kennedy and Titus, 1979).

Certain mineral factors are considered to be of importance in the flowering process. Fe u c h t (1966, 1967) and Fe u c h t and Arancibia (1968) found a high organic phosphorus content in buds of fruit trees in the period of differentiation as well as before and during blossoming. Bould and Parfit (1973) demonstrated even a simple relation between the number of flower buds set on apple trees and the phosphorus level in the leaves. Bielińska and Włodek (1958) however, did not confirm such a relation.

Calcium is always present in larger quantities in the spurs than in the long shoots (Hołubowicz, 1970; 1971; Bielińska and Włodek, 1958; Barrera-Guerra and Słowik, 1978), this seemingly indicating its role in the process of flowering.

Phosphorus like calcium and other mineral elements such as iron, magnesium, manganese, boron and zinc play an important role in the cell metabolism. It is difficult to establish their role in the transition from the vegetative to the generative phase since the level of each of these elements is dependent on the content of other mineral ones and on the rate of growth of the tested plant as well as on other factors. This might explain the frequent divergent results of various

investigators. The results obtained so far indicate that for setting of flower buds a high level of both organic and mineral compounds is necessary in the shoots.

Hormones doubtlessly have an important role in the chain of processes lead the physiological state in which the plant is capable of passing to the generative phase. Fulford (1966) and Abbott (1970; 1977) considered that the apple bud is capable of differentiation in dependence on the development of a minimal number of leaf primordia and on the length of the plastochrone that is the time period between the formation of two successive nodes. The same authors proved that the number of nodes preceding the formation of flower elements in the 'Cox Orange' cv. is 20. If the plastochrone is longer than seven days, the critical number of nodes is not reached before the end of the vegetation season and the bud remains vegetative. Very rapid node formation (short plastochrone) may cause the bud to grow into a vegetative shoot in the same year. An intermediate length of the plastochrone is favourable for flower bud formation. L u c k w i 11 (1974) believed that growth regulators which stimulate or inhibit flowering initiation in apple trees may exert an influence on the rate of node formation in the bud, and thus their number. Later investigations of Luckwill and Silva (1979), however, demonstrated that nodes formed at the same rate at first in all the buds of the apple tree 'Golden Delicious' during 10 weeks from full blossoming, independently whether the buds were to become flower buds or remain vegetative, and independently whether the shoots were sprayed with GA<sub>3</sub> or SADH or not treated at all. The subsequent nodes formed slower reaching the number of 16 in vegetative buds. In buds which became flower buds, nodes formed rapidly to an end number of about 21. The increase in the rate of node formation started one to two weeks before flower bud differentiation. GA<sub>3</sub> and SADH applied on the 14th and 39th day after full blossoming only affected the number of buds showing a vegetative or flowering character of development. SADH stimulated and gibberellin inhibited flower bud formation. These substances also had a distinct influence on the date of initiation of flower bud differentiation, but only on long shoots, no such effect was observed on spurs. SADH speeded up differentiation of the apical flower bud and GA<sub>3</sub> delayed differentiation of lateral buds on long shoots.

According to L u c k w i l l and S i l v a (1979) the mechanism of action of SADH in the stimulation of flowering initiation of fruit trees is not clear as yet. It seems possible, however, that the retardant depresses the physiological "sink power" of the long shoot apex. This results in the reversal of the direction of assimilate flow from the long shoot apex to the lateral buds and causes a shortening of the plastochrone which precedes blossoming initiation. Gibberellin enhances the "sink power" at the shoot apex and counteracts flower bud formation. S a c h s (1977) considers that the hypothesis of change in the direction of flow of nutrient substances suggested by Knight as early as 1820 is the

best explanation of the favourable influence of various treatments on flower bud formation in fruit trees, for instance ringing, good illumination, water stress, stem elongation inhibition, biosynthesis or gibberellin transport inhibition, removal of young leaves and the use of dwarf stock. The essence of this hypothesis is the relation between vegetative and reproductive development. S a c h s (1977) supposes that activation of the central zone of the meristem by enhanced flow of nutrient substances into it is the condition for transition in this meristem in the bud from the vegetative to the generative phase. According to S a c h s (1977) the hypothesis of change in the direction of flow of nutrient substances, although it does not rule out the existance of a specific flowering induction stimulator, encourages to a search for other potential factors controlling blossoming and connected with plant growth. Chemical compounds, climatic conditions or management practices which stimulate mobilization of nutrient substances in the bud meristem tissue or limit the competitive physiological sink for assimilates in the course of flowering initiation are of great importance according to this hypothesis. Auxins, cytokinins and gibberellins may enhance assimilate transport in the direction of the treated tissue, probably by increasing the growth or metabolism of the cells and stimulating in this way or inhibiting flowering, in dependence on the function of the treated tissue. Some growth inhibitors which during flowering initiation do not inhibit the activity of the apical meristem, but depress that of the competing tissues, may stimulate flowering. It would seem, therefore, that plant hormones have only an indirect influence on flower initiation in fruit trees.

Gibberellins are considered as flowering inhibitors in fruit trees. They appear in high concentration in apple seeds between the 5th and 6th week after full blossoming. Therefore, young fruitlets become inhibitors of flower bud formation at this time, and their removal causes better flowering in the next year. Removal of parthenocarpic fruits does not give a similar effect (C h a n and C a i n, 1967; Luck will et al., 1969; Luck will, 1970). Exogenous gibberellin applied as a foliar spray immediately after flowering reduces or completely inhibits bud setting for the next year in apple, pear, sour cherry, plum trees and strawberries. GA<sub>3</sub> applied to apple trees of alternately fruiting varieties in the year of their fruiting reduces flowering in the following year, but, when applied in the year when the trees bear no fruit or to trees with removed flowers it produces no such effect (Werthheim, 1966; quoted by Jonkers, 1979; Fulford, 1973). In long-day rosette plants gibberellin evokes flowering induction in those plant species which form flowers on long shoots and inhibits this process in species which form flower buds on short shoots. Thus, gibberellin has no direct influence on flowering initiation, but acts on the vegetative stage which precedes the latter.

Auxins are not so distinctly connected with initiation of flowering as gibberellins. We rzilov et al. (1978) noted different IAA levels in vegetative

and flower buds of apple trees. According to these authors, a depression of the auxin level in the meristematic tissues favours transition of vegetative buds to generative ones. J i n d a l et al. (1974) report that in the period of elongation growth the auxin level in normal apple tree shoots is higher than in those of dwarf mutations.

It seems that the main role of hormones in flowering initiation of fruit trees consists in regulation of growth by directing nutrient substances transport. Auxin and gibberellin, considered as main stimulators of fruit tree growth, are synthesized in young parts of shoots and seeds of developing fruits (J o n e s and Phillips, 1966; Luckwill et al., 1969; Grauslund, 1972) and they are readily translocated to other parts of the tree (G r o c h o w s k a. 1968; 1974; Sińska et al., 1973; Hoad, 1978). Local concentration maxima of endogenous hormones occurring in shoot apexes and fruits form a physiological sink, directing assimilates, organic nitrogen compounds and mineral compounds to these growth regions. Cytokinins synthesized in the roots play a major role in the regulation of cell division. They are capable of stimulating RNA and protein synthesis in plant cells. Together with gibberellin and auxin they contribute to the formation of a strong physiological sink power (Bollard, 1957; Zimmermann, 1960; Shindy and Weaver, 1967; Wareing and Phillips, 1978; Starck, 1979). Hatch and Powell (1971a) found that plant hormones such as auxins, gibberellins and cytokinins, especially when applied in various mixtures to apple seedlings after removal of leaves and the apical part of the stem mobilise <sup>32</sup>P flow through the phloem in acropetal and basipetal direction from leaves and to leaves, in dependence on the surface treated with hormones. The same hormones (Hatch and Powell, 1971b) mobilized also flow of organic substances with <sup>14</sup>C in acropetal direction when root competition was eliminated by ringing of seedlings. Gil-Albert and Marin (1978) achieved assimilate transport reduction of assimilates labelled with 14C from the middle leaf of shoots in acropetal direction after elongation growth inhibition of shoots sprayed with chlormequate. Gibberellins like auxins are involved in processes associated with internode elongation and thus with elongation growth of the stem. Gibberellin or a mixture of gibberellin with auxin causes elongation of decapitated apple tree shoots. Auxin applied alone stimulate very weakly internode elongation. Cytokinins in mixture with auxin and gibberellin exhibit a synergistic action stimulating growth (J a n k i e w i c z et al., 1972; Plich, 1972; Moraszczyk et al., 1974).

The influence of exogenous inhibitors such as chlormequate, SADH and ethephon on elongation growth of apple and pear trees and on flower bud formation is obvious. Jindal and Dalbro (1977) reported after Lang (1970) that growth retardants such as Amo 1618 and chlormequate reduce the gibberellin level in various plants. Volynetz and Polchenko (1977)

proved that chlormequate inhibits gibberellin and auxin biosynthesis in *Lupinus* L. Hoad and Monselise (1976) and Fontana-Degradiand Visai (1978) demonstrated that SADH depresses gibberellin content in growing apple shoots. Jindal and Dalbro (1977) observed a reduction of auxin content in the apple shoot apexes after spraying with SADH. This reduction is explained by the authors by an increased production of ethylene under the influence of SADH and the possibility of an influence of retardants on auxin catabolism by increasing the peroxidase content and the IAA activity of oxidase. An enhanced ethylene production may cause blocking of tryptophane transition to IAA by inhibiting tryptophane oxidase during IAA biosyntehsis.

On the basis of the experiments performed the influence of chlormequate may be summarised as follows:

- 1. Inhibition of vegetative shoots growth by inhibition of elongation growth of the internodes, inhibition of formation of new leaf primordia and internodes and acceleration of bud development that is of scales formation.
- 2. Stimulation of development of parenchymatous tissues in the stem and accumulation in them of storage material (saccharides proteins and mineral compounds).
- 3. Stimulation of generative bud development, that is transformation of vegetative buds to flower buds.

In the light of the present author's own results and the available literature the sequence of the processes occurring in the shoots, leading to initiation of the generative phase in the pear tree after chlormequate application may be established as follows:

- 1. Depression of the gibberellin level and disturbance of the hormonal equilibrium in the apexes of long shoots causes growth inhibition in these shoots preceded by a shortening of the internodes.
- 2. Enhanced growth of cortex parenchyma and weakened xylem development due to reduced auxin content leads to an increased proportion of parenchymatous tissue in the stem.
- 3. An enhanced accumulation of storage substances in the stem because of the large number of cells capable of their storage and a better assimilate supply by a larger number of leaves per shoot length unit and a weaker activity of the apical sink.
- 4. More intensive development of buds and formation of flower primordia, occurring with a high nutrient substances level probably with an unchanged cytokinin content and depressed gibberellin and auxin levels.

It may be supposed that the above presented scheme of chlormequate action applies to all growth retardants causing abundant bud formation on fruit trees. It also seems probable that the high level of storage substances is a condition for the transition of the bud from the vegetative to the generative phase. The actual mechanism of this transition is so far not known.

## REFERENCES

- A b b o t t D. L., 1970. The role of budscales in the morphogenesis and dormancy of apple fruit bud. [In:] Luckwill L. C. (ed.) Physiol. of Tree Crops. Acad. Press, London.
- A b b o t t D. L., 1977. Fruit bud formation in Cox's Orange Pippin. Report of Long Ashton Research Station for 1976, 167-176.
- Barrera-Guerra L. J., Słowik K., 1978. Akumulacja składników pokarmowych w liściach jabłoni odmiany 'McIntosh' w zależności od ich pozycji w koronie drzew. Pr. Inst. Sad. C. 1, 2 (61/62): 40-43.
- B a x t e r P., 1972. The flowering process a new theory. [In:] Carr D. J. (ed.) Plant Growth Substances. Spring-Verlag, 775-779.
- B i e l i ń s k a M., 1956. Chemical investigations on biennial bearing of apple trees. Bull. Acad. Polon. Sci. Ser. biol. 4: 179-181.
- B i e l i ń s k a M., 1957. Studia nad zawartością azotu i węglowodanów w liściach i pędach jabłoni przemiennie owocujących. Rocz. Nauk Rol. 75A (3): 433 520.
- B i e l i ń s k a M., W ł o d e k L., 1958. Zawartość fosforu i wapnia w liściach i pędach jabłoni owocujących przemiennie. Pr. Inst. Sad. 2: 5-28.
- Bielińska-Czarnecka M., DZięcioł U., Dawydko B., 1964. Comparison of spurs and leaves of annually and biennially bearing apple trees of Perkins variety in respect to their contents of nitrogen compounds. Acta Agrobot. 16: 133-144.
- B o 11 a r d E. G., 1953. Nitrogenous metabolism of apple trees. Nature 171: 571-573.
- B o l l a r d E. G., 1956. Nitrogenous compounds in xylem sap. Nature 178: 1189 1190.
- B o 11 a r d E. G., 1957. Composition of nitrogen fraction of apple tracheal sap. Austral J. Biol. Sci. 10: 279 287.
- B o r y s M. W., 1979. Żywienie mineralne. [In:] L. S. Jankiewicz: Fizjologia Roślin Sadowniczych. PWN, Warszawa, 178 264.
- Bould C., Parfitt R. J., 1973. Leaf analysis as a guide to the nutrition of fruit crops. X. Magnesium and phosphorus sand culture experiments with apple. J. Sci. Food. Agric. 24: 175-185.
- C h a n B. G., C a i n J. C., 1967. The effect of seed formation on subsequent flowering in apple. Proc. Amer. Soc. Hort. Sci. 91: 63 68.
- Drake M., Bramlage W. J., Baker J. H., 1974. Correlations of calcium content of Baldwin apples with leaf calcium, tree yield and occurrences of physiological disorders. J. Amer. Soc. Hort. Sci. 99: 379-380.
- De l a p A. V., 1967. The effect of supplying nitrate at different seasons of the growth, blossoming and nitrogen content of young apple trees in sand culture. J. Hort. Sci. 42: 149-167.
- E a t o n W. G., L u i A., 1970. Leaf anatomy and shoot growth in a compact strain of 'Delicious' apple compared to a normal strain treated with succinic acid 2,2-dimethylhydrazide. Hort. Sci. 5: 479 480.
- Feucht W., 1966. Mineralstoffgehalt alternierende Kurztriebe von Apfel und Birne. Mitt. Klosterneuburg 16: 146-151.
- F e u c h t W., 1967. Daten zum Stoffwechsel des Phosphors, Calciums und Stickstoff in den Endknospen des Apfelbaumes zur Zeit der floral Inductionsperiode. Arch. Gartenb. 3: 175 182.
- Feucht W., Arancibia M., 1968. La induccion floral en naranjos y su relacion con los minerales. Agrochimica 12: 89-99.
- Filipovich S.D., Rowe R.N., 1977. Effect of succinic acid 2,2-dimethylhydrazide (SADH) on starch accumulation in young apple trees. J. Hort. Sci. 52: 367-370.
- Font an a-Degradi C., Visai C., 1978. Correlation between growth of apple shoots and content of gibberellin like substances. Acta Hort. 80: 63-66.
- Fritzsche R., Kropt B., Huber L., 1964. Exakte Düngungsversuche mit Apfel und Kirschbaumen in Gefässen. Schweiz. Zeitschr. Obst- and Weinb. 73: 531-590.

- F u I f o r d R. M., 1962. The development of apple spur buds in relation to leaves and fruits. XVI the Intern. Hort. Congress, Brussels, 343.
- F u I f o r d R. M., 1966. The morphogenesis of apple buds. III. The inception of flowers. Annals of Botany 30: 209 219.
- F u l f o r d R. M., 1973. Flower initiation. Effect of gibberellin sprays. Rep. East Malling Res. Stn. for 1972, 47: 71 82.
- Gil-Albert F., Marin P., 1978. Effects of CCC on the translocation of photosynthates in young pear trees. Acta Hort. 80: 67-70.
- G r a u s l u n d J., 1972. Gibberellins in diffusates from shoots of apple trees. Physiol. Plant. 27: 65-70.
- Grochowska M. J., 1968. Translocation of indole-3-acetic acid-2 <sup>14</sup>C injected into seeds of five-week-old apple fruits. Bull. Acad. Polon. Sci., ser. biol. 16: 577 580.
- Grochowska M. J., 1973. Comparative studies on physiological and morphological features of bearing and non-bearing spurs of the apple trees. I. Changes in starch content during growth. J. Hort. Sci. 48: 347-356.
- G r o c h o w s k a M. J., 1974. Photolytic decarboxylation of carboxyl-14C-labelled indol-3yl-acetic acid in leaves of the apple tree. J. Exp. Bot. 25: 638-645.
- Halfacre G. R., Barden A. J., Rollins J. R., 1968. Effects of Alar on morphology, chlorphyll content, and net CO<sub>2</sub> assimilation rate of young apple trees. Proc. Amer. Soc. Hort. Sci. 93: 40-51.
- Halfacre G. R., Barden A. J., 1968. Anatomical responses of apple leaf and stem tissues to succinic acid 2,2-dimethyl-hydrazide (Alar). Proc. Amer. Soc. Hort. Sci. 93: 25-32.
- Harrley C. P., Magness J. R., Masure M. P., Fletcher L. A., 1942. Investigation on the cause and control of biennial bearing of apple trees. U. S. Dept. Agric. Techn. Bull. 792: 1-58.
- Harley C. P., Regeimbal L. O., Moon H. H., 1958. The role of nitrogen reserves in new growth of apple and the transport of <sup>32</sup>P from roots to leaves during early spring growth. Proc. Amer. Soc. Hort. Sci. 72: 57-63.
- Hatch A. H., Powell L. E., 1971a. Hormone-directed transport of <sup>32</sup>P in *Malus sylvestris* seedlings. J. Amer. Soc. Hort. Sci. 96: 230-234.
- Hatch A. H., Powell L. E., 1971b. Hormone-direct transport of certain organic compounds in *Malus sylvestris* seedlings. J. Amer. Soc. Hort. Sci. 96: 399-400.
- Heinickie A. J., 1930. Composition of fruit bud spur tissues of Wealthy apples under different conditions of nutritions. Proc. Amer. Soc. Hort. Sci. 27: 190-198.
- H o a d G. V., 1978. The role of seed derived hormones in the control of flowering in apple. Acta Hort. 80: 93-103.
- Hoad G.V., Monselise S.P., 1976. Effects of succinic acid 2,2-dimethylhydrazide (SADH) on the gibberellin and abscisic acid levels in stem tips of M 26 apple rootstocks. Scientia Hort. 4: 41-47.
- H o ł u b o w i c z T., 1970. Dynamika niektórych związków mineralnych w pąkach krótkopędów jabłoni odmian corocznie i przemiennie owocujących. Rocz. WSR w Poznaniu 25: 5 37.
- H o ł u b o w i c z T., 1971. Zmiany w zawartości składników pokarmowych w liściach krótkopędów corocznie i przemiennie owocujących drzew jabłoni. Rocz. WSR w Poznaniu 84-103.
- Hook er H. D., 1920. Seasonal changes in the chemical composition of the apple spurs. Res. Bull. Mo. Agric. Exp. Stn. No. 40.
- Jankiewicz L.S., Plich H., Borkowska B., Moraszczyk A., 1972. Growth correlations and the shape of young trees and shrubs. Acta Hort. 34: 107-116.
- Jindal K. K., Dalbro S., 1977. Effect of succinic acid-2,2-dimethylhydrazide on endogenous auxin level in apple shoots. Physiol. Plant. 39: 119-122.

- Jindal K. K., Dalbro S., Andersen A. S., Poll L., 1974. Endogenous growth substances in normal and dwarf mutants of Cortland and Golden Delicious apple shoots. Physiol. Plant. 32: 71-77.
- Jones R. L., Phillips I. D. J., 1966. Organs of gibberellin synthesis in light-grown sunflower plants. Plant Physiol. 41: 1381-1386.
- Jonkers H., 1979. Biennial bearing in apple and pear: A literature survey. Scientia Hort. 11: 303-317.
- K ą c z k o w s k i J., T o c z k o M., 1969. Instrukcja do ćwiczeń z biochemii. Dział Wydawnictw SGGW, Warszawa.
- L u c k w i 11 L. C., 1970. The control of growth and fruitfulness of apple trees. [In:] Luckwill L. C. i Cutting C. V. (ed.) Physiology of Tree Crops. Academic Press, London.
- Luck will L. C., 1974. A new look at the process of fruit bud formation in apple. Proc. XIX Intern. Hort. Congress, Warszawa, 237-245.
- Luck will L.C., Whyte P., 1968. Hormones in the xylem sap of apple trees. S. C. I. Monogr. 31: 87-101.
- L u c k w i 11 L. C., S i 1 v a J. M., 1979. The effects of daminozide and gibberelic acid on flower initiation, growth and fruiting of apple of 'Golden Delicious'. J. Hort. Sci. 54: 217-223.
- Luck will L.C., Weaver P., Mac Millan J., 1969. Gibberellins and other growth hormones in apple seeds. J. Hort. Sci. 44: 413-424.
- Monselise S. P., Luckwill L. C., 1974. Effects of SADH on the translocation of assimilates in apple. Scientia Hort. 2: 185-192.
- Moraszczyk A., Jankiewicz L. S., Plich H., 1974. Role of growth regulators in apple internode elongation and in the formation of secondary structure. Proc. XIX Intern. Hort. Congress, Warszawa, 1A: 416.
- O'K e n n e d y B. T., T i t u s J. S., 1979. Isolation and metabolism of storage proteins from apple shoot bark. Physiol. Plant. 45: 419-424.
- O'K ennedy B.T., Hennerty M.J., Titus J.S., 1975. Changes in the nitrogen reserves of apple shoots during the dormant season. J. Hort. Sci. 50: 321-329.
- O I a n d K., 1959. Nitrogenous reserve of apple trees. Physiol. Plant. 12: 594-648.
- P Li c h. H., 1972. Korelacje wzrostowe i wydłużanie się międzywęźli jabłoni. Ph. D. thesis, Inst. Sad., Skierniewice.
- Potter G. F., Phyllips T. G., 1930. Composition and fruit bud formation in non-bearing spurs of the Baldwin apple. N. H. Agric. Exp. Stat. Techn. Bull. 42: 1-41.
- P o w e 11 I. E., 1978. Vegetative growth in apple with reference to abscisic acid. Acta Hort. 80: 27-38.
- R o b i n s o n J. B. D., 1975. The influence of some growth-regulating compounds on the uptake, translocation and concentration of mineral nutrients in plants. Hort. Abstr. 45: 611-618.
- S a c h s R. M., 1977. Nutrient diversion: an hypothesis to explain the chemical control of flowering. Hort. Sci. 12: 220 222.
- Schumacher R., Fankhauser F., Schläpfer F. 1967. Einfluss des Heminstoffes Alar auf Fruchtentwicklung Schweiz. Landw. Forschung 6: 148-169.
- Shindy W., Weaver R. J., 1967. Plant regulators alter translocation of photosynthetic products. Nature 214: 1024-1025.
- Sińska I., Grochowska M. J., Lewak S., 1973. Changes in the endogenous gibberellins contents in immature apple seeds. Bull. Acad. Polon. Sci., ser. biol 21: 291-295.
- Spencer P. W., Titus J. S., 1971. Translocation of glutamate-<sup>14</sup>C and aspartate-<sup>14</sup>C by intact apple trees, J. Amer. Soc. Hort. Sci. 96: 131-133.
- S p e n c e r P. W., T i t u s J. S., 1972. Biochemical and enzymatic changes in apple leaf tissue during autumnal senescence. Plant Physiol. 49: 749-750.

- S t a r c k Z., 1979. Transport asymilatów w roślinach sadowniczych. [In:] L. S. Jankiewicz. Fizjologia roślin sadowniczych. PWN, Warszawa, 298-321.
- S w a r b r i c k T., 1928. Studies in the physiology of fruit trees. II. The effects of ringing, double ringing and dis-budding upon the starch content and cambial activity of two-year-old apple shoots. J. Pom. Hort. Sci. 6: 296-312.
- Tromp J., 1970. Storage and mobilization of nitrogenous compounds in apple trees with special reference to arginine. [In:] L. C. Luckwill, C. V. Cutting (ed.) Physiology of Tree Crops. Academic Press, London, New York.
- Tromp J., Ova a J.C., 1967. Seasonal variations in the amino acid composition of xylem sap of apple. Z. Pflanzenphysiol. 57: 11-21.
- Tromp J., Ova a J. C., 1971a. Spring mobilization of storage nitrogen in isolated shoot section of apple. Physiol. Plant. 25: 16-22.
- Tromp J., Ovaa J. C., 1971b. Phloem translocation of storage nitrogen in apple. Physiol. Plant. 25: 407-413.
- Tromp J., Ova a J. C., 1973. Spring mobilization of protein nitrogen in apple bark. Physiol. Plant. 29: 1-5.
- U r s u l e n k o P. K., 1955. Biologicheskie osnovy agrotekhniki i ezhegodnogo plodonosheniya yabloni. Ezhegodnoe plodonoshenie yabloni. Moskva, Sielchozgiz.
- Velarde F. G., Salamanca F. M., 1978. Effects of N-dimethylaminosuccinamic acid on mineral nutrition of one-year-old apple trees. Acta Hort. 80: 71-73.
- Volynetz A. P., Polchenko L. A., 1977. Chlorocholine chloride as a possible inhibitor of auxin biosynthesis and a factor limiting its utilization in growth processes. Fiziol. Rast. 24: 1021-1025.
- Wareing P.F., Phillips I.D.J., 1978. The control of growth and differentiation in plant. Pergamon Press., Oxford, New York, Toronto, Sydney, Paris, Frankfurt.
- Werzilov W. F., Plotnikova I. V., Alexandrova W. S., 1978. Growth regulators in relation to apple bud differentiation. Acta Hort. 80: 175.
- Włodek L., Bielińska-Czarnecka M., 1963. Zawartość skrobi oraz stosunek skrobi do azotu w krótkopędach jabłoni przemiennie owocujących przy różnym poziomie odżywiania. Pr. Inst. Sad. 7: 31-49.
- Y a d a v a U. L., D a y t o n D. F., 1972. Effect of exogenously supplied abscisic acid on a vigorous clonal apple rootstock. Hort. Sci. 7: 261 262.
- Y s t a a s J., 1972. Pear tree nutrition. I. Seasonal trend of major nutrients in pear leaves and the effect of different potassium supply on yield, fruit size and fruit quality. Meldinger Norges Landbrukshgskole 50: 1-16.
- Zimmermann M. H., 1960. Transport in the phloem. Ann. Rev. Plant Physiol. 11: 167-190.

Czynniki wpływające na tworzenie się pąków kwiatowych u gruszy odm. 'Komisówka'. III. Zawartość cukrowców, związków azotowych i niektórych składników mineralnych w liściach i łodygach gruszy

#### Streszczenie

Wpływ zahamowania wzrostu elongacyjnego długopędów gruszy odmiany 'Komisówka' na akumulację substancji pokarmowych po opryskaniu chlormequat badano w latach 1972-1974. Suchą masę, zawartość cukrów redukujących oraz azotu ogólnego, azotu związków rozpuszczalnych i azotu białkowego w liściach i osiach długopędów i krótkopędów drzew nie traktowanych i traktowanych chloromequat, oznaczano w ciągu 3 lat. W 1973 roku oznaczano zawartość argininy,

kwasu asparaginowego, glutaminy, seryny z kwasem glutaminowym i treoniny. Zawartość Fe, Mn, B, Zn, K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, Ca, Mg oznaczano w 1974 roku. Próbki do badań chemicznych pobierano każdego roku w dwóch terminach, w połowie czerwca i w połowie lipca, z sześciu drzew opryskanych chlormequat i z sześciu drzew kontrolnych. Pędy o osłabionym wzroście elongacyjnym mają większą zdolność gromadzenia substancji zapasowych w łodydze. Pędy opryskane chlormequat zawierały w połowie lipca istotnie więcej skrobi, cukrów redukujących, azotu ogólnego, azotu białkowego a także argininy oraz innych aminokwasów i ich amidów w porównaniu z długopędami drzew kontrolnych. Pędy o osłabionym przez chlormequat wzroście zawierały również więcej wapnia, magnezu, żelaza i cynku niż długopędy drzew kontrolnych. Pędy zahamowane we wzroście elongacyjnym przez chlormequat są bardzo podobne w części podwierzchołkowej, w której tworzą się pąki kwiatowe, do krótkopędów z pąkiem kwiatowym, tworzącym się na szczycie, tak pod względem budowy anatomicznej łodygi jak i zawartości składników pokarmowych. Wyniki te sugerują, że wysoki poziom, nie jednej a prawdopodobnie wszystkich substancji zapasowych ma bezpośredni wpływ na przejście pąka od fazy wegetatywnej do fazy generatywnej.