Inhibition of ^{3}H-Leucine Incorporation by Trichothecene Mycotoxins in Maize and Wheat Tissue

W. L. Casale and L. P. Hart

Graduate research assistant and associate professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

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ABSTRACT

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The trichothecenes, deoxynivalenol (DON, vomitoxin) and T-2 toxin, inhibited ^{3}H-leucine incorporation into acetone:ethanol insoluble material by maize and wheat tissue (leaf disks and kernel sections). These compounds are known to inhibit protein synthesis in animals and yeast. The toxin concentrations that gave ID_{50} (50% reduction) for ^{3}H-leucine incorporation by several maize varieties were 0.9 μM T-2 toxin and 9–22 μM DON. ID_{50} values for wheat were 0.26 μM T-2 toxin and 4.5 μM DON. T-2 toxin gave near-maximum inhibition in leaf tissue within 5 min after exposure to the toxin. T-2 toxin or its effects on ^{3}H-leucine incorporation persisted at least 120 min after removal of leaf disks from toxin solutions. Sensitivity to DON was not correlated with susceptibility to ear rot by a DON-producing strain of *Gibberella zeae* (anamorph = *Fusarium graminearum*) for six maize lines with a range of disease reactions from highly susceptible to highly resistant. However, the ID_{50} for one moderately resistant line (A509) was 2.3 times greater than the ID_{50} of the most susceptible line (B79). ^{3}H-Leucine incorporation by wheat and maize was inhibited by DON and T-2 toxin at concentrations occurring in naturally infected tissue, suggesting the need for further evaluation of these compounds as plant disease determinants.

The 12,13-epoxytrichothecene mycotoxins belong to a group of related esters of sesquiterpene alcohols possessing the tricyclic trichothecane structure (4). These compounds are elaborated by plant-parasitic and saprophytic fungi in the genera *Fusarium*, *Myrothecium*, *Trichoderma*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys* (15), and their toxicity to animals has been reviewed (18,23).

Although several trichothecene-producing fungi are plant pathogens, there have been no reports of the effects of trichothecene mycotoxins on specific biochemical mechanisms in plants. Some studies on the phytotoxicity of these compounds have been concerned with gross effects such as wilting, chlorosis, necrosis, or inhibited growth of whole plants or plant organs (2). Trichothecenes were reported to inhibit cell division (13) and to reduce growth regulator-enhanced expansion of plant tissue, apparently by inhibiting the action of indoleacetic acid (2,5,7,21).

Trichothecenes are potent inhibitors of protein synthesis in animals and fungi (16,23); they block peptidyl transferase activity in yeast (1,3) and have been divided into two groups based on whether they inhibit protein synthesis at initiation (I-types, e.g., nivalenol, T-2 toxin, and verrucarin A) or elongation/termination (E- or T-types, e.g., deoxynivalenol [DON], trichodermin, and trichothecin) (6). Some of the differences between these types may, however, be concentration dependent (6). Modifications at specific positions in the trichothecane molecule appear to be responsible for determining whether trichothecenes are I-, E-, or T-types, as well as for their potency (6,16,22). One trichothecene, trichodermin, binds to a single site on the 60 S ribosomal subunit of *Saccharomyces cerevisiae* (1,8,11,20).

This study was part of a project to determine whether or not DON and T-2 toxin will inhibit protein synthesis in wheat and maize, hosts for trichothecene-producing strains of *Gibberella zeae* (anamorph = *Fusarium graminearum*) and *F. sporotrichioides*, which cause wheat head scab and corn ear rot (15). Demonstration of a specific mechanism of phytotoxicity may suggest a role for trichothecenes in plant disease.

MATERIALS AND METHODS

Chemicals. L-[3,4,5-^{3}H(N)] leucine (sp. act. 147 Ci/mmol) and Protosol from New England Nuclear (Boston, MA), 1,4-bis [2-(4-methyl-5-phenyloxazolyl) benzene (= dimethyl-POPOP) and 2,5.diphenyloxazole (= PPO) from Research Products International Corp. (Elk Grove Village, IL); cycloheximide, streptomycin sulfate, and T-2 toxin from Sigma Chemical Co. (St. Louis, MO); and chloramphenicol from United States Biochemical Corp. (Cleveland, OH). DON was kindly supplied by Mary Witt, Department of Food Science and Human Nutrition, Michigan State University.

Plant material. The experiments used inbred maize lines, Pa347, MS74, A509, B73, Mo17, and B79 (most resistant to most susceptible to Gibberella ear rot as indicated by the abscissa in Fig. 1) (10), and the inbred wheat line, Ionia (susceptible to Gibberella head scab).

Plants used for leaf disk assays were grown in the greenhouse at 26 ± 4 C. Disks (5 mm diameter) were cut from the third expanded leaf of 3-wk-old plants with a sharp cork borer. Maize plants used for kernel section assays were grown in the field, and the ears harvested at the early dough stage and used immediately. Longitudinal sections (2 mm thick) were cut from kernels with a razor blade so that each contained a portion of the embryo. As they were cut, leaf disks or kernel sections were floated on 20 mM Tris-Cl buffer (pH 6.5) until all tissue for an experiment was prepared; they were then used immediately in the assays described below.

Incorporation of ^{3}H-leucine by leaf disks and kernel sections. The following assay was used to monitor incorporation of ^{3}H-leucine into acetone:ethanol-insoluble material by plant tissues. Leaf disks or kernel sections were rinsed briefly in 20 mM Tris-Cl buffer (pH 6.5) containing 1% Tween 20; they were then blotted dry and weighed, and 10 leaf disks, or five kernel sections, were placed in each 20-ml scintillation vial containing 950 μl of the Tris buffer (without Tween 20). Preliminary experiments showed a significant contribution to incorporated radioactivity by contaminating bacteria and leaf organelles; therefore, chloramphenicol (an inhibitor of protein synthesis in prokaryotes) was included at 100 μg/ml in the incubation buffer. After all samples were prepared, 50

μl of the Tris buffer containing 1×10^6 DPM ^{3}H-leucine was added to each vial and the tissue incubated at room temperature (22±2 C) for the appropriate time. Amino acid incorporation was stopped by placing the vials in an ice bath, immediately drawing off the liquid and rinsing the disks with 5 ml of the ice-cold Tris buffer. The rinse buffer was discarded, and 10 ml of ice-cold acetone:ethanol:water (100:95:5), elsewhere referred to as acetone:ethanol, was added to each vial that was then stored overnight at 4 C. The solvent was discarded and the tissue washed twice, 3 hr at 4 C each time, with 5 ml of fresh acetone:ethanol. Residual water was removed from the samples by placing them in 3 ml of diethyl ether:ethanol (1:1) for 10 min, then in 3 ml of diethyl ether for 10 min, at room temperature. After the solvent was drawn off and the tissue allowed to air-dry for approximately 15 min in open vials, 0.5 ml of Protosol:toluene:water (45:50:5) was added and the sealed vials incubated at 55 C for 90 min. After the vials had cooled, 5 ml of scintillation cocktail (11 g of PPO, 0.6 g of dimethyl-POPOP, 1 L of toluene, 1 L of ethylene glycol monomethyl ether [methyl cellusolve]) was added to each vial, and radioactivity measured with a Beta Trac 6895 liquid scintillation counter (Tm Analytic, Elk Grove Village, IL). Each treatment was replicated three times, and the experiments were repeated at least once. This assay was a modification of a procedure by Gardner et al (9).

Evaluating the leaf disk assay. A preliminary experiment demonstrated the sensitivity of the leaf disk assay to alterations in net protein synthesis. Twenty vials containing 10 leaf disks each were incubated in 1 ml of 20 mM Tris-Cl buffer (pH 6.5) containing 1×10^6 of DPM ^{3}H-leucine. At 30-min intervals, incorporation of radioisotope-labeled amino acid was stopped in two replicate vials and measured as described above. Incorporation by equivalent disks incubated in buffer containing cycloheximide (60 μg/ml) was determined at 60-min intervals. Differences in insoluble radioactivity between cycloheximide-treated and control disks represent incorporation of ^{3}H-leucine into protein, while residual radioactivity in the cycloheximide-treated disks is probably due to aminoacyl tRNA and other nonprotein sources of ^{3}H-leucine in the acetone:ethanol insoluble fraction. Other experiments reported here included cycloheximide-treated controls, and values for these controls were subtracted from the values of the other treatments to correct for these nonprotein sources of insoluble radioactivity.

The use of Protosol to solubilize the amino acids to avoid quenching was evaluated, as was the contribution of amino acyl-tRNA to insoluble radioactivity by hydrolysis with 1.0 N NaOH.

Inhibition of ^{3}H-leucine incorporation by trichothecenes. The sensitivity of plant protein synthesis to inhibition by trichothecenes was tested. Leaf disks or kernel sections were incubated in 950 μl of buffer containing T-2 toxin or DON for 60 min before addition of ^{3}H-leucine (0.5 μCi in 50 μl of buffer). T-2 toxin, which is less hydrophilic than DON, was first dissolved in methanol and then diluted so that the final buffer solution contained 0.1% methanol (controls without toxin also contained 0.1% methanol). After a 1–3-hr radiolabeling period, disks were washed and measured for incorporated radioactivity, as described above.

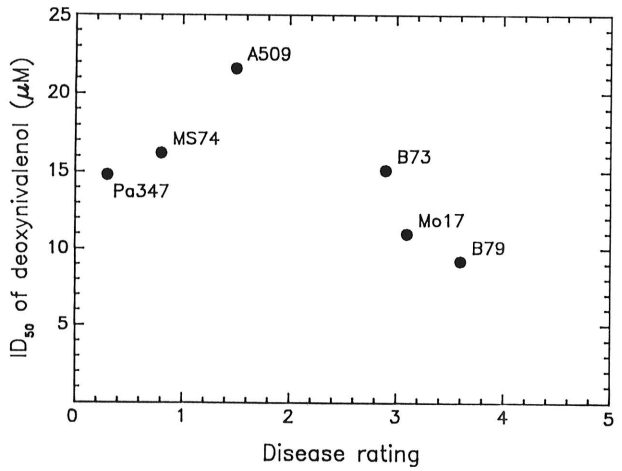

Fig. 1. Sensitivity of maize inbred lines to deoxynivalenol (ID_{50}) versus susceptibility to ear rot caused by *Gibberella zeae* (anamorph *Fusarium graminearum*). Disease ratings are on a scale of increasing severity from 0 (no disease) to 5 (76–100% of the kernels rotted) based on 2–3 yr average of field inoculations (10). ID_{50} is the concentration of deoxynivalenol inhibiting 50% of ^{3}H-leucine based on regression analysis of data shown in Figure 4.

Because epiphytic yeasts might have contributed to the observed levels of protein synthesis in the presence of chloramphenicol, we compared the incorporation of ^{3}H-leucine by surface sterilized and untreated leaf disks. Whole leaves were placed in 10% commercial bleach in buffer, or in buffer alone, for 5 min. The leaves were rinsed in fresh sterile buffer. Sixty representative disks (5 mm diameter) for each treatment were cut from the leaves (half of the disks intact and half ground in buffer), then placed on potato-dextrose agar containing streptomycin (100 μg/ml) and incubated at room temperature. Other similarly treated disks were assayed for ^{3}H-leucine incorporation either in the presence or absence of T-2 toxin (10 μg/ml) as described in the preceding paragraph.

Because virulent strains of *G. zeae* produce DON, the correlation between susceptibility to the fungus and sensitivity to trichothecenes was examined. The sensitivity of the most susceptible (B79) and most resistant (Pa347) maize lines to T-2 toxin was tested. Six maize varieties representing the range of disease reactions (10) were screened for sensitivity to DON. Because there were no available wheat lines resistant to *G. zeae* infection, only a single variety of wheat was tested for sensitivity to T-2 and DON.

Dynamics of toxin movement into maize leaf tissue. Because we found it to be of greater toxicity than DON in this system, T-2 toxin was used as a model to study the movement of trichothecenes into tissue and cells and the persistence of the toxins in plant tissue.

In an experiment designed to determine how quickly T-2 toxin reached inhibitory levels in tissue exposed to the toxin, 10 maize leaf disks per vial were incubated in 1 ml of buffer containing T-2 toxin (10 μg/ml). At 10-min intervals from 0 to 60 min, the toxin solution in sample vials (three replicates) was removed, and the leaf disks were rinsed with 5 ml of Tris buffer. Another 5 ml of fresh buffer was added and after 10 min was removed. The leaf disks were rinsed with 5 ml of buffer solution and incubated in 1 ml of buffer containing ^{3}H-leucine for 120 min. The radioisotope-labeled leaf disks were then washed, and radioactivity was determined as before.

To determine whether or not the toxin or its inhibitory effects persisted after removal of tissue from toxin solutions, the following two experiments were performed. First, 10 leaf disks per vial were incubated in 1 ml of buffer, or buffer containing T-2 toxin (10 μg/ml) for 60 min. The leaf disks were then rinsed two times, each time with 5 ml of fresh buffer. Then 1 ml of buffer containing 0.5 μCi ^{3}H-leucine, with or without T-2 toxin (10 μg/ml), was added to each vial. Incorporation of ^{3}H-leucine by leaf disks in sample vials (three replicates) was stopped at 30-min intervals between 0 and 120 min. Disks were washed and radioactivity measured as before. The second experiment differed from the first one in that after the first 60 min of incubation, leaf disks were incubated in fresh buffer solution to allow T-2 toxin (bound or unbound) to diffuse out of the leaf disks before radioisotope-labeling. Leaf disks were exposed to T-2 toxin (10 μg/ml) for 60 min, and disks washed two times with 5 ml of buffer as in the previous experiment. Control leaf disks were treated similarly, but incubated in buffer without T-2 toxin. Each group of 10 disks was then floated on 50 ml of buffer. At 30-min intervals from 0 to 120 min, sample groups (three replicates of 10 disks each) were rinsed twice with 5 ml of buffer, and transferred to 1 ml of buffer containing 0.5 ml ^{3}H-leucine. Incorporation of ^{3}H-leucine was stopped after 120 min. Leaf disks were washed and radioactivity measured as described.

RESULTS

Incorporation of ^{3}H-leucine by leaf disks. There was significant

quenching of radioactivity by leaf tissue. Radioisotope-labeled leaf disks in toluene-based scintillation cocktail had 3.2 times the apparent DPM for disks solubilized with Protosol (1491 DPM/mg fr wt) as for equivalent disks without Protosol treatment. Solubilization of tissue (leaf disks and kernel sections) with Protosol was, therefore, routinely included in the preparation of leaf disks to increase the efficiency of counting. The reduction in radioactivity of leaf disks by hydrolysis of aminoacyl-tRNA with 1.0 N NaOH was not statistically significant. In addition, leaf disks treated with NaOH retained chlorophyll after washing, which added false counts due to chemiluminescence. Although we could correct for false counts by subtracting counts of prepared control disks not exposed to ^{3}H-leucine, we decided not to treat leaf disks with NaOH as part of the preparation for counting. Incorporation of ^{3}H-leucine into acetone:ethanol insoluble material by leaf disks was linear for at least 5 hr (Fig. 2). Cycloheximide (60 μg/ml) reduced by 80% the amount of ^{3}H-leucine incorporated, demonstrating that a major portion of the incorporation was due to plant protein synthesis. Subtraction of values for cycloheximide-treated controls corrected for nonprotein sources of ^{3}H-leucine incorporation in other experiments.

Inhibition of ^{3}H-leucine incorporation by trichothecenes. Protein synthesis in leaf disks was inhibited by both trichothecenes tested. There was no significant difference in ^{3}H-leucine incorporation between surface-sterilized and non-surface-sterilized leaf disks, whereas T-2 toxin inhibited incorporation in both treatments (Table 1). In addition, no microorganisms grew from either surface-sterilized or non-surface-sterilized leaf disks incubated for 10 days on potato-dextrose agar amended with streptomycin. These data indicate that epiphytic yeasts did not contribute to the observed protein synthesis by leaf disks.

The concentration of T-2 toxin required to inhibit 50% of ^{3}H-leucine incorporation (ID_{50}) was 261, 919, and 919 nM (1 nM = 467 pg/ml) for wheat (cultivar Ionia), Pa347 maize, and B79 maize, respectively (Fig. 3). ID_{50} values were determined from linear regressions from 2.1 nM to 21 μM T-2 toxin; the r^2-values for the signicant linear regressions were: 0.880 (Ionia), 0.919 (Pa347), and 0.952 (B79). There was no significant difference between B79 maize leaf disks and kernel sections in sensitivity of protein synthesis to T-2 toxin (Table 2).

DON was less effective than T-2 toxin at inhibiting protein synthesis. ID_{50} of DON was 9.2, 11.0, 15.1, 21.6, 10.3, and 14.8 μM (1 μM = 296 ng/ml) for maize lines B79, Mo17, B73, A509, MS74, and Pa347, respectively (Fig. 4). The only significantly different ID_{50} at $P = 0.05$ were for B79 and A509. ID_{50} values were determined from linear regressions using DON concentrations from 1 to 34 μM DON; the r^2 values for the significant linear regressions were: 0.946 (B79), 0.994 (Mo17), 0.990 (B73), 0.946 (A509), 0.994 (MS74), and 0.983 (Pa347). Susceptibility to *G. zeae* ear rot was not correlated with sensitivity to DON for all maize lines, but A509, a moderately resistant variety, was less sensitive to DON than were the other varieties (Fig. 1). ID_{50} of DON for Ionia wheat leaf disks was 4.5 μM ($r^2 = 0.931$).

Dynamics of toxin movement into maize tissue and cells. T-2 toxin entered maize leaf tissue quickly. Near-maximum inhibition of ^{3}H-leucine incorporation was achieved after a 5-min exposure to T-2 toxin (Fig. 5).

The toxin or toxic effects persisted in maize leaf tissue after leaf disks were removed from toxin solutions. Inhibition of ^{3}H-leucine incorporation in leaf disks was the same for disks pretreated in T-2 toxin for 60 min, and then radioisotope-labeled in the presence or absence of T-2 toxin for up to 120 min (Fig. 6). Even when toxin-treated leaf disks were incubated in fresh buffer to remove toxin before radioisotope-labeling, ^{3}H-leucine incorporation increased only 22% when leaf disks were removed from toxin solution for 60 min; there was no further increase for recovery periods up to 120 min (Fig. 7).

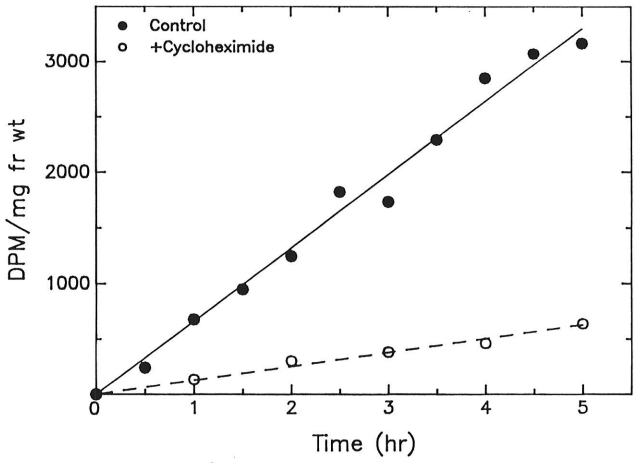

Fig. 2. Incorporation of ^{3}H-leucine into acetone:ethanol insoluble material by maize leaf disks (approximately 20 mg fr wt tissue/replicate). Leaf disks (5 mm) were floated on buffer containing ^{3}H-leucine (+ chloramphenicol, 100 μg/ml), with or without cycloheximide (60 μg/ml), for the appropriate time, then washed with cold acetone:ethanol (1:1), then diethyl ether, and finally treated with Protosol before measuring radioactivity remaining in the tissue.

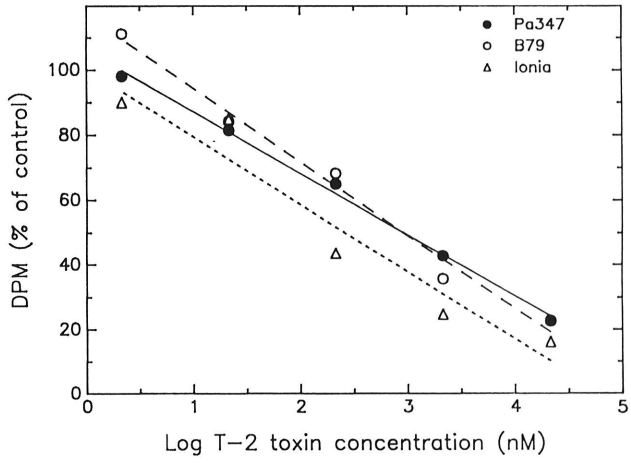

Fig. 3. Sensitivity of two maize inbreds, Pa347 (——) and B79 (- - - -), and Ionia wheat (·····) to T-2 toxin (1 nM T-2 toxin = 467 pg/ml), as determined by ^{3}H-leucine incorporation into acetone:ethanol insoluble material by leaf disks for 120 min (see Fig. 1). Control DPM/mg fr wt: wheat, 3918; Pa347, 810; B79, 967.

TABLE 1. Incorporation of ^{3}H-leucine by surface-sterilized (10% commercial bleach for 5 min) and nonsterilized B79 maize leaf disks (3 replicates × 10 disks/replicate)

	^{3}H-leucine incorporation (DPM/mg fr wt)[z]	
Treatment	Buffer	T-2 toxin (10 μg/ml)
Surface-sterilized	312 a	91 b
Nonsterilized	339 a	92 b

[z]Means with a common letter are not significantly different, by Tukey's procedure ($w_{0.01}$ = 48.8).

TABLE 2. Inhibition of protein synthesis in B79 maize leaf disks and kernel sections by T-2 toxin and cycloheximide

	DPM (% of control)[z]		
Tissue	T-2 toxin (2.1 μM)	T-2 toxin (21 μM)	Cycloheximide (178 μM)
Leaf disks	35.6 a	22.4 bc	16.2 c
Kernel sections	28.0 ab	25.1 abc	20.4 bc

[z]Means with a common letter are not significantly different by Tukey's procedure ($w_{0.05}$ = 11.26). Control DPM/mg fr wt (approximately 20 mg fr wt per replicate): Kernels = 106; leaf disks = 3460.

DISCUSSION

A number of low molecular weight compounds that are toxic to plants have been isolated from cultures of *Fusarium* spp.: naphthazarins (fusarubin, marticin, isomarticin, javanicin, norjavanicin, and novarubin) from *F. solani*, fusaric acid and lycomarasmin from *F. oxysporum*, enniatins from several *Fusarium* spp. (12,19), and trichothecenes. However, none of these phytotoxic compounds have yet been demonstrated to be involved in disease development (19). Neither have previous studies shown trichothecenes to be determinants of plant disease (2,14). However, because of their high toxicity in eukaryotic systems studied, we believe the role of trichothecenes in plant disease requires further evaluation. The experiments presented here demonstrated that trichothecenes produced by plant pathogens, specifically T-2 toxin and DON, are potent inhibitors of amino acid incorporation by tissue from hosts of the toxigenic fungi.

Concentrations of DON that inhibited ^{3}H-leucine incorporation in wheat and maize are well below those that have been reported to occur in infected plants. Up to 580 μg of DON per gram of grain has been reported in infected maize kernels (17), although the toxin may build up after colonization and death of the plant cells. To our knowledge, there is no information on trichothecene production on leaves, although we have been able to infect maize leaves with *G. zeae* under conditions of high humidity (unpublished). There is also no detailed data on toxin concentrations in localized areas adjacent to and in advance of invading hyphae where toxin concentrations may be expected to be much higher than in the surrounding tissues. Additional information on toxin levels and toxic effects in host tissue adjacent to the toxigenic pathogen will be important in evaluating trichothecenes as disease determinants, since this tissue is where resistance or susceptibility could be expressed.

The concentrations of T-2 toxin and DON that inhibited amino acid incorporation in wheat and maize were comparable, or in some cases higher, than concentrations reported for protein synthesis inhibition in other systems. ID_{50} of T-2 toxin for wheat and maize leaf disks were 122 and 429 ng/ml, respectively, whereas the ID_{40} of T-2 toxin for intact human HeLa cells was 100 ng/ml (6). Intact rat spleen lymphocytes and Vero cells (ID_{50} of T-2 toxin equaled 3.0 and 6.7 ng/ml, respectively) were more sensitive than other cell types to trichothecenes (22). The ID_{50} of DON for wheat

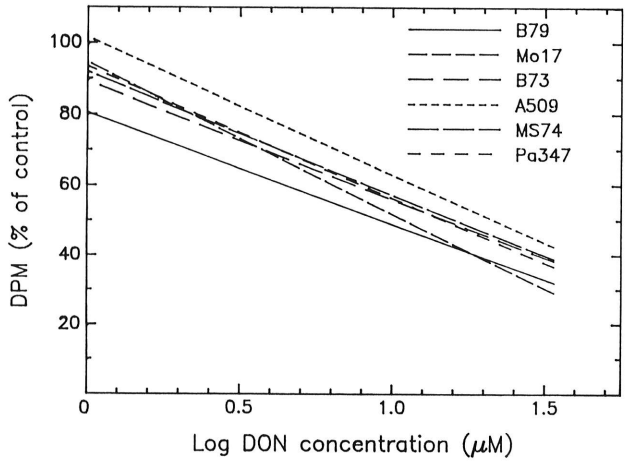

Fig. 4. Sensitivity of six maize inbred lines to deoxynivalenol (DON), as determined by ^{3}H-leucine incorporation into acetone:ethanol insoluble material by leaf disks (see Fig. 2) for 180 min (1 μM deoxynivalenol = 296 ng/ml). Control DPM/mg fr wt: B79, 2253; Mo17, 1598; B73, 2174; A509, 2163; MS74, 2137; Pa347, 2033.

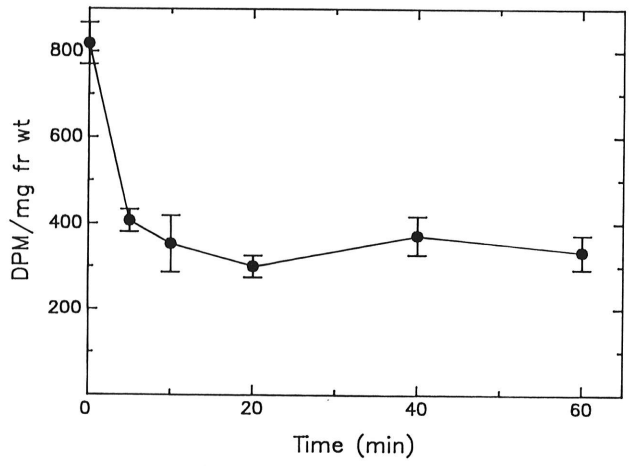

Fig. 5. Incorporation of ^{3}H-leucine into acetone:ethanol insoluble material by maize (Pa347) leaf disks (see Fig. 2) as a function of the duration of exposure to T-2 toxin (10 μg/ml) before incubation for 120 min in fresh buffer (no T-2 toxin) containing ^{3}H-leucine. Vertical bars represent 2 standard deviations.

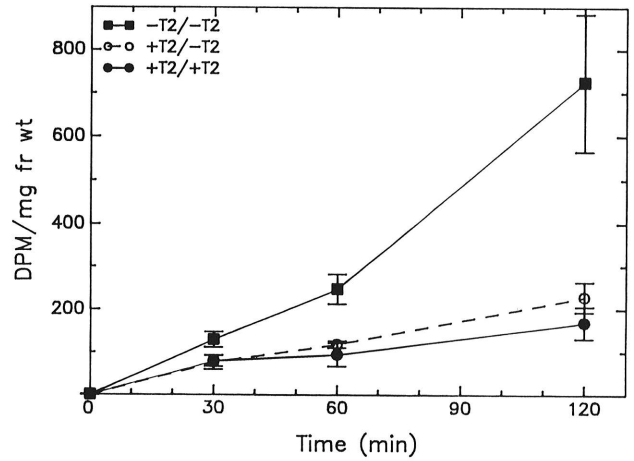

Fig. 6. Incorporation of ^{3}H-leucine into acetone:ethanol insoluble material by maize (Pa347) leaf disks (see Fig. 2) after exposure to T-2 toxin, as a function of radiolabeling period. Leaf disks were floated for 60 min on buffer containing T-2 toxin (10 μg/ml); these disks were then transferred to buffer containing ^{3}H-leucine plus T-2 toxin (+T2/+T2), or minus T-2 toxin (+T2/−T2). Control disks were treated in buffer only (−T2/−T2). Vertical bars represent 2 standard deviations.

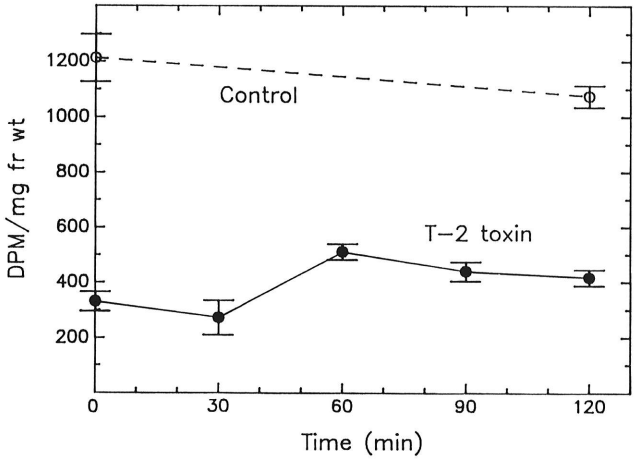

Fig. 7. Incorporation of ^{3}H-leucine into acetone:ethanol insoluble material by maize (Pa347) leaf disks (see Fig. 2) as a function of recovery period subsequent to exposure to T-2 toxin. Leaf disks were floated on buffer containing T-2 toxin (10 μg/ml) for 60 min. Before radioisotope-labeling, the disks were transferred to fresh buffer (no T-2 toxin) and incubated for various times from 0 to 120 min. Finally the disks were transferred to fresh buffer containing ^{3}H-leucine and incubated for 120 min. Control leaf disks were treated similarly, but without exposure to T-2 toxin. Vertical bars represent 2 standard deviations.

leaf disks was 1.3 μg/ml, which is again higher than the ID_{50} of DON for intact rat spleen lymphocytes and Vero cells at 252 and 444 ng/ml, respectively (22). Protein synthesis in Ionia wheat is inhibited by these two trichothecenes, although there are previous reports of the relative insensitivity of wheat to trichothecenes (2). However, these previous studies examined gross effects (chlorosis, necrosis, stunting) of trichothecenes, did not measure effects on protein synthesis, and used different wheat varieties.

If all maize inbreds are considered, there is no correlation between sensitivity to DON or T-2 toxin and susceptibility to a DON-producing *Fusarium* strain. However, this does not necessarily rule out a role for these compounds in disease resistance. Resistance in the most disease-resistant lines (Pa347 and MS74) may be independent of sensitivity to DON. Because the fungus grows very little, if at all, in these maize lines, the production of toxin may not become a factor in virulence. However, in less resistant inbreds (lacking the high-level resistance mechanism) DON may be a factor in disease development. If the highly resistant inbreds Pa347 and MS74 are ignored, there is apparently a good correlation between DON sensitivity and susceptibility to the fungus. However, since the only significantly different ID_{50} of DON were for B79 and A509, these may be the only two lines tested for which differences in susceptibility are based on differences in sensitivity to DON.

Uptake of T-2 toxin by leaf tissue occurred rapidly (reaching nearly maximum inhibitory levels within 5 min); whether active transport of toxin or simple diffusion into cells was involved is not apparent. The inhibition of amino acid incorporation continued in tissue removed from the toxin solutions for at least 120 min. The persistence of the inhibition could have resulted from the toxin binding tightly to the active site or from irreversible damage to the cell by the toxin.

Leaf tissue was used for these experiments because it is easily produced in the greenhouse and because it readily takes up radioisotope-labeled amino acids and incorporates them into protein. Although kernels are more important tissues for colonization of toxigenic *Fusarium* spp., it is more difficult to produce and maintain a supply of young kernels in the greenhouse. Also, kernels must be sectioned for uniform uptake of radioisotope-labeled amino acids, and this probably damages the tissue more than does cutting leaf disks. It was shown, however, that protein synthesis in maize kernels was inhibited at the same toxin levels as in leaf tissue. Also, because it is unlikely that leaf ribosomes differ from kernel ribosomes, leaf tissue was more suitable for these studies on inhibition of protein synthesis.

Although these experiments showed that T-2 toxin and DON inhibited ^{3}H-leucine incorporation by intact tissue, this in vivo approach is incapable of conclusively demonstrating a direct action on protein synthesis. Although inhibition of protein synthesis by trichothecenes has been well documented in other eukaryotes, it is possible that decreased incorporation of ^{3}H-leucine by leaf disks may have resulted from reduced uptake of the radioisotope-labeled amino acid, altered amino acid pools, or toxic effects on other cellular components. Results of studies using cell-free translations systems from corn and wheat are forthcoming.

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