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Effect of nitrogen form and application method on incidence and severity of *Phytophthora* crown and root rot of apple trees

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Abstract

The effect of ammonium nitrate broadcast as a soil or through irrigation, urea applied as a foliar spray, and monoammonium phosphate applied as a planting hole treatment on the incidence of *Phytophthora* crown and root rot of apple trees was determined under orchard conditions in the Okanagan Valley of British Columbia, Canada. Results from the eight year study showed that ammonium nitrate applied as a single dose in spring at 240 g tree^{-1} year^{-1}, as a split dose at 120 g tree^{-1} each in spring and early autumn, and in irrigation water (fertigation) at 7.5 g tree^{-1} wk^{-1} for 10 wk year^{-1} significantly increased *Phytophthora* crown and root rot of Macspur on MM106 rootstock. There was no significant difference in *P. cactorum* infection between the unfertilized control and treatments with urea applied as a foliar spray at 1.0 kg 100 l^{-1} of water in spring and early autumn, and monoammonium phosphate applied as a planting hole treatment at 1 g l^{-1} of soil at planting time.

Introduction

Phytophthora crown and root rot (PCRR) is one of the most serious soilborne diseases of apple trees (*Malus domestica* Borkh.), in the Okanagan Valley of British Columbia [Utkhede, 1986]. *Phytophthora cactorum* (Lebert and Cohn) J. Schroet. is the major causal agent of PCRR of apple trees in British Columbia [McIntosh, 1975; Utkhede, 1987]. Other species of *Phytophthora* also have been associated with PCRR in other parts of the world [Jeffers *et al.*, 1982; Mircetich and Browne, 1987; Jeffers and Aldwinckle, 1986; Matheron *et al.*, 1988; Tidball and Linderman, 1990; Harris, 1991; Wilcox, 1993].

Controversy exists over the relationship between nitrogen fertilizers applied to soil and the severity of plant diseases [Huber and Watson, 1974]. Apple [1961] demonstrated that both ammonium nitrogen and nitrate predisposed a resistant tobacco cultivar to *Phytophthora parasitica*. Ammonium nitrogen increased the severity of *P. citrophthora* and *P. parasitica* root rot of citrus while nitrate nitrogen decreased it [Klotz *et al.*, 1958]. Similarly, Pal and Grewal [1976] showed that high levels of ammonium nitrate increased pigeon pea blight caused by *P. drechsleri* var. *cajani*. Broadbent [1977], on the other hand, showed that high levels of ammonium nitrogen reduced avocado root rot in pot culture. Lee and Zentmyer [1982] reported that ammonium sulphate reduced *P. cinnamomi* root rot of avocado under greenhouse conditions. Soils suppressive to *P. cinnamomi* root rot of avocado have been found to have high levels of ammonium nitrogen and nitrate nitrogen [Broadbent, 1977]. Utkhede [1984] demonstrated under field conditions that soil application of nitrogen fertilizers such as ammonium sulphate, ammonium nitrate, calcium nitrate, urea, and sewage sludge increased the percentage of apple trees on MM106 rootstock affected by *P. cactorum*.

Rainfall during summer months in the Okanagan Valley of British Columbia is not enough to sustain minimum growth of apple trees. Water is applied to these orchards using one of three irrigation methods: sprinkler, microjet, and drip irrigations. With sprinkler irrigation, a large volume of the soil becomes saturated, while drip and microjets apply water to a smaller volume of soil. The use of drip irrigation is rapidly increasing all over the world [Elfving, 1982]. Injection of nitrogen fertilizers through the drip irrigation system (fertigation) is becoming increasingly popular in major fruit growing areas of the would [Haynes, 1985]. High rates of monoammonium phosphate (MAP) incorporated directly into planting holes have improved vigour [Neilsen and Yorston, 1991] and precocity of apply trees [Neilsen *et al.*, 1990]. Recently, Neilsen *et al.* [1993] showed that apple trees fertigated with calcium nitrate had increased early vigour compared to trees fertigated with urea or ammonium nitrate.

Information is lacking on the effect of nitrogen form and application methods (as a soil, foliar treatment, or through drip irrigation systems) on the incidence and severity of PCRR. Field experiments were therefore conducted to examine the effect of ammonium nitrate applied regularly to soil or through a drip irrigation system, urea applied regularly as a foliar treatment, and monoammonium phosphate applied only at the time of planting on the incidence of PCRR in the Okanagan Valley of British Columbia, Canada.

Materials and methods

The field test was conducted in a sandy loam soil at the Agriculture Canada Summerland Research Station. The soil (pH 6.2) in the field trial area contained 32 mg kg^{-1} N, 73 mg kg^{-1} K, 232 mg kg^{-1} Mg, 1211 mg kg^{-1} Ca, 30 mg kg^{-1} Na, and 2.8% organic matter. One year old MM106 rootstocks were planted on 15 April 1985 with spacings of 3.5 m between rows and 1 m between trees. The rootstocks were budded with Macspur in August 1985. Six treatments were arranged in a randomized complete block design with 4 replications. Each plot consisted of 8 trees. The treatments were: (1) ammonium nitrate (34-0-0 NPK) broadcast in spring (April) at 240 g $tree^{-1}$ $year^{-1}$ (233 kg ha^{-1} N $year^{-1}$); (2) ammonium nitrate broadcast in spring and early autumn (September) at 120 g $tree^{-1}$ $year^{-1}$ (233 kg ha^{-1} N $year^{-1}$); (3) ammonium nitrate applied in the irrigation water at the rate of 7.5 g $tree^{-1}$ wk^{-1} (73 kg ha^{-1} N $year^{-1}$) for 10 wk $year^{-1}$; (4) urea (46-0-0 NPK) applied to run off as a foliar spray in spring and early autumn each year at the rate of 1.00 kg 100 l^{-1} of water (28 kg N ha^{-1} yr^{-1}); (5) monoammonium phosphate (11-55-0 NPK) applied as a planting hole treatment at the rate of 1 g l^{-1} of soil (31 kg N ha^{-1}) at planting, and (6) unfertilized control.

Soil around each tree was infested with isolate SPHO3 of *P. cactorum* annually in mid-June. To produce inoculum, isolate SPHO3 was grown on corn meal agar (CMA) for 1 wk at 18 °C. Mycelium with agar from these cultures was blended to a fine consistency in a tissue culture grinder with 5 ml of clarified V-8 broth [Ribeiro, 1978] under sterile conditions. The supernatant was diluted with distilled water in the proportion 1:4 and then antoclaved (121 °C, 20 min). The ground material was poured into culture bottles containing 100 ml V-8 broth and thoroughly mixed; 5 ml aliquots were pipetted into 60 × 15 mm petri plates. These cultures were incubated for 2 days at 25 °C and then at 18 °C for 3–4 wk in darkness. Mycelial mats were removed and blended with 100 ml sterile water in a sterile blender at 10 s intervals for 2 min to obtain suspensions of mycelium and sporangia. The stock suspensions were diluted to obtain approximately 1350 colony forming units (CFU) ml^{-1}. For soil infestation, the stock suspensions were diluted with sterile water to 270 CFU ml^{-1}. The soil was removed from the crown region to a depth of *c.* 4 cm 50 ml of the diluted suspension poured evenly around the base of each tree and the soil immediately replaced. The pathogenicity of this isolate was maintained by regularly infecting apple seedings grown in sterile soil under greenhouse conditions and recovering the isolate.

The trees were irrigated with microjects for 4 h immediately after soil infestation with *P. cactorum*. During the growing season the test plot was irrigated with 50 l water delivered for 4 h at 2 day intervals. Weeds, insects, and foliar diseases

were controlled by standard orchard practices [Anonymous, 1985–1993].

The presence or absence of *P. cactorum* in the bark or roots of symptomatic trees was confirmed every year following the method used by Matheron *et al.* [1988], plating 10 bark or root pieces per tree on plates, using a selective medium containing corn meal agar amended with 5 mg of pimaricin, 300 mg vancomycin hydrochloride, and 25 mg of pentachloronitrobenzene litre^{-1}. The plates were kept in the dark at 21 °C and examined daily for 5–7 days for growth of *P. cactorum*. Colonies typical of *P. cactorum* were examined under a microscope to confirm their identity.

A disease rating (1 = healthy, 2 = initial, 3 = intermediate, 4 = terminal, 5 = dead) was assigned to each tree in late September of years 1987–1993. The extent of PCRR infection was determined by removing about 15 cm of soil from the base of trees. The PCRR infections were rated as follows: 1 = healthy – no infection; 2 = initial – less than one-fourth of the bark/roots at the crown region infected; 3 = intermediate – about one-fourth to one-half of the bark/roots infected; 4 = terminal – more than half the bark/roots infected; 5 = dead – tree trunk completely girdled, all roots infected, tree dead. The final percentages of dead and diseased trees were determined in late September 1993 based on isolation of *P. cactorum*. All data were analyzed by General Linear Model (GLM) procedures (SAS Institute Cary, N.C.). All percentages were transformed into arcsine values and analyzed for statistical significance by the GLM procedure. Duncan's new multiple range test was used to compare treatments after an ANOVA showed significant differences among means.

Results

Phytophthora cactorum was isolated from bark and root samples of all trees showing symptoms typical of crown and root rot, but not from healthy symptomless trees.

The General Linear Model procedure showed that the effects of treatments were significant (Table 1). Significantly higher disease ratings were observed in trees fertilized with ammonium nitrate applied as a single broadcast in the spring at the rate of 240 g tree^{-1} year^{-1} compared with the unfertilized control for all years except 1987 and 1988 (Table 2). Similarly, higher disease ratings were observed in trees fertilized at the same annual rate but in two doses applied in spring and early autumn, for all years except 1987 when compared to the control. No significant difference in disease ratings between fertigated and unfertilized control trees were observed until 1992 and 1993. There was no significant difference between trees treated with urea as a foliar application or trees planted in soil treated with monoammonium phosphate and unfertilized control trees in any year. When the data were pooled for all years, ammonium nitrate applied either as broadcast soil treatment (single or split doses) or applied as fertigation treatment resulted in a significantly higher disease rating by *P. cactorum* compared with the unfertilized control (Table 2). No significant differences in disease ratings were observed between urea applied as a foliar treatment, monoammonium phosphate applied as a planting hole treatment and the unfertilized control. The disease ratings were significantly higher for ammonium nitrate applied as a broadcast in spring compared with ammonium nitrate applied as a broadcast in spring and early autumn. This latter treatment, however, showed

Table 1. Analysis of variance of the data on disease rating (1 = healthy, 5 = dead)

		Mean squares						
Source	D.F.	1987	1988	1989	1990	1991	1992	1993
Replication	3	0.12	0.09	1.64	1.75	1.70	0.11	1.37
Treatment (T)	5	1.02*	4.68**	10.70**	14.40**	14.40**	16.52**	20.89**
RXT	15	0.29	0.92	1.48	2.54	2.66	2.74	2.73
Error	168	0.45	1.50	2.35	2.91	3.04	2.81	2.21

*, ** Significant at 5 and 1% level, respectively.

Table 2. Effect of nitrogen and phosphorus applied as a broadcast, foliar spray, planting hole treatment, and fertigation on the incidence of crown and root rot caused by *P. cactorum* under field conditions

Treatment	Rate tree^{-1}	Mode and timing	Disease rating (1–5)							
			1987	1988	1989	1990	1991	1992	1993	Mean
1. Ammonium nitrate	240 g	broadcast (spring)	1.47 ab*	2.00 ab	2.75 a	3.34 a	3.37 a	3.66 a	4.16 a	2.53 a
2. Urea	1 kg 100 l^{-1}	foliar (spring, autumn)	1.12 b	1.20 b	1.47 b	1.62 c	1.66 c	1.91 c	2.28 c	1.47 d
3. Ammonium nitrate	75g	fertigation (weekly)	1.44 ab	1.87 abc	2.16 ab	2.44 bc	2.50 abc	2.91 ab	3.16 b	2.05 c
4. Ammonium nitrate	120 + 120 g	broadcast (spring, autumn)	1.56 a	2.16 a	2.72 a	2.78 ab	2.81 ab	3.16 a	3.53 ab	2.30 b
5. Monoammonium phosphate**	100g	planting hole only	1.19 b	1.37 bc	1.59 b	1.91 bc	2.0 bc	2.12 bc	2.28 c	1.62 d
6. Control			1.22 ab	1.44 bc	1.62 b	1.75 c	1.75 c	2.00 c	2.22 c	1.55 d
S.E.			0.12	0.22	0.27	0.30	0.31	0.29	0.26	0.07

* Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

** Only applied at planting

significantly higher disease ratings when compared with the ammonium nitrate treatment injected through irrigation.

A significantly ($P \leq 0.05$) higher percentage of trees was infected by *P. cactorum* when trees were fertilized with ammonium nitrate compared with the untreated control (Fig. 1) irrespective of the method of application. No significant differences in percentages of infected trees were observed between the urea treatment applied as a foliar spray, monoammonium phosphate applied as a planting hole treatment, and the unfertilized control. No significant difference in percent infected trees was observed between the urea treatment applied as a foliar spray, ammonium nitrate applied as a fertigation treatment or split application of ammonium nitrate. A significantly higher percentage of trees died during the eight year period by *P. cactorum* infection when ammonium nitrate treatment was applied either as a broadcast (single or split dose) or fertigation treatment compared with urea applied as a foliar spray. No significant differences in tree death caused by *P. cactorum* were observed between urea applied as a foliar spray, monoammonium phosphate applied as a planting hole treatment, and the unfertilized control.

Discussion

This study has shown that the application of nitrogen fertilizer (ammonium nitrate), when applied as a broadcast in spring, in spring and early autumn, or by fertigation, caused significantly higher infection by *P. cactorum* compared with the untreated control. Similar results were obtained by Apple [1961] with *P. parasitica* var. *nicotianae* in tobacco, Klotz *et al.* [1958] with *P. citrophthora* and *P. parasitica* in citrus, and by Pal and Grewal [1976] with *P. drechlseri* var. *cajani* in pigeon pea. This study also confirms our earlier results which showed that the broadcast application of ammonium nitrate in the spring at the rate of 240 g tree^{-1} increased the percentage of apple trees on MM106 rootstock affected by *P. cactorum* [Utkhede, 1984].

The highest incidence of infection by *P. cactorum* was observed when ammonium nitrate was applied as a broadcast in a single dose (240

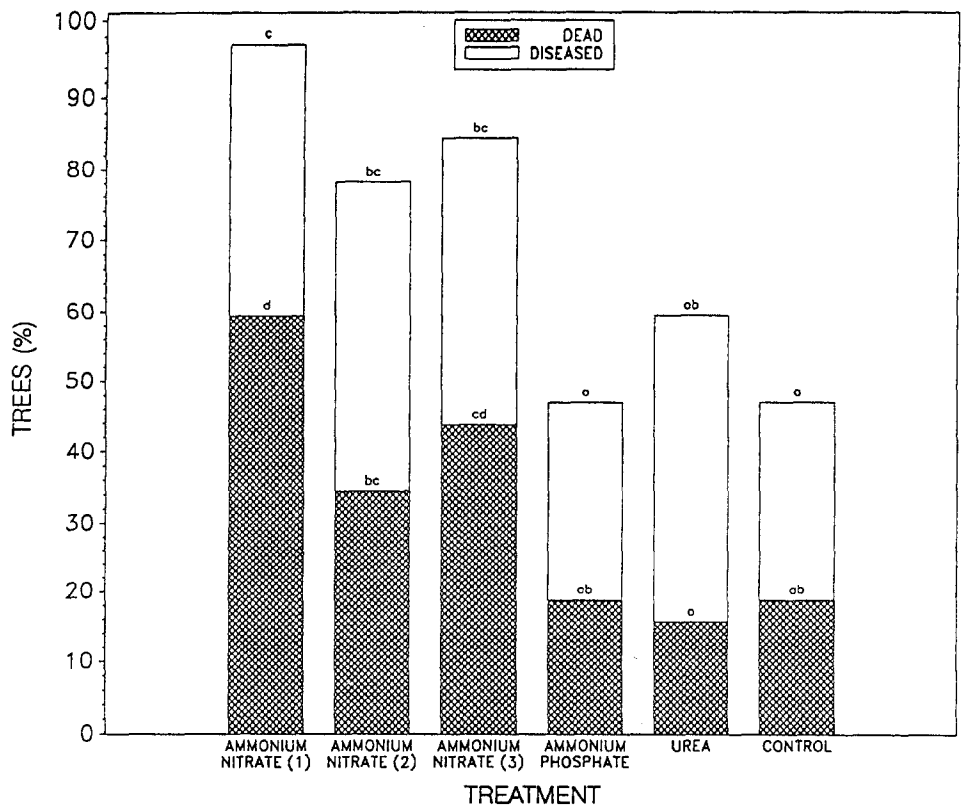

Fig. 1. Percentage of diseased and dead trees infected with *P. cactorum*. Ammonium nitrate (1) – 240 g tree^{-1} applied as a broadcast in the spring in a single dose. Ammonium nitrate (2) – 120 g tree^{-1} each applied as a broadcast in the spring and early autumn. Ammonium nitrate (3) – fertigated at 7.5 g tree^{-1} wk^{-1} for 10 wk. Monoammonium phosphate applied as a plating hole treatment at 1 g l^{-1} of soil. Urea applied as a foliar spray at 1.0 kg 100 l^{-1} of water in spring and early autumn. The values of percentages of diseased or dead trees followed by the same letter are not significantly ($P \leq 0.05$) different according to the Duncan's multiple range test.

g tree^{-1} year^{-1}) in the spring. It appears that the application of nitrogen may be causing plant tissue to become soft and succulent. This condition probably favours easy penetration of *P. cactorum* into the trunk and root tissues, which leads to infection. As expected, when the same amount of nitrogen was applied in two doses, the infection by *P. cactorum* was reduced. However, the disease severity caused by this treatment was still significantly higher than if nitrogen had not been used.

Fertigation is becoming increasingly popular in high density apple plantings. Application of nitrogen through fertigation increase disease severity by *P. cactorum* compared to the unfertilized control. However, the disease rating by this treatment was lower than the broadcast nitrogen treatment. Therefore, application of nitrogen through fertigation in *P. cactorum* infested soil should be used with caution.

In the present study, planting hole soil treatment with monoammonium phosphate at the rate of 1 g l^{-1} did not affect the disease incidence in apple by *P. cactorum*. This confirms our earlier results from the study conducted in the Kootenay Valley of British Columbia [Utkhede and Smith, 1991a]. Phosphorus fertilizers have been observed to reduce *Pythium* root rot in wheat, *Thielaviopsis* root rot in tobacco and ginseng, septoria leaf spot in tomato, eye spot in sugar cane, dodder on clover, vetch downy mildew in cabbage, and *Gibberella saubinetti* root rot in corn [Huber,

1981a]. An *in vitro* study has shown an increase in antagonistic bacterial population when nitrogen and phosphorus were added to the media [Utkhede and Smith, 1991b].

Nitrogen applied as a foliar spray in spring and early fall as urea at the rate of 1.0 kg 100 l^{-1} of water did not increase the disease incidence. The requirement of nitrogen for plant growth, its limited availability in soil, and its effect on cell size and wall thickness has been discussed by Huber [1981a]. Nitrogen promotes vigorous growth, delays maturity, and is required for the production of amino acids, proteins, growth hormones, and new protoplasm. Many plant constituents altered by nitrogen have been correlated with either resistance or susceptibility to disease. The effect of nitrogen on plant diseases has also been discussed by Huber [1981b].

In summary, good cultural practices are essential to reduce the effects of *P. cactorum* crown and root rot of apple trees. The application of ammonium nitrate either as a soil broadcast or by fertigation could be damaging to apple trees where *P. cactorum* is a problem. Urea applied as a foliar spray in the spring and early autumn or monoammonium phosphate applied as a planting hole treatment did not affect the disease severity in apple trees where *P. cactorum* is present in soil.

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