Influence of Form of Nitrogen Fertiliser on Infection of Annual Ryegrass by Conidia of *Gloeotinia temulenta*

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ABSTRACT

The effect of form of nitrogen fertiliser, including calcium nitrate, ammonium nitrate, ammonium chloride, ammonium sulphate, and urea+DCD, on infection of annual ryegrass by conidia of *Gloeotinia temulenta* was examined in field plots in 1991 and 1992. One to three percent of ryegrass seeds were infected in plots sprayed with conidial inoculum and 0% in plots sprayed with only water. Significant differences among N treatments were not detected. In greenhouse studies, level of infection among plants fertilised with various forms of N were not significantly different. Conidial germination and length of germ tubes were greater in solutions amended with calcium nitrate than in solutions amended with ammonium forms of nitrogen.

***Additional index words*: blind seed disease, *Gloeotinia granigena*.**

INTRODUCTION

Gloeotinia temulenta Wilson, Noble, and Gray (=*Gloeotinia granigena* (Quelet) Schumacher), causal agent of blind seed disease, is a flower infecting fungal pathogen of forage grasses (Hardison, 1962), especially perennial ryegrass and tall fescue (Alderman, 1991). The fungus overwinters as mycelium in infected seed. Apothecia are produced in the spring and release of ascospores coincides with flowering in the grasses. Conidia are produced about 10 days after infection and serve as secondary inoculum (Wilson, Noble and Gray, 1945).

Blind seed was a serious problem in the US during the late 1930's and early 1940's, until the discovery that post harvest field burning could control the disease (Hardison, 1957). In 1991, state legislation was passed to phase out field burning in Oregon by 1997. This has raised concerns over the possible re–occurrence of the disease.

In New Zealand blind seed was a widespread problem, but during the 1960's the disease declined to the point where pre–harvest testing for the pathogen was discontinued (Scott, 1974). Hampton and Scott (1980a, 1980b, 1981) extensively reviewed and summarised the literature relating nitrogen fertility to blind seed disease and established an association between the application or increasing use of nitrogen fertilisers and reductions in blind seed disease. They demonstrated a reduction in blind seed in response to nitrogen fertiliser and suggested that nitrogen enhanced resistance of perennial ryegrass to infection by *G. temulenta*.

Nitrogen is an important component in the management of grasses for seed. Understanding the effect of nitrogen on various components of the life cycle of *G. temulenta* could be important in understanding the impact of nitrogen on blind seed development. Several forms of N are commercially available, although it is not clear if form of nitrogen would differentially affect blind seed development. Hampton and Scott (1980b) found urea was more effective than ammonium sulphate in reducing blind seed incidence.

Research is needed to determine if form of nitrogen would differentially affect secondary spread of *G. temulenta* in annual ryegrass. The objectives of this study were to 1) determine the effect of form of nitrogen on infection of annual ryegrass by conidia of *G. temulenta* under field conditions, 2) evaluate the effect of form and concentration of nitrogen on blind seed disease under controlled conditions in a greenhouse, and 3) determine if form or concentration of nitrogen differentially affects conidial germination of *G. temulenta* in vitro.

MATERIALS AND METHODS

Effect of nitrogen on blind seed disease under field conditions

Field plots (6m by 6m) of annual ryegrass (*Lolium multiflorum* cv. Marshall) were established at the Hyslop Experimental Farm near Corvallis, OR during September, 1991 and 1992. The plots were arranged in a randomised block design with four replications. Treatments included calcium nitrate ($Ca(NO_3)_2$), ammonium nitrate (NH_4NO_3), ammonium sulphate ($(NH_4)_2SO_4$), ammonium chloride (NH_4Cl), and urea + dicyandiamide (urea+DCD). Fertiliser was surface broadcast in the autumn at 22.4 kg Nha^{-1} and at 37.6 kg Nha^{-1} in the spring. A duplicate set of plots were established as non–inoculated controls.

The centre 3m by 3m area within each field plot was inoculated by applying a conidial suspension (1 x $10^5 ml^{-1}$, 250 ml per plot) twice weekly during the flowering period with a portable sprayer (6 applications in each year). Inoculum was prepared from ryegrass plants grown in a greenhouse and inoculated 12–20 days before conidial harvest. Germination of conidia used as inoculum was 90% or greater on glass slides incubated at 20°C for 24–48 hours.

At harvest, the centre 3m x 3m area of each plot was cut by hand and threshed in a laboratory scale scarifier (Kamus

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Westrup model LA–H). Germination was evaluated as outlined in the OASA Rules for Testing Seeds (Copeland, 1981). Seed infections were determined based on a well plate assay. For each of four replicate groups of 96 seeds, seeds were placed in wells of 96–well plates (one seed per well) and to each well was added 200 µl water. After 2 hour incubation at room temperature, wells were examined for a pinkish deposit (conidia and slime) characteristic of infection by *G. temulenta*. Microscopic examination confirmed the presence of conidia of *G. temulenta*. The percentage of infected seeds was determined.

Effect of nitrogen on blind seed disease under greenhouse conditions

In all greenhouse studies annual ryegrass, cv. Marshall, was planted in a non–fertilised greenhouse potting mix. Beginning one week after emergence half–strength Hoagland's solution (Dingra and Sinclair, 1987) amended with 40 mM calcium nitrate, ammonium nitrate, ammonium sulphate, or ammonium chloride was applied to saturation twice weekly. At 20% flowering, seed heads were inoculated by dipping each head in a 100 ml graduated cylinder containing a suspension of 5 x 10^4 conidia ml^{-1}. A corresponding set of non– inoculated control treatments were also included. Three weeks after inoculation, seeds from each of eight replicate seed heads per treatment were placed individually in wells of 96 well plates and covered with 200 µl water. After 2 hours incubation at room temperature, wells were examined for a pinkish deposit, as described above. The percent of infected seeds per head was determined.

To determine the effect of N concentration on blind seed incidence, annual ryegrass plants were fertilised twice weekly with one half strength Hoagland's solution amended with ammonium sulphate at 20, 40, or 80 mM N. Plants were inoculated at 20% flowering by dipping heads in a 100 ml cylinder containing 1 x 10^5 conidia per ml and 2 drops Tween 20. After three weeks the number of infected blind seeds in each of eight replicate seed heads was determined.

Effect of nitrogen on In–vitro germination and growth

To determine the effect of N on conidial germination and germ tube growth 2 ml aliquots of a 2 x 10^5 conidial suspension were mixed with equal volumes of N solutions, including NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3 or $Ca(NO_3)_2$, to achieve a conidial concentration of 1 x 10^5 at N concentrations of 3, 6, 12, 24, or 48 mM. Four replicate 5 ml drops were placed on a 22 mm square cover glass and incubated in a covered petri dish with saturated tissue at 20°C. After 24 hr, 2 ml of 2% aequeous $CuSO_4$. $5H_20$ was added to each drop to prevent further germination or growth. Conidia were observed under a microscope at 150 X. Germination was based on the first 50 conidia encountered. Germ tubes of the first 20 germinated conidia encountered were measured with an ocular reticule (calibrated against a stage micrometer). All experiments were repeated at least once.

RESULTS

In 1991 and 1992 annual ryegrass seed germination from plots inoculated with *G. temulenta* was 92–95% (Table 1). Germination in non–inoculated plots was 99% or greater in 1991 and 97% or greater in 1992. Percent seed infected with *G. temulenta* among nitrogen treatments ranged from 1.7 to 2.5 in 1991 and 0.2 to 1.7 in 1992 with no significant differences among treatments (Table 1). Blind seed was not detected in any of the non– inoculated control plots.

Table 1. Percent germination of annual ryegrass and percentage of seed infected by *Gloeotinia temulenta* under various nitrogen treatments in 1991 and 1992.

Nitrogen source	1991 Germination (%)	1991 Infection (%)	1992 Germination (%)	1992 Infection (%)
$Ca(NO_3)_2$	95.0±1.8[1]	2.5±0.8[2]	95.3±2.1	1.0±0.6
NH_4C1	94.4±1.7	1.7±0.6	94.3±0.7	0.7±0.8
NH_4NO_3	92.7±3.0	2.5±0.8	95.3±8.5	1.2±0.7
$(NH_4)_2SO_4$	92.9±3.1	2.0±2.0	93.2±1.8	1.1±0.5
Urea	95.1±1.6	2.0±2.0	92.3±1.6	1.0±0.5

[1] *Mean and standard deviation based on four 100–seed samples.*
[2] *Mean and standard deviation based on four 384–seed samples.*

Blind seed incidence among plants treated with various forms of N under greenhouse conditions ranged from 28 to 33 percent infected seeds per head (data not presented). No significant differences among nitrogen treatments were detected. Similarly, no significant differences in blind seed were detected among plants treated with varying concentrations of nitrogen. Percent ± standard deviation blind seed among plants fertilised with 20, 40, and 80 mM N were 38±17, 29±18, and 33±13, respectively. Blind seed was not present in the non–inoculated controls.

Germination of conidia ranged from 82 to 96% in the presence of calcium nitrate and from 4 to 34% in the presence of ammonium forms of N. (Table 2). Germ tube length ranged from 3.9 to 7.6 µm in the presence of calcium nitrate and from 1 to 2.6 µm in the presence of ammonium forms of N (Table 3). In the presence of calcium nitrate, length of germ tubes decreased with increasing concentration of N from 3 to 48 mM.

Table 2. Effect of form of N on percentage germination of *Gloeotinia temulenta*.

Nitrogen source	Concentration (mM) 3	6	12	24	48
$Ca(NO_3)_2$	96.0±1.4[1]	91.0±3.0	82.5±3.0	86.0±4.2	96.5±0.9
NH_4Cl	13.5±2.5	10.5±0.9	4.0±1.6	14.5±14.1	25.0±4.4
NH_4NO_3	34.0±5.1	11.5±1.7	7.0±3.6	3.5±0.9	9.0±1.7
$(NH_4)_2SO_4$	30.0±10.0	8.0±1.4	12.5±5.4	20.0±3.7	25.5±2.6

[1] *Mean and standard deviation based on 50 conidia in each of four replicate drops. Mean and standard deviation of unamended treatment was 95.5±1.7.*

Table 3. Effect of form of N on germ tube length (μm) of *Gloeotinia temulenta*.

Nitrogen source	Concentration (mM) 3	6	12	24	48
$Ca(NO_3)_2$	7.6±0.4[1]	7.1±0.7	7.0±0.0	6.3±1.1	3.9±0.8
NH_4Cl	1.2±0.3	1.0±0.0	1.0±0.0	1.0±1.0	1.0±1.0
NH_4NO_3	1.7±0.5	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
$(NH_4)_2SO_4$	1.8±0.3	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0

[1] *Mean and standard deviation based on 20 germ tubes in each of 4 replicate drops. Mean and standard deviation of germ tubes in unamended drops was 1.*

DISCUSSION

Inoculation of annual ryegrass under field conditions resulted in a reduction in germination of 2–4% with about 2% seeds infected in 1991 and 1992. Greater levels of infection were reported by Hampton and Scott (1980, 1981) and Hampton (1987) under treatments of high N, most likely a result of higher inoculum pressure from overwintered blind seeds. Differences among forms of N were not apparent, suggesting that the N forms used in this study would have no differential effect on blind seed level.

Under greenhouse conditions no significant difference was observed among form of N or nitrogen concentration. The mechanism of increased resistance to blind seed with increasing concentration of N, as reported by Hampton and Scott (1980) under field conditions was not observed under greenhouse conditions. However, the physiological response of annual ryegrass to nitrogen under field conditions may not necessarily be duplicated under the greenhouse conditions.

Soil N levels or N–form may have an effect on apothecial production from overwintered blind seeds. Hampton and Scott (1980) reported reduced germination of apothecia in the presence of nitrogen. Thus, a combination of reduced fungal vigour and enhanced plant resistance in response to nitrogen fertilisation may account for reduced blind seed incidence. Ammonium N is the most abundant N source in the upper soil layers of western Oregon due to reduced mobility of ammonium ions.

Conidial germination of *G. temulenta* was differentially affected by form of nitrogen. Lower germination and shorter germ tubes in the presence of ammonium forms of nitrogen may have resulted from ammonium toxicity. These observations were consistent within each of three experimental runs. High ammonium levels found in western Oregon soils could play a role in restricting blind seed germination (apothecial production).

Although field plots were sprayed twice weekly, little disease developed. A low infection level observed in field plots may have been due to unfavourable (dry) atmospheric conditions. The slime matrix of *G. temulenta* becomes a hardened mass under dry conditions but dissolves when placed in water, suggesting that rain splash may be an important means of distributing conidia of *G. temulenta*. However, the epidemiological significance of conidial inoculum, compared with ascospore inoculum, needs further study under a range of environmental conditions.

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