MELiSSA

Technical Note

Memorandum of Understanding 19071/05/NL/CP

UNIVERSITEIT GENT

FACULTEIT WETENSCHAPPEN

TECHNICAL NOTE: 89.12

PLANT STRESS RESPONSE: DEFINITION OF PRELIMINARY REQUIREMENTS

prepared by/*préparé par*	D. Hagenbeek, L. Chaerle, D. Van Der Straeten
reference/*réference*	Contract number 19787/06/NL/PA
issue/*édition*	1
revision/*révision*	0
date of issue/*date d'édition*	13Nov06
status/*état*	Final
Document type/*type dedocument*	Technical Note
Distribution/*distribution*	

CONFIDENTIAL DOCUMENT

APPROVAL

Title *titre*	issue *issue* 1	revision *revision* 0

author *auteur*	date *date* 13Nov 06

approved by *approuvé by*	date *date*

CHANGE LOG

reason for change /*raison du changement*	issue/*issue*	revision/*revision*	date/*date*

CHANGE RECORD

Issue: 1 Revision: 0

reason for change/*raison du changement*	page(s)/*page(s)*	paragraph(s)/*paragraph(s)*

Table Of Contents

1. Ranking of stress factors *1*
1.1. Light availability **1**
1.2. Temperature control **1**
1.3. CO_2 supply **1**
1.4. Water and nutrient availability **2**
1.5. Allelopathic factors **2**
1.6. Pathogen avoidance **3**
1.7. Stress prevalence under space conditions **4**
2. Detection requirements of most critical stresses *4*
2.1. Conclusions **7**
3. References *7*

List of Tables

Table 1: Estimates for suboptimal growth or stress factors regarding importance, chance of occurrence and impact upon happening. 4
Table 2: Overview of the most critical stresses and the associated detection requirements 6

1. Ranking of stress factors

The aim of this study was to assign weighing factors to the stress factors described in TN89.11, to be able to rank them based on criticality. However, for many stress factors limited quantitative data is available, which link the occurrence of these stresses to productivity. The criticality will depend on how much the respective stress factors influence crop development and growth, what the chance is for occurrence of the stress, and what the impact will be if the stress occurs (see Table 1).

1.1. Light availability

Alternative life support systems need to be under tight control and therefore will rely on environmental control systems. However these environmental control systems routinely have control failures, and learning how to gracefully recover from such failures will be one of the main challenges (Bugbee, 2003). Failures of the power supply system are among the most common and most detrimental of all system failures (Bugbee, 2003).
Power failure will severely impact light availability, and absence of light means no growth (a minimal amount of light is required for the photosynthetic carbon fixation to outweigh respiratory losses, the so called light compensation point; Taiz and Zeiger, 2006). This will extend the duration of the crop cycle and if the dark (or minimal light) period lasts too long, the crop will be lost entirely (within 14 days, unless temperature is lowered; Bugbee, 2003). The damage can be limited by providing some light from a backup energy supply, the crop cycle time will be enlarged but the final yield can be the same. (see paragraph 2.1 of TN89.11).

1.2. Temperature control

Power failure will also have its consequences on temperature control but if the growth space is well insulated, effects on temperature will be markedly slower than on light supply. However, the optimum temperature range for most crops is quite narrow and 5 degrees above or below the optimum temperature will already negatively influence the yield; in the given example of spinach by 30% (see paragraph 2.3 of TN89.11). It will be useful to learn how big temperature fluctuations are on board of space crafts or stations (e.g. ISS), in order to know to which temperature the growth space will adapt upon failure of the temperature of the plant growth chamber, and to be able to estimate daily yield losses due to corresponding suboptimal temperatures. Only when temperatures drop till just above zero or lower, crops will entirely cease growing and wither all together. However, cold sensitivity of plants is species dependent and is related to the relative abundance of unsaturated fatty acids in the membranes, determining the transition temperature (Sung *et al*., 2003). Temperatures of more than 15 degrees above the optimum will also dramatically reduce crop yield. The chance these extreme temperatures might occur needs to be assessed.

1.3. CO_2 supply

CO_2 supply on earth seldom drops. Only in greenhouses on cold but sunny days, when windows are closed and photosynthesis is running optimally, CO_2 levels are known to drop and become limiting (see paragraph 2.2 of TN89.11). The effects of CO_2 levels below ambient on yield are logarithmic and therefore it is important to maintain adequate levels. Since the crew generates sufficient CO_2 the quantity available should not be a problem, but the transport to the plants might be more critical in space, since temperature induced convective mixing of gasses is virtually eliminated in microgravity (Ferl *et al*., 2002).

1.4. Water and nutrient availability

Water and nutrient supply to the root is of utmost importance but normally does not become critical in a hydroponic system operating on earth (see paragraph 2.4 and 2.5 of TN89.11). In space this will be a much bigger issue. Technical adaptations are required to provide plants with sufficient water and these systems might be more liable to failure (Ferl *et al*., 2002). Plant roots do not need to be submerged in the water, 100% air humidity, as used in aeroponics (Kratsch *et al*., 2006), is sufficient.
Oxygen supply to the roots might be a bigger problem in space. The maximal level of oxygen soluble in water (approximately 10 mg/ l at 20°C; http://www.lenntech.com/why_the_oxygen_dissolved_is_important.htm) is already much lower than oxygen in air (21%, corresponding to ~250 mg/ l) and should be kept high in order not to become limiting for crop yield. Precise data (relating oxygen levels in solution to yield) is not available for the MELiSSA crops. For cucumber the respiration of the roots is inhibited by oxygen levels below 5 mg/ l (http://www.fytagoras.nl).
The nutrient needs are well defined in general and optimal formulations have been developed for monocots and dicots (http://www.usu.edu/cpl/research_hydroponics.htm). If the plant water relations (water and oxygen supply to roots of the plant and proper air humidity for optimal leaf-level evaporation) are managed well, the total nutrient supply should not be a problem. Nevertheless, the nutrient solutions proposed by USU should be experimentally tested on the different MELISSA crops to confirm crop performance under the given conditions. There is one recurrent problem, mentioned in greenhouse horticulture (pers. com. Belgium Research Centres) as well as in controlled environment crop production (Bugbee, 1997): the lack of calcium. Due to low mobility of calcium ions in the plant it often gets limiting in leaf tips. In wheat, this leads to a reduced flag leaf (the last leaf before the ear) and consequently reduced seed filling (Bugbee, 1997). In lettuce it leads to cosmetic damage and in severe cases to reduced yield due to hampered emergence of young leaves (called tip burn). In tomato it is called blossom end rot and causes fruits to rot from the tip onward. These physiological disorders render the affected tissues more susceptible to pathogens.

1.5. Allelopathic factors

From the allelopathic factors ethylene is certainly important. It accumulates in closed environments and it is difficult to maintain at non-physiologically active levels (Campbell *et* *al*., 2001; Klassen and Bugbee, 2002; Klassen and Bugbee, 2004). Upon stress plants are known to release specific volatile compounds (Moalemiyan et al., 2006; Loreto et al., 2006), and detection techniques are being optimised (Dudareva et al., 2006). Effects of root-secreted allelopathic factors are not obvious to characterize (see chapter 4 of TN89.11), but after frequent recycling of nutrient solution it can not be excluded that there might be root-secreted water soluble allelopathic factors reducing yield. For cucumber for example, yields were significantly higher when activated charcoal, which presumably bound root exudates which are detrimental to growth, was added to the hydroponic culture solution (Asao *et al*, 1999). Especially mixed cropping might give problems due to differences in production of these factors and sensitivity to these factors for the different crops.

1.6. Pathogen avoidance

For pathogens the motto is to prevent contamination. It remains to be seen however in how far it is possible to totally exclude this factor. Furthermore, in case of infection, sensitivity in space might be higher (Leach *et al*., 2001; Ryba-Whyte *et al*., 2001). This could for instance be due to easier spread of the pathogen resulting from reduced strength of plant cell walls in microgravity, or due to lack of competition by other (non-harmful) pathogens normally present in the phyllo- and rhizosphere (Schuhegger *et al*., 2006). There is little if any quantitative data available from controlled studies relating presence of pathogens to crop losses, rather averages are taken for vast geographical regions of field based agriculture (Oerke *et al*., 2004). Viruses should not be a problem because they rely on hosts to be spread efficiently carried around. These in general are plants themselves, insects or nematodes (refs), all of which should be easily kept out with proper sanitation methods (pre-launch). Plant culture should only be started with certified virus-free material (examples) or resistant cultivars
Bacteria and fungi will be harder to get rid off. Bacteria can cause considerable damage, but especially fungi cause major damage in horticulture, including in hydroponic cultures. Botrytis, Pythium and Phytophtora are mentioned as the economically important pathogens (fungi; personal communication Belgium Research Center). If humidity levels are maintained low (60-70%, and ventilation to avoid local spots of high humidity) Botrytis can be easily controlled but in humid conditions it can be rampant in e.g. tomatoes. Although in general plant pathogen pressure is much less in hydroponic systems compared to soil bound systems there is one main exception, the so called zoospore forming fungi (oomycetes or water moulds, kingdom Chromista) from which Pythium is the most common (Herrero *et al*., 2003; Johnstone *et al*, 2005). Phytophtora might also need to be followed up as important member of this group of fungi (http://annual.sp2000.org/2006/show_species_details.php?record_id=1355116).

2. Detection requirements of most critical stresses

Table 1: Estimates for suboptimal growth or stress factors regarding importance, chance of occurrence and impact upon incidence.

Suboptimal or Stress factor	Subfactor	Importance	Chance for failure/ occurrence	Impact of failure
Light		+++	+++	+++
Temperature		+++	+++	++
CO_2 *		+++	+	+++
Water *	Root zone	+++	+	+++
	Humidity	++	+	+
Oxygen (roots)*		+++	+	++
Nutrients *	All	+++	+	++
	Calcium	+++	++	+
Allelopathic factors **	ethylene	++	+++	++
	others	+	+	+
Pathogen ***	General	+++	+	+++
	Viruses	+	+	+
	Bacteria	++	+	++
	Fungi	+++	++	+++

* Supply under normal (earth) conditions easily controlled but in microgravity (space) or reduced gravity (moon or mars surface) supply needs special technical adaptations and will be much more liable to failure
** Under reduced gravity sufficient airflow and mixing around the plant leaves is critical and might cause accumulation of allelopathic factors
*** Some reports (see paragraph 1.6) indicate that pathogenicity might be increased in micro gravity

1.7. Stress prevalence under space conditions

Some of the mentioned possible stresses are in general not a problem on earth but might become critical in space depending on the technical possibilities. These are related to altered physical properties regarding gas and liquid flows in reduced gravity. The possible problems include water, nutrient and oxygen supply to the roots, maintaining air humidity and sufficient air flow (Ferl *et al.*, 2002). Plant developmental processes and general plant architecture are also depending on gravity as steering force. All these problems will need to be addressed in microgravity and fall outside the scope of this project.

For the physical environmental factors (light, temperature, CO_2 and oxygen in solution) optimal growth curves will need to be determined experimentally. Minimum and maximum limits should be determined experimentally for each crop and cultivar. There are guiding experiments from which to determine the optimal ranges to work with but complete growth response curves in relation to the stress factors are not available for closed environment cultures; in some cases (spinach, lettuce, wheat and potato see TN 89.11) experimental results were obtained, recording response curves for a single stress factor. To develop predictive models, more extensive data is needed. Besides determining growth curves at stable conditions (reference or baseline data), short and longer term interruptions of optimal values of these factors will also need to be tested. Here again only preliminary work has been conducted.
Among nutrient supply linked factors, lack of calcium is ranked as most likely stress. This is best tested in relation to light levels, humidity and air flow in the controlled environment, since lack of calcium in the plant is often not due to lack of calcium in the nutrient solution but related to growth rate and water/ nutrient transport within the plant (Franz *et al*, 2004; Bugbee and Koerner, 1997; personal communication Belgium Research Center).
Hydroponic solutions can be checked for allelopathic factors with a bioassay (e.g. germination of lettuce seeds; Rimando *et al*., 2001) or with use of filtering systems like active charcoal (Asao *et al*., 1999). Although these factors can not be excluded, especially not with frequent recycling and mixed cropping, they probably are of minor importance. Gaseous contaminants and especially ethylene can however not be ignored. The first plant growth experiment in space yielded only sterile wheat seeds due to accumulated levels of ethylene (Campbell *et al*., 2001). Technically ethylene might be removed, by scrubbing or photocatalytic oxidation, but to what extend this can be achieved remains to be tested, since ethylene is physiologically active (affecting yield) in low concentration ranges. Production levels of ethylene for the MELiSSA crops as well as sensitivity to ethylene for the respective crops needs to be experimentally determined. Quantitative relations between ethylene levels and yield of a crop also have not been documented. Trade off between energy cost and air circulation over a catalyst in order to reduce ethylene to a certain threshold with corresponding predicted yield loss will be necessary.
Since pathogen occurrence can not be excluded it will be important to test the impact of at least to most important ones (Botrytis and Pythium; Kan van J.A.L, 2006, Johnstone *et al*, 2005).
Summarizing, a shortlist of most likely stress factors can be deducted (see table 2). For these stresses the needed sensitivity for detection of the allowable threshold remains to be determined.

Table 2: Overview of the most critical stresses and the associated detection requirements

	Environmental factors (light, temperature, CO_2 and oxygen in solution)	Lack of Ca	Ethylene	Pathogens (Botrytis, Pythium)
Duration before visual symptoms	1 day of less than optimal values	1 week	1 day	2-3 days after inoculation
Parameters to be detected/ known	Respective parameter Yield or stature of crop Growth retardation point for min and max of each factor	Ca in solution Ca in leaves Necrotic/ chlorotic symtoms at leaf tips Leaf transpiration rate	ethylene production yield reduction or crop stature reduction	Botrytis: Spots at inoculation site and fungal mass at infected site Pythium: reduced growth of young seedlings
Measurement range	40-2000 PPF 1-40 °C temp 50-1500 ppm CO_2	0-3 mM in solution With and without airflow Low and high humidity	10ppb-1 ppm	botrytis $2x10^7$ spores/ ml
Measurement frequency	daily	weekly	daily	daily
Measurement accuracy	PPF 1PPF T 0.1C CO_2 1ppm	In solution 0.1mM (0.002mM attainable by ICP-ES*)	5ppb Photoacoustic determination	Hours by imaging techniques (e.g. chlorophyll fluorescence) PCR or other assays
Upscale	Plant level	Leaf level	Several plants	Leaf level

* ICP-ES = Inductively Coupled Plasma Emission Spectrophotometry (http://www.usu.edu/cpl/research_hydroponics3.htm)

2.1.Conclusions

Fundamental plant physiological research on MELiSSA crops will be needed to be able to design reliable predictive models, since the required data –in hydroponic culture- are lacking in literature. Dose response relations of individual growth and stress factors in relation to yield are most often not available as well. Furthermore these factors are interrelated and quantitative data regarding these interactions are again limited.

3. References

Asao T., Ohba Y., Tomita k., Ohta K. and Hosoki T. (1999) Effects of activated charcoal and dissolved oxygen levels in the hydroponic solution on the growth and yield of cucumber plants. J. of the Japanese Soc. for Horticultural Science 68:1194-1196.

Bugbee B. and Koerner G. (1997) Yield comparisons and unique characteristics of the dwarf wheat cultivar 'USU-Apogee'. Adv. Space Res. 20:1891-1894

Bugbee B., Chard J. and Akula G. (2003) Crop production on the lunar surface: mitigating the effects of prolonged darkness with reduced temperature and low light. NASA Bioastronautics Investigator's Workshop Jan. 13-15, 2003; Galveston, TX

Campbell W.F., Salisbury F.B., Bugbee B., et al. (2001) Comparative floral development of Mir-grown and ethylene-treated, earth-grown Super Dwarf wheat. J. of PlantPhysiology 158: 1051-1060

Cloutier G. and Dixon M.A. (1997) Culture strategies for higher plants in closed systems. MELISSA Technical Note: 40.1

Dudareva N., Negre F., Nagegowda D.A. and Orlova I. (2006) Plant volatiles: Recent advances and future perspectives. Critical Reviews in Plant Sciences 25: 417-440

Ferl R., Wheeler R., Levine H.G. and Paul A-L. (2002) Plants in space. Current Opinion in Plant biology 5:258-263

Frantz J., Ritchie G., Cometti N., Robinson J., and Bugbee B. (2004) Exploring the Limits of Crop Productivity: Beyond the Limits of Tipburn in Lettuce. Jour. ASHS 129: 331-338

Herrero M.L., Hermansen A. and Elen O.N. (2003) Occurance of Pythium spp. And Phytophtera spp. In Norwegian Greenhouses and their pathogenicity on cucumber seedlings. J. Phytopathology 151: 36-41

Johnstone M., Chatterton S, Sutton J.C. and Grodzinski (2005) Net carbon gain and growth of bell peppers, *Capsicum annuum* 'Cubico', following root infection by *Pythium aphanidermatum* Phytopathology 95:354-361

Kan van J.A.L (2006) Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends in Plant Science 11: 247-253

Klassen S.P., and Bugbee B. (2002) Sensitivity of wheat and rice to low levels of atmospheric ethylene. Crop Science 42: 746-753.

Klassen S.P. and Bugbee B. (2004) Ethylene synthesis and sensitivity in crop plants. HortScience 39: 1546-1552

Kratsch H.A., Graves W.R. and Gladon R.J (2006) Aeroponic system for control of root-zone atmosphere. Environmental and Experimental Botany 55: 70–76

Leach J.E., Ryba-White M., Sun Q., Wu C.J., Hilaire E., Gartner C., Nedukha O., Kordyum E., Keck M., Leung H. and Guikema J.A. (2001) Plants, plant pathogens, and microgravity, a deadly trio. Gravitational and Space biology Bulletin 14:15-23

Loreto F., Barta C., Brilli F. and Nogues I. (2006) On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant Cell and Environment 29: 1820-1828

Moalemiyan M., Vikram A., Kushalappa A.C. and Yaylayan V. (2006) Volatile metabolite profiling to detect and discriminate stem-end rot and anthracnose diseases of mango fruits. Plant Pathology 55: 792-802

Oerke E.C. and Dehne H.W. (2004) Safeguarding production – losses in major crops and the role of crop protection. Crop Protection 23: 275-285

Rimando A.M., Olofsdotter M., Dayan F.E., *et al.* (2001) Searching for rice allelochemicals: An example of bioassay-guided isolation Agronomy journal 93:16-20

Ryba-White M., Nedukha O., Hilaire E., Guikema J.A., Kordyum E. and Leach J.E. (2001) Growth in microgravity increases susceptibility of soybean to a fungal pathogen. Plant and Cell Physiology 42:657-664

Schuhegger R., Ihring A., Gantner S., Bahnweg G., Knappe C., Vogg G., Hutzler P., Schmid M., Van Breusegem F., Eberl L.E.O., Hartmann A. and Langebartels C. (2006) Induction of systemic resistance in tomato by N-acyl-L-homoserine lactone-producing rhizosphere bacteria. Plant, Cell and Environment 29: 909-918

Sung D.Y., Kaplan F., Lee K.J. and Guy C.L. (2003) Acquired tolerance to temperature extremes. Trends in Plant Science 8:179-187

Taiz and Zeiger (2006), Plant Physiology, 4th edition, Sinauer associates, Sunderland (Mass.) 690pp.

Wheeler R.M. (2006) Potato and human exploration of space: some observations from NASA-sponsored controlled environment studies. Potato research 49: 67-90