

Growing super-dwarf wheat on the Russian space station Mir (Project "Greenhouse")

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Introduction

There are two primary reasons to grow plants in an orbiting satellite: 1) To study basic gravitational biology; that is, to study possible plant responses to the near absence of accelerational forces (usually called microgravity - although accelerational forces are closer to microgravity). 2) To achieve maximum yields of edible plant products for future use in a bioregenerative life support system (a Controlled Ecological Life Support System - CELSS). In America and Western Europe, most space research with plants has been motivated by basic

gravitational biology although the work is often justified with reference to a CELSS application. In the former Soviet Union and apparently also in Bulgaria, studies with plants in space have been more strongly motivated by the CELSS challenge than by that of fundamental biology.

Although abnormal affects have been noted in short-duration plant experiments in space (downward bending of leaf petioles and blades called *epinasty*, spindly growth, chromosome aberrations, etc.), many plants have appeared to grow quite normally during short duration experiments on the U.S. Shuttle and on Soviet or Russian space craft although detailed studies often discover subtle abnormalities. This seems to be especially true for seedlings that have obtained most of their nutrients (energy, minerals) from the seed.

On the other hand long-duration plant experiments in space, carried out only by Soviet, Russian, and Bulgarian investigators, have never produced completely normal plants that have grown through a complete life cycle. *Arabidopsis* did grow through a life cycle (i.e., "from seed-to-seed"), but growth was quite retarded and generally poor [1]. The plants were grown in a *Phyton Three* device in *Salyte Seven* in 1982 for 69 days from sowing to return to earth. Plants were under continuous light. Upon return to earth, 5 plants produced 22 normal pods, and 2 plants produced 11 sterile pods. There were about 200 seeds, half of them immature. Forty-two percent germinated to produce normal plants. The good news is that the life cycle was indeed completed (we might say there were no "show stoppers"). The bad news is that plant growth was considerably less vigorous and, healthy than was achieved with ground controls in the same plant-growth devices.

Several studies were carried out with wheat in various devices in Mir [2,3]. Again, plants were far less vigorous than comparable plants on earth. Chlorophylls were reduced producing chlorosis, carotenoid levels also declined, and there were various mineral imbalances: phosphorus increased, calcium decreased, and other minerals changed. Total lipids decreased, and fatty-acid ratios changed. Super-Dwarf wheat was grown in Svetoblock-M in Mir for 167 days during 1991 [2]. Growth ceased at about day 40 but began again after 90–100 days, producing new shoots. Three heads, each surrounded by a leaf (i.e., in the "boot"), developed from 100–132 days but matured on earth under somewhat higher light. Twenty eight seeds were produced, and 8 out of 12 given to us germinated normally in Utah.

In 1990, radish and cabbage were grown in the Bulgarian-Russian growth chamber Svet [4,5]. Plants were grown for 54 days. Again, although plants grew during the 54-day period, many abnormalities were observed. For example the radishes produced 2.6 g of dry mass in space and 21.6 g of dry mass on earth; Chinese cabbage produced 2.65 g in space and 35.80 g on earth.

Is the poor growth observed in the long-term space experiment a direct plant response to microgravity? We cannot conclude that this is the case because space plants were usually exposed to stresses that were not always duplicated in the ground controls. For example CO₂ levels in Mir are much higher than those in most of the control studies, and they fluctuated over a wide range. Plants are known to be highly sensitive to CO₂ levels [6]. Temperatures have fluctuated in the Svet experiment reaching 37°C at one point, for example (Tania Ivanova, personal communication). The substrate conditions are especially critical because they cannot be duplicated on earth. The drainage caused by gravity creates a totally different substrate environment from that in space [7]. Lack of drainage in space could lead to water logging or even to regions of dry substrate if capillary forces are not sufficient to bring water to such regions.

Goals of our experiment

Two approaches must be taken to solve the problem of environmental stresses in space experiments: First, the environmental conditions and the stresses and strains that they produce must be carefully documented. Irradiance levels are typically low and may or may not be the same as those used in ground controls. Although many environmental measurements have been made, the desirable level of documentation has never been achieved. Second, available technology should be applied to reduce the levels of stress as much as possible.

With these ideas in mind, our current experiment on Mir has the following goals:

1. To grow Super-Dwarf wheat through a complete life cycle in the Russian-Bulgarian plant growth chamber Svet in the Krystal module of Mir. As noted above in relation to *Arabidopsis*, it has long been a goal of space biologists to grow a plant through a complete life cycle in microgravity. Our current experiment did not achieve this goal, but we will strive to achieve it during 1996 (see below).
2. To document the environmental parameters that might impact plant growth (in addition to microgravity): CO₂ concentrations (potentially allowing measurement of photosynthesis and respiration), water vapor (allowing measurement of transpiration), irradiance levels, leaf (infrared) and air temperatures, oxygen, total cabin pressures, and substrate moisture conditions (16 moisture probes in the root module).
3. To collect various samples and photographs for analysis on the ground. This goal should allow us to document the strains experienced in response to whatever stresses are measured.
4. To improve conditions for plant growth as much as possible. Paramount in this goal is to utilize the 16 moisture probes to maintain ideal moisture levels in the root module. We have also added Mylar (mirror) reflectors to the walls of Svet to increase irradiance levels. In general, however, future effort will be required to further improve conditions for plant growth, specifically the high levels of CO₂ and the relatively low irradiance.

Hardware for "Project Greenhouse"

Svet was designed jointly by scientists at the Institute of Biomedical Problems in Moscow and the Bulgarian Academy of Sciences, Space Research Institute. There is about 0.1 m² of plant growing area. A modified computer controls the photoperiod and the automatic watering system. Air circulates to cool the lamps and to hold temperatures close to those in the cabin. An improved version of Svet was developed and delivered in 1995. In addition to many technological improvements, it allows a wider range of photoperiods and has about twice as much irradiance as the original version of Svet.

Under the direction of Cail Bingham in the Space Dynamics Laboratory at Utah State University, an environmental data system has been developed. This

* Stress can be defined as any environmental parameter that limits plant growth below its genetic potential (or less rigorously, below the yield and quality levels observed under "normal" conditions on earth). By analogy to physical science, the actual reduction in yields or other measured plant parameters may be referred to as strain.

has involved the major part of the U.S. budget and human effort. The biggest challenge was to develop infrared CO₂ analyzers that could measure CO₂ levels up to 2 % with an accuracy of ± 0.035 %. To measure photosynthesis, plants are enclosed in a transparent plastic bag (actually two cuvettes, each one above half of the root module, which is also divided into two compartments). CO₂ and humidity levels of air entering and exiting each cuvette are measured. This requires four infrared analyzers. Because removal of humidity was not feasible in this project, the CO₂ measurement had to be carried out with an open system rather than closed cuvettes in which the humidity would increase to high levels, producing condensate on many surfaces. Such an open system to measure photosynthesis requires a much higher level of accuracy than would be the case in a closed system in which the plants could be allowed to draw down the CO₂ as it was being measured. The necessary levels of accuracy were achieved.

In addition to the infrared sensors for CO₂ and water vapor, the environmental data system includes sensors for leaf (infrared) and air temperatures, irradiance, pressure, oxygen, and substrate moisture (16 probes based on thermal conductivity). Leaf area must be estimated to provide photosynthesis, respiration, and transpiration readings, and this was to be achieved by leaf scanners when plants were sampled and by estimates based on photographs. All of this instrumentation was flight qualified and is presently installed on Svet in Mir and on ground control versions of Svet. (The leaf scanners were not used by the cosmonauts in the 1995 experiment; for the 1996 experiment, we are considering measurements of samples after they are returned to earth.)

Much attention has been given to the root module because of the difficulties mentioned above in achieving ideal moisture, nutrient, and oxygen levels in the substrate for plant growth in microgravity. In the current experiment, changes were made in the root module compared with its status in the 1990 experiment. Hydraulic conductivity was improved by using substrate particles of smaller average diameter and by more dense packing to assure firm particle-to-particle contact. The substrate is a zeolite called Balkanine. It was precharged by Bulgarian scientists with the nutrient elements required for ideal plant growth, and indeed such plant growth has been achieved with Balkanine in many earth-based experiments. To improve our understanding of water and oxygen availability to roots, a mathematical model of water movement based on concepts of soil physics and thermodynamics (free energy) was developed by a graduate student, Scott Jones, working with a soil physicist, Dani Or, at Utah State University.

Table 1 shows the schedule for "Project Greenhouse". The equipment was delivered to Mir during June and July of 1995, and an experiment was carried out on Mir-19 and Mir-20 from August to November 1995. The goal of this first experiment was to test the hardware and to obtain plant data that will be compared not only with data obtained in ground experiments but also with data obtained during the 1996 experiment. That experiment will be carried out between March and December as indicated in Table 1. It also involves an attempt to grow plants through a complete life cycle with environmental measurement and sampling. In addition, a second crop of plants will be harvested when they are approximately 35 d old and returned to earth at liquid nitrogen temperature in the GN₂ freezer. These plants will be analyzed for various chemical parameters including a suite of plant hormones that are known to be extremely sensitive to environmental stresses.

Table 1

Schedule for "Project Greenhouse"

Mission	Date	Objective
Spektr	Docked with Mir June 1, 1995	Equipment delivered
STS-71	July 7, 1995	Delivered root module and seeds
Mir 19 and Mir 20	August to November, 1995	Experiment "Greenhouse 2a" a proof-of-concept experiment to test the hardware, obtain plant data
STS-74	November, 1995	Return of root module and plant samples
STS-76	March 21, 1996	Launch: root module, fixation kit, logbook kit
Priroda Module	April, 1996	Launch new light block, controller, and spare parts
NASA-3	July to December, 1996	Seed-to-seed experiment; second planting (35 d)
STS-79	August 1, 1996	Launch hard disc №2
STS-81	December 5, 1996	Recover samples from seed-to-seed experiment plus the second planting (35-day-old plants frozen in GN ₂ , freezer for subsequent chemical analysis)

The experiment involved four kinds of procedures carried out in space:

1. **Recording of environmental data.** This was discussed above. As noted, the parameters included soil moisture status (16 probes plus two sensors that are part of the Bulgarian root module), leaf (infrared) and air temperatures, irradiance (two sensors placed below the lights and near the top of the leaf cuvettes), carbon dioxide and oxygen concentrations, water vapor concentration, and cabin pressure.

2. **Photography.** A camera bracket was designed to be attached to Svet in such a way that nine photographs could be taken at each photography session. Three photographs were to be taken at each of three positions: looking directly into the unit at a low level, a higher level, and the same higher level but with the camera tipped toward the substrate by a few degrees. At each position, a photograph was to be taken from a center position, from the left of center, and from the right of center. These were to provide stereo views of the growing plants that might help in estimating leaf areas. Unfortunately, the camera bracket was misplaced before the experiment began. It was located only after the experiment was complete.

3. **Plant samples.** Samples were to be (and will be in 1996) taken according to the schedule shown in Table 2. Although the stages did not occur as hoped (see discussion below), plants were sampled five times. Each time, some plants were fixed in chemical fixative for subsequent anatomical analysis on earth, and some

plants (first two stages) or excess materials were placed into desiccant bags where they were dried; dried plant material can be analyzed for such parameters as mineral element content, cellulose, and lignin. At the time the samples were taken, plants were to be placed on a leaf scanner that measures leaf area. This would allow us to document growth rate and would also provide areas necessary in the expression of photosynthetic and respiratory data. As noted this procedure was not carried out in 1995; we are working on ways to measure leaf areas of the returned fixed samples.

4. Final harvest. In the seed-to-seed experiments, the goal was to harvest mature plants with viable seeds. These plants were to be placed in desiccant bags for final drying and to prevent growth of fungi. Because mature plants are dry anyway, it is not necessary, to use chemical fixatives. There were no seeds or even sterile heads produced in the 1995 experiment (see discussion below), but the available plants were placed in the desiccant bags as planned. (Some fungal growth was detected on one of the young plants in a desiccant bag; apparently the sides of the bag had been pressed together so that water vapor could not move from the plant to the desiccant.) In the case, of 35-day old plants (1996 experiment), fresh plants will be placed in the GN_2 freezer and returned to earth. Excess material if there is any, will be placed in desiccant bags.

As shown in Table 2, plants in the seed-to-seed experiment are to be sampled at five stages. In a workshop with American and Russian participants in September, 1995, it was agreed that our original schedule should be changed slightly. Of great interest to plant anatomists and cytologists are the events that occur during gametogenesis (formation of pollen and the embryo sac) as well as the growth of the pollen tube and fertilization, which is the combining of the sperm nuclei from the pollen tube with the egg and the endosperm nuclei. We realized, on the other hand, that seed filling is a relatively less interesting stage in the plant's life cycle, albeit an important stage in the production of the final seeds. Thus it was decided to take the last three samples during the period of gametogenesis to fertilization, hoping to catch some of these events in progress.

Table 2

Sampling schedule for the seed-to-seed experiments

Original sampling schedule	Modified sampling schedule (As of September 12, 1995 in Moscow)
Prefloral: 6-day-old seedlings	Prefloral: 6-day-old seedlings
Early floral: 13-day-old, young plants	Early floral: 13-day-old, young plants
Boot stage: ca. 30-day-old plants (crew determines)	Boot stage: ca. 30-day-old plants (crew determines)
Anthesis: ca. 45-day-old plants	Gametogenesis/Anthesis: ca. 42-day-old plants
Seed Filling: ca. 60-day-old plants	Pollination/Fertilization: ca. 49-day-old plants

The 1995 Experiment (Mir 19-20)

As this manuscript is being prepared, "Project Greenhouse, 1995" has just been completed. To summarize, there were several failures of the Svet hardware sent to Mir in 1990, but enough of the problems were overcome to keep the plants growing during the 90-day interval from seed planting to harvest. No heads were formed, however, so sampling times were arbitrary and not based on visible, developmental stages. The following outlines the experiment as events were reported to us (or as we participated):

Events during Greenhouse 2

1. August 7. Cosmonauts initiate installation of the Utah equipment for "Greenhouse 2." We planned a daily transfer of data to earth via MIPS (Mir Interface to Payload Systems), but the MIPS system was tested and failed.
2. August 8. The setup for Svet and the Gas Exchange Measurement System (GEMS) was completed.
3. August 9. Svet and GEMS were provided with power.
4. August 10. "Program 1" was run to test the Svet hardware.
5. August 11. Cosmonaut Nikolai Budarin, who was assigned to our project, initiated "Program 2" on the Svet controller; this program adds water to the root module. The moisture sensors reported that cuvette №1 received too little water; cuvette №2 received more but only about half of the desired amount.
6. August 13. (Aug. 13 is assumed to be day zero.) Program 2 had been initiated again, providing sufficient water to cuvette №1 but too much to cuvette water to cuvette №2. *Seeds were planted.*
7. August 14 (day 1). The lights were turned on ("Program 3"), and three of the six lamps in the light unit, specifically those over cuvette №1, shut down after a brief functioning period. These lamps remained off, and a fourth lamp also failed although we are not sure exactly when this occurred. Continued tests of the MIPS system failed.
8. August 15 (day 2). The Balkanine was still too wet in cuvette №2 although cuvette №1 had about the right amount of water. The excess water in cuvette №2 may have inhibited germination. It was concluded that the leaf bags (properly called cuvettes, but this term has also been applied to the two compartments that make up the root module) were restricting the flow of cooling air by the lamps and probably caused the malfunctioning of half of the lamps. Thus the leaf bags were removed, and the GEMS was hooked up to run in looped-back mode. It provided analytical data of CO₂ levels in the Krystral module, but photosynthesis measurements were not possible for this experiment (Modified bags have been designed and constructed to allow more airflow past the lamps. They will be sent to Mir for the 1996 experiment.) It is not clear, however, that the lamp failure was caused by restricted air flow caused by the leaf bags. Temperature data indicate that the lamps overheated twice after the leaf bags were removed (ca. days 6 and 40). This apparently occurred because the fan that was supposed to cool the lamps when off and had to be restarted by the cosmonauts. In any case, plants were subjected to high temperatures (at least 45°C) twice during their growth cycle, and this may have had profound effects on the course of their development.
9. August 16 (day 3). The Svet automatic controller failed because of an overloaded power supply caused by the non-functioning lamps. Hence, the system had to be operated in manual mode (i.e., lamps and the water supply system had to be turned on and off by the cosmonauts). The lamps over cuvette №1 were confirmed dead.
10. August 17 (day 4). Germinating seedlings were about 1 cm tall. The Svet controller still failed to function, and lights were turned off when the cosmonaut retired and on again upon arising. Thus plants received about 16 h of light and 8 h of darkness each day – a photoperiod too short to promote rapid development of flowers and seed heads. (Lights were left on over night five times: ca. days 51, 64, 76, 82 and 89.) Plants were watered for about 5 s in the morning; the root modules were very wet. The GEMS moisture sensors were used to determine the daily water doses. Data from the computer screen were radioed orally to earth. These data indicated that all sensors were functional and properly installed. Actually, the equipment had not been properly grounded, and this led to erroneous data, noted in the next entry.
11. August 18 (day 5). Cuvette №1 had 36 healthy plants (reduced-light side); cuvette №1 had 10 plants, and a fungal growth was noted on the wicks (determined to be harmless). (The seed strips sent to Mir had 26 seed; thus, each cuvette had 52 seeds for a total of 104 seeds in Svet.) The light sensors suggested that light levels, in spite of the lamp failures, were higher than in the ground studies. It now appears that the overheating that caused the lamps to fail also upset the irradiance sensors because the controller was not electrically grounded. When a ground was hooked up (ca. day 36), irradiance was seen to be very low. (There is a possibility that lamps function more efficiently in microgravity because of the lack of internal convection, but such an effect was not sufficient to provide ample light in this experiment.) The relative humidity in the core module of Mir was about 65 % and about 50 % in the Krystral module where Svet is

located. Temperature increased to about 28°C because of an oven experiment located next to Svet (23°C is ideal for wheat). The cosmonauts reported that the camera bracket we had specially designed and constructed to take stereo photographs was lost somewhere on Mir. It was subsequently located but too late to take the desired pictures.

12. August 21 (day 8). The root modules were both drying out. There was a question about whether seeds should be replanted in cuvette №2; the decision was delayed pending more information.

13. August 22 (day 9). More seeds germinated mostly in cuvette №1 as the over-wet substrate dried out. The irradiance sensors reported 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in cuvette №1 and 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in cuvette №2; this made us very optimistic until the sensor malfunction was finally deduced. Because corrections must still be made to the recorded data, exact figures for irradiance at the plant level are not available; irradiance was probably about 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or less. In ground tests, irradiance levels were 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which was sufficient but not as high as might be desired. Photographs were taken but unfortunately without the camera bracket.

14. August 24 (day 11). There were 40 plants in cuvette №1 (20/row) and 17 plants in cuvette №2 (row 1 = 6; row 2 = 11). The plants in cuvette №2 that germinated later than those in cuvette №1 seemed to be catching up. This is probably because they were receiving somewhat more light. The decision was made not to replant seeds in cuvette №2.

15. August 26 (day 13). Plants were about 8 cm tall and appeared quite healthy. They had reached the 3-leaf stage. Six plants were placed in chemical fixative and three were placed into desiccant bags (first sampling). This stage was to have been the prefloral stage, but 13-day old plants usually have passed that stage.

16. August 31 (day 18). Four plants were fixed and three dried at the 3 or 4-leaf stage (second sampling). This was to be the early floral stage, but only examination of samples will tell us if the plants had initiated floral primordia.

17. September 3 (day 21). The Svet controller completely failed so that it became impossible to turn on the lights or to add water to the substrate. This led to a frantic weekend in which Bingham at the Space Dynamics Laboratory communicated with Ivanova in Bulgaria and with our Russian coinvestigators. The problem was finally diagnosed as low voltage (9 V) from the power supply. A way was devised to provide 12 V from the Utah power supply by connecting the appropriate plugs with paper clips. After 33 h of darkness, the lights were back on, everything was working, and the plants were growing. A video recording was telemetered to earth, but reception was poor. Nevertheless, it was possible to see that the plants did indeed appear healthy.

18. September 6 or 7 (day 24 or 25). Soyuz docked with Mir, and Yuri Gázenko replacing Budarin.

19. September 11 or 12 (day 29 or 30). Budarin returned the hard disc from the notebook controller, allowing us to examine complete environmental data for the first month of the experiment (data that should have been sent to earth via MIPS).

20. September 16 (day 34). Four plants were fixed and two dried. At this age, plants would normally have been in the boot stage (third sampling).

21. September 18 (day 36). A video was taken showing the equipment and the plants, which appeared to be a normal green color (although color balance of the lights makes this somewhat uncertain). The plants were growing *in all directions, more like crab grass than wheat!* Of course they did not have the directional force of gravity to orient them; apparently they did not grow directly toward the lamps because Svet was lined with mirror Mylar so light came from essentially all directions (as we had planned with our Russian colleagues to increase the light the plants received).

22. October 5 (day 53). The stems and flag leaves (attached to the head, if a head had formed) of six plants were chemically fixed and the remaining leaves were placed in desiccant bags (fourth sampling). This was to be the gametogenesis stage if plants had developed as they do on earth, but as noted, heads failed to form.

23. October 21 (day 69). Another video was taken, again showing a fairly good green color of the plants.

24. October 25 (day 73). Six stems and flag leaves were fixed and excess material dried at what would have been the pollination/fertilization stage (fifth sampling).

25. November 8 (day 87). The experiment was terminated, and plants were harvested into drying bags. No heads were visible to the cosmonauts.

26. November 12. The U.S. Shuttle Atlantis, STS-74, was launched to dock with Mir. During subsequent days, samples and equipment (root module, lamp bank, controller) were transferred to STS-74.

27. November 20. Samples were returned to earth at Kennedy Space Center and divided among U.S. and Russian investigators.

28. November 27 to December 1, 1995. A science meeting was held at the NASA Ames Research Center, Moffett Field, California, to discuss the 1995 experiment and to plan ground and space studies for 1996. Attending the meeting were the following U.S. and Russian investigators: Salisbury, Bingham, Carman, Campbell, Walkyria Goncalves (graduate student), Sytchev, Levinskikh, and Podolsliy (Chernova and Ivanova were present at Kennedy Space Center. Personnel from Ames included Bubenheim, Yendler, Greenawalt, Jahns, Lagel, Patterson, Pletcher, Savage, Schnepf, and Tverskaya (interpreter).

Synchronized ground control and post-flight experiment procedures

After the hard discs from the controlling notebook computer were returned to earth, it became possible to work up detailed sets of data on the environmental parameters that were recorded. This has been accomplished to a large extent. We have documented when the lights were actually functioning, levels of moisture in the root module, air and plant (infrared) temperatures, and other factors. These data make it possible to establish a synchronized ground control in which temperatures, CO₂ levels, light, etc. are duplicated. Such a control was discussed in some detail among U.S. and Russian investigators at the meeting at the NASA Ames Research Center following return of the samples and data. The synchronized ground control will be carried out in Moscow and a similar study will be done at NASA Ames under the direction of Bubenheim, who will not have a copy of Svet to use but who has capabilities to control pressure and gas composition somewhat more accurately than will be possible in Moscow.

When the root module was returned to earth, it was examined by several team members (Yendler, Bingham, Podolsky, Levinskikh, Ivanova, Chernova, Salisbury Bubenheim, and Campbell). The goal was to examine distribution of water and the presence of microorganisms. The Balkanine in cuvette №2 was wet enough (about 21 to 27 % of dry mass) that water might have been redistributed during the accelerational forces of landing and while standing before delivery to us; bottom layers were wetter than upper layers, suggesting that this was the case. The Balkanine in cuvette №1 appeared only slightly wet; moisture content varied from about 17 to 19 % of the dry mass. (Note the rather narrow range of wetness from "slightly wet" to "very wet" appearance; this range roughly represents most of the water that is available to plants.) Analysis of the data from the soil-moisture probes (recorded on the hard discs) indicated that there was a sharp range in moisture content, with the Balkanine close to the wicks where the seeds were planted being very wet, essentially at full saturation, whereas the Balkanine some distance away was fairly dry. This was not apparent in the module returned to earth, but there was much time (about two weeks) for water to be redistributed after the final watering.

Some chemical analysis will be carried out to study the available nutrients and other soil characteristics of the Balkanine. Procedures have been discussed at length, but budgetary considerations may limit how much can be done.

Fixed and dried samples were distributed to U.S. and Russian investigators for microscopic analysis. Some of the dried material will be examined for nutrient elements, cellulose, lignin, etc. These analyses will be carried out in Russia, Utah and NASA Ames Research Center. The frozen material, to be returned in late 1996, will be analyzed for plant hormones (abscisic acid, indoleacetic acid, several cytokinins), free amino acids, and other chemicals. Although at the time of this writing the samples have not been examined anatomically or biochemically as planned, a striking initial observation was that there were no visible heads although the plants remained alive in the low light conditions for 90 days until the final harvest. Under such low light conditions on earth, plants have always formed heads even though there were often no seeds in the heads. We plan to study at least three factors that could have led to the continued vegetative state in Mir: (1) Our ground studies under low light have always had continuous light, and it is known that long photoperiods strongly promote flowering in wheat, including Super-dwarf. (2) Brief treatment with high temperature. Such temperatures (45°C or higher) occurred twice the first time when the plants were only seedlings; such temperatures could have destroyed the flowering process. (3) Water logged substrate. Lack

of oxygen around the roots can lead to ethylene production, and ethylene inhibits flowering in many species. As noted above, de Balkanine was super-saturated much of the time, especially where the roots were located.

Experiment verification test

An experiment verification test was carried out at the Institute of Biomedical Problems from February 21 to May 24, 1995. The Bulgarian Svet was used with the lights and controls comparable to those expected to be used in the 1990 experiment (but, because of equipment failures, not comparable to those in the 1995 experiment). The substrate was Balkanine, and the full Utah instrumentation was attached. All scheduled samples and photographs were taken. For the first time in six or seven similar but somewhat less elaborate trials, plants were healthy all the way to harvests. It was felt that the soil-moisture probes made it possible to maintain a near ideal substrate moisture level. Samples and photographs are not being analyzed. The Utah instrumentation functioned well except that there was too much CO₂, enrichment noise to allow accurate measurement of photosynthesis and respiration. Ways were devised to overcome some of these limitations, and rough photosynthesis measurements were obtained. In a somewhat more stable environment, continuous photosynthesis measurements should be possible as had been previously planned.

Seven kits were tested in the experiment verification test, and some modifications were suggested. These kits were and will be used on Mir to carry out "Project Greenhouse". The kits are: fixative, harvest, leaf-area-measurement, glove bag, dry stowage, log book, and observation (photography). (As noted, the leaf-area-measurement may be dropped if we can obtain reliable measurements on fixed plants.)

Some ground-based research

Many studies that cannot be described here have been carried out in Moscow, Utah State University, and NASA Ames Research Center as preparation for interpretation of results obtained from the flight experiments [8-10]. For example, at Utah State University six, foam-board Svet mockups are located in each of two temperature-controlled rooms. Various substrates have been used in these studies including zeolite, perlite/peatmos (ca 80/20 %), and in the near future hydroponics. Each mockup has two plastic bags (cuvettes) as in the flight and ground control Svets. Photoperiod is controlled individually in each mockup, and CO₂ levels are measured as gas enters each cuvette. Levels, including levels as high as those in Mir, can be set manually.

In studies with this facility we have documented the uniformity among the various mockups and we have demonstrated that, at the light levels expected in Svet in Mir, plant growth is much better at 22 °C than at 27 °C. The substrate is clearly a serious problem even on earth at 1 g_n. In addition, we have clearly documented the promotive effect of long photoperiods on the rate of flower development.

In other studies we have grown Super-dwarf wheat under a range of low irradiance levels. We have studied ways to measure leaf areas. We have developed fixatives that remain stable for many months. We have learned to break a post-harvest dormancy of Super-dwarf wheat seeds by treating moist seeds with cold. We have validated the GN₂ freezer for storage. At NASA Ames, we have carried out preliminary studies with high CO₂, (Bubenheim), and Yendler has studied moisture distribution in Balkanine and other substrates (e.g., glass beads).

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**Отглеждане на пшеница сорт “Супер-джудже”
на руската орбитална станция “МИР”
(Проект “Оранжерия”)**

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(Резюме)

В статията е описан експериментът “Оранжерия 2”, проведен на орбиталната станция “Мир”, по фундаменталния биологичен проект Мир-Шатъл’95. Експериментът е стартиран на 10 август 1995 г. от 19-тия и продължен от 20-тия екипаж на “Мир”. Той е опит да се отгледа пшеница сорт “Супер-джудже” по време на един цял вегетационен цикъл и да се експериментира апаратурата за контролиране параметрите на средата, създадена в университета в щат Юта, САЩ (Лаборатория по космическа динамика) и монтирана към българската космическа оранжерия “Свет”. Статията засяга общата организация и научните резултати от експеримента. Представено е подробно описание на експеримента “Оранжерия 2”, точно както събитията са били предавани на Земята. Дадена е също програмата на Руско-Американо-Българския проект “Оранжерия”.