Inhibition Radioimmunoassay for *Aspergillus repens* Compared with Other Indices of Fungal Growth in Stored Corn[1]

S. L. MARTIN,[2] J. TUITE,[2] and M. A. DIEKMAN[3]

ABSTRACT

Cereal Chem. 66(3):139-144

An inhibition radioimmunoassay (IRIA) was developed to measure the growth of *Aspergillus repens* in corn kernels. *A. repens* in kernel extracts can be measured over a range of 0.25–100 μg per determination, equivalent to 10–4,000 μg per gram of corn. High-quality seed corn adjusted to 17.0% moisture (wet basis) was stored at 26° C for 52 days after inoculation with 1×10^3 conidia of *A. repens* per gram. Quantitation of *A. repens* by IRIA was compared with eight other indices of fungal proliferation and seed deterioration: internal seed infection, number of fungal propagules, blue eye, sporulation visible at 3× and 10× magnification, evolved CO_2, seed germination, and ergosterol content. Results of the IRIA were significantly correlated with all indices; the highest correlations were with ergosterol content and evolved CO_2 ($r = 0.96$ and 0.99, respectively).

Because fungi are important in the deterioration of stored corn (*Zea mays* L.), a rapid, sensitive and objective measurement of fungal biomass in corn kernels would be valuable in research and commerce. Fungal invasion of stored corn reduces quality and grade through loss of dry matter, discoloration, heating, caking, mustiness, and contamination by mycotoxins (Christensen and Sauer 1982). The microbiological indices of fungal invasion and seed deterioration which have been investigated for corn include visual inspection, seed infection, number of fungal propagules, evolved CO_2, decrease in seed germination and ergosterol content (Saul and Steele 1966; Perez et al 1982; Seitz et al 1982a,b; Cantone et al 1983; Fernandez et al 1985). Immunological techniques such as radioimmunological assay (RIA) and enzyme-linked immunosorbent assay (ELISA) may offer an alternative to these indices. ELISA techniques have detected *Plasmopara halstedii* in sunflower seeds (Liese et al 1982), *Acremonium coenphialum* (*Epichloe typhina*) in tall fescue seeds (Johnson et al 1982), and an endophyte in perennial ryegrass seeds (Funk et al 1983). ELISA and RIA have been developed for mycotoxins in grains and grain products (El-Nakib et al 1981; Lee and Chu 1981, 1984; Pestka et al 1981; Rousseau et al 1985; Fan and Chu 1984; Gendloff et al 1984; Liu et al 1985). However, neither RIA nor ELISA have measured fungal biomass in seeds.

In the present study, an inhibition radioimmunoassay (IRIA) was developed for *Aspergillus repens* De Bary. Measurements obtained from IRIA were compared to eight other indices of fungal development and seed deterioration for corn stored under controlled conditions after inoculation with *A. repens*. *A. repens* is a member of the *A. glaucus* group that, with various *Penicillium* and *Aspergillus* species, is important in the deterioration of low-moisture corn as measured by decrease in germination and increase in blue eye (visible sporulation on the germ). *A. repens* was chosen because isolates of this species generally produce abundant conidia, and it is commonly isolated from stored midwestern corn.

MATERIALS AND METHODS

Corn

Seed corn (hybrid 29097-1803, Funks Seed International, Bloomington, IL) was used. It did not contain material passing a 12/64 sieve. Germination was 96%. By visual inspection only 2.4% by weight of the kernels in four 100-g samples were physically damaged. Damage was 12.1 on the scale for the fast green method where 10 represents all sound kernels and 1,000 represents all kernels crushed or fragmented (Chowdhury and Buchele 1976).

Moisture Content

Moisture content (mc) was determined by the official whole seed method (USDA 1978) and is reported on a wet basis. The initial 11.1% mc was increased to 16.0% by spraying the corn with water and equilibrating at 4° C for four days. A water suspension of inoculum brought the corn to 17.0% mc. Moisture content was determined whenever the corn was sampled for fungal development.

Source and Enumeration of Fungi

Twelve fungal species were isolated from corn kernels. *Aspergillus repens* 2178, 2238, 2486; *A. ruber* 2072, 2239; *A. amstelodami* 2219, 2240; and *A. restrictus* 2217 were cultured on Czapek solution agar with 20% sucrose (CZXA; this and other media are fully described in Tuite 1969). *A. flavus* 2435, *Alternaria alternata* 715, *Cephalosporium acremonium* 2211, *Fusarium moniliforme* 747, *Gibberella zeae* 2002, *Nigrospora oryzae* 2215, *Penicillium cyclopium* 2313, and *P. oxalicum* 2204 were cultured on potato dextrose agar (PDA) with 100 ppm Tergitol NPX (Union Carbide, New York) and 30 ppm chlortetracycline, both added to molten agar before pouring. After growth covered the plates, the fungi were scraped or brushed from the agar and suspended in minimal volumes of phosphate buffered saline (PBS, pH 7.4) with 0.8% bovine serum albumin (BSA, Sigma, St. Louis, MO) and 0.4% Tween 20. Triplicate 100-μl samples of the suspensions were placed on membranes (Type GS, Millipore, Bedford, MA), dried at 103° C overnight, and weighed.

Inoculum

Conidia of *A. repens* from 11-day cultures on CZXA covered with water-permeable cellophane were suspended in deionized water with 0.005% Tween 80. Conidia were sprayed on the corn to give 1×10^3 conidia per gram. Corn was equilibrated at 4° C for six days before the storage test.

Storage Apparatus and Procedures

Apparatus for controlling storage conditions and measuring evolved CO_2 was modified from Fernandez et al (1985). Four 1.4-kg replicates of inoculated corn were incubated in 4 cm o.d. × 122 cm Plexiglass columns in a controlled temperature room set at 26° C. Air flow of 1.0 standard cubic foot per hour (500 ml/min) was measured by purge meters (Lab Supplies, Hicksville, NC) at the inlet and outlet and monitored daily with a Gilmont no. 12 meter. Carbon dioxide was removed from incoming air by passing it through KOH (30%, w/v) in a Fisher-Mulligan gas washing bottle. Molds were removed by bubbling the air through 1% $CuSO_4$. The air was then passed through a saturated KCl solution followed by a glycerol solution with a refractive index of 1.388 to give a relative humidity of 86%, in equilibrium with 17.0% mc corn.

[1]Journal paper no. 11,689 of the Purdue Agricultural Experiment Station.
[2]Former graduate assistant and professor, respectively, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.
[3]Associate professor, Department of Animal Science, Purdue University, West Lafayette, IN 47907.

Evolved CO_2

Air containing the evolved CO_2 was dried by 6-16 mesh indicating silica gel topped with Drierite (calcium sulfate with indicator, W. A. Hammond, Xenia, OH) and then by anhydrous magnesium perchlorate. Two U-tubes containing Ascarite II (8-20 mesh asbestos and NaOH particles with indicator (Thomas Co., Philadelphia, PA) absorbed the evolved CO_2. Magnesium perchlorate was layered on the Ascarite II to absorb water given off by the CO_2 absorption. The U-tubes were weighed daily, and the Ascarite and magnesium perchlorate were replaced when the gain exceeded 5 g per tube. The cumulative weight of absorbed CO_2 was divided by the dry weight of the stored corn to calculate total evolved CO_2 per gram of dry weight. CO_2 was converted to dry matter loss by using 14.7 mg CO_2 production for oxidation of 10 mg of carbohydrate (Steele et al 1969).

Visible Mold

Inspection of the corn in situ indicated that mold growth appeared uniform throughout the storage columns. After 10, 17, 24, 31, 38, 45, and 52 days of storage, all corn was transferred from each chamber to a plastic bag. The corn was mixed gently before sampling for fungal measurement and seed germination. At each sampling 50 kernels were examined for blue eye and sporulation visible at 3× and 10× magnification.

Biological Indices

Seed infection. Prior to inoculation, kernels were surface disinfected with 1% NaOCl (Clorox disinfectant bleach) in a 250-ml flask for 1 min, shaken dry, and placed on malt salt agar with 7.5% NaCl (MSA) and PDA. Fungi were identified after seven to 14 days at room temperature. Immediately after inoculation, four replicates of 100 kernels were plated on MSA and three on PDA. MSA was used exclusively for later samplings.

Number of fungal propagules. Kernels (20 g) were blended in 500 ml of sterile 0.1% agar with 0.1% peptone for 1 min in a Waring Blendor. Serial dilutions (5 ml in 45 ml) were made in the same solution, and triplicate 1-ml samples were cultured in seven media (MSA, PDA with 100 ppm Tergitol NPX and 9, 12.5, or 18% glycerol, PDA with 2% NaCl or 40% sucrose or both 18% glycerol and 30 ppm chlortetracycline). Dilutions of inoculated samples were added to cooled molten MSA. Colonies were identified and counted after 14 days.

Seed germination. Fifty surface-disinfected kernels were placed on filter paper moistened with sterile deionized water. Seed germination was determined after seven days.

Ergosterol Assay

Samples of corn (30 g) were ground for 30 sec in an electric food mill (Moulinex, Virginia Beach, VA). The nonsaponifiable lipids were extracted according to Seitz et al (1977) and redissolved in 1 ml of methylene chloride-isopropanol (99:1, v/v). Ergosterol was quantified by high-performance liquid chromatography (HPLC). The mobile phase was methylene chloride-isopropanol at 1.7 ml/min. The normal phase system consisted of a 600A solvent delivery system, a 440 absorbance detector, a U6K injector and a μ-Porasil column (Waters Associates, Milford, MA), and a Hewlett-Packard 3390A data analyzer.

Inhibition Solid-Phase Radioimmunoassay

Rabbit immunization. Antisera to *A. repens* 2178 were generated in four New Zealand white rabbits by intravenous injections of aqueous suspensions of 1×10^6 intact conidia per animal. Booster injections were given two, four, and seven weeks after the initial injection. Rabbits were bled from the ear vein immediately before the initial immunization and weekly thereafter. Antisera were compared by the IRIA procedure described below to determine the rabbit whose immune serum resulted in the highest level of counts per minute (cpm) bound to the microtiter wells. This rabbit received additional boosters at 17 and 22 weeks and was last bled at 24 weeks after the initial immunization.

Preparation of plates. The inhibition radioimmunoassay (IRIA) was derived from the inhibition enzyme-linked immunosorbent assay method of Voller et al (1980). Buffers and incubation times and conditions were adapted from the RIA of Reardon et al (1982).

Polyvinyl microtiter plates (Costar, Cambridge, MA) were sensitized with aqueous extract of *A. repens* 2178 from CZXA culture. Approximately 98% of the fungal biomass was conidial, and 2% was fragments of hyphae and cleistothecia. The fungus was disrupted for 2 min in PBS containing 0.01% Tween 20 and 1 m*M* phenylmethylsulfonylfluoride with 0.45 mm glass beads in a Braun ball mill cooled with liquid CO_2. Conidial disruption was verified microscopically. Four volumes of 0.05*M* carbonate-bicarbonate buffer (pH 9.6) were added to the suspension, which was stirred gently at 4° C overnight. The pH of extract was adjusted to 7.0 with 10% HCl, filtered through a sterile 0.2-μm pore membrane (Sybron/Nalge Co., Rochester, NY) and stored at −20° C. The extract was diluted with nine volumes of carbonate-bicarbonate buffer (pH 9.6) before use. Each well on the microtiter plate received 100 μl of the diluted extract equivalent to 500 μg of conidia. The plates were rinsed five times with glass-distilled deionized water. Each well was treated with 200 μl of 1% BSA in PBS for 60 min at 35° C to block unbound sites. The plates were stored at −20° C without emptying the wells, or emptied, rinsed, and used immediately.

Assay. Storage test samples and uninoculated kernels were ground for ergosterol extraction. Five grams of ground kernels from each sample was suspended in 15 ml of PBS, stirred for 5 min, and 360 μl of extract removed to a 3-ml tube. For the infected corn dose-response curve, the sample extracts were diluted with extract of uninoculated corn. PBS with 0.8% BSA and 0.4% Tween 20 was added to all extracts (1:6, v/v). Serial dilutions of fungi in the same volume (60 ml) of this second buffer were added to extract of uninfected corn to generate the standard curve for *A. repens* and other fungal dose-response curves.

Immune serum drawn 24 weeks after the initial immunization was used for all assays. Crude immune and preimmune antisera were incubated simultaneously with the diluted corn extracts with or without fungi (1:7, v/v) for 60 min in a shaker bath at 35° C. After incubation and resuspension, 100 μl from each tube was placed in each of four wells previously sensitized with *A. repens* extract, i.e., quadruplicate determinations were made for each sample, standard, and control. The plates were incubated for 60 min at 35° C. Contents of the wells were aspirated, and unbound antibodies were removed by rinsing the plates five times with PBS with 0.5% Tween 20.

The bound antibodies were reacted with Protein A (Pharmacia, Piscataway, NY), which had conjugated to ^{125}I (New England Nuclear, Boston, MA) by using Iodo-Gen (Pierce, Rockford, IL). The initial activity was approximately 10 μCi per microgram of protein. Approximately 1×10^5 cpm of radioiodinated Protein A in 100 μl of PBS with 0.1% BSA was added to each well. The plates were incubated as described above, rinsed five times with glass distilled water, and air-dried. The wells were counted individually on a Hewlett-Packard Auto Gamma scintillation spectrometer for 1 min.

Data transformation. Counts were averaged for the quadruplicate determinations with the omission of any determination that differed by more than 2.92 standard deviations from the other three. Nonspecific binding, which was determined by assaying preimmune serum, was subtracted from the cpm. Percentage of cpm bound in the presence of infected corn samples of fungus was calculated relative to the counts bound in the presence of the corn extract containing no fungus (zero standard). Percentage of cpm bound, P, was then converted by the logit function, $\ln(P/100 - P)$, which straightens sigmoidal curves (Rodbard and Munson 1980). Regression curves were calculated separately for each sampling date using Regression I (Dynacomp, Rochester NY). If the slopes agreed within 95% confidence levels, a single curve was fitted to the combined data. Correlation coefficients among the indices of fungal growth and seed deterioration were calculated using Regression I.

RESULTS

Standard Curve for *A. repens*

The standard curve for *A. repens* 2178 cultured on CZXA is shown in Figure 1. Concentrations of *A. repens* ranged from 0.1 to 100 μg fungal dry weight per determination. One microgram dry weight was the equivalent of approximately 1×10^4 conidia. Nonspecific binding averaged 1%.

Regression lines were fitted to the data from each of five sampling dates. Because the slopes of these lines were not significantly different, data were combined and a single line fitted to the 36 points (means of quadruplicate determinations for fungal dry weight of 0.25–100 μg per determination). The regression line was expressed as

$$Y = 0.30 - 1.37 \times X$$

where X was the log of the fungal dry weight in micrograms per determination, and Y was the logit transformation of the specific cpm bound. Correlation coefficient was 0.98. Sensitivity of the assay was 0.25 μg of fungus per determination, corresponding to 25% inhibition of binding specific antibodies (and thus radioiodinated Protein A) to the sensitized plate. The quantity of fungal biomass that inhibited 50% of binding was 1.66 μg.

Cross-Reactivities of Other Fungi

Similar curves were prepared for the other 14 fungal isolates. From each of these curves, the dry weight of fungus associated with 50% inhibition of binding was determined. The reactivities of the isolates relative to *A. repens* 2178 were calculated as the ratios of 1.66 μg to these dry weights (Table I). Except for *A. repens* 2238, a cleistothecial isolate, which had a relative reactivity of 0.42, the relative reactivities followed taxonomic groups. The highest values (0.20–1.05) were measured for isolates of *A. ruber* and *A. amstelodami*. *A. ruber* and *A. amstelodami* are closely related to *A. repens*; these three species and others are in the *A. glaucus* group. *Penicillium cyclopium* was the only storage species tested not in the *A. glaucus* group that exhibited a detectable cross-reaction (0.07).

Measurement of *A. repens* in Infected Corn

Because the biomass of *A. repens* in infected corn could not be measured directly, curves for *A. repens* in infected corn were computed for comparison to the standard curve for *A. repens* 2178 cultured on CZXA (Fig. 2). Samples from each replicate stored for 10 or 52 days at 86% rh and 26° C were diluted to the equivalent of 25, 5, 1, and 0.2 mg of infected corn per determination. The regression line for the 10-day samples was expressed as

$$Y = 0.22 - 1.21 \times X$$

where X was the log of the weight of corn in milligrams per determination, and Y was the logit transformation of the specific cpm bound. The regression line for the 52-day samples was expressed as

$$Y = 1.72 - 1.11 \times X$$

for the same parameters. Because the slopes for the 10- and 52-day samples fell within the 95% confidence interval for the slope of the standard curve (−1.08 to −1.71) the standard curve was used to convert IRIA results for *A. repens* in infected corn to micrograms of *A. repens*.

TABLE I
Inhibition Radioimmunoassay Reactivities of Fungi Isolated from Corn Kernels Relative to *Aspergillus repens* 2178

Fungal Species and Isolate	Reactivity
Aspergillus repens 2178	1.00
A. repens 2486	1.20
A. repens 2238[a]	0.42
A. ruber 2072	1.05
A. ruber 2239	0.38
A. amstelodami 2219	0.20
A. amstelodami 2240	0.23
Penicillium cyclopium 2313	0.07
A. restrictus 2217	<0.02
A. flavus 1383	<0.02
Cephalosporium acremonium 2211	<0.02
Gibberella zeae 2002	<0.02
P. oxalicum 2204	<0.02
Fusarium moniliforme 747	<0.02
Nigrospora oryzae 2215	<0.02

[a] Cleistothecial isolate.

Changes in Indices with Time in Storage

Corn selected for the storage test and for the control extract was substantially free of fungal invasion. Two of 100 surface-disinfected kernels cultured on MSA before inoculation yielded *A. glaucus*. None of the 400 kernels cultured on MSA immediately after inoculation yielded *A. glaucus*. Of 300 kernels cultured on other media either before or immediately after inoculation, 12% were infected by other fungi (most frequently by *Chaetomium* sp., but also by *Nigrospora oryzae* and *Fusarium moniliforme*). The only propagules detected by culturing dilutions of uninoculated kernels were *Cephalosporium acremonium*. No ergosterol was detected by HPLC in either uninoculated corn or corn tested immediately after inoculation.

Indices of seed deterioration and fungal invasion as the means of the four replicates (from the four storage chambers) were plotted against the time in controlled storage (Figs. 3 and 4). The mean moisture content for all sampling dates was 16.9% ± 0.1 and therefore not plotted.

Seed infection, number of propagules, evolved CO_2, ergosterol, and concentration of *A. repens* increased during the first 10 days of

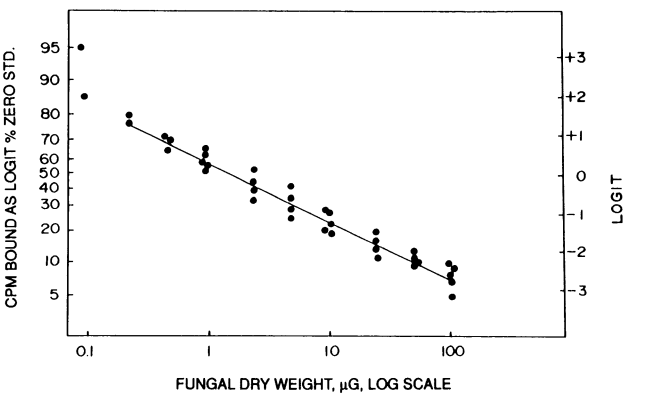

Fig. 1. Standard curve for *Aspergillus repens* 2178. Solid line indicates regression line drawn through means of quadruplicate determinations.

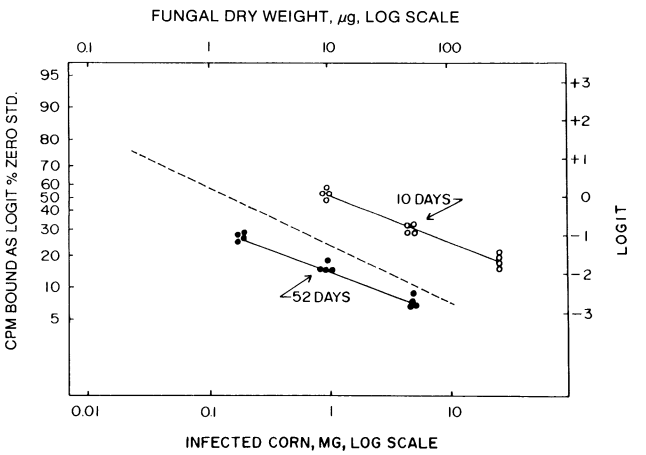

Fig. 2. Curves for *Aspergillus repens*-infected corn samples stored for 10 and 52 days at 86% relative humidity and 26° C. Solid lines indicate regression lines drawn through means of quadruplicate determinations.

storage (Figs. 3 and 4). Light sporulation (not blue eye) was visible in situ on 5% of the kernels on day 10, but no mold was visible even at 3× magnification after removing the corn from the storage chambers. Seed germination began to decrease by day 24 and blue eye appeared by day 31.

Blue eye was the least sensitive of the measures tested. As in previous studies (Seitz et al 1982b, Fernandez et al 1985) even gentle handling reduced the sensitivity of the visual indices, because the increases in sporulation visible with the unaided eye and with 3× magnification lagged behind the sporulation observed in the undisturbed storage columns prior to sampling. Also, blue eye is not obvious unless there is heavy sporulation on the germ.

Evolved CO_2, ergosterol, and IRIA continued to increase through day 52, reflecting continued fungal growth. Respiration by the corn may have contributed significantly to the evolved CO_2 early in the test. During the first eight days of storage, evolved CO_2 was virtually constant at 0.036 mg per gram of dry weight per day but kernel infection was 60% after eight days. On day 9 the rate of CO_2 evolution increased to 0.082 mg per gram of dry matter per day and sporulation appeared in situ.

Correlations

A. repens biomass as measured by IRIA was correlated with days of storage and with all of the indices of fungal growth and kernel deterioration (Table II). *A. repens* as measured by IRIA correlated most highly with days of storage, evolved CO_2, and ergosterol (r = 0.99, 0.99, and 0.96, respectively; $P < 0.001$). Measurement of *A. repens* correlated very highly with seed germination loss and with mold visible at 3× magnification ($r = 0.91$, both indices; $P < 0.01$) and moderately with mold visible at 10× magnification, blue eye, number of fungal propagules, and percentage of infected seeds ($r = 0.82, 0.80, 0.79, 0.71$, respectively; $P < 0.05$).

IRIA, evolved CO_2, ergosterol, number of propagules, kernel infection, and visible mold at 10× magnification were sensitive to the initial stages of mold growth. Because the ergosterol assay is not species specific, its ability to detect early growth in storage may be limited by preharvest invasion (Seitz et al 1982b). However, the corn used for this test had little preharvest invasion.

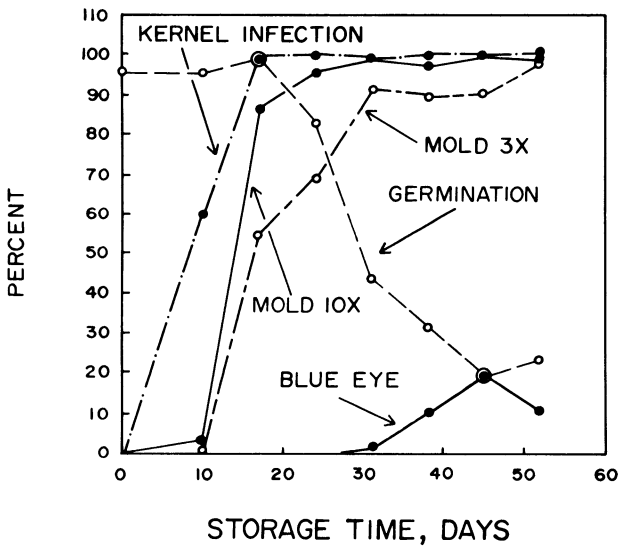

Fig. 3. Percentages of seed germination, kernel infection, visible mold at 10× and 3× magnification and blue eye in corn samples stored at 86% relative humidity and 26° C. Points represent means of indices for four samples.

CONCLUSIONS

IRIA is potentially useful for the study of *A. repens* in stored corn because of its sensitivity and high correlations with other measures of fungal invasion. IRIA is not subject to the high nonspecific binding, which was a persistent problem with previously tested types of immunoassays for *A. repens* (*unpublished data*). IRIA had a detection limit of 250 ng of *A. repens* per determination (approximately 2.5×10^3 conidia), equivalent to 2 μg per milliliter or 10 μg per gram of corn kernels. This limit was greater than the detection limits reported for other immunoassays for fungi in buffer rather than plant tissue extracts. Savage and Sall (1981) and Johnson et al (1982) reported limits of 100 ng per milliliter for *Botrytis cinerea* and *Epichloe typhina*,

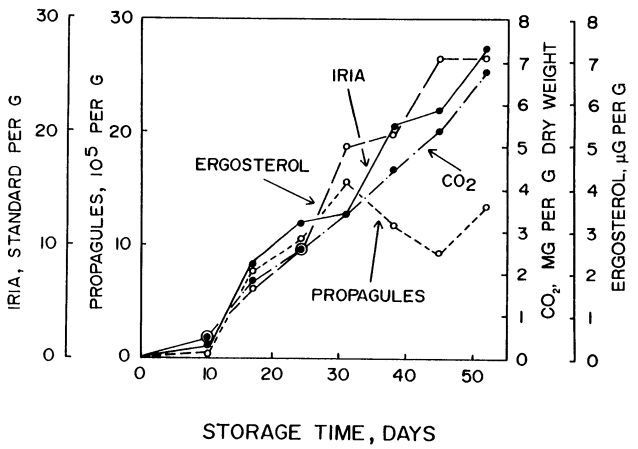

Fig. 4. Concentrations of *Aspergillus repens*, ergosterol, evolved CO_2, and number of fungal propagules in corn samples stored at 86% relative humidity and 26° C. Points represent means of indices for four samples.

TABLE II
Correlation Coefficients Among Storage Time, Decrease in Seed Germination, and Measures of Fungal Growth or Sporulation

Parameter	Germ. Dec.	Blue Eye	Visible Mold 3×	Visible Mold 10×	Seed Infection	No. Propagules	CO_2 Evolved	Ergosterol	IRIA[a]
Storage time, days	0.93 d[b]	0.89 b	0.91 c	0.80 b	0.74 b	0.91 c	0.99 d	0.98 d	0.99 d
Seed germination, % decrease	···	0.86 c	0.83 b	0.66 a	0.54	0.71 b	0.93 d	0.97 d	0.91 c
Blue eye, %		···	0.61	0.48	0.39	0.42	0.80 b	0.84 c	0.80 b
Visible mold at 3×, %			···	0.96 d	0.83 b	0.96 d	0.90 c	0.91 c	0.91 c
Visible mold at 10×, %				···	0.89 c	0.93 d	0.79 b	0.78 b	0.82 b
Seed infection, %					···	0.81 b	0.69 a	0.67 a	0.71 b
Number of fungal propagules per g						···	0.79 b	0.79 b	0.79 b
CO_2 evolved, mg per g dry weight							···	0.98 d	0.99 d
Ergosterol, μg per g								···	0.96 d

[a] Inhibition radioimmunoassay.
[b] Letters a, b, c, and d indicate significance at the levels of $P < 0.10$, 0.05, 0.01, and 0.001, respectively.

respectively; Liese et al (1982) reported a limit of 180 spores per milliliter for *Plasmopara halstedii*. The limit of the IRIA was comparable to or lower than the detection limits of other immunoassays for fungi in plant tissue. Leach and Swinburne (1984) reported a limit of 5 mg of *Verticillium albo-atrum* per gram of hops; Lin et al (1986) reported from 1 to 10 μg of fungus (depending on species) per milliliter of tomato puree.

Cross-reaction of other fungi in the IRIA generally followed taxonomic groups. The highest cross-reactions occurred for *A. ruber* and *A. amstelodami*, which are closely related to *A. repens* as members of the *A. glaucus* group. Cross-reaction by these fungi might be advantageous in storage studies, since the three species are generally found together and may have similar effects on seed deterioration. The low reactivity for a cleistothecial isolate of *A. repens* was not unexpected because the fungal biomass used for immunization and for sensitization of the microtiter plates was mostly conidial. Other immunoassays for fungi have also had much higher reactivities for the standard isolate than for another isolate of the same species (Savage and Sall 1982, Johnson et al 1982).

Saul and Steel (1966) advocated 0.5% dry matter loss (DML) as a guideline for safe storage of U.S. no. 2 corn. Although samples were not rated by a licensed inspector, commercial grade could be estimated from ratings for blue eye. After 52 days of storage in this test, evolved CO_2 was equivalent to 0.46% DML but the corn would have graded U.S. no. 4 because of blue eye on 11% of the kernels. This finding confirms the need for caution in applying the 0.5% DML criterion for low moisture corn (Seitz et al 1982 a,b; Fernandez et al 1985).

Correlations among IRIA, CO_2, and ergosterol were very high presumably because they measured fungal growth. Lower correlations of these measures with the number of propagules per gram may have resulted because some of the biomass did not consist of viable propagules. The high number of propagules and the high coefficients of variation could also have affected these correlations; these factors may have accounted for the low correlation of blue eye with number of propagules. The low correlation of decrease in seed germination with the percentage of seed infection (0.54) was consistent with previous reports that members of the *A. glaucus* group are weak pathogens (Cantone et al 1983, Sauer 1984, Tuite et al 1985).

IRIA may be useful as a rapid and quantitative supplement to microbiological tests. Ecological, host resistance, and chemical preservative studies could utilize IRIA. Results can be obtained as rapidly by IRIA as by any other measure except visual inspection. Unlike IRIA, visual inspection is usually not species specific (although some types of discoloration are associated with certain groups of fungi, and fruiting structures can be identified if present) and it is not quantitative. IRIA appears to be highly correlated with two useful non-species specific quantitative indices, ergosterol content and evolved CO_2.

ACKNOWLEDGMENTS

The authors thank Cindy Koh-Knox for technical support, Gary Krouse for help with the statistical analysis, and Joe Griffith for advice on HPLC. We also thank Ronald Sprouse for assisting in raising the antisera and developing the IRIA.

LITERATURE CITED

CANTONE, F. A., TUITE, J., BAUMAN, L. F., and STROSHINE, R. 1983. Genotypic differences in reaction of stored corn kernels to attack by selected *Aspergillus* and *Penicillium* spp. Phytopathology 73:1250.

CHOWDHURY, M. H., and BUCHELE, W. F. 1976. Development of a numerical damage index for critical evaluation of mechanical damage of corn. Trans. ASAE 19:428.

CHRISTENSEN, C. M., and SAUER, D. B. 1982. Microflora. Pages 219-240 in: Storage of Cereal Grains and Their Products, 2nd ed. C. M. Christensen, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

EL-NAKIB, O., PESTKA, J. J., and CHU, R. S. 1981. Determination of aflatoxin B_1 in corn, wheat, and peanut butter by enzyme-linked immunosorbent assay and solid phase radioimmunoassay. J. Assoc. Off. Anal. Chem. 64:1077.

FAN, T. S. L., and CHU, F. S. 1984. Indirect enzyme-linked immunosorbent assay (ELISA) for T-2 toxin in biological fluids. J. Food Prot. 47:964.

FERNANDEZ, A., STROSHINE, R., and TUITE, J. 1985. Mold growth and carbon dioxide production during storage of high-moisture corn. Cereal Chem. 62:137.

FUNK, C. R., HALISKE, P. M., JOHNSON, M. C., SIEGEL, M. R., STEWART, A. V., AHMAD, S., HURLEY, R. H., and HARVEY, I. C. 1983. An endophytic fungus and resistance to sod webworms: Association in *Lolium perenne* L. Bio/Technology 1:189.

GENDLOFF, E. H., PESTKA, J. J., SWANSON, S. P., and HART, L. P. 1984. Detection of T-2 toxin in *Fusarium sporotrichiodes*-infected corn by enzyme-linked immunosorbent assay. Appl. Environ. Microbiol. 47:1161.

JOHNSON, M. C., PIRONE, T. P., SIEGEL, M. R., and VARNEY, D. R. 1982. Detection of *Epichloe typhina* in tall fescue by means of an enzyme-linked immunosorbent assay. Phytopathology 72:647.

LEACH, J. E., and SWINBURNE, T. R. 1984. An indirect ELISA for quantitative estimation of *Verticillium albo-atrum* in hops. (Abstr.) Phytopathology 74:845.

LEE, S. C., and CHU, F. S. 1981. Radioimmunoassay of T-2 toxin in corn and wheat. J. Assoc. Off. Anal. Chem. 64:156.

LEE, S. C., and CHU, F. S. 1984. Enzyme-linked immunosorbent assay of ochratoxin A in wheat. J. Assoc. Off. Anal. Chem. 67:45.

LIESE, A., GOTTLIEB, A. R., and SACKSTON, W. E. 1982. Use of the enzyme-linked immunosorbent assay (ELISA) for the detection of downy mildew (*Plasmopora halstedii*) in sunflower. Proc. Int. Sunflower Conference 10th. Australian Sunflower Assoc. and International Sunflower Assoc., Australia.

LIN, H., LISTER, R. M., and COUSIN, M. A. 1986. Enzyme-linked immunosorbent assays for detection of mold in tomato puree. J. Food Sci. 51:180.

LIU, M. T., RAM, B. P., HART, L. P., and PESTKA, J. J. 1985. Indirect enzyme-linked immunosorbent assay for the mycotoxin zearalenone. Appl. Environ. Microbiol. 50:332.

PEREZ, R. A., TUITE, J., and BAKER, K. 1982. Effect of moisture, temperature, and storage time on subsequent storability of shelled corn. Cereal Chem. 59:205.

PESTKA, J. J., LEE, S., LAU, J. P., and CHU, F. S. 1981. Enzyme-linked immunosorbent assay for T-2 toxin. J. Am. Oil Chem. Soc. 58:940A.

REARDON, T. P., SPROUSE, R. F., and GERNER, H. E. 1982. Radioimmunoassay for the detection of antigen-specific IgM, IgG, and IgA in equine sera. Am. J Vet. Res. 43:294.

RODBARD, D., and MUNSON, P. J. 1980. Radioimmunoassay data processing. Pages 243-349 in: Manual of Clinical Immunology, 2nd ed. N. R. Rose and H. Friedman, eds. Am. Soc. for Microbiol.: Washington, DC.

ROUSSEAU, D. M., SLEGERS, G. A., and PETEGHEM, C. H. 1985. Radioimmunoassay of ochratoxin A in barley. Appl. Environ. Microbiol. 50:529.

SAUER, D. B. 1984. Growth of *Aspergillus* and *Penicillium* species following mixed inoculation in stored grain. (Abstr.) Phytopathology 74:879.

SAUL, R. A., and STEELE, J. L. 1966. Why damaged corn costs more to dry. Agric. Eng. 47:376.

SAVAGE, S. D., and SALL, M. A. 1981. A radio-immunosorbent assay for *Botrytis cinerea*. Phytopathology 71:411.

SAVAGE, S. D., and SALL, M. A. 1982. The use of a radio-absorbent assay for *Botrytis cinerea*. EPPO Bull. 12:49.

SEITZ, L. M., MOHR, H. E., BURROUGHS, R., and SAUER, D. B. 1977. Ergosterol as an indicator of fungal invasion in grains. Cereal Chem. 54:1207.

SEITZ, L. M., SAUER, D. B., MOHR, H. E., and ALDIS, D. E. 1982a. Fungal growth and dry matter loss during bin storage of high-moisture corn. Cereal Chem. 59:9.

SEITZ, L. M., SAUER, D. B., and MOHR, H. E. 1982b. Storage of high-moisture corn: Fungal growth and dry matter loss. Cereal Chem. 59:100.

STEELE, J. L., SAUL, R. A., and HULKILL, W. V. 1969. Deterioration of shelled corn as measured by carbon dioxide production. Trans. ASAE 12:685.

TUITE, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publ. Co.: Minneapolis, MN.

TUITE, J., KOH-KNOX, C., STROSHINE, R., CANTONE, F. A., and BAUMAN, L. F. 1985. Effect of physical damage to corn kernels on the development of *Penicillium* species and *Aspergillus glaucus* in storage. Phytopathology 75:1137.

USDA. 1978. Official Grain Standards of the United States. Federal Grain

Inspection Service, U.S. Dept. Agric. U.S. Govt. Printing Office: Washington, DC.
VOLLER, A., BIDWELL, D., and BARTLETT, A. 1980. Enzyme-linked immunosorbent assays. Page 359 in: Manual of Clinical Immunology, 2nd ed. N. R. Rose and H. Friedman, eds. Am. Soc. for Microbiol.: Washington, DC.

[Received June 2, 1988. Revision received November 29, 1988. Accepted December 1, 1988.]