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SOME RESULTS FROM STUDIES ON THE EFFECTS OF WEIGHTLESSNESS ON THE GROWTH OF EPIPHYTIC ORCHIDS

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16. Abstract

Epidendrum orchids were placed in a "Malakhit-2" micro-greenhouse aboard the Soyuz-36-Salyut-6 space station to test their growth under weightless conditions. Growth occurred but was less than in control plants left on Earth; cells were smaller and parenchymal development slowed in all tissues. Stems, roots, and leaves were smaller. The number of stomas on the leaves was about the same as in the controls, but, because of the smaller leaf size, there were more per unit area. A modeling experiment using a clinostat revealed a large decrease in gibberellin activity and auxin activity. It was assumed that weightlessness primarily affects gibberellin biosynthesis, inhibiting cell growth. Reestablishment of growth compound activity upon return of the plants to Earth was indicated by the fact that the orchids resumed growth thereafter.

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SOME RESULTS FROM STUDIES ON THE EFFECTS OF WEIGHTLESSNESS ON THE GROWTH OF EPIPHYTIC ORCHIDS

T. M. Cherevchenko and T. K. Mayko

Study of higher plants under weightless conditions is interesting /31* not only in a biological regard, but also because they represent an important part in a total life-support system for humans spending long periods in closed ecological conditions. Plants are needed on manned space flights as a source of oxygen and carbohydrate-vitamin nutrition, and they help provide more esthetic surroundings and enrich the environment with air.

Many researchers have studied the growth and development of seedlings from numerous agricultural plants in dynamic weightlessness [1-3]. The obtained data indicate that pea, lettuce, and corn seedlings do not differ from controls in the initial periods of growth. According to A. I. Merkis [4], this is because of the determined nature of the intial phases of seedling growth and morphogenesis. Further growth of seedlings slows, however, and they die at various stages of development.

Since experimental cultures have been studied for a short period, we decided that epiphytic plants, in which the geotropic reaction is considerably weaker, could be a subject for study on long-duration space flights because of their biological peculiarities. For this purpose we chose epiphytic tropical orchids, in view of the following biological features:

1. The epiphytic life style of orchids has weakened their geotropic reaction, because their roots attach to cracks in the trunks of trees, the forks of branches, and hollows, primarily under the influence of chemo- and hydrotropism. The roots of epiphytic orchids grow laterally, and even upwards, in search of the required substrate.

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*Numbers in the margin indicate pagination in the foreign text.

As opposed to the roots of plants normally growing on the ground, theirs are not capable of negative phototropism.

2. Orchids are not ecologially demanding, they settle on impoverished substrates, do not require great depth for roots, and are satisfied by low concentrations of humus.

3. Because of their tuberous, thick stems, which can accumulate moisture and nutrient liquids, and the thickened leaf and stem cuticles, orchids survive dry air and live up to two or three weeks without watering.

4. As epiphytes, orchids for long periods attach to trees high above the ground, withstanding high winds, heavy rain, severe solar radiation, and high heat, i.e., they often survive under extreme conditions. Also important is that their optimal growth and development temperature is close to that comfortable for humans in the cabin of a space craft.

5. Many species of orchids are capable of blossoming for long periods of time. For example, one Cattleya flower lasts up to two weeks, Cymbidium and Lady's Slipper up to four months, and Phalenopsis up to six months. Even if orchids are not capable of renewed growth under weightless conditions, they can be used for color and mood in the spacecraft cabin.

Before sending orchids into space, we had to be satisfied about their safety for humans. Studies by allergists at the UkSSR Academy of Sciences Oncology Institute showed that orchid pollen and preparations do not have allergenic effects.

Since the most notable reaction of plants to changes in ecological factors is the nature of growth, we set out to study the effects of long-term stays of plants in space craft on the growth and anatomical-morphological features of their vegetative organs. As the subject of study we took the epiphytic, sheath-leaf orchid Epidendrum, most extensively studied in extreme conditions. /32

To carry out the experiment on cultivation in the cabin of the Soyuz-36-Salyut-6 complex, orchids were planted in a cell of the special "Malakhit-2" container. This apparatus (micro-greenhouse) has a rectilinear area measuring 45×35×10 cm, mounting four cells. The temperature in the micro-greenhouse was kept at 20-22°C, the relative humidity of the air at 50%; for ventilation, they were opened up twice a day for 15-20 minutes.

It turned out that, after 171 days aboard the orbiting station Salyut-6, Epidendrum plants not only had not lost their decorativeness, but also had experienced marked growth -- 9-11 cm, with 5-7 new leaves and 2-4 air roots. But the growth processes were slowed under dynamic weightlessness conditions: internodule and air root growth was significantly less and the leaves smaller than on the control plants on Earth (table 1).

TABLE 1. BIOMETRIC FIGURES OF EPIDENDRUM GROWTH ON EARTH AND IN SPACE

Parameters, mm	Earth				Space					
Length:										
air roots	70	100	50	65	52	50	45	45		
internodules	28	16	15	22	15	14	15	15	18	20
Leaf size:										
length	70	60	75	72	50	42	41	52	—	35
width	15	13	13	13	10	14	12	10	—	9

No substantial changes in the anatomy of their vegetative organs were found to have occurred during the flight. Well-developed xylem and phloem elements were characteristic of the major stem and rootlet systems; sheath tissue thickness underwent minor changes. However, cell size was smaller and parenchymal development inhibited in all tissues; for these reasons, the stems and air roots of the plants grown in space were noticeably thinner (tables 2 & 3).

TABLE 2. ANATOMICAL STRUCTURE OF EPIDENDRUM AXIAL SHOOT AFTER 171 DAYS IN SPACE

Internodules	Shoot diameter mm	Cell diameters, mμ parenchyma	sclerenchyma	xylem vessels
Left on Earth	2,9	90±5	7±0,8	14±2
On flight:				
first	2,6	85±3	6±0,5	11±2
second	2,1	77±4	4±1	13±2
third	2,6	79±5	7±1	19±3
fourth	2,2	77±4	10±2	15±2

TABLE 3. ANATOMICAL STRUCTURE OF EPIDENDRUM ROOTLETS AFTER 171 DAYS IN SPACE

Air root	diameter mm	Including bulb thickness, mμ velamen	ectoderm	parenchyma	endoderm	main tiss.	pith
Left on Earth	2,5	208±10	38±2	608±33	15±3	110±19	255±8
On flight							
first	2,1	250±12	40±3	470±14	20±2	95±12	150±10
second	1,5	154±14	36±4	372±12	15±3	110±10	50±12
third	1,5	188±8	50±5	315±20	25±4	108±10	96±8

Air root	Cell diameter, mμ velamen	ectoderm	endoderm	xylem vessels
Left on Earth	55±3	49±2	31±5	34±4
On flight:				
first	60±4	49±2	23±4	29±3
second	50±4	43±3	21±2	22±2
third	52±2	41±4	20±2	26±6

Analyzing the data of tables 2 and 3, one can see that in the internodules and rootlets there was a later tendency for development of larger cell sizes in certain tissues, primarily in the xylem. This likely is a sign of metabolic restructuring and adaptation to dynamic weightlessness conditions.

The long stay of the Epidendrum plants in space most strongly affected leaf growth. The leaves of plants which spent time in space were considerably smaller than those which stayed on Earth. Table 4 shows that, because of the weak growth of leaf parenchyma, leaf thickness

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TABLE 4. ANATOMICAL STRUCTURE OF EPIDENDRUM LEAVES AFTER 171 DAYS IN SPACE

Leaf	leaf blade thickness, mm	cells/mm² epidermis		stomas/mm² epidermis	stomal size		stomal coefficnt	distance between leaf veins, mμ
		upper	lower		lnth	wdth		
Left on Earth	745	519±8	703±10	29±1	29±2	25±2	20	179
On flight								
first	578	530±4	717±6	41±4	31±2	30±2	18	140
second	540	532±2	709±4	40±1	33±2	30±2	18	148
third	391	558±8	813±10	44±2	30±1	28±2	18	124
fourth	410	471±6	783±8	39±1	31±1	28±1	20	87
fifth leaf vestigial								
sixth	325	584±12	845±10	45±4	28±1	25±2	18	111
seventh	300	617±15	934±12	54±5	27±1	24±1	17	90

decreased, and each successive leaf growing on the main stem was thinner than its predecessor. Furthermore, cells in the epidermis were smaller, /33 as a result of which there was less distance between the leaf veins (figs. 1 and 2). We should note that the size of the stomas and the stomal coefficient (ratio between the number of epidermal cells and stomas) remained virtually unchanged. However, because the epidermal cells were smaller, the number of stomas per unit surface area on the leaves developing during the flight was much larger, and in the later leaves was almost twice the same index for leaves developing on Earth (see table 4).

Thus long-term flight conditions led to a reduction in the size of cells in the main tissues of axial organs and leaves of the Epidendrum. This is evidence that dynamic weightlessness inhibits cell elongation processes. The features of cell growth and differentiation depend primarily on the balance of the plant phytohormonal complex [5,6].

Literature data indicate that the chief role in cell elongation is played by gibberellin-like compounds, and deficits in them cause smaller internodules and leaves [7-9]. We assumed that weightless con- /34 ditions have a negative effect on plant growth because they disrupt biosynthesis of native growth stimulants. A model experiment was set up, therefore: Epidendrum plants were grown for two and four months on a horizontal clinostat rotating at 3 r.p.m. The centrifugal force developed by the clinostat's rotation was 7×10^{-5} g. Lighting and temperature were kept about the same as on the orbiting station (1500 lux, 20-22°C).

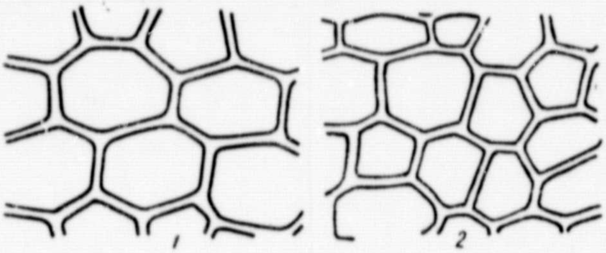

Fig. 1. Upper epidermis of Epidendrum leaf: 1 -- later leaf developing on Earth; 2 -- sixth leaf in space, 15×10.

Despite the fact that many other factors affected the plants during space flight, the clinostat to some extent imitated the effects of dynamic weightlessness and made it possible to study enough plants to determine growth compounds. We were most interested in the effects of long-term weightlessness on the activity of free gibberellins and auxins and the time of their aftereffects.

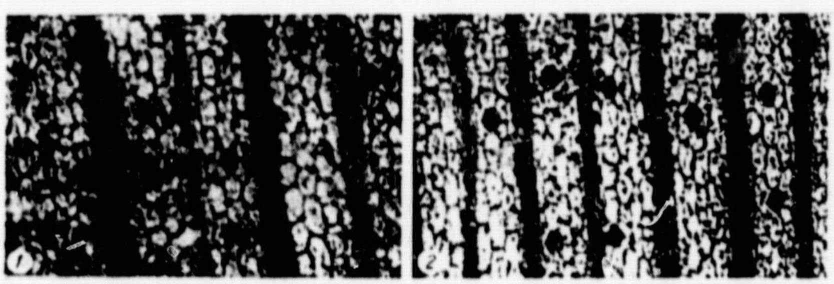

Fig. 2. Veined leaf of Epidendrum: 1 -- later leaf, developing on Earth; 2 -- fourth leaf developing in space.

The activity of gibberellins and auxins was established, using the generally accepted methodology [10], in the vegetative masses immediately after removal from the clinostat and 24 and 48 hours after the experiment The controls were plants grown for the same period right next to the clinostat. The results of these studies are shown in fig. 3.

After the plants had been on the clinostat for two months, gibberellin activity was so diminished that our methods could not detect them,

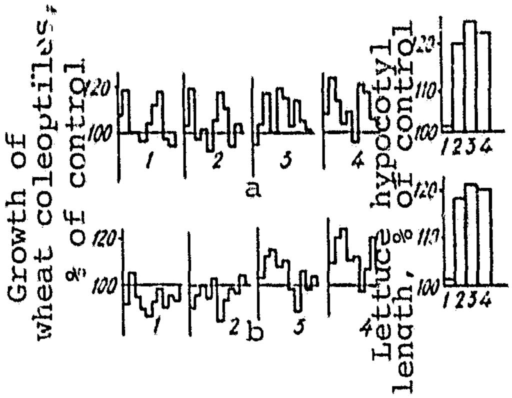

Fig. 3. Histograms of auxin activity (left) and gibberellin-like compound activity (right) in vegetative mass of Epidendrum after two (a) and four (b) months of plant growth on clinostat; 1 -- immediately after removing plants from clinostat; 2 -- 24 hrs later; 3 -- 48 hours later; 4 -- control.

but the activity of auxins was not decreased so severely. Only increasing the stay of the Epidendrums on the clinostat to four months resulted in a perceptible reduction in the amount of compounds with auxin activity and the appearance of β-inhibitors in the plants. Study of growth compounds in the experimental plants after 24 hours revealed that the activity of gibberellins recovered rapidly after removing the weightlessness conditions, but auxin activity remained at a low level. Two days later, gibberellin activity was even higher than in the controls, but auxin activity recovered more slowly: after four months on the clinostat, the plants had auxin activity which still had not reached the level in the controls at this time.

Analyzing the data obtained in space flight and after the model /35
experiment, we believe that dynamic weightlessness conditions have a negative effect primarily on the biosynthesis of gibberellins; this inhibits cell growth. In accord with the theory of plant development hormonal factors [11, 12], it is assumed that a gibberellin deficit is thus one cause of reduced plant blossoming under space flight conditions. Rapid recovery of the growth compound balance and even increased activity of growth stimulants after removing weightlessness can explain the fact that the epiphytic orchids immediately resumed growth upon Earth.

Thus, the results of the studies confirmed our hypothesis that epiphytic orchids can grow on space craft for long periods and are a promising subject of experiments with higher plants. They permit study of the physiological, morphological, and other reactions of plants to weightlessness. In addition, highly decorative tropical orchids can be used in the interior design of space craft.

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