

# Relationship Between Take-all of Wheat and Rhizosphere pH in Soils Fertilized with Ammonium vs. Nitrate-Nitrogen

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## ABSTRACT

Take-all of wheat caused by *Ophiobolus graminis* was reduced by ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) supplemented with 2-chloro-6-(trichloromethyl) pyridine (N-Serve 24) to slow nitrification, but was severe with no added N, or with  $\text{Ca}(\text{NO}_3)_2$  at N rates equivalent to that supplied by  $\text{NH}_4\text{-N}$ . The addition of lime (CaO) negated control with  $\text{NH}_4\text{-N}$ .

The correlation between disease severity and bulk soil pH ( $\text{pH}_b$ ) was relatively poor. A higher correlation existed between disease severity and rhizosphere pH ( $\text{pH}_r$ ). The  $\text{pH}_r$  dropped with uptake of  $\text{NH}_4\text{-N}$  by roots, increased with uptake of  $\text{NO}_3\text{-N}$ , and remained generally unchanged

with no added N. Disease severity in nonsterile soil was progressively less as the  $\text{pH}_r$  decreased below 7.0 and was greatly reduced at  $\text{pH}_r$  values below 6.6. In comparable soil treated with methyl bromide, disease was controlled only when  $\text{pH}_r$  dropped below 5.0. Pathogen growth was nil in sterile and nonsterile soil at pH less than 5.0.

Reduced disease apparently resulted from direct inhibition of the pathogen at  $\text{pH}_r$  less than 5.0 and indirect inhibition (possibly a biological control) above 5.0. Best control in field plots occurred when  $(\text{NH}_4)_2\text{SO}_4$  was mixed into the tilled layer rather than broadcast.

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*Additional key words:* biological control, soil fumigation.

Take-all of wheat (*Triticum aestivum* L.), caused by *Ophiobolus graminis* Sacc., [= *Gaeumannomyces graminis* (Sacc.) v. Arx & Olivier var. *tritici* Walker (33)] has been controlled in field plots with soil applications of ammonium sulfate (17), particularly when applied in combination with 2-chloro-6-(trichloromethyl) pyridine (N-Serve) to slow or prevent nitrification. Ammonium sulfate had little or no effect on the disease if nitrification was largely completed by time of planting (16), and take-all was not reduced by soil applications of nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) (17). Ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) also suppresses *Ophiobolus*-patch of turf regardless of the combined anion (14).

Glynn (11) and Salt (26) obtained partial control of take-all with N, but in later experiments (12, 27) take-all increased with increased rates of N. Their first experiments were made with  $\text{NH}_4\text{-N}$ , and the latter with Nitrochalk (ammonium nitrate and limestone, 3:2; 20.5% N). Garrett (9) reduced take-all with N in the field but was unable to do so in greenhouse tests. He used  $\text{NO}_3\text{-N}$  in his greenhouse study and  $\text{NH}_4\text{-N}$  in the field study. It is expected that small concentrations of  $\text{NO}_3\text{-N}$  exist in  $\text{NH}_4\text{-N}$  fertilized soils, with the quantity of  $\text{NO}_3\text{-N}$  increasing as  $\text{NH}_4\text{-N}$  is oxidized. Hornby & Goring (15) have shown that  $\text{NH}_4 + \text{NO}_3\text{-N}$  are conducive to take-all control, but that individually they are not. Thus,

there is good evidence to support the form-of-N hypothesis of Huber et al. (17) namely, that  $\text{NH}_4\text{-N}$  reduces and  $\text{NO}_3\text{-N}$  increases take-all in the field. Thus far, however, there is no satisfactory explanation for the effect.

Absorption of  $\text{NH}_4\text{-N}$  by roots reduces the rhizosphere pH ( $\text{pH}_r$ ) whereas absorption of  $\text{NO}_3\text{-N}$  raises it, even in the absence of changes in the bulk soil pH ( $\text{pH}_b$ ) (6, 7, 21, 22, 24). This results from the electrochemical equilibrium maintained during absorption of cations and anions by roots (2, 18, 20, 22). Acidity, reduces take-all of wheat and *Ophiobolus*-patch of turf, and alkalinity favors these diseases (5, 10, 19, 34, 35). This paper provides evidence that the form-of-N effect on take-all of wheat is indeed a  $\text{pH}_r$  effect. A brief account of this work has been presented (28).

**MATERIALS AND METHODS.**—*Fertilizers.*—Calcium nitrate, ammonium nitrate, ammonium sulfate, ammonium phosphate, and urea were used as N sources for wheat. The granular fertilizers were passed through screens and fractions having approximately 0.5 mg N and 400 particles/g were used to ensure a uniform geometric distribution of fertilizer particles in soil, and a uniform N content per particle. Urea was foliarly applied to wheat. N-Serve 24 was applied (2% w/w based on N) directly to the fertilizer crystals (sand particles in the case of checks).

*Soils.*—A slightly alkaline Ritzville silt loam (RSL) from the surface 10 cm of a virgin clean-tilled, nonfertilized area [located at the Washington State University Dryland Research Unit, Lind (Adams County), Washington] was used for one soil. The second soil, an acid Puyallup fine sandy loam (PFSL), was collected from the surface 15-cm of an area with no recent history of cereal crops at the Western Washington Research and Extension Center, Puyallup (Pierce County), Washington. Each soil was thoroughly mixed and stored outdoors, and kept slightly moist, under plastic. Their respective properties were: saturated paste pH values (using 0.01 M CaCl<sub>2</sub>) of 7.1 and 5.6; 0.9 and 1.8% organic matter; 13.1 and 7.5 ppm P; 11 and 9 meq/100 g cation exchange capacity at pH 7; 95 and 71% base saturated; and exchangeable cation concentration of 6.4 and 4.5 ppm Ca, 2.4 and 1.5 ppm Mg, 1.2 and 0.4 ppm K, and 0.5 and 0.2 ppm Na.

Each soil was used in a nontreated state (native pH) or adjusted to a pH similar to that of the other, using dilute sulfuric acid for the RSL, or burned lime (CaO) for the PFSL. All soils were then kept moist for 7-14 days, remixed, and then each lot was divided further for fertilizer treatments with and without inoculum of *O. graminis*. Treble superphosphate or gypsum was blended with the soil to supply equivalent quantities of P and S to all fertilizer trials. Dried, rolled or whole oat culture media, colonized by *O. graminis*, was blended (0.25% w/w) with the soil or, in some cases, introduced as a point source of inoculum. In the field, the infested oat kernels were sown directly with the wheat seed.

*Measurement of rhizosphere and soil pH, and concentrations of NH<sub>4</sub>- and NO<sub>3</sub>-N.*—Roots were removed from the soil mass of a pot or field plot by gentle uplifting and shaking. Twenty-g samples of "bulk" soil were placed in plastic vials with tightly fitting caps, and stored moist for subsequent analysis. Several g of tightly adhering "rhizosphere" soil was shaken from the roots and stored in paper envelopes under high humidity. Roots were then washed and placed in plastic bags for disease assessment. All soil and plant materials were refrigerated (4 C) immediately after sampling. The pH<sub>T</sub> was generally measured the day of sampling, and pH<sub>B</sub> within one day of sampling. The bulk soil was then frozen until NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> analyses were made.

Measurements of soil pH were in 0.01 M CaCl<sub>2</sub> (29). This method of pH measurement is especially valuable, in view of recent evidence for salt accumulation in the rhizosphere (1,3,25). The pH<sub>B</sub> was measured in a saturated paste (using about 15 g soil) and the pH<sub>T</sub> in a 1:2 (v/v) suspension of soil in 0.01 M CaCl<sub>2</sub> (using 0.25 g soil). Reproducibility of the suspension pH method on 50 well-mixed soil samples ranging from pH 3.6 to 8.2, was nearly always ± 0.1 unit. The relationship of the pH measured by the two methods was [pH of the saturated paste] = 1.08 [pH of the suspension - 0.64], with a correlation coefficient (r) of 0.986. All suspension pH data were corrected to the saturated paste equivalent. The

rhizosphere soil suspensions were prepared and measured in a series of wells in a 25 X 25 X 300-mm block of Plexiglas. Each well was drilled slightly larger than, and then shaped to, a Corning combination electrode (No. 476051).

Equilibrium extracts of 1:10 suspensions of soil in 2N KCl were analyzed for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations by the method of Bremner (3).

*Pathogen growth and disease assessment.*—Unless stated otherwise, early disease readings were based on counts of lesions/root, with no distinction between seminal and crown roots. Later, when many lesions had coalesced, visual infection ratings were made on a 0-4 scale in which 0 = no infected roots; 1 = a few small lesions on few roots; 2 = multiple small lesions on few roots; 3 = multiple small to large lesions on most roots; and 4 = multiple large lesions on all roots. The percentage of blackened culms (stem bases) at later stages of plant growth and disease development was also determined.

**RESULTS.**—*Influence of form of N on soil and rhizosphere pH.*—The two soils at native and adjusted pH values were infested with oat inoculum of *O. graminis* (0.25% w/w), and either unamended (checks) or amended with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, or NH<sub>4</sub>NO<sub>3</sub> (each with N-Serve 24 added) or Ca(NO<sub>3</sub>)<sub>2</sub>; all at 0.1 g N/kg soil. The untreated soils were then dispensed into 15-cm diam plastic pots (1.5 kg soil/pot) seeded within 1 day with 'Idaed' spring wheat, and incubated in the greenhouse with a day-night temperature regime ranging about 22-10 C. The stand was thinned after emergence to 6-8 seedlings per pot. Each treatment was replicated five times in this experiment, and three times in a repeat study. Sufficient pots were prepared to provide sampling 3, 6, and 9 weeks after planting. The entire experiment was in duplication, one-half of the soil having been fumigated with methyl bromide prior to pH adjustment, fertilization, and inoculation; the other half left in the natural condition.

At three weeks pH<sub>T</sub> was lower than pH<sub>B</sub> in soils fertilized with NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and higher than pH<sub>B</sub> in soils fertilized with Ca(NO<sub>3</sub>)<sub>2</sub> (Table 1). The pH<sub>T</sub> was essentially the same as pH<sub>B</sub> in nonfertilized soils and in those fertilized with NH<sub>4</sub>NO<sub>3</sub>. The elevated pH<sub>T</sub> in NO<sub>3</sub>-fertilized soils persisted for 6 weeks while the depressed pH<sub>T</sub> in NH<sub>4</sub>-fertilized soils tended to return more nearly to that of the checks. Nevertheless, at 6 weeks, the difference in pH<sub>T</sub> in NO<sub>3</sub>- vs. NH<sub>4</sub>-fertilized soils was 1.5 units in PFSL. The difference in pH<sub>T</sub> in RSL was 1.2 units at 3 weeks, but only about 0.3 units at 6 weeks. The pH<sub>B</sub>, on the other hand, remained essentially unchanged or rose very slightly with both forms of N. The drop in pH<sub>B</sub> with NH<sub>4</sub>-N in the nonfumigated soil, but not in fumigated soil, also indicates that no nitrification occurred in the latter. Lime apparently buffered effectively against pH changes in the rhizosphere and bulk PFSL for all forms of N.

*Influence of rhizosphere pH on take-all.*—Take-all severity was correlated with pH<sub>B</sub> for both soils at all three sampling dates. The two soils differed however,

TABLE 1. Acidity of bulk soil (pH<sub>b</sub>) and rhizosphere soil (pH<sub>r</sub>) as influenced by wheat plant age, form of N, fumigation, and soil type

| pH adjustment                   | Fumigated with CH <sub>3</sub> Br | pH <sub>r</sub> and pH <sub>b</sub> <sup>a</sup> per nitrogen fertilization <sup>b</sup> and sampling at 3 versus 6 weeks after planting |                 |                 |                 |                                   |                 |                 |                 |                                 |                 |                 |                 |  |                 |                 |                 |   |                 |                 |                 |
|---------------------------------|-----------------------------------|--|-----------------|-----------------|-----------------|-----------------------------------|-----------------|-----------------|-----------------|---------------------------------|-----------------|-----------------|-----------------|--|-----------------|-----------------|-----------------|---|-----------------|-----------------|-----------------|
|                                 |                                   | Control  |                 |                 |                 | Ca(NO <sub>3</sub> ) <sub>2</sub> |                 |                 |                 | NH <sub>4</sub> NO <sub>3</sub> |                 |                 |                 | NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> |                 |                 |                 | (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> |                 |                 |                 |
|                                 |                                   | 3  |                 | 6               |                 | 3                                 |                 | 6               |                 | 3                               |                 | 6               |                 | 3  |                 | 6               |                 | 3   |                 | 6               |                 |
|                                 |                                   | pH <sub>r</sub>  | pH <sub>b</sub> | pH <sub>r</sub> | pH <sub>b</sub> | pH <sub>r</sub>                   | pH <sub>b</sub> | pH <sub>r</sub> | pH <sub>b</sub> | pH <sub>r</sub>                 | pH <sub>b</sub> | pH <sub>r</sub> | pH <sub>b</sub> | pH <sub>r</sub>                                | pH <sub>b</sub> | pH <sub>r</sub> | pH <sub>b</sub> | pH <sub>r</sub>                                 | pH <sub>b</sub> | pH <sub>r</sub> | pH <sub>b</sub> |
| <u>Puyallup fine sandy loam</u> |                                   |  |                 |                 |                 |                                   |                 |                 |                 |                                 |                 |                 |                 |  |                 |                 |                 |   |                 |                 |                 |
| None                            | +                                 | 5.5  | 5.1             | 5.4             | 5.0             | 6.0                               | 5.1             | 6.3             | 5.5             |                                 |                 |                 |                 |  |                 |                 |                 | 5.2   | 5.2             | 4.9             | 5.3             |
|                                 | -                                 | 5.3  | 5.2             | 5.6             | 5.4             | 5.7                               | 5.3             | 6.3             | 5.7             | 5.1                             | 5.2             | 5.5             | 5.5             | 4.4  | 5.0             | 4.9             | 5.0             | 4.3   | 5.0             | 4.8             | 4.9             |
| Lime                            | +                                 | 8.2  | 7.9             | 8.1             | 7.9             | 8.0                               | 7.9             | 8.1             | 8.0             |                                 |                 |                 |                 |  |                 |                 |                 | 7.9   | 8.0             | 8.1             | 8.0             |
|                                 | -                                 | 8.2  | 8.0             | 8.3             | 8.1             | 8.3                               | 8.0             | 8.3             | 8.1             | 8.2                             | 8.0             | 8.2             | 8.1             | 8.2  | 8.0             | 8.2             | 8.0             | 8.1   | 8.0             | 8.3             | 8.0             |
| <u>Ritzville silt loam</u>      |                                   |  |                 |                 |                 |                                   |                 |                 |                 |                                 |                 |                 |                 |  |                 |                 |                 |   |                 |                 |                 |
| None                            | +                                 | 7.4  | 7.4             | 7.3             | 7.3             | 7.6                               | 7.4             | 7.5             | 7.4             |                                 |                 |                 |                 |  |                 |                 |                 | 6.8   | 7.3             | 6.8             | 7.3             |
|                                 | -                                 | 7.3  | 7.1             | 7.4             | 7.5             | 7.4                               | 7.1             | 8.0             | 7.7             | 7.2                             | 7.1             | 8.0             | 7.5             | 6.0  | 6.7             | 7.5             | 6.9             | 6.2   | 6.7             | 7.7             | 7.0             |
| H <sub>2</sub> SO <sub>4</sub>  | +                                 | 5.4  | 5.0             | 5.2             | 5.1             | 6.0                               | 5.3             | 5.4             | 5.2             |                                 |                 |                 |                 |  |                 |                 |                 | 4.9   | 5.3             | 4.3             | 5.4             |
|                                 | -                                 | 5.0  | 5.2             | 5.2             | 5.5             | 5.8                               | 5.4             | 6.5             | 6.0             | 5.3                             | 5.4             | 6.2             | 5.9             | 4.7  | 5.3             | 5.3             | 5.6             | 4.5   | 5.3             | 5.6             | 5.8             |

<sup>a</sup> Each pH value is the average of four and five replicates for nonfumigated and fumigated soils, respectively. Values of pH<sub>r</sub> have been corrected to remove differences due to methods of measurement [pH<sub>sat'd. paste</sub> = 1.08(pH<sub>suspension</sub> - 0.64), except where free carbonates were present (limed Puyallup soil).

<sup>b</sup> 0.1 g N added/kg soil (equivalent to 224 kg N/ha soil). All NH<sub>4</sub>-containing fertilizers were treated with 2% (w/w, based on N) N-Serve 24. Fertilizers were mixed with soil immediately before planting to wheat.

in that  $pH_b$  6.0 was suppressive to disease in RSL, whereas a  $pH_b$  of 5.0 was needed to suppress disease in PFSL (Fig. 1-A). When the disease readings were plotted against  $pH_r$ , disease severity was progressively less as the  $pH_r$  dropped below 6.8 - 7.0 regardless of the form of N, soil type, or amendment. There was also a high correlation ( $r = 0.960$ ) between  $pH_r$  at 3 weeks and disease severity at 6 weeks. N-Serve 24 caused no detectable differences in take-all or plant growth if applied with  $Ca(NO_3)_2$  or with coarse sand checks.

Lesion numbers were only slightly affected by  $pH_r$ ; i.e., there were nearly as many lesions at low as at high  $pH_r$  at 3 weeks. The effect of  $pH_r$  was apparently on disease progression after lesion establishment. Consequently, the data given in this paper are for disease severity, and not disease incidence. Lesions on roots washed from  $NH_4$ -treated soils were smaller than those in  $NO_3$ -treated and nonfertilized soils. In addition, when roots grew through plant debris infested with *O. graminis*, the lesions were generally restricted to root segments within the inoculum in  $NH_4$ -N treated soil, but spread extensively away from the inoculum in  $NO_3$ -treated and nonfertilized soils.

The correlation between disease severity and  $pH_r$  was less striking in the repeat experiment in which the temperature was higher (30-18 C, day-night) but again,  $pH_r$  was the only parameter that correlated (1% level) with take-all. Disease ratings were slightly higher for a given  $pH_r$  value in soils at higher temperatures and the  $pH_r$  tended to be lower for a given treatment.

The correlation was particularly high between magnitude change in  $pH_r$  at 3 weeks and magnitude change in disease severity at 6 weeks. Thus, the extent of root damage for older plants appeared to depend largely upon the level of acidity in their rhizospheres at an earlier stage of growth. Absolute disease values with  $Ca(NO_3)_2$  and  $(NH_4)_2SO_4$  were variable for N rates of 25, 50, and 100 mg N/kg soil, but a highly significant relationship occurred between change in  $pH_r$  and change in disease severity for these rates and forms of N ( $r = 0.854$ ). Thus, increasing rates of  $NH_4$ -N caused increasingly larger reductions in both  $pH_r$  and disease rating.

In methyl bromide-fumigated soils, extreme acidity in the rhizosphere ( $pH_r$  less than 4.9) was the only factor associated with reduced take-all severity. All plants of treatments with  $pH_r$  greater than 5.0 (Table 1) showed severe disease by 6 weeks. Disease was reduced in the acidified RSL fertilized with  $(NH_4)_2SO_4$  where the  $pH_r$  dropped to 4.9 and 4.3 after 3 and 6 weeks respectively (Table 1). Noninoculated control plants grew as well or better in the fumigated soil, compared with those in nonfumigated soil.

*Influence of pH on pathogen growth on sterile wheat straw.*—The influence of pH and form of N-source on growth of runner hyphae of *O. graminis* was measured on sterile straws inoculated with the pathogen and buried in soil at a soil water potential of approximately -1 bar, using the method of Cook

et al. (4). The sterile soils were amended aseptically with sufficient dilute  $H_2SO_4$  or KOH to adjust pH over the range 3.5 - 8.2. Duplicate series were prepared with sufficient  $(NH_4)_2SO_4$  added to one, and  $Ca(NO_3)_2$  to the other, to supply 0.1 g N/kg soil. Controls received no N. The soil with adjusted pH

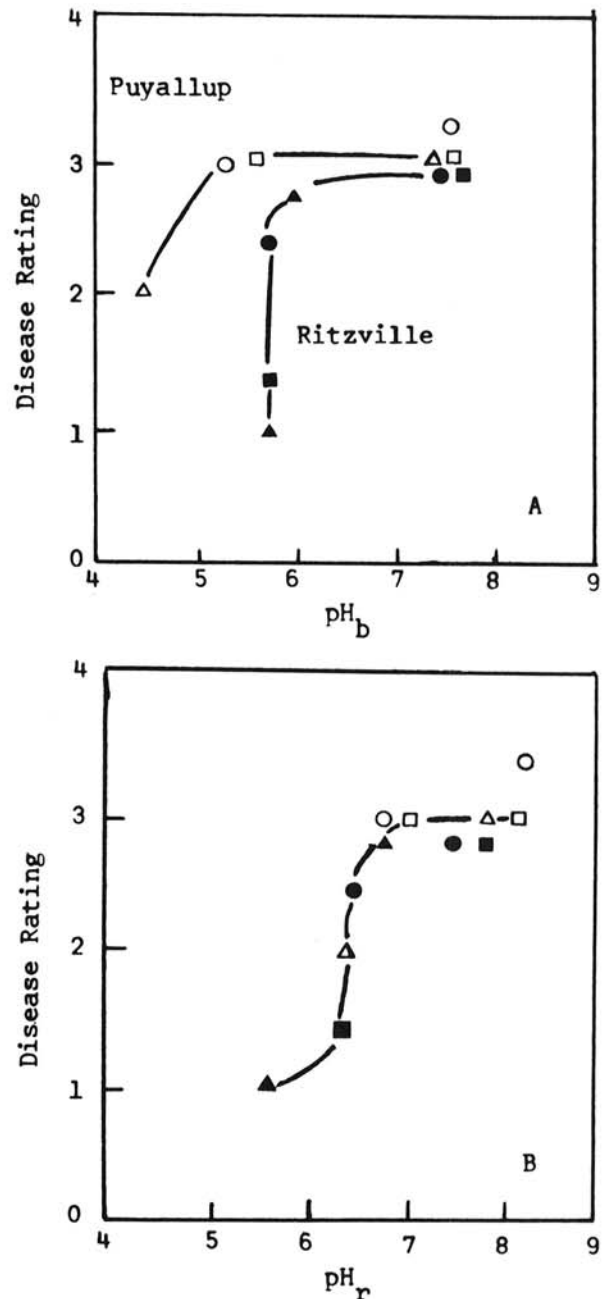


Fig. 1. Relationship between take-all severity (0-4 scale) and A) bulk soil pH ( $pH_b$ ) and B) rhizosphere soil pH ( $pH_r$ ) 3 weeks after planting in  $NH_4$ -N- and  $NO_3$ -N-treated soils. Puyallup (open symbols) and Ritzville (solid symbols) soils were treated with no added N (●),  $Ca(NO_3)_2$  (■), or  $(NH_4)_2SO_4$  (▲), and were incubated at 10-22 C.

was stored at 4 C for several days and remixed periodically during storage. Soil pH measurements (saturated paste) were made before straws were placed in soil, and again after their removal, to insure that the  $\text{pH}_b$  remained within 0.3 units of the original value.

Hyphal growth was greatest at the higher soil pH values (Fig. 2-A). None was detected on the straw at soil pH values less than 4.8. Differences in growth were apparent between PFSL and RSL, but reasons for this were not determined.

*Influence of pH on pathogen growth on living wheat roots.*—The influence of form of N and pH on growth of runner hyphae of *O. graminis* was also studied on roots of wheat seedlings (5 plants/pot) growing in nonsterile soil (1.5 kg soil/pot). Inoculum of *O. graminis* was supplied by mycelium in a 5-mm diam agar disk from a 10-day PDA culture (incubated at 20 C) placed 1-2 cm beneath the seeds at the time of planting. Roots were removed from soil at 18 days and soil samples were retained for measurement of  $\text{pH}_b$ ,  $\text{pH}_r$ , and concentration of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The roots were then soaked and rinsed in water, killed by sterilization with propylene oxide, and stored at 4 C. Lineal extension of runner hyphae was measured using a technique developed by Garrett (8). Each seminal root was severed at the seed and at the

hypha terminus, and the section of root then measured. Three replicates of three roots/plant and three plants/treatment were measured for each of four soils (RSL, acidified RSL, PFSL, and limed PFSL), two temperatures (15 and 24 C), and three fertilizer treatments (no N,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ ).

Lineal extension of runner hyphae of *O. graminis* on wheat roots growing during the 18 days of the experiment was retarded at  $\text{pH}_r$  less than 5.0 (Fig. 2-B). There was no influence of soil type, amount (or form) of N, or soil temperature on growth of the fungus. The plot of data for hyphal growth vs.  $\text{pH}_b$  (not shown) was much more irregular than that shown here for hyphal growth vs.  $\text{pH}_r$ . The partial pressure of  $\text{CO}_2$  in the bulk soil air in this experiment was always less than the detection limit of 0.002 atm as measured by gas chromatography.

*Fertilizer placement and localization of N-form effect.*—Tests were made without the pathogen to determine whether the form of N near one portion of a root system, or N applied foliarly, influenced  $\text{pH}_r$  in another region of the same root system. Each soil was amended with 0.1 g N/kg soil as  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{NH}_4\text{NO}_3$ , and  $(\text{NH}_4)_2\text{SO}_4$ , or with equivalent mass of sand, all treated with N-Serve 24 at 2% (w/w) of the N rate (or a similar quantity on sand). Soils were contained in pairs of  $8 \times 8 \times 10$ -cm plastic pots, one unseeded and one seeded to two *Idaed* spring wheat plants. The pairs were arranged one on top of the other (the bottom of the top pot was removed to allow full contact of soil) as follows: fertilized soil over fertilized soil, fertilized over control, and control over fertilized. Measurements of  $\text{pH}_b$  and  $\text{pH}_r$  were made, with separate  $\text{pH}_r$  measurement being made for root segments in the upper and lower pots.

The  $\text{pH}_b$  was not affected by  $\text{Ca}(\text{NO}_3)_2$ , but was decreased by  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  (Table 2). There was no evidence for a transfer of the N-form effect on  $\text{pH}_r$  along the root. The  $\text{pH}_r$  of soil from younger root segments equaled or exceeded that from older roots. The  $\text{pH}_r$  and  $\text{pH}_b$  values differed only slightly in soils treated with  $\text{NH}_4\text{NO}_3$  and  $\text{Ca}(\text{NO}_3)_2$ , but reductions of up to 1.5 and 1.0 units, respectively, occurred in  $(\text{NH}_4)_2\text{SO}_4$ -treated soil.

In another study, 10-gal cans were filled with 35 kg of RSL and *O. graminis* inoculum was blended with the surface 15 cm. Triplicate series were prepared in which  $\text{NH}_4\text{NO}_3$  (67 or 134 mg N/kg soil) was mixed either into the surface 15-cm or into the subsurface 20 cm of soil in the following arrangements: fertilized soil over control, control over fertilized, and fertilized or control soil throughout. Twenty *Idaed* wheat plants were grown in each can. Another treatment consisted of foliarly applying urea to wheat (applied on alternate days as 50 ml of aqueous mist, 0.5 g N/liter) growing in nonfertilized soil which was covered during the spraying operation. Take-all incidence and severity was measured on the mature wheat plants and  $\text{pH}_b$  measurements were also made at that time.

Application of  $\text{NH}_4\text{NO}_3$  markedly reduced the  $\text{pH}_b$  but only in the layer to which it was applied. Infection ratings were reduced when N was placed in

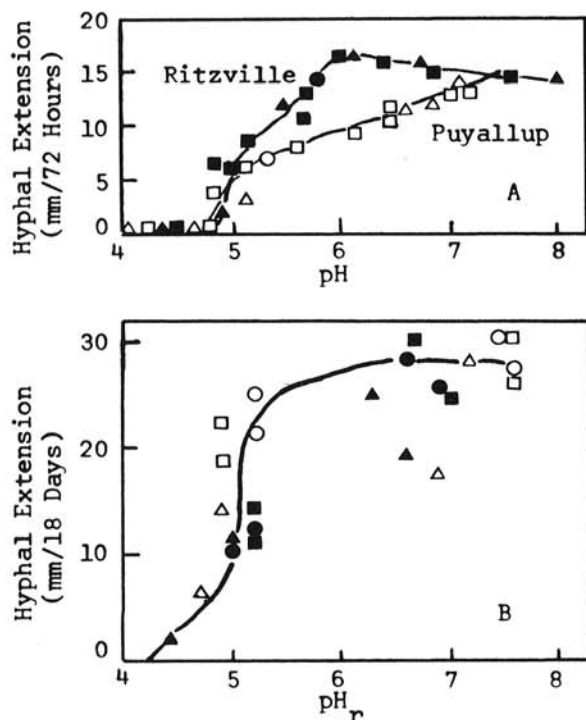


Fig. 2. Growth of *Ophiobolus graminis* hyphae A) on sterile wheat straw in sterile fertilized soils at 20 C; and B) on wheat roots in nonsterile, fertilized soils at 24-15 C day-night temperature in Puyallup (open symbols) and Ritzville (solid symbols) soils which were treated with no added N (●), or with  $(\text{NH}_4)_2\text{SO}_4$  (▲),  $\text{Ca}(\text{NO}_3)_2$  (■) at the rate of 0.1 g N/kg soil.

the surface 15-cm of soil; i.e., when it came in contact with the inoculum, but not when roots were supplied with adequate N at a point distant from the infection court. At surface soil  $pH_b$  values of 7.1, 6.7, 5.5, and 4.1, the respective infection ratings were 2.5, 1.0, 0.6, and 0.3. Neither the incidence nor severity of take-all was reduced when urea was foliarly applied to plants throughout the growing season.

*Field studies.*—Winter wheat plots were established in the fall of 1970 on acid soil (5.0-5.5) at Puyallup, Washington where the humid mild climate is favorable to take-all. Treatments included broadcast applications of  $Ca(NO_3)_2$  and  $(NH_4)_2SO_4$  + N-Serve 24 at rates of 90 kg N/ha on areas limed (90 kg  $CaCO_3$  per 100  $m^2$ ), fumigated (400 g methyl bromide/10  $m^2$ ), limed + fumigated, and nontreated. The lime was applied first and rototilled to 15 cm

depth and then the fumigant was applied under a plastic tarp. Fertilization and seeding was done about 3 weeks after fumigation. All fertilizers were mixed into the surface 5-cm of soil by hand raking. Supplements of  $CaSO_4$  were applied to the  $Ca(NO_3)_2$  treatments to supply sulfur at a rate equivalent to that supplied by the  $(NH_4)_2SO_4$ . Seeding was with 'Nugaines' wheat mixed in the drillbox 2:1 (w/w) with oat kernel inoculum of *O. graminis*. Individual plot size was about 1.5 m X 2.75 m in each of five reps.

Applications of  $NH_4$ -N resulted in a lower percentage of infected roots, and ultimate disease severity ratings (0-5 basis) were about one-half those in soil fertilized with  $NO_3$ -N (Table 3). The  $pH_T$  of these two treatments, respectively, was consistently below 5.0, where take-all was controlled, and above

TABLE 2. Rhizosphere soil pH ( $pH_T$ ) of 5-week-old wheat plants grown in fertilized and nonfertilized soils in pots stacked in various arrangements, one upon the other

| Fertilizer                      | $pH_T$ per treatment arrangement |                            |       |                         |       |                         |       |                    |
|---------------------------------|----------------------------------|----------------------------|-------|-------------------------|-------|-------------------------|-------|--------------------|
|                                 | Fertilized Soil                  | Fertilized over fertilized |       | Fertilized over control |       | Control over fertilized |       | Noncropped Soil pH |
| <u>Puyallup fine sandy loam</u> |                                  |                            |       |                         |       |                         |       |                    |
|                                 |                                  | upper                      | lower | upper                   | lower | upper                   | lower |                    |
| Control                         | 6.2                              | 5.5                        | 6.1   | 5.4                     | 6.0   | 5.7                     | 6.3   | 5.6                |
| $NH_4NO_3$                      | 5.8                              | 5.6                        | 6.2   | 5.6                     | 5.5   | 5.4                     | 5.6   | 5.4                |
| $Ca(NO_3)_2$                    | 6.0                              | 5.6                        | 5.9   | 5.4                     | 5.7   | 5.5                     | 6.0   | 5.6                |
| $(NH_4)_2SO_4$                  | 4.9                              | 4.8                        | 5.5   | 4.9                     | 5.9   | 5.9                     | 4.8   | 5.1                |
| <u>Ritzville silt loam</u>      |                                  |                            |       |                         |       |                         |       |                    |
| Control                         | 7.5                              | 7.5                        | 7.3   | 7.7                     | 7.3   | 7.4                     | 7.5   | 7.6                |
| $NH_4NO_3$                      | 7.4                              | 7.4                        | 7.4   | 7.3                     | 7.3   | 7.5                     | 7.5   | 7.1                |
| $Ca(NO_3)_2$                    | 8.2                              | 7.5                        | 7.6   | 7.5                     | 7.5   | 7.4                     | 7.7   | 7.6                |
| $(NH_4)_2SO_4$                  | 6.7                              | 6.7                        | 6.8   | 6.2                     | 7.6   | 7.4                     | 6.5   | 6.6                |

TABLE 3. Take-all severity, rhizosphere soil pH ( $pH_T$ ) and bulk soil pH ( $pH_b$ ) in field plots (Puyallup, 1970-71) fertilized with ammonium sulfate, calcium nitrate, or no N, and in the nontreated native state, fumigated with methyl bromide, limed, or fumigated and limed

| Soil treatment      | Fertilizer <sup>a</sup> | pH   |      | pH   |      | Disease <sup>b</sup> |      |       |
|---------------------|-------------------------|------|------|------|------|----------------------|------|-------|
|                     |                         | Nov. | Apr. | Nov. | Apr. | Nov.                 | Apr. | June  |
| None                | None                    | 5.0  | 5.5  | 5.5  | 5.3  | %                    | %    | Index |
|                     | $NO_3$ -N               | 4.7  | 5.6  | 5.8  | 5.1  | 26                   | 46   | 3.6   |
|                     | $NH_4$ -N               | 4.7  | 4.8  | 4.6  | 4.6  | 35                   | 47   | 4.2   |
| Fumigated           | None                    | 4.8  | 5.4  | 5.2  | 5.3  | 15                   | 27   | 1.8   |
|                     | $NO_3$ -N               | 4.7  | 5.5  | 5.1  | 5.2  | 20                   | 75   | 3.6   |
|                     | $NH_4$ -N               | 4.8  | 5.2  | 4.8  | 5.1  | 34                   | 74   | 3.8   |
| Limed               | None                    | 6.4  | 7.1  | 7.0  | 7.1  | 36                   | 72   | 2.4   |
|                     | $NO_3$ -N               | 6.3  | 7.1  | 6.5  | 7.3  | 20                   | 67   | 4.8   |
|                     | $NH_4$ -N               | 6.7  | 7.0  | 6.3  | 7.2  | 22                   | 68   | 4.8   |
| Limed and fumigated | None                    | 6.3  | 7.1  | 7.0  | 6.8  | 23                   | 65   | 4.0   |
|                     | $NO_3$ -N               | 6.6  | 7.0  | 6.7  | 7.3  | 36                   | 53   | 4.4   |
|                     | $NH_4$ -N               | 6.5  | 6.9  | 6.7  | 6.8  | 30                   | 56   | 5.0   |

<sup>a</sup> Fertilizers applied at 90 kg N/ha, mixed into the surface 5-cm of soil.  $NH_4$ -N treated with N-Serve 24 (1% w/w based on N).

<sup>b</sup> Based on percentage infected roots in Nov. and Apr. and disease index (0-5) in June.

6.0 where take-all was not controlled. Control was best in the nonfumigated, nonlimed, soil. Liming in particular, and fumigation to some extent, tended to negate the control with  $\text{NH}_4\text{-N}$  in natural soil. As with greenhouse studies, some control was associated with reduced  $\text{pH}_b$ , but the best correlation was between disease rating and  $\text{pH}_r$  (Fig. 3).

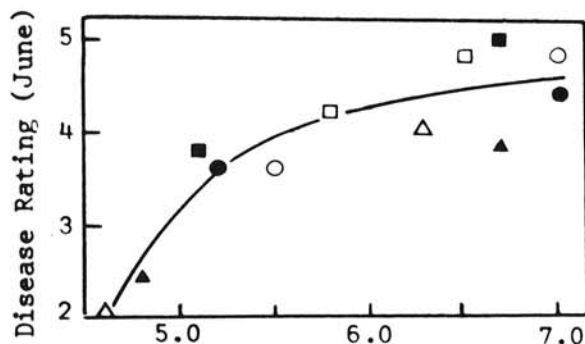


Fig. 3. Take-all severity (0-5 rating) in June as related to rhizosphere soil pH ( $\text{pH}_r$ ), measured the previous November in field plots at Puyallup, Washington (1970-1971), fertilized with calcium nitrate (■), ammonium sulfate (▲), or no fertilizer (●); with no previous treatment (open symbols) or fumigated (methyl bromide) or limed (solid symbols).

After harvest, the plot area was prepared for a second seeding in the fall of 1971. Fertilizer treatments included  $(\text{NH}_4)_2\text{HPO}_4$  and  $(\text{NH}_4)_2\text{SO}_4$  mixed into the surface 15 cm with the final preplanting tillage operation, and  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{Ca}(\text{NO}_3)_2$  as preplant broadcast applications just prior to seeding. Each fertilizer was applied to provide about 110 kg/ha. The  $\text{Ca}(\text{NO}_3)_2$  was applied at 33 kg N/ha in the fall before planting and 67 kg N/ha in the spring, to minimize loss to the crop from winter leaching. Each plot received magnesium oxide, magnesium sulfate, and triple superphosphate to insure a balanced nutrition for the crop. Nugaines wheat was again planted, but this time without additional inoculum.

Ammonium-N again resulted in reduced numbers of infected plants in nonlimed soil, especially where the fertilizer was mixed into the soil rather than broadcast. Disease reduction was less in soil that had been fumigated one year earlier.

*Influence of N-form on root growth.*—Healthy wheat plants in noninoculated soil produced more roots (seminal plus coronal roots) in nonamended, fertilized soil at 6 weeks (9-11 roots) than in nonfertilized soil (8-9 roots). Inoculated plants tended to produce slightly more roots (average 9.2) than healthy plants (average 8.6). In all cases, more roots were produced in  $\text{Ca}(\text{NO}_3)_2$ -treated soil than in  $(\text{NH}_4)_2\text{SO}_4$ -treated soil, although differences averaged less than one root per plant. Differences in number of roots represented differences in numbers of coronal roots. Assessments at 9 weeks indicated

considerable root pruning on diseased plants (average reduced to 6.2).

**DISCUSSION.**—These results confirm the observations of others (5, 19, 34, 35) that *O. graminis* pathogenesis is greatest in an alkaline environment. In addition, it would now appear that the form-of-N effect reported by Huber et al. (17) for take-all is also a pH effect, with  $\text{NH}_4\text{-N}$  reducing disease through rhizosphere acidity, and  $\text{NO}_3\text{-N}$  increasing disease severity by making the rhizosphere more alkaline.

The uniform severity of disease at all  $\text{pH}_r$  above 5.0 in the fumigated treatments, suggests that control may operate directly on the fungus at  $\text{pH}_r$  below 5.0, and indirectly, perhaps through the aid of antagonistic microorganisms, at  $\text{pH}_r$  between 5.0 and 6.6. Growth of the pathogen decreased progressively as pH values dropped below the optimum of 6-7. This reduced vigor of the fungus would reduce its inoculum potential (*sensu* Garrett); i.e.; its capacity for rapid growth and tolerance of antagonism. On the other hand, where the resident rhizosphere microflora was altered by soil fumigation, growth of *O. graminis* was probably reduced at the lower pH values, but still could incite severe infection as long as growth was not prevented entirely. This suggests that manipulation of the environment at the infection court by the form of N absorbed by wheat roots has potential for biological control of take-all, by shifting the balance of active organisms to the detriment of *O. graminis*. Studies on the microbiological aspects of this hypothesis are in progress.

Reduced  $\text{pH}_r$  in this study was induced by a high  $\text{NH}_4\text{:NO}_3$  ratio in soil rather than by  $\text{NH}_4\text{-N}$  alone. Commercially available fertilizer particles were treated with N-Serve and mixed with nonleached soils to simulate field practices. Ammonium-N alone, predisposes wheat to severe take-all, at least when it is mixed as a solution into magnesium-deficient acid soils that were leached to remove all  $\text{NO}_3\text{-N}$  and then treated uniformly with N-Serve (15). This may relate to the impairment of root metabolism that is inherent in plants grown for prolonged periods on  $\text{NH}_4\text{-N}$  alone (30). Plants grown on  $\text{NO}_3\text{-N}$  were also more subject to take-all than plants grown with  $(\text{NH}_4 + \text{NO}_3)\text{-N}$ . Thiels (31) has suggested that  $\text{NH}_4\text{-N}$  toxicity is most likely to occur in acid soils that are devoid of  $\text{NO}_3\text{-N}$ , and that toxicity is reduced or eliminated in the presence of even a small quantity of  $\text{NO}_3\text{-N}$ . Results presented here; e.g., suppression of take-all by  $\text{NH}_4\text{-N}$ , are compatible with those of Hornby & Goring (15).

Host resistance was not an important factor in the form-of-N effect since control was not attained in fumigated soil treated with  $\text{NH}_4\text{-N}$  (except directly at  $\text{pH}_r$  less than 4.9), and disease control attained by  $\text{NH}_4\text{-N}$  in nonfumigated soil was largely negated by the addition of lime. In addition,  $\text{NO}_3\text{-N}$  was more favorable to wheat growth than was  $\text{NH}_4\text{-N}$  in noninoculated soils, and foliarly applied urea and placement of N at a point distant from the infection court failed to reduce the incidence or severity of take-all.

Lack of definite correlation between take-all and

soil pH in the past may relate to the fact that  $pH_b$  rather than  $pH_r$  has been measured. In this study, the  $pH_r$  was the only accurate method for assessing the influence of pH on parasitic activity of *O. graminis*. Reductions in  $pH_r$  resulted largely from  $NH_4-N$  absorption by roots and not from nitrification in the rhizosphere. Thus, the  $pH_r$  is not directly related to the  $pH_b$  and measurements of  $pH_b$  could not be used to predict  $pH_r$ . Differences in  $pH_b$  and  $pH_r$  were as large as 0.8 units and were largest with young roots growing in moderately acid, coarse-textured soil (PFSL) to which most N was supplied either as  $NH_4-N$  or  $NO_3-N$  rather than as a combination of the two ( $NH_4NO_3$ ). Differences between  $pH_b$  and  $pH_r$  decreased with time in soil treated with  $NH_4-N$  because of nitrification which increased  $pH_r$  and decreased  $pH_b$ . The differences in  $pH_r$  up to 1.5 units between soils treated with  $NH_4-N$  and  $NO_3-N$  were shown both in fumigated and nonfumigated soil, regardless of whether or not the  $pH_b$  changed.

The  $pH_r$  measurements obtained are probably conservative estimates since a composite of rhizosphere soil was used. The values measured were with a free carbonate phase at equilibrium with  $CO_2$  in the atmosphere (0.03%) during measurement. One would anticipate higher  $CO_2$  concentrations in soil and especially in the rhizosphere; thus, all  $pH_r$  values in Table 1 should probably be lower than given (29). Notwithstanding the  $CO_2$  effect, however, the most extreme  $pH_r$  differences (1.5 units) due to N-form are probably less than those actually affecting growth of *O. graminis* on the root surface. Our readings are probably representative of some point in a gradient between pH on the rhizoplane and  $pH_b$ . Breakage of root hairs and subsequent release of cellular sap into the soil is another source of error in rhizosphere samples; with a resultant buffering of the  $pH_r$  toward the internal wheat root pH of 6.2 to 6.5. Nevertheless, even these crude values seem more realistic than  $pH_b$ , as evidenced by their higher correlations with disease severity.

The dependence on soil microorganisms to assist in disease reduction below  $pH_r$  values of 6 to 7 suggests that this control will be highly variable depending on other environmental factors that affect the soil microbiota. Such variability is indicated by the fact that control in the greenhouse was detectable as the  $pH_r$  dropped below 6.6, whereas in the field no control occurred unless  $pH_r$  was below 5.0. This variability and dependence on second- and perhaps third-order effects may explain why some of our best correlations were between magnitude of disease reduction and magnitude of change in  $pH_r$ . In fertilizer trials with turf, Goss & Gould (13), reported a weak correlation between *Ophiobolus*-patch and soil pH, but reevaluation of their data similarly indicates a strong relationship between change in numbers of patches over a 4-year period vs. change in soil pH. The rate and magnitude of pH change has also been reported to exert a greater influence on certain soil microorganisms (32) and on plant growth (23) than either the initial or final pH. It seems reasonable that the greater the magnitude of change

in environment, the greater the impact of that change on the established biological balance, and on *Ophiobolus graminis*.

Biological control of take-all via selective  $NH_4-N$  fertilization is expectedly less pronounced and dependable in the field than in the greenhouse due to poorer control of nitrification and to less uniformity in fertilizer particle distribution, among other factors. However, control by  $NH_4-N$  may still hold potential since the present study was instigated because of field observations (14, 16, 17). Apparently, one can expect best results where  $NH_4^+$  is uniformly and thoroughly distributed through the infested layer rather than broadcast or placed between the rows, or in soil too deep beneath the seed zone.

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