NASA TECHNICAL MEMORANDUM NASA TM -75,281

GERMINATION OF PINE SEED IN WEIGHTLESSNESS (INVESTIGATION IN KOSMOS 782)

R. N. Platonova, G. P. Parfenov, V. P. Ol'khovenko, N. I. Karpova, and M. Ye. Pichugov

(NASA-TM-75281) GERMINATION OF PINE SEED IN WEIGHTLESSNESS (INVESTIGATION IN KOSMOS 782) (National Aeronautics and Space Administration) 15 p HC A02/MF A01 CSCL 22A N78-21172
Unclas 00/12 11912

Translation of "Prorastaniye semyan sosny v nevesomosti," Izvestiya Akademiya Nauk SSR, Seriya Biologicheskaya, No. 5, Sept. - Oct. 1977, pp. 770-776

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 MARCH 1978

15

STANDARD TITLE PAGE

1. Report No
NASA TM -75,281

2. Government Accession No

3. Recipient's Catalog No

4. Title and Subtitle
GERMINATION OF PINE SEED IN WEIGHTLESS-NESS. (INVESTIGATION IN KOSMOS 782)

5. Report Date
March 1978

6. Performing Organization Code

7. Author(s)
R. N. Platonova, G. P. Parfenov, V. P. Ol'khovenko, N. I. Karpova, and M. Ye. Pichugov

8. Performing Organization Report No

10. Work Unit No

9. Performing Organization Name and Address
Leo Kanner Associates
Redwood City, California 94063

11. Contract or Grant No
NASw-2790

13. Type of Report and Period Covered
Translation

12. Sponsoring Agency Name and Address
National Aeronautics and Space Administration, Washington, D.C. 20546

14. Sponsoring Agency Code

15. Supplementary Notes
Translation of "Prorastaniye semyan sosny v nevesomosti," Izvestiya Akademiya Nauk SSR, Seriya Biologicheskaya, No. 5, Sept. - Oct. 1977, pp. 770-776 (A78-18523)

16. Abstract
An investigation was made of the orientation of aboveground and underground organs of pine plants grown from seed in weightlessness. Orientation was found to be caused by the position of the seeds relative to the substrate surface. Normal growth was manifest only for the plants grown from seed oriented with embryo toward the substrate. Differences were noted between experiment and control as to the quantitative content of nucleoli in the meristematic cells of the rootlets and the shape of cells in the cotyledonous leaflets. No complete agreement was found between data obtained in weightlessness and when gravity was compensated (clinostat treatment with horizontal rotation).

17. Key Words (Selected by Author(s))

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified		

GERMINATION OF PINE SEED IN WEIGHTLESSNESS (INVESTIGATION IN KOSMOS 782)

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One of the principal and interesting problems in growing plants in weightlessness is clarifying their spatial orientation in uncustomary conditions for their growth and development. /770*

When this problem was investigated on Biosatellite-2 with annual pepper plants and wheat seedlings, geotropic reactions in reduced gravity of 10^{-5} were observed to be variable (Johnsson and Tebbitts, 1968; Gray and Edwards, 1968). For the pepper plant, weightlessness caused downcurling of leaf petioles (epinasty), and for wheat seedlings developing in a humid atmosphere and not in contact with a solid substrate, the orientation of rootlets and shoots changed. In addition, the statholithic starch granules in the cells of the experimental plants were randomly distributed in contrast to their distribution at the posterior cell wall in plants of the vertical control group (Lyon, 1968a, c). When an investigation was made of the influence of factors in short space flights (33-50 hr) on germination, seedling formation, and the first growth stages of lettuce and pea plants, it was found that the seedlings lose their spatial orientation. However, this did not lead to a change in the mutual order and shape of the organs, tissues, and cells typical of seedlings growing in constant gravity (Merkis et al, 1975). The experiments on Biosatellite-2 did not give a full understanding of the initial geotropic reactions of seedlings and shoots and the nature of plant growth in weightlessness. The objects used in these experiments (up-turning seeds, plant seedlings placed

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

in the vertical position, and intact plants) received their initial stimuli while still on Earth, and the brevity of the flight did not enable them to form plants in weightlessness.

In 1974, from a 21-day growing period in weightlessness on a satellite (Kosmos 690) of pine plants from seeds oriented parallel to the substrate surface, we found that a manifestation of normal geotropic response requires the presence of exogenic stimuli, that is, this process is not realized when gravity is absent (Parfenov et al, 1975). Also established was the obligate inequiorientation of the rootlets and shoots. Additionally, in this experiment it was shown that somatic cells in the leaflets of the experimental plants were more rounded compared to ground control. Additional experiments had to be arranged to clarify the spatial orientation of the underground and aboveground organs of plants growing in weightlessness and the anatomical features of their sustentacular tissues and to make a cytological analysis of the nuclear structures in the meristematic cells of growing plants. This problem was posed in the present investigation on /771 Kosmos-782, by growing in weightlessness pine seeds that had different, but fixed orientation of the embryo relative to the substrate surface.

Material and Method

Seeds of the pine (Pinus silvestris) were the object of study. Selection of this object stemmed from the fact that pine seeds at 20° germinate only on the fifth to the seventh day, and shoots and leaflets grow slowly. Pine seedlings have an erect stem and a primary taproot; these enable their mutual position to be clearly seen. These features are vital in the carrying out of this experiment. Slowed germination of the rootlets permits placing the material in time on board the spacecraft-satellite. The slow growth of stems and chlorophyll in the seeds let us utilize this object in lengthy experiments (1-1.5 months) in the absence of illumination.

Agar (1.8 percent) moistened with distilled water was the substrate for growing pine plants from seed. The seeds were treated with 3 percent hydrogen peroxide and placed in agar to a depth of 1.5 mm in four positions: embryo upward, downward, horizontally left and horizontally right, with 70 specimens in each position. At the same time with the experimental variant, the pine seeds were analogously placed in the agar and used as the synchronous control.

The synchronous control was placed in a biosatellite mockup and the goal was to differentiate the effect of weightlessness from the possible effect of specific conditions of plant maintenance on board the biosatellite.

One more variant of the experiment was set up on Earth in a horizontal clinostat, spinning at 2 rpm. This experiment was aimed at finding how closely data from weightlessness agree with data from the clinostat treatment. A vertically spinning container bearing pine seeds on the clinostat was the control for horizontal rotation.

The plants growing in weightlessness were photographed at the end of the flight in a field laboratory at the landing site. Germination and survival rate of the plants, morphological characteristics, and the spatial orientation of the aboveground and underground organs were recorded. Then the plants were fixed in acetal-alcohol (3:1), then brought to the stationary laboratory for further work. From the leaflets and rootlet tips "crushed" acetocarmine preparations were prepared for cytological and anatomical analysis. Treatment of the synchronous control followed the same procedures. A morphological analysis was made for plants grown in the clinostat.

Results and Discussion

The plants grew normally (with roots in substrate and with shoots and leaflets in the atmosphere) only in the group where the seeds were oriented with embryo vertically downward. Here the stems were light pink and each bore five to six green cotyledonous leaflets. Small rootlets (1-3 mm) were white, springy, and viable, while longer rootlets (6-10 mm) browned and decayed. The angle of both aboveground and underground organs relative to the substrate surface was 85-90°.

When the seeds were horizontal in the substrate, the plants grew parallel to its surface, and the roots and shoots were oriented in opposite directions. Some plants lifted their roots and shoots into the atmosphere at angles of 30-35°. Contrasting with the plants in the two other experimental groups, here the shoots were all white; the leaflets had less intense coloration.

In the synchronous control, at any orientation of the seeds the plants grew with their shoots vertically upward into the atmosphere (elevating above the substrate), but with the roots vertically downward (into the substrate). Here positive and negative geotropism was well-defined. The geotropic curving of the roots growing from horizontally oriented seeds and from seeds placed with their embryos above the substrate was already observable for rootlets 2-3 mm long. Right after curving they assumed a vertical position. In the group of seeds oriented with their embryos upward, several plants emerged with their roots into the atmosphere, but in contrast to the experimental variant with the same orientation of seeds, at different distances from the substrate surface they curved downward and grew into the agar at a right angle. The color of the shoots and the cotyledon in the experiment and the control did not differ. Long roots (6-10 mm) browned and decayed, while short rootlets

(2-3 mm), as in the experimental material, were viable. Growth of pine plants in weightlessness and on Earth is shown in Fig. 1, a and b and schematically in Fig. 2.

Data on the number of growing plants in all variants of experiment and control are given in the table.

Noteworthy is the fact that the number of growing plants was the largest (nearly 100 percent) in those experimental and control variants where the seeds were oriented with embryo upward relative to the substrate surface, in spite of the fact that it would appear, from the "Earth" point of view, that this position is the most unsuitable for germination. Possibly, this fact is associated with better access to oxygen for the upward-curling rootlets together with their growing shoots, and therefore, with the improvement in respiratory processes. Not to be excluded is the fact that it was hard for the rootlets to grow into the agar because it was resilient.

Examination of meristematic cells in the pine rootlet tips gowing in all the variants of seed orientation revealed that most interphasic nuclei in the control contained one to eight nucleoli. The modal class was the class that had four nucleoli in the nucleus. The number of cells with a single nucleolus was insignificant--0.39 ± 0.17 percent. The interphasic nuclei of the cells of the experimental plants had from one to five nucleoli. Here the modal class was the class that had three nucleoli. The incidence of cells that had a single nucleoli in the experiment was reliably higher ($P < 0.05$) than in the control and was 5.62 ± 0.79 percent. This latter finding can be associated with the spatial change in the chromosomes, including the nucleolus-forming chromosomes. The small number of dividing cells (2.1 ± 0.5) made it hard to prove this hypothesis. Although mitotic activity was low both in the control and in the experiment, it was noted that it was higher in the rootlets grown from

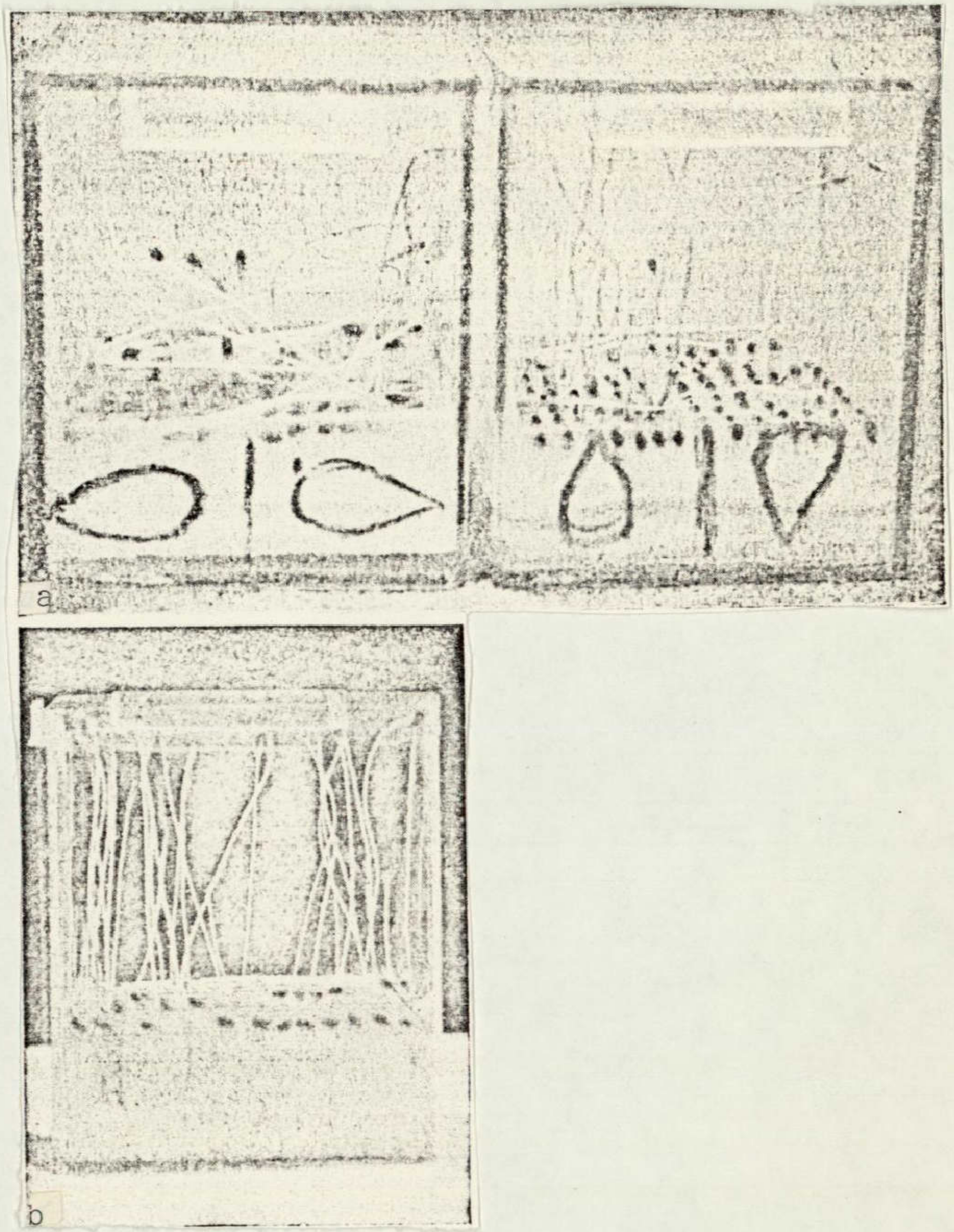

Figure 1. Growth of pine plants in weightlessness (a) and on Earth (b) for different orientations of seeds relative to substrate surface

from the seed placed in the substrate with the embryo upward and parallel to the substrate surface ($P < 0.01$). The more rapid growth of the seed can be the reason for this finding. In both control and experiment, not a single cell with chromosomal aber- /773 rations was detected in the divided cells. Cells in the leaflets of plants growing in weightlessness usually were more rounded than cells in the leaflets of synchronous control (Fig. 3, a and b). In the experiment only 11.0±0.6 percent of the cells were angular, while in the control the incidence of angular cells was 71.8±1.2 percent. The same pattern was observed also in analogous material after the flight of Kosmos 690 (Parfenov et al, 1975). The incidence in this case of cells with angular contours was smaller still, 3.53±0.9 percent. The change in the shape of cells found in the initial stages of plant development (in cotyledonous leaflets) in weightlessness compels us to assume that it is attributable to the absence of the pressure of cells on each other. And, by obeying laws of surface tension, the cells tend to adopt a spherical contour.

Survival Rate of Plants in Weightlessness and in Synchronous Control

1 Варианты (направление зародыша по отношению поверхности субстрата)	2 Опыт		3 Контроль	
	Количество 4	M±m, %	Количество 4	M±m, %
Вверх 5	68	97,2±2,0	69	98,6±1,4
Вниз 6	23	32,8±9,8	21	30,0±10 0
Параллельно влево 7	27	38,5±9,3	23	32,8±9,7
Параллельно вправо 8	27	38,5±9,3	32	45,8±8,0

Key: 1. Variants (embryo orientation relative to surface of substrate)
2. Experiment
3. Control
4. Number
5. Upward
6. Downward
7. Parallel, left
8. Parallel, right

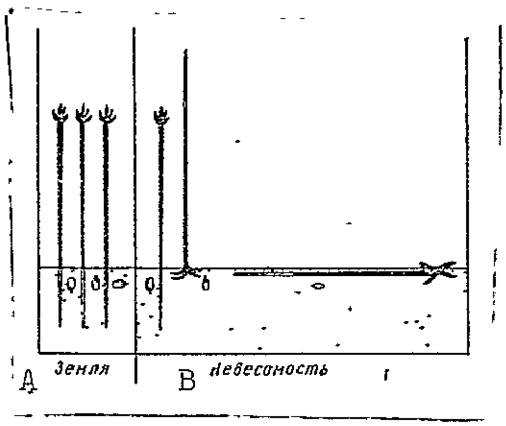

Figure 2. Schematic representation of the orientation of underground and aboveground plant organs when pine plants are grown in weightlessness and on Earth

Key: A. Earth
B. Weightlessness

Analysis of plants grown for 21 days in the clinostat spinning horizontally showed the following: in the group where the seeds were oriented with embryo upward, the plants grew root-first into the atmosphere and with shoots into the substrate. The inclination of the aboveground and underground organs was approximately 5-40°; of 23 plants that grew, only one grew at a 90° angle. In the group where the seeds were oriented with embryo downward, 15 plants grew rootfirst into the substrate, and with stems into the atmosphere. The angle of inclination in this case was approximately 45-90°. In the two variants of horizontally disposed seeds (25 and 24, respectively) the plants grew partially trailing over the surface of the substrate, and partially inclined into the atmosphere at approximately 20-60°. Generally, the shoots grew into the atmosphere; only in two cases was this true of the roots. Analysis of plants growing for 21 days on the vertical clinostat, for any seed orientation in the substrate, showed that the plants grew with their roots vertically downward--into the substrate, and with their shoots vertically upward--into the atmosphere (Figure 4, a and b). The plants growing in the clinostat in all variants were pale pink, leaves were green, and rootlets white. They decayed as the roots grew.

When flight data were compared with data from the horizontal clinostat, it was seen that the orientation of plants grown from different seed generations was similar. But weightlessness results were "purer." In weightlessness there were no exceptions and, additionally, the angle of inclination of the roots and the shoots was closer (orientation with embryos upward and downward) to the vertical. The horizontal rotation of the clinostat at a given rate compensated only incompletely for the effect of gravity.

This experiment showed that the orientation of underground and aboveground organs (roots and shoots), when there was no phototaxis, occurs not at all as on Earth. Plants in weightlessness can grow in different ways: roots "downward"--into the substrate, and with shoots "upward"--into the atmosphere; with roots "upward"--into the atmosphere, and with shoots "downward"--into the substrate; parallel to the substrate surface and at different angles to it; in this position both roots and shoots can lift into the atmosphere. This uncustomary growth and orientation in weightlessness are caused by the position of the seeds relative to the substrate surface. The roots grow in the direction where the embryo is oriented, and the shoots--in the opposite direction--along the embryonic axis. In normal conditions on Earth, however the seeds are placed in the substrate, the plants obligately grow rootfirst into the substrate, and shoots into the atmosphere. Therefore, when gravity is eliminated, /774 germinating seeds lose the ability to manifest positive and negative geotropism intrinsic to most higher plant organisms. From the Kosmos 690 and Kosmos 782 experiments, we can assume that geotropism is not a genetically determined property, but is exogenic and can be modified by changing the habitat environment. Both experiments also showed that the polarity of growth orientation of roots and shoots does not depend on whether gravity is present or not. The experiments confirmed that weightlessness affects processes that are induced under its influence and for which there exists a mechanism of gravitation reception. It is assumed that geotropic reactions in plants take place at the cellular level. Starch grains--amyloplasts--in all probability are the gravitational statholiths. Closely associated with their distribution in the cell is the transport of the growth hormone indolylacetic acid (IAA), determining the direction of geotropic curving. Disorientation of plant growth in weightlessness, it must be assumed, occurs because the amyloplasts cease "functioning" as cellular regulators of gravity. Their delocalization can bring about, it is probable, uniformity of distribution of the IAA; owing to this there are no longer conditions for sensing the geotropic curving needed for normal plant orientation.

It can be assumed that normal growth and development in weightlessness can occur only for the plants whose seeds are placed in the substrate embryo downward, if then the seeds are cultivated with sufficient nutrition, illumination, aeration, and other conditions essential for vital activity. In all other experimental variants, the young growing plants must die, even if all necessary conditions will be met. Ahead lies the study of whether in weightlessness roots beginning to grow into the atmosphere can curve around and continue growing into the substrate, if the experiment is conducted with illumination, that is, whether phototaxis can replace positive geotaxis.
In the experiment conducted it was also found that compensation for gravity achieved by effective clinostat treatment does not allow normal geotropic reactions to be manifested. Ahead lies a study of whether the effectiveness compensating for gravity (by clinostat treatment) can be 100 percent by changing the speed of rotation and providing normal growth conditions for the plants.

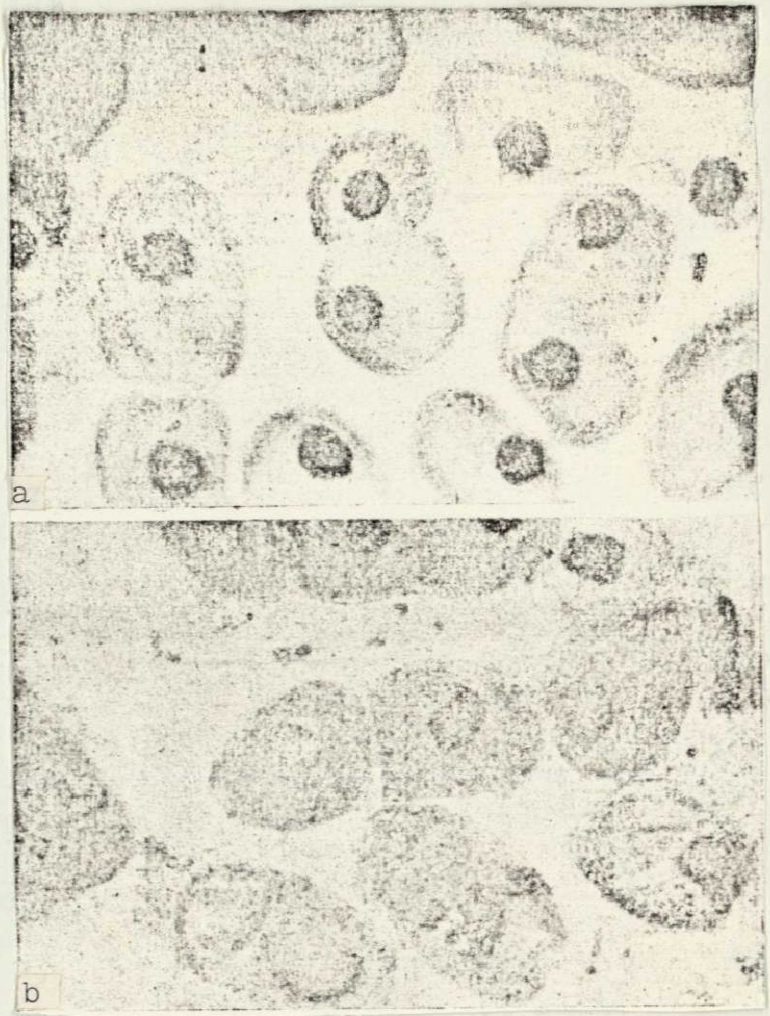

Figure 3. Shape of cells in cotyledonous leaflets of plants grown in weightlessness and on Earth:
a. experiment b. control

Data obtained on the initial geotropic reactions in plants occurring in weightlessness are of theoretical importance for gravitational biology. They are also important in practicality in building greenhouses for different kinds of investigation requiring the raising of plants in space.

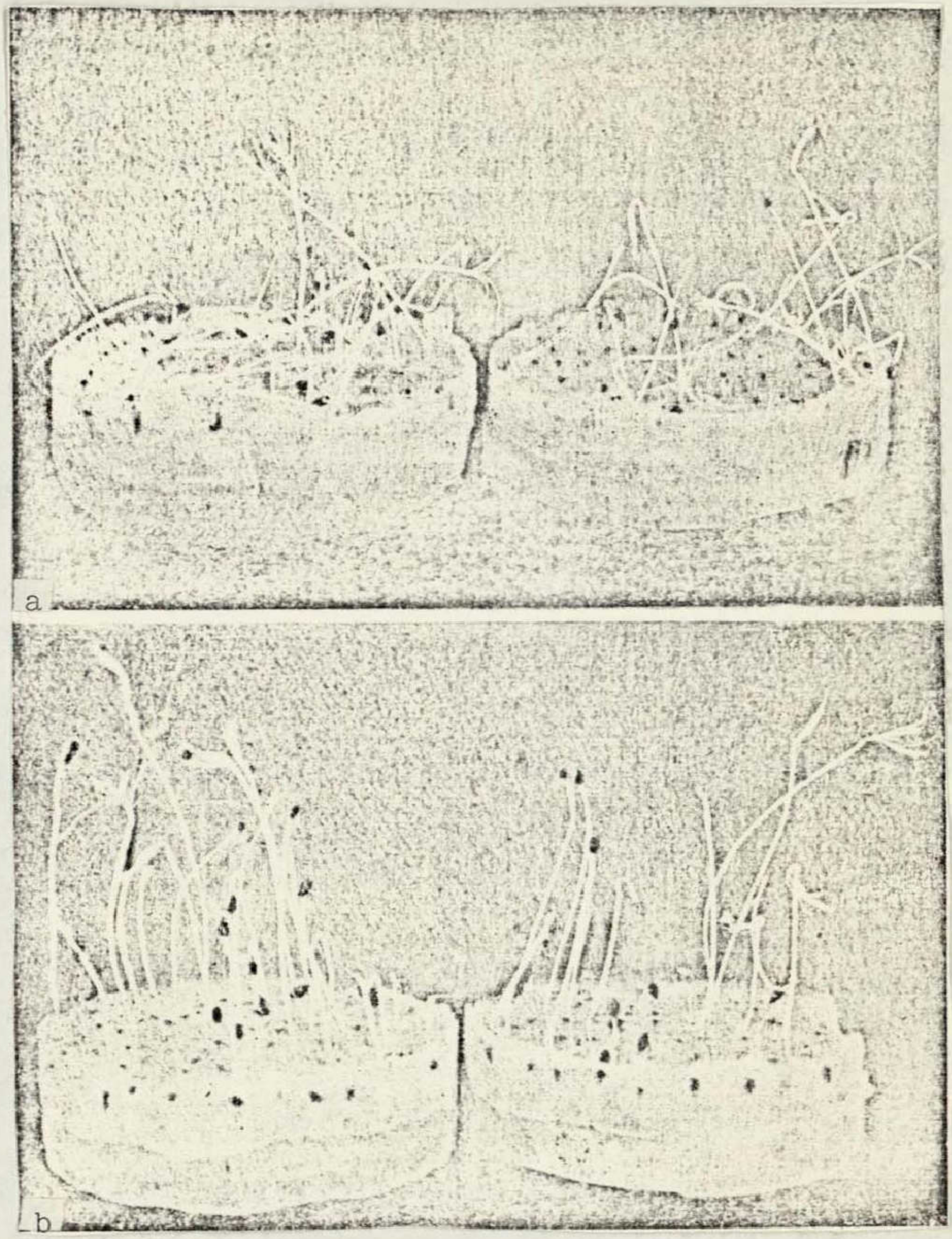

Figure 4. Growth of pine plants: a. horizontal clinostat treatment (experiment) b. vertical clinostat treatment (control)

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Received

1 Feb 77