# Temperature as a Determining Factor in the Storage Rot of Sugarbeet Caused by *Aspergillus fumigatus*

**J. M. Halloin[1,2] and D. L. Roberts[2]**

[1]*U.S. Department of Agriculture,*
*Agricultural Research Service,*
*Sugarbeet and Bean Research Unit, and*
[2]*Department of Botany and Plant Pathology,*
*Michigan State University, East Lansing, MI 48824-1312.*

## ABSTRACT

**_Aspergillus fumigatus_ was a pathogen in sugar beet roots held at temperatures of 35°C or greater. The fungus occurred as a surface contaminant on freshly harvested roots; it increased in incidence during storage of roots at 6°C, apparently infecting wounds, but caused no rot at the lower temperature. A cultivar resistant to crown and root rot caused by _Rhizoctonia solani_ (AG-2-2) was more tolerant to _A. fumigatus_ than a _R. solani_-susceptible cultivar at 35°C, but this tolerance diminished with an increase in temperature to 40°C. Roots killed by freezing were rotted rapidly at 22°C, suggesting that tolerance to the rot is more likely due to inducible rather than to constitutive inhibition of the fungus. Development of rot coincided with maximum growth of the fungus at about 40°C. Rot caused by _A. fumigatus_ is likely to be a problem only in stored roots that have undergone composting or metabolic heating. Subsequent cooling of such roots is unlikely to control further rot development.**

**Additional Key Words:** *Beta vulgaris,* bacteria, disease resistance, *Rhizoctonia solani,* Rhizoctonia crown and root rot.

Storage rots of sugarbeet *(Beta vulgaris* L.) most commonly are associated with *Penicillium* spp., *Phoma betae, Botrytis cinerea,* and *Fusarium* spp. (Bugbee, 1975, 1982; Bugbee and Nielsen, 1978). Genetic resistance to Rhizoctonia crown and root rot confers resistance to some of these rots (Bugbee and Campbell, 1990). Bacteria can rot dead sugarbeet tissue, such as that which has been frozen and thawed (W. Bugbee, personal communication), and living tissue held under anaerobic conditions (Cole and Bugbee, 1976), but usually present no problem in living tissue. Some of these bacteria, especially *Leuconostoc mesenterioides,* produce polysaccharide slimes that interfere with screening and filtering during sugar refining (Halden, 1971).

*Aspergillus fumigatus* Fresen. is observed commonly as masses of olive-green spores on cut surfaces of sugarbeet roots in cold storage at East Lansing, MI, but apparently does not cause damage under those circumstances. This fungus has been reported to occur on dried sugarbeet pulp (van Schingen, 1990) and in silage from sugarbeet press pulp (Nout, et al., 1993). While examining temperature effects on development of crown and root rot caused by *Rhizoctonia solani* Kühn, we observed a severe rot of roots caused by *A. fumigatus* in harvested roots incubated at 35°C or greater (Halloin and Roberts, 1991). This rot often was accompanied by rotting and slime production by *L. mesenterioides.* This study was done to determine when infection by *A. fumigatus* occurs, the effect of temperature and host resistance to *R. solani* on rot development, and to obtain preliminary information on the nature of resistance to the fungus. Associations of bacteria with fungal rot were noted.

## MATERIALS AND METHODS

Sugarbeet cultivar Hilleshog MonoHy E4 (MHY E4, *Rhizoctonia*-susceptible) and the germplasm line FC-701/5 *(Rhizoctonia*-resistant) were planted mid-May in field plots at East Lansing, MI. Plants with crown diameters of 8-12 cm were dug in October and November, and foliage was removed by cutting. Roots were washed to remove soil, then taken to the laboratory for immediate use or stored in perforated plastic bags at 6°C for up to 2 weeks.

*A. fumigatus* was isolated by plating cubes 0.5 cm on a side of healthy or diseased tissues on 2% water agar containing 20% (w/v) sucrose; these were incubated at 40°C. Isolated cultures were maintained on 2% potato-dextrose agar.

Inoculum of *A. fumigatus* was prepared as follows. Millet caryopses (250 g) were immersed in water for 16 hr. After removing excess water, the imbibed caryopses were placed in a 32 x 32 x 6 cm baking pan, covered with aluminum foil, and autoclaved for 30 min. A 6-day-old culture of the fungus was flooded with 30 ml of sterile water containing 0.5% polyoxyethylenesorbitan monolaurate. The resultant conidial suspension was poured over the caryopses, which then were incubated at 22°C for 2 weeks, followed by air-drying in a laminar flow hood. Dry inoculum was stored in glass bottles at -20°C.

**The effect of root storage and surface disinfection on occurrence of *A. fumigatus*.** The fungus was isolated from the exterior of healthy crowns and roots as follows: eight cubes of tissue 0.5 cm on a side were cut from each root and crown of 20 freshly harvested roots, or from roots and crowns of each of 10 roots that had been stored at 6°C for 2 weeks. Four of these pieces either were rinsed with sterile distilled water or were immersed for 1 min in aqueous 70% ethanol and rinsed with sterile distilled water before placement on water agar.

**The effect of temperature and inoculation on development of rot.** A 3 mm diameter x 1 cm deep hole was drilled into each freshly harvested root near its previous soil line. Inoculum caryopses (five per hole) were inserted into the hole. Roots were placed individually into perforated plastic bags and incubated as required for individual experiments. Control roots contained holes, but no inoculum. Five roots were used for each treatment within an experiment, and experiments were done five times. Few lesions in excess of 1.5 cm long were less than 7 cm long. Lesion length was not used to quantitate susceptibility, as lesions often extended the entire length of the root, and thus were limited by root length. Thus, roots with lesions less than 3 cm long were scored as tolerant, and rot frequency was expressed as percentage of roots with lesions longer than 3 cm. Results were evaluated by analysis of variance.

**Effect of temperature and sucrose concentration on growth of *A. fumigatus*.** Sugarbeet medium (SBM) used to evaluate the effect of sucrose concentration and temperature on growth of the fungus was made as follows: sugarbeet roots (MHY E4) were reduced to a wet pulp by maceration with a beet saw, and juice was obtained from this pulp by squeezing through a double layer of surgical gauze. Solute concentration of the juice was determined with a refractometer. Subsequent analysis (Michigan Sugar Company) revealed that the solute was mostly sucrose. Juice was stored frozen for future use. For use, 100 ml of juice was supplemented with 20 g of agar and enough sucrose to give a final concentration of either 2 or 20%, diluted to 1 L, autoclaved for 15 min, and poured into 9 cm diameter petri dishes. A single inoculum caryopsis was placed in the center of each dish, and the dishes were incubated at the required temperatures for 4 days. Results are the means of two tests with 10 dishes at each temperature.

**Effect of regimes of different temperatures on frequency of occurrence of rot.** To examine the nature of the resistances to *A. fumigatus* that are associated with temperature and genetic resistance to *R. solani,* roots were inoculated with the fungus as described previously, and exposed to three regimes of differing temperatures. Results are the means of two tests, each on five roots (-20 and 22°C) or of three to five tests, each on five to eight roots.

## RESULTS AND DISCUSSION

Rotting of sugarbeet caused by *A. fumigatus* resulted in blackening of diseased tissue (Fig. 1). Some sporulation of the fungus commonly occurred at the margin of the rotted area. Internal rot developed before noticeable appearance of the fungus on the root surface; however, extensive sporulation occurred on the surface of the root in the later stages of decomposition. When rotting was associated with slime-producing bacteria (not shown), internal tissue retained coloration similar to that of healthy tissue, and sporulation of the fungus, both internally and on the root surface, was quite limited. Slime-producing bacteria were observed in 25-30% of rotted roots, always in association with *A. fumigatus,* and were probably secondary rotters. Bacteria have been reported as internal contaminants of sugarbeet roots (Bugbee et al., 1975), but they normally cause rots only in dead tissues or under anaerobic conditions (Cole and Bugbee, 1976).

**The effect of root storage and surface disinfection on occurrence of *A. fumigatus.*** The relatively infrequent isolation of the fungus from freshly harvested roots, and its easy reduction with 70% ethanol (Table 1) indicates that it was a surface contaminant. The somewhat more frequent occurrence on crown tissues probably was related to their rougher surface. Increased frequency of isolation following storage indicates that the fungus invaded tissues following harvest, possibly at wound sites, and the greater increase in frequency of isolation from crowns than from roots of stored tissues may have been related to greater abundance of wounded tissues on the crown surfaces. The similarity in frequencies of isolation of the fungus from both R. solani-susceptible and -resistant roots demonstrated that such resistance was not a major factor in initial contamination or infection.

**Figure 1.** Median longitudinal section of a sugarbeet root rotted by *Aspergillus fumigatus.* The root was inoculated with the fungus and incubated for 7 days at 40°C before sectioning.

**The effect of temperature and inoculation on development of rot.** Evaluation of the effect of temperature on percentage rot of inoculated and noninoculated E4 and FC 701/5 roots (Table 2) showed significant effects for variety (F = 26.68, *P* = 0.0355; means, E4 = 42%, FC 701/5 = 16) and for temperature (F = 29.03, *P* = 0.0001; means, 30o = 8%, 35o = 30%, 40o = 49). No rot occurred in roots of the Rhizoctonia-resistant cultivar at 30°C. The interaction of inoculation by temperature also was significant (F = 3.89, P = 0.0273); rot occurred approximately twice as frequently in inoculated as in non-inoculated roots at all temperatures.

**Effect of temperature and sucrose concentration on growth of *A. fumigatus.*** Optimum growth of the fungus on SBM occurred near 40°C (Fig. 2). The apparent loss of tolerance at temperatures of 35°C or greater appears related to more rapid growth of *A. fumigatus* at those temperatures. With an active (inducible) resistance, the fungus may grow through tissues more rapidly than those tissues can respond. Growth rate of the fungus was only about 30% lower on 20% sucrose than on that containing 2%, demonstrating the relative tolerance of the fungus to an osmotic environment comparable to that of a mature sugarbeet root.

**Effect of regimes of different temperatures on frequency of occurrence of rot.** Roots killed by freezing at -20°C for two days showed no tolerance to the fungus, and were thoroughly rotted after four days at 22°C (Table 3). All of these roots were covered with the fungus on the surface, but the interior tissues were completely colonized by slime-producing bacteria. This rapid development of rotting organisms in dead tissue indicates that if constitutive resistance systems were involved in disease tolerance expressed by living tissue, they were destroyed upon root death. Initial incubation of live roots at 30°C failed to arrest development of rot upon transfer to 40°C in either R. solani-susceptible or -resistant roots. Thus, incubation of inoculated roots under conditions inconducive to rot development does not result in formation of materials providing continued

**Table 1.** Isolation frequency of *Aspergillus fumigatus* from root or crown tissues of field grown *Rhizoctonia solani*-resistant (FC 701/5) or -susceptible (MHY E4) sugarbeet. Eight cubes of tissue 0.5 cm on a side were cut from each root and crown of 20 freshly harvested roots, or from roots and crowns of each of 10 roots that had been stored at 6°C for 2 weeks in perforated plastic bags. Four of these pieces either were rinsed with sterile distilled water or were immersed for 1 min in aqueous 70% ethanol and rinsed with sterile distilled water before placement on water agar.

| Cultivar | Stored | Tissue | 70% ethanol | Isolation frequency |
|---|---|---|---|---|
| MHY E4 | — | Root | — | 3/80 |
| | — | Root | + | 0/80 |
| | — | Crown | — | 7/80 |
| | — | Crown | + | 5/80 |
| | + | Root | — | 11/40 |
| | + | Root | + | 6/40 |
| | + | Crown | — | 15/40 |
| | + | Crown | + | 9/40 |
| FC 701/5 | — | Root | — | 2/80 |
| | — | Root | + | 0/80 |
| | — | Crown | — | 7/80 |
| | — | Crown | + | 3/80 |
| | + | Root | — | 8/40 |
| | + | Root | + | 6/40 |
| | + | Crown | — | 19/40 |
| | + | Crown | + | 8/40 |

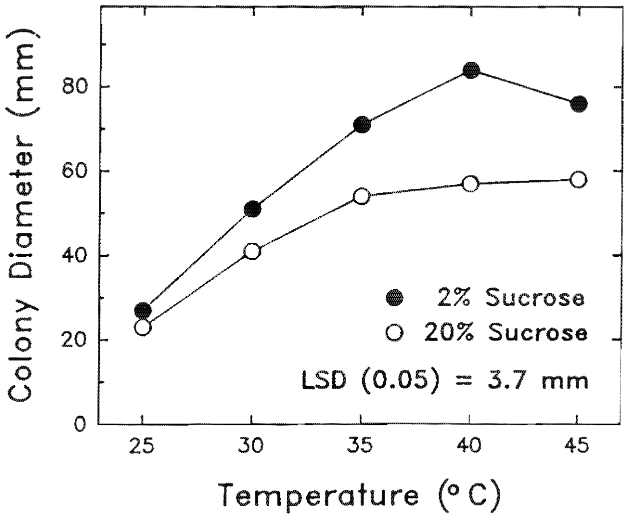

**Figure 2.** Effect of temperature and sugar concentration on radial growth of *Aspergillus fumigatus.* The fungus was grown for 4 days on 2% agar containing 1/10X extract of sugarbeet roots, with sucrose concentrations of either 2% or 20% (w/v).

**Table 2.** Effect of incubation for 10 days at various temperatures and inoculation with *Aspergillus fumigatus* on the frequency of rot in *Rhizoctonia*-resistant (FC 701/5) and -susceptible (MHY E4) sugarbeet roots.

| Cultivar | Temperature (°C) | Inoculated | Rot frequency (%)[†] |
|---|---|---|---|
| MHY E4 | 30 | — | 12 |
| | 30 | + | 20 |
| | 35 | — | 32 |
| | 35 | + | 60 |
| | 40 | — | 48 |
| | 40 | + | 80 |
| FC 701/5 | 30 | — | 0 |
| | 30 | + | 0 |
| | 35 | — | 8 |
| | 35 | + | 20 |
| | 40 | — | 16 |
| | 40 | + | 52 |

[†] Values are the means of five tests of five roots each.

**Table 3.** Effect of differing regimes of temperature and incubation duration of roots inoculated with *Aspergillus fumigatus* on the frequency of rot in *Rhizoctonia*-resistant (FC 701/5) or - susceptible (MHY E4) sugarbeet roots.

| Incubation regimes | | | Rot frequency |
|---|---|---|---|
| Temperature (°C)/Days | | | |
| First | Second | Cultivar | (%) |
| −20/2 | 22/4 | MHY E4 | 100 |
| | | FC 701/5 | 100 |
| 30/8 | 40/10 | MHY E4 | 94 |
| | | FC 701/5 | 70 |
| 40/4 | 30/14 | MHY E4 | 67 |
| | | FC 701/5 | 50 |

prophylaxis against the fungus. Rot initiated at 40°C was not halted in either the *R. solani*-susceptible or -resistant lines by transfer to 30°C. Subsamples of roots evaluated following the initial incubation at 40°C revealed only small lesions (< 2cm diameter) at the time of transfer to the cooler temperature. Either tolerance was suppressed by prior incubation at the warmer temperature, or sufficient fungus developed within the tissues that it was able to overwhelm any inhibitory systems.

*A. fumigatus* is unlikely to present a problem under normal root storage conditions; however, it is able to rapidly rot roots in which metabolic heating or composting has occurred. Subsequent cooling of partly rotted roots is unlikely to prevent further rot in storage piles. The behavior of this fungus in stored sugarbeet roots may be representative of that of opportunistic microorganisms that are both thermophilic and osmophilic, and can be associated with roots.

Human pathogenicity of *A. fumigatus* was noted by van Schingen (1990). Rogers (1980) described this pathogenicity as opportunistic, because the fungus parasitizes only tissues exhibiting other pathologies or suppression of the immune system. Mycotoxin production has never been observed with A. fumigatus (A. L. Rogers, personal communication).

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