BULBS AND BULBILS OF ORNITHOGALUM CAUDATUM AIT.

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SUMMARY

The assumption of TROLL (1939), that the bulbils attached to the abaxial side of the bulb-scales of *Ornithogalum caudatum* are stalked axillary buds grown congenitally with the next-higher situated leaf, has been confirmed by the present morphological and anatomical investigations.

The bulbils originate at the base of the abaxial side of bulb-scales. Analysis of the vascularization showed, however, that they are connected with the enveloping bulb-scales which are therefore to be considered the actual subtending leaves.

1. INTRODUCTION

Ornithogalum caudatum Ait. (Liliaceae) is a bulbous plant originating from South Africa. The bulb is composed of thick, fleshy bulb-scales and extends partially above ground. The outer bulb-scales bear groups of bulbils on their abaxial side. In the outermost scales the highest bulbil of such a group is situated very high, often higher than half the distance between the base and the upper margin of the scale. Consequently, the remaining bulbils are inserted below this level. Sometimes they seem to be arranged in two or three longitudinal rows (*figs. 1* and *2*). In very rare cases bulbils are encountered on the adaxial side of a bulb-scale. Such bulbils are very small and situated close to the disk (*fig. 2*).

At first sight the bulbils seem to originate directly from the scale surface, but the fact that from the base of each bulbil a narrow ridge or rib runs along the scale surface toward the disk suggests another origin.

TROLL (1939) described the situation as follows: "... Man stelle sich vor, dass eine Gruppe von gestielten Beiknospen, ... statt selbständig sich zu entwickeln, der Zwiebelschale, vor der sie steht, und der sie durch ihr Tragblatt dicht angepresst ist, anwächst ... Natürlich muss ... hier der Prozess des Anwachsens im kongenitalen Sinne verstanden werden." Thus, according to this author, and before him BRAUN (1860, cit. Troll), the bulbils are stalked axillary buds, which during their development fuse with the abaxial side of the next higher leaf. Testing this hypothesis by anatomical research, we attempted to find answers to the following questions:

1. Are the described bulbils to be considered as axillary buds or are they actually only adventitious buds?
2. If the bulbils are to be considered as axillary buds, it would be interesting to

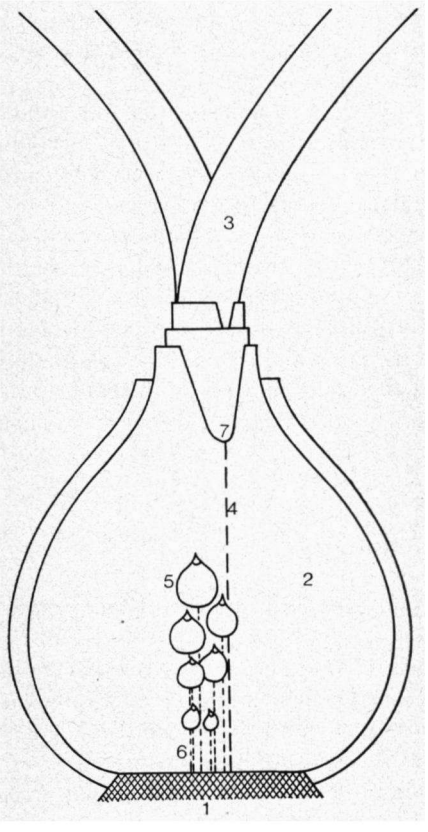

Fig. 1. Schematic drawing of the bulb of *Ornithogalum caudatum* Ait., outer bulb-scale partly removed.
1, disk; 2, bulb-scale; 3, foliage leaf; 4, ventral suture; 5, group of bulbils; 6, ridges; 7, incision.

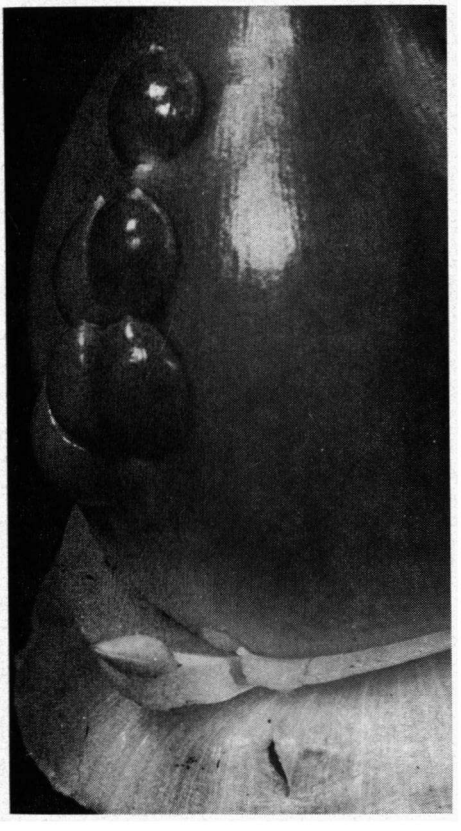

Fig. 2. Bulb of *Ornithogalum caudatum* with two bulb-scales removed. A group of bulbils can be seen on the abaxial side of the third bulb-scale. A small bulbil can be seen on the adaxial side of the second bulb-scale. About 1.5 times natural size.

learn how they become implanted on the "wrong" scales and, in addition, through which cause so high?

3. Are the described ridges running longitudinally with respect to the bulb-scales circumscribed entities?

2. MATERIAL AND METHODS

The material consisted of a number of bulbs of *Ornithogalum caudatum* grown in the Leiden Botanic Garden and all belonging to the same clone.

For the morphological investigations several bulbs were dissected.

For the investigation of anatomical structures several methods were used. To

study the gross anatomy of the vascularization, fragments of bulb-scales with bulbils attached and exhibiting the characteristic ridges were cleared in a mixture of 8 parts of chloralhydrate and 5 parts of distilled water.

For the preparation of slides, fragments were killed and fixed for three days *in vacuo* in F.A.A. (50 ml ethanol 95%, 10 ml glacial acetic acid, 10 ml formalin and 35 ml distilled water). After dehydration in an ethanol series (50, 70, 80 and 95%) the fragments were embedded in diglycolstearate. Sections were cut 15μ thick on a rotatory microtome, mounted on slides with Haupt's adhesive, dried in an oven over formalin fumes, and transferred to distilled water through an ethanol series (95%–10%). Staining was done according to MAÁCZ & VÁGÁS (1963): 2 minutes in Astra-blue (0.5 g in 100 g of a 2% solution of tartaric acid) and rinsed with distilled water, 2 minutes in Auramin (a saturated aqueous solution) and rinsed with distilled water and, finally, placed for 2 minutes in a 1% aqueous solution of safranin. Via tertiary butanol, phenolbenzene and xylene the sections were mounted in D.P.X. (E. Gurr).

3. MORPHOLOGY

3.1. Structure of the bulb

The bulb of *Ornithogalum caudatum* is composed of a number of bulb-scales, the disk, and numerous roots. The thick fleshy bulb-scales are inserted as entirely closed circular structures on the disk. The outermost bulb-scales loose their fleshy character and are present as desiccated membranes enclosing the green bulb. The green colour is caused by the fleshy outer scales which are green except for the colourless subterranean parts. The pale inner bulb-scales bear very long ribbon-like "foliage leaves" which die back at the tip. Each scale shows an incision (*fig. 1.7*). The part distal to this incision can be considered as the lamina of a foliage leaf, the scale as the vagina. A line drawn from the incision to the disk will be called the ventral suture of the bulb-scale (*fig. 1.4*). *Table 1* gives some data on the component parts of a bulb, starting at the outside and moving towards the centre, as they were found in February. These parts can be roughly divided into three categories, viz. bulb-scales with or without long ribbon-like laminae, flowerstalks or their desiccated remnants, and, lastly, leafy organs so young that the vaginal and laminar parts cannot yet be distinguished in them.

Fig. 3 shows how the different organs are inserted. The diagram gives information on the location of the ventral suture, the main vein, and the points of insertion of the highest situated bulbils. The main vein could be easily located in cases in which the "foliage leaves" were still present by tracing the distinct midrib of the laminar part into the scaly vaginal part. In the cases in which "foliage leaves" were no longer present the location of the main vein could be approximately fixed by drawing a straight line in the diagram from the site of the ventral suture through the centre of the circle to its opposite side. From *fig. 3* it is clear that the bulb-scales are spirally inserted.

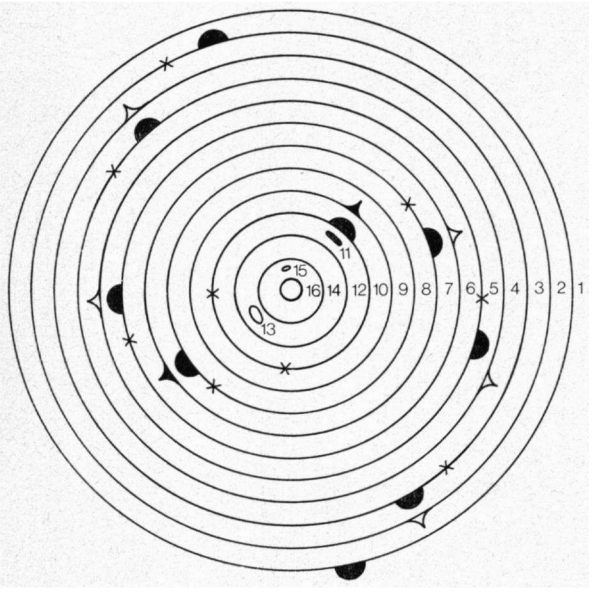

Fig. 3. Diagram of a bulb of *Ornithogalum caudatum*, dissected in February. (For details see *Table 1*.) Circles 1–10, 12, 14, and 16 represent bulb-scales and foliage leaves, nrs. 11, 13, and 15 the remnants of an inflorescence of the preceding year, the present inflorescence and an inflorescence primordium, respectively. The solid triangles represent the main veins of the foliage leaves, the open triangles the main veins of the outer scales (the latter arrived at by deduction). The crosses indicate the ventral sutures and the filled hemicycles the insertion site of the highest bulbils on the respective scales.

3.2. The insertion of the bulbils

It became apparent that the groups of bulbils are always to be found to the left of the ventral suture (see *fig. 1*). Furthermore, the highest inserted bulbil of a group proved to be inserted opposite the main vein of the leaf with the next-lower insertion. *Fig. 4* gives some data on the bulbils found on the four outer scales of a bulb dissected in February. Several points are apparent:

a. In general the length and diameter of the bulbils gradually decrease moving downwards on the scale;
b. The colour changes from green to white in the same sense.

From a comparison of the data of individual groups of bulbils deriving from different scales, starting at the outside of the bulb and moving towards the centre (see *table 1*), it is conspicuous that:

1. The number of bulbils per group decreases.
2. The distance between the point of insertion of the individual bulbils and the disk decreases.
3. The average length and diameter of the bulbils decrease.
4. On the outer bulb-scales the bulbils are predominantly green, on the inner scales white.

4. ANATOMY

According to ESAU (1953) the vascular system of a bulb is of the Palm type. The small traces originating in the scales follow a peripheral course in the disk. The larger bundles, however, approach the central part of the disk and return to the periphery on a lower level where they sometimes become united with other

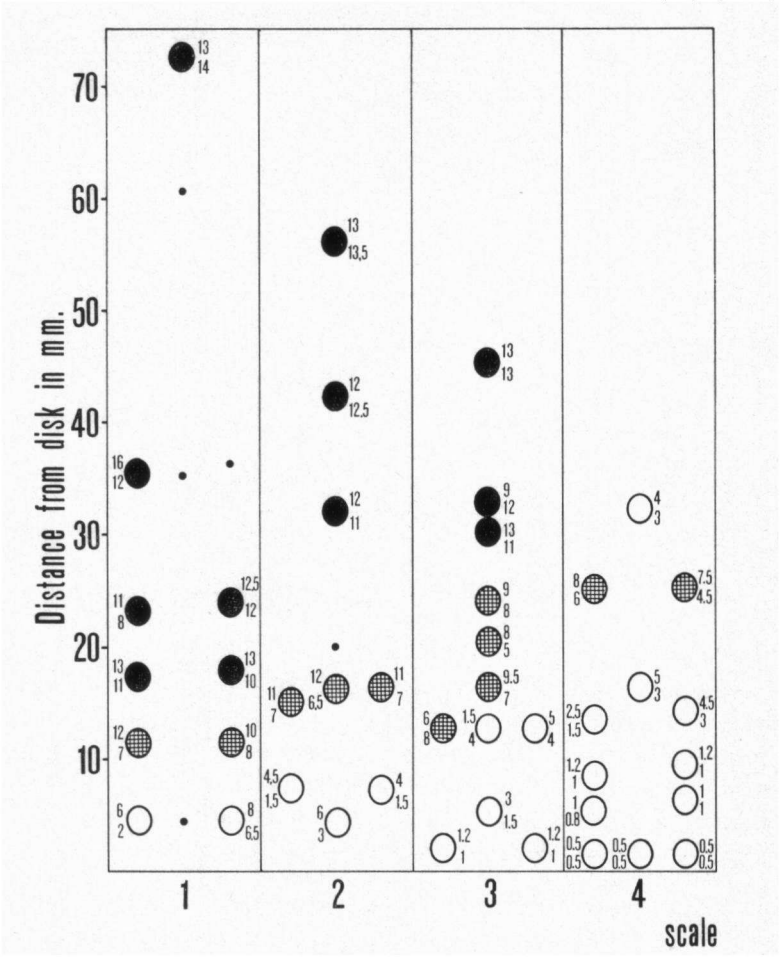

Fig. 4. Schematic drawing of the insertion site of the bulbils on the 4 outer scales of the bulb of *fig. 3* (see also *table 1*). Length and diameter in mm.
Solid circles = green bulbils; hatched circles = pale-green bulbils; open circles = white bulbils. Dots indicate the position of abscised bulbils of unknown colour.

peripheral bundles. According to MANN (1952) once they are united the bundles anastomose with other bundles, resulting in a complex network. The vascularization of the bulb of *Ornithogalum caudatum* also appears to follow the Palm type.

Fig. 5A shows a transverse section from a series originating from a young, still growing bulb-scale bearing three small white bulbils. This section shows the lowest-inserted bulbil and the vascular supply of the ribs belonging to two bulbils situated higher on the scale.

The epidermis of the scale covers both the bulbil and the ribs. No difference

Table 1.

No.	Bulb parts	"Foliage leaf" present or absent	Diameter of the remaining bulb (mm)	Total length (mm)	Distance incision to disk (mm)	Number of bulbils	Distance highest-inserted bulbil to disk (mm)	Distance lowest-inserted bulbil to disk (mm)
1.	green bulb-scale	−	54	95	95	14	72	4
2.	green bulb-scale	−	49	96	93	10	56	4
3.	green bulb-scale	−	45	95	83	12	35	1.5
4.	green bulb-scale	−	39	93	70	13	32	1
5.	pale green bulb-scale	−	31	92	60	4	14	1
6.	pale green bulb-scale	−	24	108	46	3	4	0.5
7.	pale green bulb-scale	+	16	−[1]	27	1	0.2	0.2
8.	white bulb-scale	+	12	−[1]	16	1	+[2]	+[2]
9.	white bulb-scale	+	9	−[1]	7.5			
10.	white bulb-scale	+	6	−[1]	4			
11.	inflorescence							
12.	undifferentiated leaf		4	156	4			
13.	inflorescence			104				
14.	undifferentiated leaf		3	10	+[2]			
15.	inflorescence			1.5				
16.	undifferentiated leaf		1.5	4	+[2]			

Some data pertaining to a bulb dissected in February
The numbers of column 1 correspond with those in Fig. 3.
−[1] not measured, "foliage leaf" dying back at the distal end
+[2] too small to be measured

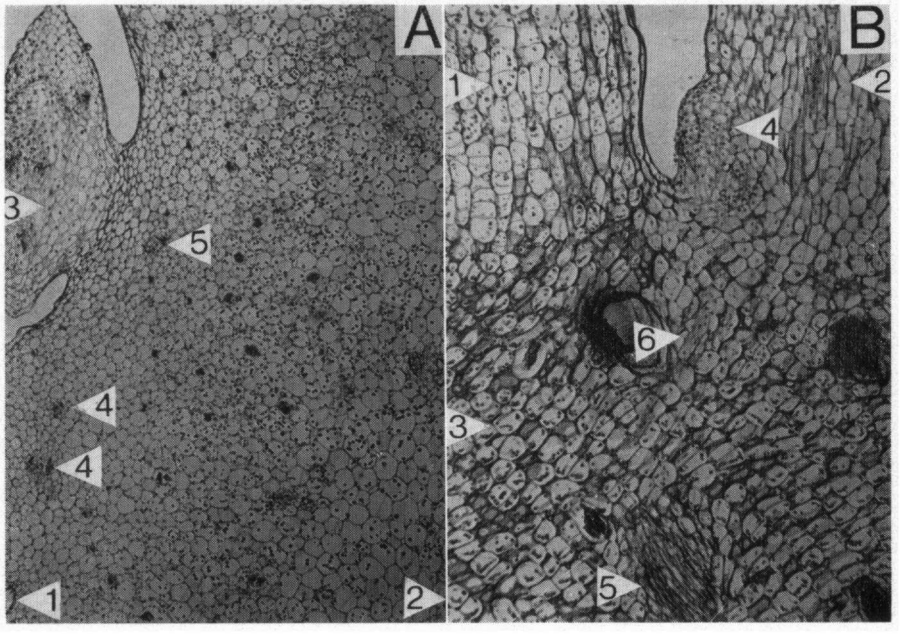

Fig. 5. A. Transverse section of a scale cut at a distance of 1 mm from the disk.
1, abaxial side of the scale; 2, adaxial side; 3, transverse section of a bulbil; 4 and 5, vascular bundle(s) in the rib of bulbils situated at 1.3 and 2 mm respectively from the disk. F.A.A.; Maácz-Vágás. × 40.

B. Longitudinal section through the basal parts of two adjacent bulb-scales and part of the disk.
1, older and 2, younger bulb-scales; 3, disk; 4, bulbil primordium situated at the abaxial side of scale 2; 5, vascular bundle belonging to scale 1; 6, procambium strand of the bulbil primordium (4). F.A.A.; Maácz-Vágás. × 100.

could be found between the parenchyma of the scale and of the ribs. In the scale two types of vascular bundles are present. Larger bundles possessing both xylem and phloem are found only in the adaxial part of the scale. Xylem is oriented adaxially, phloem abaxially. Slender procambium bundles are encountered everywhere in the scale.

These two types of bundles are also present in the ribs. The serial sections show clearly that the bundles run directly from the bulbils through the ribs to the disk; they do not anastomose with scale bundles.

Other serial sections indicate that the number of bundles in the ribs varies from 1 to 4. No regularities regarding the orientations of xylem and phloem were found. In almost all cases only one bundle, exhibiting abaxial xylem and adaxial phloem, runs downwards from the highest bulbil.

Fig. 5B shows a longitudinal section from a series comprising part of the disk and the basal parts of two adjacent scales. On the abaxial side of the base of the highest inserted bulb-scale a very young bulbil (4) is present. Analysis of the

complete series showed that the vascular bundle (5) containing xylem and phloem belongs to the adaxial part of the bulb-scale with the next lower insertion, and the procambium bundle (6) to the bulbil (4). It could also be established that bundles (5) and (6) anastomose. From other series of slides and in fragments cleared in a chloralhydrate solution it was also possible to demonstrate that vascular bundles running downward from bulbils on certain scales always unite, in the disk, with bundles of lower inserted scales.

Fig. 6 shows a case in which there are seven bulbils present, the scale being the fourth, starting from the outside of a bulb. A series of cross sections was pre-

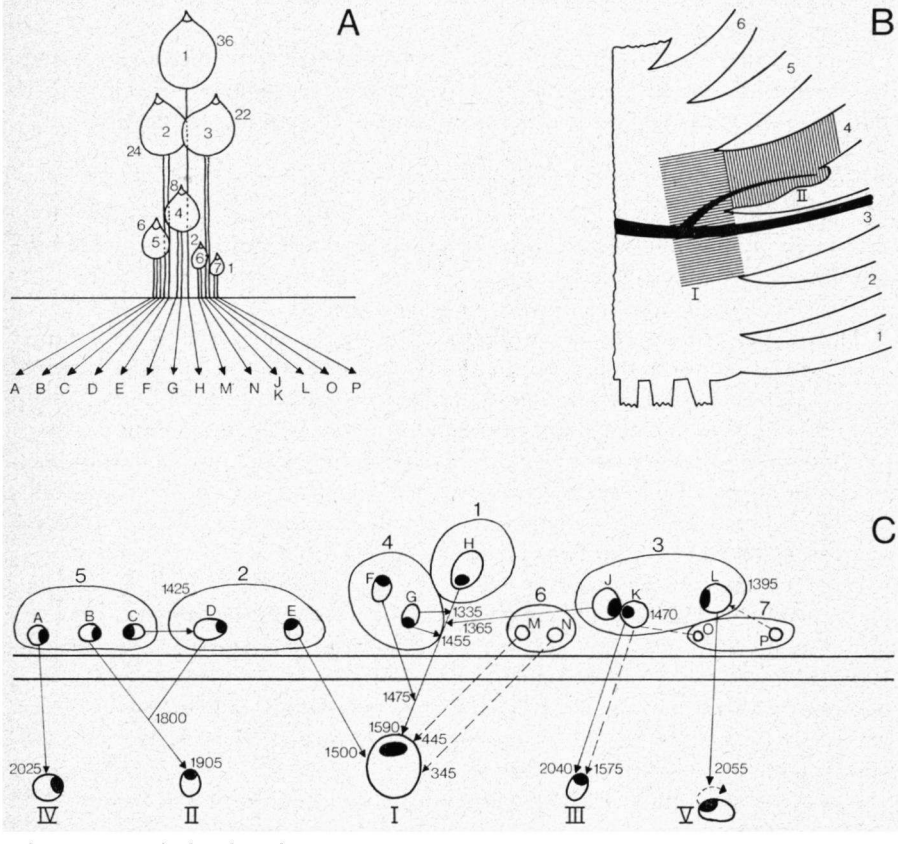

Fig. 6. Schematic drawing of the vascular bundle pattern of bulbils on a scale.

A. Insertion of the bulbils on the scale, the distance from the disk in mm. A-P indicate bundles running from the bulbils downwards.

B. Longitudinal section showing disk with roots and with 6 scales. In black the vascular system of scale 3 and of the bulbils on the abaxial side of scale 4. Hatched area: I was sectioned and II was cleared in a chloralhydrate solution.

C. Vascular connections between the 7 bulbils on scale 4 (bundles A-P) and scale 3 (bundles I-V). Bundles with a black spot, indicating xylem, are vascular bundles complete with xylem and phloem (A-L). M-P are procambium strands.

pared from the basal part of the scale together with the corresponding part of the disk, i.e., the hatched part I in *fig. 6B*. A second fragment, that on which the bulbils were inserted and which is represented by the hatched part II, was cleared in a chloralhydrate solution. The bundles present in this fragment could be traced in the series of slides prepared from part I. Bundles A-L (see *fig. 6A* and *C*) exhibit both xylem and phloem over a long distance. Bundles M-P are procambium bundles. Many bundles of the bulbils proved to be interconnected but ultimately they all joined with bundles from the adaxial part of scale 3. *Fig. 6C* shows the bundle system. Bundle I appears to be the main vein of scale 3. Many bundles proved to be connected with it, either directly or indirectly. Anastomosis with the main vein generally took place less deeply in the disk than was the case for bundles II-V.

Except with regard to the main vein complex, where some relationship might be present, no correlation seems to exist between the level of insertion of the bulbils on the scale and the site of anastomosis of the bundles in the disk.

5. DISCUSSION

The information obtained in this study may make it possible to answer the questions posed in the introduction.

1. *Are the bulbils axillary buds or adventitious buds*?

Three types of buds can be distinguished in the Angiosperms, viz. terminal buds, axillary buds, and adventitious buds. It is clear that in our case terminal buds may be excluded. Axillary buds and adventitious buds differ in that axillary buds originate in the axillary meristem of a leaf, whereas adventitious buds develop on full-grown parts of a plant. At first sight, the bulbils of *Ornithogalum caudatum* appear to be true adventitious buds. Two types of adventitious buds can be distinguished, one originating normally at places characteristic of the species and the other originating artificially as the result of some treatment.

As an example of the first type TROLL (1939) mentioned *Hyacinthus fastigiatus*, in which bulbils normally originate on the leaves. In the majority of cases, however, adventitious buds are formed as a result of some unnatural event. KERNER VON MARILAUN (1898) mentioned a number of examples. LENSKI (1958) described the formation of artificially induced bulbils in *Drimiopsis kirkii*, whereas RIVIÈRE (1967a and b) investigated the formation of bulbils on bulb-scale fragments of *Lilium candidum*.

Almost all the authors discussing the formation of adventitious buds pointed to the fact that their vascular bundles become united with the vascular system of the organ on which they arise. It is therefore evident that the bulbils of *Ornithogalum caudatum* are not adventitious buds, but rather serial axillary buds, since they show vascular connection with bundles of the leafy organ situated one place lower on the axis, and chiefly with its main vein.

Table 1 clearly shows that the outer, older bulb-scales bear more bulbils than inner, younger ones. Furthermore, the bulbils on the inner scales are inserted at a shorter distance from the disk. This makes it conceivable that the bulbils

originate in the central part of the bulb at the base of bulb-scales and that both bulb-scales and bulbils grow and gradually move to the periphery of the bulb by the successive formation of new bulb-scales and the desiccation and disintegration of the oldest bulb-scales. Meanwhile the distance between the insertion of the bulbils and the base of the scales increases and new bulbils are formed basipetally.

2. *Through which cause are the bulbils attached to the abaxial side of the leaf inserted higher than the subtending leaf*?

The youngest stages of bulbils that could be found, amounting to not more than a group of small meristematic cells (Fig. 5B), are situated at the base of bulb-scales at the abaxial side. According to ESAU (1953) and VON GUTTENBERG (1960), in many representatives of the Monocotyledones axillary buds originate close to or at the base of the leaf situated above the subtending leaf. The bud becomes separated from its subtending leaf by intercalary growth. In this way the bulbils become situated at the abaxial side of the leaf above the subtending leaf. The vascular system betrays the actual state of affairs.

3. *Are the ridges to be regarded as discrete entities*?

The ridges or ribs are indeed to be considered as discrete entities, despite their complete concrescence with the scales. An important argument is that the ribs have their own vascular system of bundles with an orientation of xylem and phloem different from that of the scale bundles. Second, in very rare cases bulbils are found on the adaxial side of a bulb-scale. Troll described stalked bulbils of *Allium scorodoprasum* in which the stalks are free and not united with the bulb-scales. The situation of *Ornithogalum caudatum* can, with Troll, be described as a congenital concrescence of bulbil stalks with bulb-scales.

4. *What causes the bulbils to be situated at such high levels above the place of insertion of the bulb-scales on the disk*?

Little attention was given to this problem in the present study. The basal parts of the bulb-scales show a relatively undifferentiated structure suggesting a basal meristem. We could not find indications for a pronounced frequency of cell divisions in those regions, but again, as already mentioned, no special attention has been given to this problem. Nevertheless it is feasible that the observed phenomenon could be realized by intercalary or basal growth, by a combination of cell multiplication and cell expansion in the bulb-scale proper as well as in the bulbillic ribs.

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