# PLANT GROWTH CONDITIONS INSPACEFLIGHT AND PATHOGENICITY OF MICROBES

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## ABSTRACT

In spaceflight conditions, plants are subjected to a variety of environmental stresses which may promote microbial growth and potential pathogenicity. Among the changes that may occur in the plant is a decrease in freely available carbohydrates, resulting in the microbe having to look for alternative carbon sources. A seed-borne fungal endophyte, an *Acremonium* species, was identified as the symptom-causing agent in Super Dwarf seedlings grown in a spaceflight mission in 1995. Plants bearing the endophyte grew without symptoms in the greenhouse in open conditions. The isolated *Acremonium* grew well on different carbohydrates available in wheat leaf apoplastic fluids as well as isolated wheat leaf cell walls and a common component of plant walls, pectin. Invertase, an enzyme that degrades sucrose, a major carbon molecule transported in plants, was detected in *Acremonium* grown in sucrose medium. The requirement of sucrose for invertase induction by sucrose and growth of *Acremonium* on isolated wheat leaf cell walls suggest that the fungus may turn to degradation of the plant cell wall when the plant becomes stressed. Avoidance of plant stress during spaceflight may help the plants defend themselves against pathogens.

## INTRODUCTION

A stress phenomenon that has been little studied in connection with spaceflight is the response of plants to pathogen attack. Microbes will always be present in spaceflight (Ahearn et al., 1995) and some may be able to adapt to become pathogenic. Others that are pathogenic may be imported into flight. For example, wheat grown aboard an eight-day NASA shuttle flight mission in 1995 was severely infected by a fungal pathogen (Bishop et al., 1997). Contamination of the wheat was from a seed-borne infection (Bishop et al., 1997) by a fungus, an *Acremonium* species. *Acremonium* infection of grasses normally is asymptomatic (An et al., 1993). However, under enclosed conditions of spaceflight, in which plants may be subjected to a variety of environmental stresses, including elevated carbon dioxide, low photosynthetic photon flux density, and high relative humidity, the *Acremonium* fungus grew more aggressively in the wheat. This fungal growth resulted in stunting, chlorosis and girdling of the plant sheath by fungal hyphae that sporulated at the leaf surface. The wheat seedling died without producing new seed. Microscopic and biochemical examination show that the plant cell wall in the diseased wheat are modified, with some degradation of the outer sheath (Bishop and Anderson, unpublished). In contrast, the same seed, bearing the *Acremonium* fungus, grew under open greenhouse conditions to produce seed without causing symptoms.

Experiments in this report were designed to initiate our understanding of the factors that govern the switch of the *Acremonium* fungus that normally does not cause disease in wheat to one that causes symptoms.

There is evidence that fungal infection of plants leads to changes in invertase activity. Invertase is an enzyme that degrades sucrose into glucose and fructose. Because sucrose is the major sugar that is circulated in plants, it is an important source of carbon nutrition for invading pathogens. Benhamou et al. (1991) found that tomato challenged with the pathogenic fungus *Fusarium oxysporum* accumulated a plant-produced invertase in tomato root cell walls. A plant cell wall-bound invertase was induced similarly in carrots upon infection by *Erwinia carotovora* (Sturm and Chrispeels, 1990). Invertase activity also increased in wheat after infection with the stem rust fungus, *Puccinia graminis* (Heisteruber et al., 1994).

The microbe may produce additional invertase activity. Lam et al. (1994) showed that *Acremonium typhinum*, an nonpathogenic endophyte of the grass, *Festuca rubra*, -

displayed both cytosolic and cell-wall bound invertase activity. The production of fungal invertase may be regulated by the carbon source. For example, invertase activity was high in *Aspergillus nidulans* that was grown on raffinose and sucrose, but was repressed by the addition of glucose to sucrose medium (Vainstein and Perberdy, 1991).

We propose that under optimal plant growth, the *Acremonium* fungal hyphae grow within the plant cell wall, presumably utilizing the sugars that are in the apoplastic space (Bishop et al., 1997). We suggest under stressed growth conditions, sucrose becomes limited (Bishop et al., 1997) and the fungus is forced to employ other enzymes to degrade plant cell wall materials to gain nutrition. This act is responsible for the onset of symptoms in the *Acremonium*-infected wheat.

Our goals in this report were to find whether:

1) the fungus will grow on the simple sugars present in wheat leaf apoplastic fluids, such as sucrose, glucose, fructose, and myoinositol (Bishop et al., unpublished)
2) the fungus would grow, like a pathogen, on isolated wheat leaf cell walls and a common component of plant walls, pectin
3) the fungus produced invertase and to initiate characterization of its isozymes and their regulation
4) invertase activity differed in *Acremonium*-inoculated and noninoculated plants.

## METHODS AND MATERIALS

### Fungal growth and protein extraction

The *Acremonium* isolate was obtained from the *Acremonium*-infected wheat from the NASA space shuttle flight as an outgrowth from infected leaves on potato dextrose agar (PDA) (Bishop et al., 1997). Spores were obtained by growing *Acremonium* for seven days on PDA at 28°C. A spore suspension was made by the addition of sterile, distilled water and was stored at -20°C in 15% glycerol.

Medium for growth of *Acremonium* contained in one liter: 1.5 g of $KH_2PO_4$, 0.5 g of $MgSO_4.7H_2O$, 1.44 g $KNO_3$, 1% final concentration of different carbon sources (0.5% of each carbon source if two carbon sources were used), and 1 ml/100 ml of trace elements (1.5 g nitrilotriacetic acid, adjusted to pH 6.0 with HCl, 3.0g $MgSO_4.7H_2O$, 0.5g $MgSO_4.H_2O$, 1.0g NaCl, 0.1g $FeSO_4.7H_2O$, 0.1g Co $SO_4$, 0.1g $CaCl_2.2H_2O$, 0.01g $NaMoO_4.2H_2O$, 0.1g $ZnSO_4.7H_2O$, 0.01g $CuSO_4.5H_2O$, 0.01g, $AlK(SO_4)_2.12H_2O$, 0.01g $H_3BO_3$/liter).

For plate cultures, the medium was supplemented with 2% agar and the plates were inoculated in the center with $10^4$ spores. Growth was measured using a ruler after incubation at 28°C. For liquid culture, 40 ml of medium in 250 ml flasks were inoculated with $10^4$ spores/ml. Flasks were shaken at 110 rpm and incubated at 28°C.

At known times, liquid cultures were removed and mycelium was collected by vacuum filtration through 3MM Whatman paper. The culture medium from the filtration was dialyzed overnight against distilled water in 6-8,000 MW cut-off membranes and saved as the "culture filtrate" for further analysis. The mycelium was split into two equal weights. One portion was used to measure dry weight by drying at 80°C in a vacuum chamber for 12 h (15 pounds). The other portion was used for protein extraction.

Mycelia were ground in liquid nitrogen and the powder resuspended in lysis buffer [50 mM $PO_4$ pH 7.0, 0.1 mM EDTA, 1 mM $Na_2SO_3$, 1 mM β-mercaptoethanol, 5 mM PMSF] in Eppendorf microcentrifuge tubes and centrifuged at 13,000 g for 30 min at 4°C. The supernatant was removed and stored at -20°C until analysis. This was assayed as the cytosolic fraction. The pellet was washed three times by centrifugation in lysis buffer, resuspended in 4:1 (v/v) 1M NaCl:lysis buffer, and incubated overnight at 4°C. The suspension was centrifuged at 13,000 g for 10 min at 4°C. The supernatant was desalted and concentrated by centrifugation using Amicon concentrators with a cut-off of 10 kDa. The cell wall-bound proteins were resuspended in 200 µL of lysis buffer.

### Seed surface sterilization treatments

Seeds of Super Dwarf wheat, obtained from Dr. Bruce Bugbee, Plants, Soils

and Biometeorology Department, Utah State University, were submersed in sterile, double-deionized water containing 0.2% Triton-X 100 and incubated with vigorous shaking at 150 rpm for 40 min at 50°C. The procedure was repeated twice. The seeds were washed three times with sterile double deionized water equilibrated to 50°C. The seeds were immersed in 12.5% sodium hypochlorite containing 0.2% Triton X-100, washed five times with sterile water and transferred to agar plates. The seeds were vernalized at 4°C for four days. Seeds were inoculated with spores ($10^3$ spores/ml in 1% carboxy methylcellulose for 2 min) or planted without fungal inoculation as controls. Seeds were planted in Magenta boxes containing sterilized vermiculite (300 ml) and water (150 ml). The closed boxes were placed at 30°C under a 24 h photoperiod by incandescent lighting (110 $\mu mol\ m^{-2}s^{-1}$) for 14 days.

Plants were harvested and the section above the seed and the root sectioned to provide one cm lengths. These sections were extracted to yield the apoplastic fluids by the method of MacAdam et al, 1992.

**Invertase activity assay**

Invertase assays were performed by adding 50 µL of sample (cytosolic, cell wall-bound or dialyzed culture filtrate) by incubation for one hour at 26°C in a final volume of 1 mL containing 0.1 mL sodium acetate buffer, pH 6.0, and 0.1 mL of 1% sucrose. Samples incubated in the absence of sucrose served as controls. Reducing sugars were assayed using 0.1 ml aliquots of the reaction mixture, measured spectrophotometrically at 520 nm according to Nelson, 1944, and Somogyi, 1936. One unit of activity was defined as the amount of enzyme that generated 1 µmol of reducing group (glucose equivalent)/min. Specific activities were defined as U/mg dry mass for the preparations from the fungal mycelia and U/mg protein for the apoplastic materials.

The determination of pH and temperature optima was done using cytoplasmic fractions from two-day old cultures grown on 1% sucrose. For the testing of pH optima, a sodium acetate buffer (0.1 M) was used for pH 3-6 while a potassium phosphate buffer (0.1M) was utilized for pH 7-9. Incubation was done at room temperature. The temperature optima study was accomplished by performing the reaction for invertase activity in cytoplasmic fractions in sodium acetate buffer, pH 6.0, containing 0.1 ml of 1% sucrose at known temperatures, between 7°C and 35°C

**Protein determination**

Protein determination was done using the BCA protein assay according to manufacturer's recommendation (Pierce, Rockford, IL).

**Detection of invertase isozymes**

Aliquots of samples (5-25 µg protein) were subjected to electrophoresis at 4°C for 2 hours at 200 volts using non-denaturing 7.5% polyacrylamide gels in a minigel apparatus (Anderson and Katsuwon, 1990). Immediately after electrophoresis, the gel was washed at least three times in distilled water, and incubated in 0.1 M sodium acetate buffer, pH 5.0, containing 0.1 M sucrose for 30 minutes at 26°C. The gel was washed at least three times in distilled water and immersed in freshly made solution of 1 N NaOH containing 0.2% (w/v) 2,3,5 tetrazolium chloride (Sigma Chemical Co.) The gel was heated in a boiling water bath until red-colored bands appeared indicating the formation of formazan. The gels were rinsed in water and fixed in 7.5% acetic acid for 30 minutes.

## RESULTS

**Growth of *Acremonium* on plant components**

*Acremonium* on grew on solid medium plates containing glucose, sucrose or fructose equally well, although growth on myoinositol was slower (Table 1). The addition of either glucose, sucrose or fructose to myoinositol restored the faster growth rate.

The increase in mass of *Acremonium* in liquid cultures was similar on glucose, sucrose, or fructose and stationary phase growth was reached by day two post inoculation (Figure 1). Myoinositol supported least growth also in liquid culture. Addition of glucose, sucrose or fructose to the myoinositol resulted in increased rate and level of growth (Figure 2).

The *Acremonium* fungus was observed to grow on slurry of isolated wheat leaf cell walls. Mycelia was observed associated with the walls and a purple pigmentation, similar to that produced during growth of myoinositol or on the roots of

infected wheat, was observed. The fungus also grew well on 1% citrus pectin (Figure 3).

**Table 1**

**Growth of *Acremonium* on minimal medium plates containing different carbon sources**

| Carbon source | Radius of growth measured in cm | | | |
|---|---|---|---|---|
| | day 3 | day 4 | day 7 | day 10 |
| Glucose | 0.50 +/- 0.02 | 1.19 +/- 0.03 | 2.15 +/- 0.06 | 3.66 +/- 0.10 |
| Sucrose | 0.49 +/- 0.02 | 1.20 +/- 0.02 | 2.08 +/- 0.07 | 3.61 +/- 0.10 |
| Fructose | 0.48 +/- 0.02 | 1.18 +/- 0.03 | 2.04 +/- 0.06 | 3.57 +/- 0.12 |
| Myoinositol | 0.36 +/- 0.02 | 0.88 +/- 0.10 | 1.32 +/- 0.60 | 2.95 +/- 0.65 |
| Myoinositol + glucose | 0.49 +/- 0.02 | 1.18 +/- 0.04 | 1.95 +/- 0.08 | 3.58 +/- 0.11 |
| Myoinositol + sucrose | 0.50 +/- 0.02 | 1.17 +/- 0.03 | 2.05 +/- 0.07 | 3.57 +/- 0.09 |
| Myoinositol + fructose | 0.50 +/- 0.02 | 1.18 +/- 0.04 | 1.90 +/- 0.08 | 3.59 +/- 0.11 |

**Figure 1**

**Dry mass of mycelia of Acremonium grown on different carbon sources**

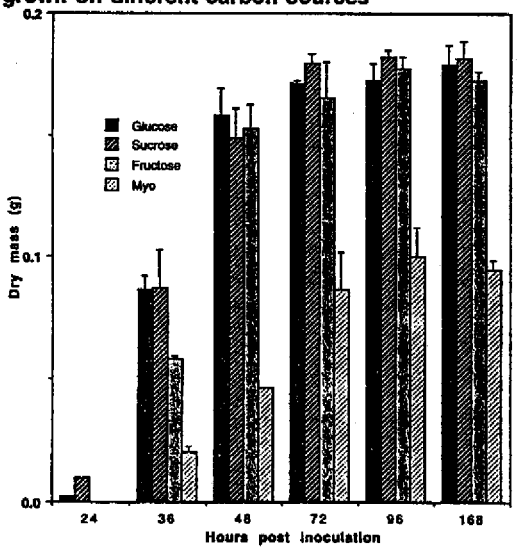



**Figure 2**

**Dry mass of mycelia of Acremonium grown on combined carbon sources**

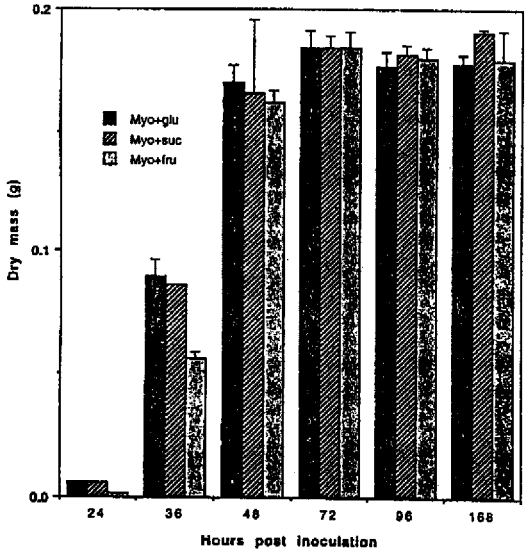
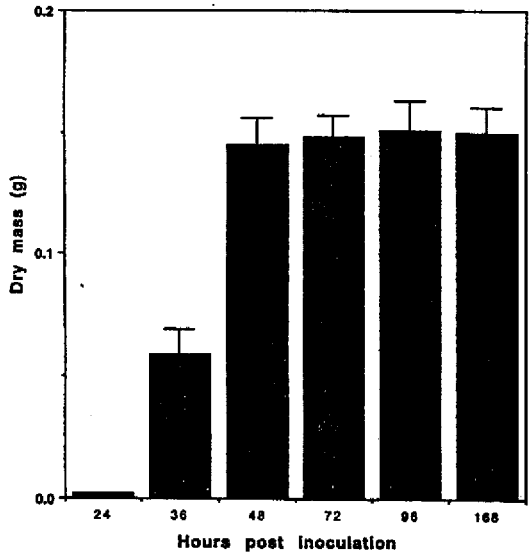



**Figure 3**

**Dry mass of mycelia of Acremonium grown on pectin**



## *Acremonium* invertase activity

Cytoplasmic fractions from *Acremonium* mycelia grown on 1% glucose, fructose or myoinositol displayed a basal level of invertase activity (Table 2). However, *Acremonium* myelia grown on 1% sucrose showed a greater invertase specific activity. At days two, four and six, activity in the sucrose-grown *Acremonium* was as much as a ten-fold greater than activity detected in *Acremonium* grown on other carbon sources. Growth on the combination of 0.5% myoinositol and 0.5% sucrose, *Acremonium* produced invertase activity about 80% that of the activity detected in mycelia grown on 1% sucrose. Growth on raffinose as a sole carbon source also produced a high invertase activity, whereas growth on cellobiose or maltose produced low activity (Tables 3a, b).

Invertase activity was detected in the culture filtrates and cell wall-bound fractions but only when the *Acremonium* fungus was grown on sucrose or sucrose and myoinositol. Table 4 shows that the proportion of activity in the three fractions varies with the age of the culture. The highest specific activity was observed in the culture filtrate at day four (Table 2).

**Table 2**

**Invertase activity from Acremonium grown in liquid media on different carbon sources**

**Culture filtrate fractions**

| Carbon source | Specific activity (U/mg dry mass)[a] 48 h | 4 days | 6 days |
|---|---|---|---|
| Glucose | 0 | 0 | 0 |
| Sucrose | 1.30 +/- 0.27 | 11.93 +/- 1.59 | 4.40 +/- 1.83 |
| Fructose | 0 | 0 | 0 |
| Myoinositol | 0 | 0 | 0 |
| Myo + glucose | 0 | 0 | 0 |
| Myo + sucrose | 1.00 +/- 0.67 | 5.46 +/- 0.63 | 3.33 +/- 1.60 |
| Myo + fructose | 0 | 0 | 0 |

**Cytoplasmic fractions**

| Carbon source | Specific activity (U/mg dry mass)[a] 48 h | 4 days | 6 days |
|---|---|---|---|
| Glucose | 0.07 +/- 0.01 | 0.37 +/- 0.06 | 0.15 +/- 0.02 |
| Sucrose | 2.44 +/- 0.31 | 4.02 +/- 0.23 | 3.20 +/- 0.22 |
| Fructose | 0.26 +/- 0.02 | 0.70 +/- 0.10 | 0.24 +/- 0.00 |
| Myoinositol | 0.16 +/- 0.02 | 0.42 +/- 0.00 | 0.20 +/- 0.03 |
| Myo + glucose | 0.26 +/- 0.01 | 1.01 +/- 0.10 | 0.16 +/- 0.06 |
| Myo + sucrose | 1.96 +/- 0.21 | 3.35 +/- 0.06 | 2.45 +/- 0.18 |
| Myo + fructose | 0.28 +/- 0.02 | 0.76 +/- 0.03 | 0.27 +/- 0.04 |

**Cell-wall bound fractions**

| Carbon source | Specific activity (U/mg dry mass)[a] 48 h | 4 days | 6 days |
|---|---|---|---|
| Glucose | 0 | 0 | 0 |
| Sucrose | 0.20 +/- 0.02 | 0.92 +/- 0.12 | 1.05 +/- 0.00 |
| Fructose | 0 | 0 | 0 |
| Myoinositol | 0 | 0 | 0 |
| Myo + glucose | 0 | 0 | 0 |
| Myo + sucrose | 0.01 +/- 0.00 | 1.03+/- 0.03 | 1.15 +/- 0.07 |
| Myo + fructose | 0 | 0 | 0 |

a +/- standard deviation

**Table 3a**

**Relative invertase activity of *Acremonium* grown for two days on raffinose vs sucrose**

| Extract source | *Acremonium* grown on: Sucrose | Raffinose |
|---|---|---|
| Culture filtrate | 100% | 68% |
| Cytoplasmic | 100% | 79% |
| Cell-wall bound | 100% | 112% |

Invertase activity from *Acremonium* grown on sucrose was arbitrarily set at 100%. Invertase activity from *Acremonium* grown on raffinose was expressed as a percentage of the invertase activity from *Acremonium* grown on sucrose.

**Table 3b**

**Relative invertase activity of *Acremonium* grown for two days on maltose or cellobiose vs glucose**

| Extract source | *Acremonium* grown on: glucose | maltose | cellobiose |
|---|---|---|---|
| Cytoplasmic | 100% | 112% | 118% |

Invertase from *Acremonium* grown on glucose was arbitrarily set at 100%. Invertase activity from *Acremonium* grown on maltose or cellobiose was expressed as a percentage of the invertase activity from *Acremonium* grown on glucose.

**Table 4**

**Percentages of invertase activity from *Acremonium* in the culture filtrate, cytoplasmic, and cell-wall bound fractions**

| Carbon source | % of activity Culture filtrate | Cytoplasm | Cell wall-bound |
|---|---|---|---|
| Sucrose | | | |
| Day 2 | 33 | 62 | 5 |
| Day 4 | 71 | 24 | 5 |
| Day 6 | 51 | 37 | 12 |
| Myoinositol + sucrose | | | |
| Day 2 | 34 | 66 | 0 |
| Day 4 | 55 | 34 | 11 |
| Day 6 | 48 | 35 | 17 |

### Electrophoresis of fungal invertase

Two isozymes of invertase activity with the same mobility were revealed by nondenaturing gel electrophoresis with both the cell wall-bound and cytoplasmic sources (Figure 4). The isozymes were identical whether the fungus was grown on sucrose or sucrose and myoinositol (Figure 4). The faster moving species was the major band of activity.

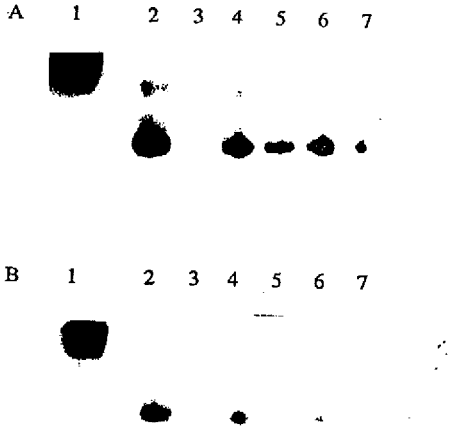



Figure 4 Native gel electrophoresis of invertase from *Acremonium* grown on (A) sucrose or (B) sucrose and myoinositol. Lane 1, control invertase from *Saccharomyces cerevisiae*. Lane 2, cytoplasmic fraction, 48 h. Lane 3, cell-wall bound, 48 h. Lane 4, cytoplasmic, 96 h. Lane 5, cell-wall bound, 96 h. Lane 6, cytoplasmic, 144 h. Lane 7, cell-wall bound, 144 h. Cytoplasmic samples were loaded with 25 µg protein. Cell-wall samples were loaded with 5 µg protein.

### Characterization of *Acremonium* invertase activity

The activity present in the cytoplasm from sucrose-grown mycelium had an acidic pH optimum between pH 5-6 (Figure 5). Invertase activity was maximal at 26°C (Table 5) but was able to retain 35% of this activity at the cooler temperature of 7°C, and 75% activity at 35°C.

**Figure 5**

**pH optima for invertase activity of cytoplasmic fraction from Acremonium grown on 1% sucrose for two days**

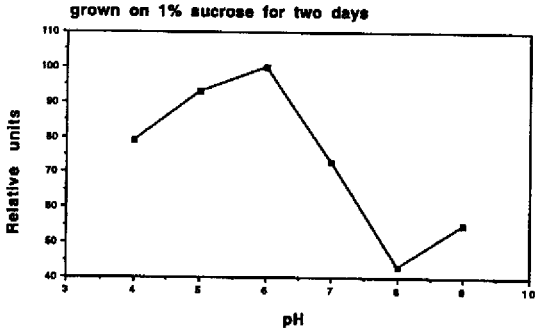



**Table 5**

**Effect of temperature on invertase activity of cytoplasmic fraction from *Acremonium* grown on 1% sucrose for two days**

| Temperature (°C) | Relative Units |
|---|---|
| 7 | 56.1 +/- 12.2 |
| 13 | 63.4 +/- 4.9 |
| 22 | 73.2 +/- 0.0 |
| 26 | 100.0 +/- 2.4 |
| 30 | 78.0 +/- 0.0 |
| 35 | 75.6 +/- 2.4 |

### Invertase activity in wheat

Invertase activity was detected in apoplastic fluids obtained from the sheath and leaves as to a lesser extent the roots (Table 6). Specific activity in the sheath and leave extracts was not signigicantly different upon infection with *Acremonium*. However, in the infected root, invertase activity increased seven-fold.

**Table 6**

**Invertase activity from *Acremonium*-infected and control wheat seedlings**

| | Specific activity (U/mg protein) |
|---|---|
| control sheath and leaf | 10.4 +/- 1.8 |
| infected sheath and leaf | 12.3 +/- 1.1 |
| control root | 1.4 +/- 0.7 |
| infected root | 8.5 +/- 0.9 |

Tissue was harvested 14 days post germination and apoplastic fluid was isolated for control and infected roots or sheath and the inner leaves.

## CONCLUSIONS

The *Acremonium* fungus isolated from wheat is versatile in its ability to grow on components in the wheat shoot apoplast and the wall. The fungus utilizes the simple sugars in the apoplast, sucrose, glucose, fructose and myoinositol. However, it also grows on isolated plant cell walls that have no simple-

sugars available, but rather offers a complex of polymers of carbohydrates, proteins and phenolics. Growth on pectin, an acidic structural polymer in the wall, indicates that the fungus should be able to degrade the pectic layer found in the middle lamella between cells and that this degradation may permit the degradation of additional wall polymers. Studies with other pathogens suggest that removal of the pectin is needed before access to the other polysaccharides can be gained (Dean and Timberlake, 1989, Roberts et al., 1986). This speculation is consistent with the observed cell maceration of the sheath layer as the *Acremonium* infection proceeds in the wheat (Bishop et al., 1997). We propose to study the regulation of enzymes required for cell wall degradation in the next year.

The utilization by the *Acremonium* fungus of the simple sugar, sucrose, that is the major carbon molecule transported in the plant, involves two isozymes of invertase. Production of invertase during growth on raffinose but not on maltose or cellobiose suggests to us that the *Acremonium* activity is a β-fructosidase rather than an α-glucosidase. Furthermore, the lack of invertase activity from *Acremonium* grown on cellobiose indicated that there were no general non-specific β-glycosidases produced. The activity is detected both within the fungal cell as well as being secreted from the mycelium. A secreted enzyme would be appropriate to degrade the plant's apoplastic sucrose to the monomers to be taken up by the fungus for further metabolism. The cytosolic location of invertase suggests that the fungus may also be able to import sucrose directly. These two methods may help the fungus compete for the sucrose with the plant cell. In the next year, we will examine whether the secreted invertase activity is due to the same or different isozymes than those in the cytoplasm. Infection-enhanced invertase activity was detected in the plant roots but not the plant shoot and leaves. We propose to use electrophoresis to determine whether the fungal invertases are produced *in planta* or whether only wheat activities are present.

Expression of the invertase by the fungus is regulated: sucrose is required for invertase to be maximally produced. A low constitutive level of activity is observed in the cytoplasm of the fungal mycelia. This finding suggests that the fungus also regulates the secretion of the invertase.

The invertase has a broad acidic pH optimum, with maximum activity at pH 5-pH 6, which is appropriate for its expected activity in the plant cell wall. This pH optima is consistent with invertases from other fungi. Invertases from *Aspergillus niger* (Boddy et al., 1993) as well as the yeast, *Candida utilis* (Chavez et al., 1997), have similar pH optima at 5.5.

Our findings to date suggest that the *Acremonium* fungus is well adapted to securing nutrients within the plant. The observation that production of invertase requires sucrose to maximize activity adds support to our hypothesis that the fungus turns to degradation of the plant cell wall when the plant becomes stressed. Bishop et al. (unpublished) find that the plants grown under stressful enclosed conditions have less sucrose in the leaf/sheath apoplast than plants grown in open conditions. Bishop et al. (unpublished) also find that plants grown under high carbon dioxide, as expected in the spaceflight conditions have low apoplastic sugar levels. Thus, we predict that these spaceflight conditions would enhance the pathogenic potential of the fungus.

**Relevance to spaceflight**

Our studies show that the interactions between an apparently benign microbe and a plant may be influenced by spaceflight conditions. High carbon dioxide, low light intensities and high relative humidity or ethylene could enhance pathogenicity, as is observed with the *Acremonium*-fungal interaction. Thus, it is important to be studying ways in which plants grown in space can combat potential pathogens.

A second point is that the pathogen in this example was imported into space within the seed, even though preventive measures were taken to eliminate surface-borne microbes from the seed at the time of planting. Thus, careful screening of plant material is essential prior to their use in space.

Third, understanding of the basis of plant-pathogen interactions may be important in modeling the likelihood that some microbe will mutate or adapt to be a pathogen. Surveys of spacecraft, or the plant growth simulators used on earth show that they are far from sterile. Our studies with an *Acremonium*

species are also of interest in that *Acremonium* isolates are one of the most common of the fungi to be detected on returning missions or in ground base simulators (Ahearn et al., 1995). The possibility that some *Acremonium* isolates are human pathogens and allergens indicates that this study is pertinent.

**Acknowledgements**

This research was supported by the Rocky Mountain NASA Space Grant consortium.

**REFERENCES**

Ahearn, D.G., Simmons, R.B., Price, D.L., Crow, S.A., Mishra, S.K., Peirson, D.L. 1995. Fungal colonization of synthetic substrates for use in space craft. *Journal of Industrial Microbiology*. 14: 26-30.

An, Z.Q., Siegel, M.R., Hollin, W., Tsai, H.F., Schmidt, D., Schardle, C.L. 1993. Relationships among non-*Acremonium* sp. fungal endophytes in five grass species. *Appl. Environ. Microbiol.* 59: 1540-1548.

Benhamou, N., Grenier, J., Chrispeels, M.J. 1991. Accumulation of β-fructosidase in the cell walls of tomato roots following infection by a fungal wilt pathogen. *Plant Physiology*. 97: 739-750.

Bishop, D.L., Levine, H.G., Kropp, B.R., Anderson, A.J. 1997. Seedborne fungal contamination: consequences in space-grown wheat. *Phytopathology*. 87: 1125-1133.

Boddy, L.M., Berges, T., Barreau, C., Vainstein, M.H., Dobson, M.J., Ballance, D.J., Peberdy, J.F. 1993 Purification and characterisation of an *Aspergillus niger* invertase and its DNA sequence. Current Genetics. 24: 60-66.

Chavez, F.., Rodriguez, L., Diaz, J., Delgado, J.M., Cremata, J.A. 1997. Purification and characterization of an invertase from *Candida utilis*: comparison with natural and recombinant yeast invertases. *Biotechnology*. 53: 67-74.

Dean, R.A., Timberlake, W.E. 1989 Production of cell wall-degrading enzymes by *Aspergillus nidulans*: a model system for fungal pathogenesis of plants. *Plant Cell*. 1: 265-273.

Heisteruber, D., Schulte, P., Moerschbacher, B.M. 1994. Soluble carbohydrates and invertase activity in stem rust-infected, resistant and susceptible near-isogenic wheat leaves. *Physiological and Molecular Plant Pathology*. 44: 111-123.

Katsuwon, J., Anderson, A.J. 1990. Catalase and superoxide dismutase of root-colonizing saprophytic fluorescent pseudomonads. *Applied and Environmental Microbiology*. 56: 3576-3582.

Lam, Cuong K., White, James F., Jr., Daie, Jaleh. 1994 Mechanism and rate of sugar uptake by *Acremonium typhinum*, an endophytic fungus infecting *Festuca rubra*: Evidence of presence of a cell wall invertase in endophytic fungi. *Mycologia*. 86: 408-415.

MacAdam, J.W., Sharp, R.E., Nelson, C.J. 1992. Peroxidase activity in the leaf elongation zone of tall fescue. Spatial distribution of apoplastic peroxidase activity in genotype differing in length of the elongation zone. *Plant Phys*. 99: 879-885.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biol. Chem.*. 153: 375-379.

Roberts, D.P., Berman, P.M., Allen, C., Stromberg, V.K., Lacy, G.H., Mount, M.S. 1986. Requirement for two or more *Erwinia carotovora* subsp. carotovora pectolytic gene products for maceration of potato tuber tissue by *Escherichia coli*. *Journal of Bacteriology*. 167: 279-284.

Somogyi, M. 1937. A reagent for the copper-iodometric determination of very small amounts of sugar. *Journal of Biol. Chem.* 117: 771-776.

Sturm, A., Chrispeels, M.J. 1990. cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection. *The Plant Cell*. 2: 1107-1119.

Vainstein, H. and Peberdy F. 1991. Regulation of invertase in *Aspergillus nidulans*: effect of different carbon sources. *Journal of General Microbiology*. 137: 315-321.