Patulin Production in Apples Decayed by

*Penicillium expansum*¹

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Sixty isolates of *Penicillium expansum* were tested for patulin production in decaying apples. All the isolates were found to produce the mycotoxin patulin as determined by thin-layer chromatography. Since patulin is known to be stable in many apple products, the results indicate that apple products made partially from apples decayed by *P. expansum* will contain patulin which may present a health hazard. The results also suggest that patulin may be important in the decay of apples by *P. expansum.*

*Penicillium expansum* Link causes the most common storage rots of apples. Apples rotted by *P. expansum* can contaminate apple cider and other apple products. *P. expansum* is known to produce the mycotoxin patulin [4-hydroxy-4H-furo[3,2-C]-pyran-2(6H)-one] (1). Patulin has been shown to be carcinogenic to rats when administered by subcutaneous injection (3) and is acutely toxic to animals (2). The properties of patulin were most recently reviewed by Ciegler et al. (2). The purpose of this study was to determine how much patulin may occur in apples rotted by *P. expansum.*

*P. expansum* was isolated from apples decayed in refrigerated storage at 0 C. The isolates were collected in 1971 and 1972 from one orchard in Burlington, Vt. Sound McIntosh apples were wounded with a sterilized glass rod and then mycelial plugs from cultures growing on potato dextrose agar were placed in the wounds. The inoculated apples were incubated in perforated plastic bags at 25 C. When the apples were mostly decayed, 100 g of decayed tissue from each apple was blended for 3 min and then filtered through Whatman no. 1 filter paper. Fifty milliliters of this filtrate or 50 ml of cider was extracted with three 50-ml portions of ethyl acetate. After the ethyl acetate extract was evaporated to dryness, the residue was dissolved in 5.0 ml of ethyl acetate (6). To determine the amount recovered, authentic patulin was added to apple juice and extracted in the same manner. There was a 55% recovery of patulin with this extraction procedure. Twenty uliters of this extract or 1 to 25 uliters of the patulin standard (1 mg/ml) was spotted onto Mallinckrodt 7 GF silica gel plates. The patulin was a gift from Lederle Laboratories, lot no. 1129 C-127-1. The plates were developed with benzene-methanol-acetic acid (90:5:5) in unequilibrated, unlined tanks and then inspected under shortwave ultraviolet. The patulin appeared as a dark spot against a fluorescent background (4). The Rf value (approximately 0.47), size, and difference in intensity of the standard spots and the background were recorded. Each sample spot was placed in a range between two standard spots by comparing size and difference in intensity.

All isolates of *P. expansum* tested produced patulin. The amounts varied from a low range of 9 to 18 mg/liter (26.7% of the isolates) to a high range of 120 to 150 mg/liter (1.7% of the isolates). Table 1 shows that, in general, the number of isolates per range decreased as patulin production increased. In selected cases, two or three apples were inoculated with the same isolate and were assayed for patulin. In these tests patulin production was similar in all but two cases.

One hundred samples of fresh apple cider purchased from several cider mills were also assayed for patulin. Up to 45 mg of patulin per liter of apple cider was detected in five samples of so-called "organic" apple cider. Inspection of the mill revealed that the organic apple cider
Table 1. Patulin recovered from apples decayed by 60 different isolates of *P. expansum*.

| Range of patulin/liter of expressed apple juice (mg) | No. of isolates in this range |
|-----------------------------------------------------|-------------------------------|
| 9–18                                                | 16                            |
| 18–27                                               | 7                             |
| 27–36                                               | 6                             |
| 36–45                                               | 6                             |
| 45–54                                               | 7                             |
| 54–72                                               | 7                             |
| 72–90                                               | 4                             |
| 90–108                                              | 4                             |
| 108–126                                             | 2                             |
| 126–146                                             | 1                             |

*The values are corrected for a 55% recovery from expressed juice.*

was made from wild, unsprayed apples that were insect damaged and decayed. In other ciders tested, the estimated amount of patulin detected ranged from undetectable in 91 samples up to 25 mg/liter in four samples in 1971. The ciders containing the appreciable amounts of patulin were produced by cider mills where decayed apples were not sorted out or where the apples were stored in large bins for extended periods. These practices were changed, and no patulin was detected in these sample mills in 1972.

The results show that all the isolates of *P. expansum* tested produced patulin, often in appreciable quantities. Since patulin is stable in apple products (5, 7), the results indicate that products made from apples rotted by *P. expansum* will contain patulin which may present a health hazard. Up to 45 mg of patulin per liter in purchased apple cider was found only in cider produced by cider mills where decayed apples had not been sorted or where apples were stored in bins for extended periods. This allowed *P. expansum* ample time to decay the apples in the bins.

Since patulin is highly toxic to plants as well as animals (2), it is possible that the patulin produced by *P. expansum* may play a role in plant pathogenesis. Brian et al. (1) stated that patulin is not always present in *P. expansum*-rotted apples. From this they postulated that patulin is not involved in apple decay. Our results do not support this hypothesis, since all of our isolates produced at least 10 µg of patulin/ml of expressed juice from decayed apples. This amount may be sufficient to be cytotoxic to apple cells.

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