Effects of Patulin and Method of Application on Growth Stages of Wheat

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When a single, 100-μg/ml application of patulin, produced by Penicillium urticae Bainier, was applied to growth stages 7, 9, 10, and 10.1 (Feekes scale) of Lee spring wheat (Triticum aestivum L.), decreases in internodal elongation, floret number, seed weight, and seed number were observed. Yields were reduced according to the proximity of application prior to heading. Application of patulin to the soil in crystalline form and dissolved in aqueous solution were also investigated, and the solution method of application was found to be the treatment of choice. A single exposure of growing wheat plants to patulin can produce yield reductions similar to those observed in stubble-mulch farming.

Penicillium urticae Bainier, a fungus which produces patulin, has been found in large numbers in the soil in the Great Plains area. In some cases, the P. urticae population comprises 90% of the total fungal population in stubble-mulch wheat farming (J. R. Ellis and T. M. McCalla, Unpublished data). Wheat roots have been found to stimulate the growth of P. urticae over other fungi species (6). Other soil fungi have been reported to produce patulin; however, primarily, P. urticae has been found where plant residues and mulches have been left near the soil surface (11, 12, 14).

The toxicity of patulin has been demonstrated on animals, plants, fungi, and bacteria. In the early 1930's, patulin was used as a broad-spectrum antibiotic but was found to be toxic to humans; consequently, its use was discontinued. In recent years, interest has been centered on its toxicity to plants. It was implicated in problems concerned with apple seedling transplants (3, 4). The toxic effect of patulin has been observed on young seedlings, germinating seed, isolated plant tissue, and plants which had continuous applications of patulin until maturity. The effect of patulin on root development, cell division, cell wall development, and enzyme inhibition has also been reported (10, 15).

The effect of a single patulin application on wheat plants grown to maturity or the effect of the method of its application has not been demonstrated previously. In the experiments reported here, patulin was applied at specific growth stages to simulate the effect of short-duration P. urticae Bainier blooms and subsequent patulin production on wheat plants. Two application methods were used to compare methods being used in research investigations.

MATERIALS AND METHODS

Patulin used in experiment. The patulin used in this experiment was produced and purified in the laboratory from cultures of P. urticae Bainier isolated from stubble-mulched plots (13). Purity of the antibiotic was verified by using thin-layer chromatography, melting point, and infrared spectroscopy.

Soil type. Holdrege silt loam from North Platte, Nebraska, was used in the pot experiment. Water-holding capacity of the soil at one-third bar is 24%. Physical and chemical characteristics of this soil have been described by Norstadt and McCalla (12).

Experimental procedure. Thirty-five hundred grams of oven-dried soil was placed in 3.3-liter plastic pots with a layer of 100-mesh nylon cloth covering the drain holes. Optimum fertilizer for wheat production was determined by soil tests and appropriate compounds were mixed with the soil. The following rates of the elements N, P, Mn, Zn, and Fe, respectively, expressed as millimoles per kilogram of soil, were added to the soil: 3.57, 0.352, 0.046, 0.061, and 0.082.

Lee spring wheat (Triticum aestivum L.) was pregerminated for 3 days, and 7 seedlings were placed in each pot at 2.0-cm depth within a 10-cm circle. Pots were watered to moisture-holding capacity twice weekly with distilled water. Patulin was applied to the soil to give a concentration of 100 μg/ml (oven-dry basis) (650 μmol/kg). The treatments were: control,
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Feekes stages 7, 9, and 10 (8) with one aqueous solution application; and Feekes stages 7, 9, 10, and 10.1 with one crystalline application. Three replicates per treatment were used. The solution treatment was applied with a hypodermic syringe and needle in four places around each plant 3.8 cm apart, 2.54 cm from the plant, and 2.54 cm deep. The crystalline application was applied in 0.8-cm holes bored 2.54 cm deep in four equally spaced locations, 3.8 cm apart and 2.54 cm from each plant. After patulin application, the pots were brought to field capacity with distilled water.

Pots were placed in an ISCO E-2 growth chamber in three randomized blocks, with one replicate of each treatment in a block. Pots were moved each day so that each treatment was rotated in the block every 8 days.

Temperature cycles in the growth chamber were based on Agronomy Farm (Lincoln, Nebraska) averages of bare and bromegrass-covered soil at a depth of 10.16 cm, from April through July 1966 (17). The plants were harvested at maturity (113 days) and observations were made on plant height, internodal elongation, straw weight, chaff weight, grain weight, floret number, and kernel number. The data were analyzed statistically.

RESULTS

Internodal elongation was markedly affected by the time of patulin application (Table 2; Fig. 1). When patulin was added in solution to the wheat plants at stage 7 Feekes scale (Table 1), the internodal distance was shortened (not statistically significant) between the second and third node, but normal growth occurred thereafter. A decrease was noted in total seed yield (Table 3), indicating a residual effect. In contrast, the crystalline patulin application to stage 7 reduced the growth of the third and fourth internodes (not statistically significant) but not the second internode length. A significant reduction in floret number was observed.

Solution application to stage 9 wheat plants did not affect second internode growth, but a reduction was noted in the third and fourth internodes and the stem between the last node and head (Table 2). Stage 9 treatment significantly reduced seed number and total yield. Crystalline treatment reduced fourth to fifth internode growth but not the third to fourth internode or the fifth node to head and also significantly reduced the seed yield.

Stage 10 application of solution induced the greatest reduction in seed weight, number, total yield, floret number, and chaff weight. The grain yield (Table 3) was reduced to 0.1 of the control and 0.2 of any of the crystalline applications in this experiment. Stem elongation of the fourth internode and of the upper stem was significantly reduced. The crystalline application to the same stage significantly reduced seed weight, total yield and fourth internode elongation, and elongation between fifth node and head.

Figure 1. Effect of one patulin treatment in two forms on stem elongation and plant height. A bar presents the total plant for the indicated treatment. Shaded areas indicate the internodal elongations discussed in text and Table 2. Numbers on the control bar (a) indicate node location.
Since stage 10.1 application treatment is made after stem elongation, the crystalline application had no effect on this process. However, the greatest reduction of seed weight and total yield was exhibited by this crystalline application.

DISCUSSION

The patulin solution application method affected the particular plant growth phase at the time of application, and inhibition was observable for several phases of plant elongation. A residual effect was noted as final wheat yield was reduced with solution treatment applications.

Yield reductions increased as patulin in aqueous solution was applied nearer to heading and seed production stages. There was a steady decrease in the seed number, seed weight, floret number, chaff weight, and straw weight. In contrast, the crystalline application was usually not effective on the particular growth stage to which it was applied. The treatment effects were not as great when the crystal treatments were used.

The comparison of solution and crystalline applications of patulin showed that patulin must be in solution to produce an effect on the immediate growth phase treated. The crystalline application reduced stem elongation, but the effect, compared to solution application, was delayed. The crystalline form did not reduce the seed weight and number as much as the solution application. The crystalline application had much larger least significant difference (LSD) values, which indicated the greater variability of this type of treatment.

The decreased effect of the crystalline treatment could be explained by two factors. The crystals do not dissolve rapidly, thus lowering the patulin concentration in the soil solution. In field treatments, Norstad, Ellis, and McCalla (Unpublished data) found crystalline patulin in the soil for several weeks after

| Stage     | Description                                                                 |
|-----------|------------------------------------------------------------------------------|
| 6         | First node of stem visible at base of shoot                                  |
| 7         | Second node of stem formed; next-to-last leaf just visible                   |
| 8         | Last leaf visible but still rolled up; ear beginning to swell               |
| 9         | Lingule of last leaf just visible                                            |
| 10        | Sheath of last leaf completely grown out; ear swollen but not yet visible   |
| 10.1      | First ears just visible (awns just showing in barley; ear escaping through split of sheath in wheat or oats) |

*See reference 8.

| Treatment stage (Feekes scale) | Internodal length (mm) | Straw wt (g avg/plant) |
|-------------------------------|------------------------|------------------------|
|                               | 2nd to 3rd node        | 3rd to 4th node        | 4th to 5th node | 5th node to head |                 |
| Solution control               |                        |                        |                |                |                 |
| Control                        | 50                     | 69                     | 90             | 143**          | 1.78            |
| 7                              | 41                     | 61*                    | 40**           | 22             | 1.40            |
| 9                              | 59                     | 66                     | 37**           | 122**          | 1.79            |
| 10                             | 12                     | 10                     | 37             | 22             | 1.78            |
| LSD (P = 0.05)*                |                        |                        |                |                |                 |
| 17                             | 14                     | 14                     | 52             | 31             | 0.49            |
| Crystal treatment              |                        |                        |                |                |                 |
| Control                        | 50                     | 65                     | 75             | 177            | 0.55            |
| 7                              | 49                     | 66**                   | 52**           | 157            | 0.80            |
| 9                              | 44                     | 68                     | 76*            | 116**          | 1.79            |
| 10.1                           | 36                     | 70                     | 86             | 176            | 1.79            |
| LSD (P = 0.05)                 |                        |                        |                |                |                 |
| 13                             | 10                     | 13                     | 55             | 55             | 0.55            |
| LSD (P = 0.01)                 |                        |                        |                |                |                 |
| 18                             | 14                     | 18                     | 76             | 76             | 0.55            |

* LSD, Least significant difference at 5 and 1% probability. One asterisk indicates significance at the 5% level; two asterisks indicates significance at the 1% level.
application. Patulin has a half-life in the soil of 24 h (11), and this would also decrease the soil solution concentration.

Patulin affects cell division (1, 16), which would cause the reduction in stem elongation noted in this experiment. Cereal crops which suffer damage during these rapidly growing phases often show reduced yields.

The action of patulin may take place in several ways. However, a possible mechanism has been shown. Dickens and Jones (5) found that patulin can inactivate S-H groups. They showed that a 1:1 relationship existed between the number of S-H groups blocked and the number of patulin molecules. This reaction would interfere with enzyme activity and protein synthesis (1, 2, 7), thus explaining the effect of patulin on internode length. The blocking action of important enzymes and interference with cell division processes can reduce plant vigor and yield.

This study showed that patulin does have a marked effect on plant development even when plants had only one exposure to patulin. A single exposure to patulin can produce the toxicity problems noted in stubble-mulch farming (9, 11) by reducing plant vigor and final grain yield.

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