Variation Among Isolates of *Fusarium graminearum* Associated with Fusarium Head Blight in North Carolina

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ABSTRACT

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Fusarium head blight (FHB) can reduce yield of wheat and decrease the value of harvested grain by accumulation of detrimental toxins. Understanding the variability of the fungal population associated with infection could improve disease control strategies. Sixty-six isolates of *Fusarium graminearum* associated with FHB were collected in North Carolina and tested for in vitro growth rate, in vitro production of deoxynivalenol (DON) and zearalenone, and pathogenicity on three cultivars of soft red winter wheat. Significant differences among isolates were found for all three traits. Randomly Amplified Polymorphic DNA (RAPD) analysis revealed high levels of genotypic diversity among isolates. Isolates of *F. graminearum*, *F. culmorum*, and *F. avenaceum* acquired from the Pennsylvania State University Fusarium Center were included for comparison in all tests. In vivo levels of DON were measured for the five isolates associated with the highest levels of disease and the five isolates associated with the lowest levels of disease, and no significant differences were found. However, all ten isolates produced detectable levels of DON in vivo. Mean disease ratings ranged from 3.4 to 96.4%, in vitro (DON) levels ranged from 0 to 7176.2 ppm, and zearalenone ranged from 0 to 354.7 ppm, among isolates. A multiple regression model using in vitro growth, in vitro DON, and zearalenone production, collection location, wheat cultivar of isolate origin, plot, tillage conditions, and previous crop as independent variables and percent blighted tissue as the dependent variable was developed. The cumulative R^2 value for the model equaled 0.27 with in vitro rate of growth making the largest contribution. Analysis of phenotype and genotype among isolates demonstrated diversity in a single plot, in a single location, and in North Carolina. Genotypic and phenotypic diversity were significant under both conventional and reduced tillage conditions, and diversity was high regardless of whether the previous crop had been a host or non-host for *F. graminearum*. These data indicate a variable pathogen population of *F. graminearum* exists in North Carolina, and members of this population can be both highly pathogenic on wheat and produce high levels of detrimental toxins, indicating a potential threat for problems with FHB within the state.

Additional keyword: resistance

Fusarium head blight (FHB) or wheat scab is a seed and head disease characterized by bleaching of the wheat head, shriveled kernels, and accumulation of mycotoxins in grain (1,2,5,7,12,15). Several species of *Fusarium* are associated with FHB. *F. graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) predominates in North America. *F. culmorum* and *F. avenaceum* are also responsible for FHB, but are less often implicated in the United States (5,7,12). Infection can occur

from the time of flowering to the soft dough stage, and is promoted by environmental factors such as warm temperatures and high humidity (1,2,5,7,12,15). *Gibberella zeae* is also the causal agent of ear rot in maize and rotations of wheat and maize can increase the incidence of both diseases (12).

Fusarium isolates associated with FHB can produce mycotoxins, notably deoxynivalenol (DON) or vomitoxin, which poses a threat to humans and livestock when consumed in scabbed grain (1,2,4,5,6,7,12,15). DON can cause feed refusal and higher-than-normal rates of abortion when ingested by livestock (7,12). *Fusarium* species associated with FHB may also produce the toxin zearalenone which is reportedly linked to reduced weight gain and increased rates of abortion when ingested by swine (6). High levels of these mycotoxins reduce the value of grain and infested grain must be mixed with clean grain to reduce toxins to acceptable levels

(7). DON may function as a pathogenicity factor (4).

There is little evidence for pathogenic specialization within *F. graminearum* (2,12). Differences in aggressiveness among isolates of *F. graminearum*, however, have been reported (1,2,12). Reports of genotypic diversity among isolates of *F. graminearum* vary, with Bowden and Leslie (3) reporting high levels of variation in vegetative compatibility groups among *F. graminearum* isolates collected in the Midwestern United States, while Ouellet and Seifert (11) reported a low amount of genetic diversity among isolates of *F. graminearum* collected in Canada. Genetic characterization of European isolates of *Fusarium* species associated with FHB reveals a large amount of variation among isolates of some species (9,14).

Bowden and Leslie (3) stated the population structure of *F. graminearum* is poorly understood, and a better understanding of spatial and temporal patterns of genetic diversity, potential for sexual and asexual reproduction, and selection pressures, could lead to better control strategies for FHB.

FHB is currently not a severe disease problem in North Carolina. Nevertheless, growers in the state have expressed concern over the potential for an increased problem with the disease due to the occurrence of severe outbreaks of the disease in the Midwestern United States. If an adequate source of inoculum is present, changes in cultural practices such as a trend toward reduced tillage may create the potential for FHB epidemics in areas where the disease is currently not a problem. The adequacy depends on the volume of inoculum, which is increased under reduced tillage, and its capability to cause disease. The purpose of this study was to provide data on pathogen diversity within North Carolina with regard to factors such as isolate aggressiveness and toxin production, and to determine both the diversity of the pathogen population and its potential to cause severe disease in the state.

MATERIALS AND METHODS

Isolate collection and phenotypic testing. Isolates were collected from wheat heads exhibiting symptoms of FHB in the Coastal, Piedmont, and Mountain regions of North Carolina during May and June 1996 (Table 1). Several isolates were collected at the Center for Environmental

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Table 1. Source of 66 isolates of *Fusarium graminearum* collected from wheat in North Carolina

Isolate	County	Geographical designation	Wheat cultivar from which isolate originated	Plot	Tillage conditions	Previous crop
NC 1	Ashe	Mountain	Oasis × C762 ^a	116	Conventional ^d	Maize
NC 2	Ashe	Mountain	Oasis × C762 ^a	116	Conventional	Maize
NC 3	Ashe	Mountain	C8012 × Gore ^b	78	Conventional	Maize
NC 4	Ashe	Mountain	C8012 × Gore ^b	78	Conventional	Maize
NC 5	Ashe	Mountain	C8012 × Gore ^b	78	Conventional	Maize
NC 6	Alamance	Piedmont	FFR 555	Commercial ^c	Conventional	Soybean
NC 7	Alamance	Piedmont	FFR 555	Commercial ^c	Conventional	Soybean
NC 8	Alamance	Piedmont	FFR 555	Commercial ^c	Conventional	Soybean
NC 9	Alamance	Piedmont	FFR 555	Commercial ^c	Conventional	Soybean
NC 10	Davie	Piedmont	Karl	Commercial ^c	Conventional	Maize
NC 11	Davie	Piedmont	Karl	Commercial ^c	Conventional	Maize
NC 12	Davie	Piedmont	Karl	Commercial ^c	Conventional	Maize
NC 13	Wayne	Coastal	Wakefield	1	Conventional	Maize
NC 14	Wayne	Coastal	Wakefield	1	Conventional	Maize
NC 15	Wayne	Coastal	Wakefield	1	Conventional	Maize
NC 16	Wayne	Coastal	Wakefield	2	Conventional	Maize
NC 17	Wayne	Coastal	Wakefield	2	Conventional	Maize
NC 18	Wayne	Coastal	Coker 916	3	Conventional	Soybean
NC 19	Wayne	Coastal	Wakefield	4	Conventional	Soybean
NC 20	Wayne	Coastal	Wakefield	4	Conventional	Soybean
NC 21	Wayne	Coastal	Wakefield	4	Conventional	Soybean
NC 22	Wayne	Coastal	Wakefield	4	Conventional	Soybean
NC 23	Wayne	Coastal	Wakefield	4	Conventional	Soybean
NC 24	Wayne	Coastal	Coker 916	5	No-till	Maize
NC 25	Wayne	Coastal	Coker 916	5	No-till	Maize
NC 26	Wayne	Coastal	Coker 916	5	No-till	Maize
NC 27	Wayne	Coastal	Coker 916	5	No-till	Maize
NC 28	Wayne	Coastal	Coker 916	5	No-till	Maize
NC 29	Wayne	Coastal	Coker 916	5	No-till	Maize
NC 30	Wayne	Coastal	Wakefield	6	Conventional	Soybean
NC 31	Wayne	Coastal	Wakefield	7	Conventional	Maize
NC 32	Wayne	Coastal	Wakefield	7	Conventional	Maize
NC 33	Wayne	Coastal	Wakefield	7	Conventional	Maize
NC 34	Wayne	Coastal	Wakefield	7	Conventional	Soybean
NC 35	Wayne	Coastal	Wakefield	7	Conventional	Soybean
NC 36	Wayne	Coastal	Wakefield	7	Conventional	Soybean
NC 37	Wayne	Coastal	Wakefield	7	Conventional	Soybean
NC 38	Wayne	Coastal	Coker 916	8	Conventional	Soybean
NC 39	Wayne	Coastal	Wakefield	9	Conventional	Soybean
NC 40	Wayne	Coastal	Wakefield	9	Conventional	Soybean
NC 41	Wayne	Coastal	Wakefield	9	Conventional	Soybean
NC 42	Wayne	Coastal	Wakefield	9	Conventional	Soybean
NC 43	Wayne	Coastal	Wakefield	9	Conventional	Soybean
NC 44	Wayne	Coastal	Coker 916	10	No-till	Maize
NC 45	Wayne	Coastal	Coker 916	10	No-till	Maize
NC 46	Wayne	Coastal	Coker 916	10	No-till	Maize
NC 47	Wayne	Coastal	Coker 916	10	No-till	Maize
NC 48	Wayne	Coastal	Coker 916	10	No-till	Maize
NC 49	Wayne	Coastal	Wakefield	11	Conventional	Maize
NC 50	Wayne	Coastal	Wakefield	11	Conventional	Maize
NC 51	Wayne	Coastal	Wakefield	11	Conventional	Maize
NC 52	Wayne	Coastal	Wakefield	11	Conventional	Maize
NC 53	Wayne	Coastal	Wakefield	11	Conventional	Maize
NC 54	Wayne	Coastal	Wakefield	12	No-till	Maize
NC 55	Wayne	Coastal	Wakefield	12	No-till	Maize
NC 56	Wayne	Coastal	Wakefield	12	No-till	Maize
NC 57	Wayne	Coastal	Wakefield	12	No-till	Maize
NC 58	Wayne	Coastal	Coker 916	13	Conventional	Soybean
NC 59	Wayne	Coastal	Coker 916	13	Conventional	Soybean
NC 60	Wayne	Coastal	Coker 916	13	Conventional	Soybean
NC 61	Wayne	Coastal	Wakefield	14	No-till	Maize
NC 62	Wayne	Coastal	Wakefield	14	No-till	Maize
NC 63	Wayne	Coastal	Wakefield	14	No-till	Maize
NC 64	Wayne	Coastal	Wakefield	14	No-till	Maize
NC 65	Wayne	Coastal	Coker 916	14	Conventional	Soybean
NC 66	Wayne	Coastal	Coker 916	14	Conventional	Soybean

^a Oasis × Coker 762 F₂ progeny.^b Coker 8012 × Gore F₂ progeny.^c Commercial field.^d Conventional tillage consisted of chisel plow and soil disc prior to planting.

Farming in Wayne County, NC, in the eastern part of the state where the majority of wheat production occurs. The fields at the center were adjacent to each other, with different combinations of crop rotation and tillage types in each field. Two isolates of *F. graminearum* (R-6914), (R-6925), an isolate of *F. culmorum* (R-6565), and an isolate of *F. avenaceum* (R-5314) were obtained from the Pennsylvania State University Fusarium Center (University Park, PA) for comparison to the North Carolina isolates. Seeds from infected wheat heads were surface sterilized in 0.525% sodium hypochlorite for 1 min and then washed for 1 min in sterile distilled water. Surface sterilized seeds were plated on synthetic nutrient agar (SNA) as described by Nirenberg (10). SNA is a low-nutrient medium that reduces mutations in *Fusarium* species. Single-spore isolations as described by Snyder and Hansen (8) were performed for each sample.

Growth rate was measured for each isolate in a randomized complete block design. A 5-mm agar plug was taken from each isolate and placed in the center of an 80 mm diameter Petri dish containing 15 ml of SNA. Three replicates of each isolate were incubated at 25/22°C (day/night) for 7 days. Cultures were exposed to 12 h of fluorescent light per day. Light intensity averaged 45.9 $\mu\text{mol s}^{-2} \text{m}^{-1}$ and was measured using a Li-189 Quantum/Radiometer/Photometer (Licor, Lincoln, NE). Measurements of radial growth were taken at 3, 5, and 7 days, and log transformed. An average rate of growth per day was calculated for each isolate prior to final analysis. Incubator shelves functioned as blocks. The entire test was performed twice.

Pathogenicity testing was performed in the greenhouse using wheat cultivars 'Cardinal' (PI365440), 'Wakefield' (PI547040), and 'Caldwell' (CItr17897). A completely random design was used with 70 isolates $\times$ 3 cultivars $\times$ 3 plants per cultivar. Seeds were vernalized prior to planting by placing them in moist paper towels that were kept at 5°C for 8 weeks. The vernalized seeds were grown in pot-

ting soil under greenhouse conditions. Plants were exposed to 12 h of light per day at an average intensity of 285 $\mu\text{mol s}^{-2} \text{m}^{-1}$. Individual wheat heads were inoculated in the greenhouse at mid-anthesis. Inoculations were performed using a micropipettor to apply a 20 μl drop of inoculum at a concentration of approximately 1,000 macroconidia in the center region of one central spikelet per plant. Plastic bags were misted with sterile water and placed over inoculated heads for 5 days. This technique provided the moisture required for infection and prevented movement of isolates to other plants. Controls used sterile water in place of inoculum. The percentage of blighted head tissue was recorded at 10 and 20 days after inoculation. The visual scale published by Stack and McMullen (16) was used as a reference for determining percent FHB infection. Due to differences in maturity, inoculations of the three cultivars occurred at different times. Greenhouse tests using 70 isolates were performed twice and a smaller test of the five most and least aggressive isolates was performed a third time and subsequently analyzed for in vivo DON production. Statistical analysis was performed using SAS release 6.12 (SAS Institute, Cary, NC).

RAPD analysis. Single-spore isolates from each original field isolate were grown for 7 days in 1.5 ml micro centrifuge tubes containing 500 μl Yeast-Malt (YM) broth (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g dextrose per liter, Difco, Detroit MI). After 7 days, 500 μl of sterile Tris-EDTA pH 8.0 (TE) was added and tubes were boiled at 100°C for 10 min. Tubes were then cooled on ice for 10 min and spun at 13,000 rpm for 10 min. The aqueous fraction containing DNA was removed and placed in a new tube. Samples were diluted by mixing 3 μl of the aqueous DNA fraction with 97 μl of sterile water.

Each RAPD reaction consisted of 15 μl volume composed of 5 μl diluted DNA, 1.5 μl *Taq* polymerase buffer (Promega, Madison WI), 0.9 μl 25mM MgCl_2 , 1.2 μl 1.25 mM dNTPs, 1.2 μl 100X unacetylated

bovine serum albumin (NEB, Beverly, MA), 30 ng primer (Operon Technologies, Alameda, CA, and University of British Columbia, Vancouver), and 0.2 μl *Taq* polymerase (Promega). Reactions were performed using 96-well plates and a PTC-100 thermocycler (MJ Research, Watertown, MA). Cycle parameters consisted of 1 min at 92°C, then 1 min at 35°C, followed by 2 min at 72°C repeated for a total of 40 cycles. Samples were run out in 1.5% agarose gels at 100V for approximately 4 h and visualized using EtBr and UV fluorescence. Twenty primers were screened on eight randomly selected isolates. The five primers producing the highest levels of polymorphism, UBC28 (5'-CCGGCC TTAA-3'), OPB15 (5'-GGAGGGTGT-3'), OPQ4 (5'-AGTGCCTGA-3'), OPL10 (5'-TGGGAGATGG-3'), S12 (5'-CTG GGTGAGT-3'), OPM5 (5'-GGGAAC GTGT-3'), were used for analysis of the 70 isolates. Isolates were screened twice with each primer and repeatable polymorphisms were scored as 1 for presence of a band and 0 for absence.

Multiple regression analysis. A multiple regression model was developed using disease rating as the dependent variable with the wheat cultivar from which the isolate was collected, collection location, plot, previous crop, tillage conditions, in vitro rate of growth, and in vitro production of DON and zearalenone as independent variables. The categorical values (location, cultivar, etc.) were used as dummy variables (18) and no consideration should be given to the numbers assigned to each condition (e.g., conventional versus no tillage). Interpretation is limited to the detection of differences only. The maxr and Mallow's CP functions were used to aid in model development.

Cluster analysis. Cluster analysis of isolates utilized mean ratings of the phenotypic characteristics of toxin production, rate of growth, and disease rating to form a dendrogram. The NTSYS numerical taxonomy program (Exeter Software, Setauket, NY) (13) was used for analysis. The SIMINT function for quantitative data was used to form a covariance matrix and the

Table 2. Means and ranges of in vitro growth, in vitro levels of deoxynivalenol (DON) and zearalenone produced, and disease rating of the four typed *Fusarium* isolates compared to the 66 *Fusarium graminearum* isolates collected in North Carolina

Variable	FA ^a 5314	FC ^b 6565	FG ^c 6914	FG ^b 6925	NC mean	NC minimum	NC maximum
In vitro growth (mm/day)	3.4	3.7	7.2	5.1	4.7	0.9	11.4
DON 27-4-27°C ^a (ppm)	401	1,341	952	412	641	0	7,176
DON 27°C ^b (ppm)	734	1,987	4,087	643	736	0	8,972
Zearalenone 27-4-27°C ^c (ppm)	5.6	0.2	10	1.7	26	0	355
Zearalenone 27°C ^d (ppm)	33	0	0	10	12	0	87
Disease Rating (mean percent area)	13	76	67	64	51	0	100

^a In vitro DON cultures incubated at 27°C for 7 days, then 4°C for 5 days, followed by 18 days at 27°C.

^b In vitro DON cultures incubated at 27°C for 30 days.

^c In vitro zearalenone cultures incubated at 27°C for 7 days, then 4°C for 5 days, followed by 18 days at 27°C.

^d In vitro zearalenone cultures incubated at 27°C for 30 days.

^e Typed isolate of *F. avenaceum*.

^f Typed isolate of *F. culmorum*.

^g Typed isolate of *F. graminearum*.

^h Typed isolate of *F. graminearum*.

dendrogram was generated using the SAHN clustering function. Cluster analysis was also performed on genotypic data collected from RAPD analysis. Polymorphism data were used to form a dice coefficient matrix using Win-Boot (IRRI, Manila, Philippines) (19). The resulting matrix was used to form a dendrogram using the SAHN clustering function of NTSYS.

Toxin analysis. Isolates for in vitro toxin analysis were grown on rice medium consisting of 5 g rice with 3 ml deionized water. All 70 isolates were tested for in vitro DON and zearalenone. The medium was placed in 20 ml screw-capped vials and autoclaved for 30 min before inoculation. Twelve vials were inoculated per isolate. Six were incubated after inoculation for 30 days at 27°C. The remaining six were incubated 7 days at 27°C, followed by 5 days at 4°C, and 18 days at 27°C. At 30 days, all cultures were frozen until extraction.

Fifteen ml of 84% acetonitrile were added to six cultures of each isolate (three taken from each of the two temperature treatments). A 5-ml sample of each culture extract was passed through a cleanup column and collected. Cleanup columns were composed of a filter disk pressed into the bottom of a 5 ml syringe barrel. One g of 1:3 C18RP silica gel:aluminum oxide was dispensed into the syringe barrel. A filter disk was placed on top of the packing and compressed. Columns were heated overnight at 60°C and stored in a desiccator until use.

In vitro DON was quantified by capillary gas chromatography (Model GC-14A, Shimadzu Scientific, Baltimore, MD) using techniques described by Tacke and Casper (17). Electron capture was utilized and N₂ at 4 ml/min was the carrier gas. The detector and injector were operated at 300°C and 250°C, respectively. The oven was programmed from 200 to 260°C at 4°C per min. Data were recorded and calculated relative to pure DON standards (Sigma Chemical, St. Louis, MO) using a micro-computer equipped with SS420 analog/digital converter boards and the EZCHROM 6.2 chromatography software system. Analysis was performed on a 30-m Megabore (0.53 mm) DB-1701 fused silica column (J & W Scientific, Folsom, CA). Solvent was dried under N₂, and purified fungal extract was derivatized with 100 µl Tri-Sil TBT (Pierce, Rockford, IL) for 1 h at 60°C. After cooling, 1 ml hexane was added and samples vortexed. One ml deionized water was added and vortexed for 1 min. The upper organic layer was taken and a 2 µl volume was injected onto the column.

Wheat heads infected with the five most aggressive and five least aggressive isolates were collected from greenhouse tests, threshed in a single head thresher, and analyzed individually for in vivo DON.

Samples were weighed and ground in an electric coffee grinder and a 2:1 (solvent:sample) ratio of 84% acetonitrile was added to each sample. Subsequent sample preparation and analyses were performed as described for in vitro DON analysis.

In vivo DON for the Wakefield samples was quantified by ELISA with an upper limit of 5 ppm for Tests 1 and 2. All samples from the most aggressive isolates exceeded this limit, therefore in vivo DON for the Cardinal and Caldwell samples were quantified using the previously described gas chromatography procedure. Gas chromatography was used in the third

test of in vivo DON on all three cultivars.

In vitro analysis of zearalenone was performed using the six remaining cultures of each isolate. After incubation, 15 ml of 70% methanol were added to all six. Extracts were analyzed by direct competitive enzyme-linked immunosorbent assay utilizing the Veratox Zearalenone Quantitative Test Kit (Neogen, Lansing, MI). Samples were read on a EL301 strip reader using a 650 nm filter (Bio-Tek Instruments, Winooski, VT).

RESULTS

All isolates collected in North Carolina were identified as *F. graminearum* based

A.

B.

C.

Fig. 1. Fusarium head blight rating and in vitro deoxynivalenol (DON) and zearalenone produced by 70 isolates of *Fusarium* species on three wheat cultivars. **A.** Mean percentage of blighted spikelets. **B.** Mean level of in vitro DON in ppm. **C.** Mean level of in vitro zearalenone in ppm.

on visual characteristics as described by Nelson et al. (8). Each isolate was compared to the typed isolates from the Pennsylvania State University Fusarium Center. Disease rating, in vitro, and in vivo toxin production varied greatly among isolates (Table 2, Figure 1). The *F. graminearum* isolates collected in North Carolina were similar to the two typed isolates of *F. graminearum* in pathogenicity, growth rate, and production of in vitro DON and zearalenone (Table 2). Of the four typed isolates, *F. culmorum* and *F. graminearum* were more pathogenic on wheat than *F. avenaceum*.

Disease ratings differed significantly among the three cultivars tested ($p < 0.0001$). Mean disease severities on Wakefield, Cardinal and Caldwell were 51%, 41% and 60% respectively. No symptoms of disease occurred in the control inoculations. Significant differences between greenhouse tests occurred ($p < 0.02$); however, mean disease scores among tests were highly correlated ($r = 0.96$, $p < 0.0001$) when mean disease ratings were compared for each isolate. Ranking of isolates based on pathogenicity remained constant in both tests. Analysis of the three greenhouse pathogenicity tests involving the five most and least aggressive isolates demonstrated

no significant differences among tests ($p < 0.224$), and mean disease scores between tests were highly correlated ($r = 0.98$, $p < 0.0001$) when compared on a per isolate basis.

Significant differences in pathogenicity were found among isolates from within a single plot, within a single field, and within a collection location (Table 3). No differences in pathogenicity were found among isolates due to tillage or previous crop.

In vitro DON at the constant temperature of 27°C was highly correlated to in vitro DON production at the 27°C-4°C-27°C temperature regime ($r = 0.86$, $p < 0.0001$). In vitro and in vivo DON production were not correlated.

The two temperature regimes for in vitro zearalenone production were correlated ($r = 0.31$, $p < 0.0001$), with the variable temperature regime producing a higher mean level (26 ppm) of zearalenone than the constant temperature regime (12 ppm). The standard deviation of the variable temperature regime was much higher (59.2) compared to the constant temperature regime (18.7).

No significant differences were found among the two groups of most and least aggressive isolates in terms of in vivo

DON production, nor were there significant differences among cultivars for this toxin. In vivo DON was highly variable among individual replications of seedlots, making analysis of trends difficult. Weakly pathogenic isolates tested did produce in vivo DON when averaged over several replications, with the least aggressive isolate producing 113 ppb DON on average. The mean level of symptoms produced by this isolate was 2.3% bleaching on wheat heads.

Multiple regression analysis using in vitro growth, in vitro DON and zearalenone production, collection location, the wheat cultivar from which an isolate originated, plot, tillage conditions, and previous crop as independent variables and disease rating as the dependent variable produced a cumulative $R^2 = 0.27$ with in vitro rate of

Table 3. Analysis of variance of differences in disease rating in a greenhouse test among 66 isolates of *Fusarium graminearum* in relation to collection location, field, plot within a field, tillage conditions, and previous crop

Variable	df	Type III mean square	Pr > F
Collection Location	4	6.94	0.0001
Field	6	4.32	0.0001
Plot	12	2.23	0.0001
Tillage ^a	1	0.005	0.138
Previous Crop ^b	1	0.035	0.112
Error	2,522	0.077	

^a Tillage was conventional or no-till.

^b Previous crop was maize or soybean.

MW FAFC FG FG 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 MW

Fig. 2. Randomly Amplified Polymorphic DNA profile produced by Operon primer OPB15. Lane 1 is Molecular Marker III (Boehringer, Mannheim, Germany), lane 2 is *Fusarium avenaceum* (R-5314), lane 3 is *F. culmorum* (R-6565), lane 4 and 5 are *F. graminearum* (R-6914 and R-6925), and lanes 6-24 are North Carolina *F. graminearum* isolates 1 to 24, respectively.

Fig. 3. Cluster analysis of 70 *Fusarium* spp. isolates based on disease rating, in vitro deoxynivalenol and zearalenone production, rate of isolate growth, location where isolate was collected, and cultivar of wheat where isolate originated. a = Phenotypic distance; b = Isolate number.

making the greatest contribution (0.14) to this value. RAPD analysis demonstrated a large amount of genotypic variability among the isolates (Fig. 2). Ninety-six polymorphisms were repeatable in two tests of each primer.

The phenotypic traits of mean disease scores, mean level of production of in vitro DON and zearalenone, and mean rate of growth for each isolate were used to form the dendrogram in Figure 3. Genetic data from the RAPD analysis were used to form the dendrogram presented in Figure 4 and suggests genotypic diversity was greater than phenotypic diversity among the characters measured in this population. Some isolates collected from the same plot were genetically similar such as NC 3 and NC 5, while others from the same plot were divergent such as NC 4. The typed isolates of *F. graminearum* were very similar to each other, both phenotypically and genotypically. In comparison to the North Carolina isolates, the typed *F. graminearum* isolates were more similar genetically to North Carolina isolates collected

in the mountain region, while phenotypically there were more similar to the North Carolina isolates collected in the eastern region of the state.

DISCUSSION

These data indicate the *F. graminearum* population in North Carolina is diverse. Some isolates produced on average 80 to 100% disease severity on the three wheat cultivars tested, whereas others were much less virulent (Fig. 1). Diversity among isolates for pathogenicity was found within a single plot, a single field and location, and across North Carolina. High levels of genotypic diversity within a plot, a field, and a location were found in the RAPD analysis, suggesting sexual reproduction is occurring within the surveyed *F. graminearum* population. Therefore, new genotypes are constantly being produced and diversity should remain high even in a limited spatial area.

Bowden and Leslie (3) hypothesize that high levels of genotypic diversity occurring in a small spatial area indicate several

independent infections occurred from diverse sources of inoculum, and the occurrence of multiple genotypes in a small area may provide opportunities for high levels of sexual recombination. *Gibberella zeae*, the perfect state of *F. graminearum*, is homothallic, and this may contribute to high levels of sexual reproduction. The data reported here indicate high levels of genotypic and phenotypic diversity can occur in an area where FHB has not been a severe problem.

Tillage conditions and previous crop did not have a significant effect on isolate diversity in regard to pathogenicity. These two factors, however, may have an impact on inoculum density by creating a greater reservoir of primary inoculum.

Data from this study indicate at least two components of the disease triangle, an aggressive pathogen and a susceptible host, are present in North Carolina. Warm, moist conditions can occur during the time wheat is susceptible to infection in North Carolina, so the question is raised as to why FHB epidemics occur infrequently in the state. To obtain the isolates used in this study, we had to search fields carefully to find plants showing symptoms of FHB or signs of the pathogen. Even though some isolates were highly aggressive and the host is probably at least moderately susceptible, the overall incidence of disease in a plot or field was well under 1%. We suspect the inoculum density and timing of rainfall events may have prevented wide-scale epidemics of FHB in North Carolina. Reduced tillage wheat production has not been widely adopted as of yet in the state, and the relatively warm winters accelerate degradation of any surface debris after conventional tillage. We believe these factors have kept the inoculum levels of *F. graminearum* at low enough levels to prevent an epidemic.

Data presented here indicate the presence of a pathogen population that can generate high levels of FHB on susceptible cultivars of wheat, and members of this population are able to generate high levels of detrimental toxins. Data presented here on in vivo DON levels indicate plants with very low symptomatic expression of FHB can have detectable levels of in vivo DON. Based on these data, it may be advisable to periodically survey grain elevators for DON, even if epidemics have not occurred. The high levels of in vitro zearalenone produced by some isolates indicates further research on the levels of this toxin in harvested grain are warranted.

Further investigations into naturally occurring inoculum densities may be beneficial in determining factors that limit FHB epidemics in North Carolina. This information may be useful in determining the future threat of an epidemic in the state and allow researchers and extension personnel to address the problem in a timely manner.

Fig. 4. Cluster analysis of genetic polymorphism data produced by 70 isolates of *Fusarium* spp. using the Randomly Amplified Polymorphic DNA technique.

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