Plant Pathology (2006) **55**, 246–249 Doi: 10.1111/j.1365-3059.2005.01322.x

Effects of nitrogen, phosphorus, potassium and calcium nutrition on strawberry anthracnose

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The effects of a range of concentrations of four nutrients – nitrogen, phosphorus, potassium and calcium – in fertilizer solutions on the severity of anthracnose on strawberry cv. Nyoho cultivated under a noncirculation hydroponics system were determined after inoculation with *Colletotrichum gloeosporioides*. Crop growth and tissue nitrogen, phosphorus, potassium and calcium contents of the entire above-ground parts of the plant were also investigated. Elevated nitrogen and potassium concentrations in the fertilizer solution increased disease severity in contrast to phosphorus and calcium. Treatment with either NH_4 or NO_3 nitrogen was not significantly different. The dry weight of the strawberry plants increased significantly with elevated concentrations of nitrogen (R^2 = 0·9078) and phosphorus (R^2 = 0·8842), but was not influenced by the elevated amounts of potassium (R^2 = 0·8587) and calcium (R^2 = 0·6526) concentrations.

Keywords: *Colletotrichum gloeosporioides*, disease severity, fertilizer, macronutrients, strawberry anthracnose

Introduction

In Korea, strawberries (*Fragaria* × *ananassa*) are favoured for winter cultivation when the production of other vegetables and fruits is relatively low. In 2003 strawberries were planted on a total area of 7503 ha in Korea, with plastic greenhouse cultivation comprising 7172 ha and production for fresh market totalling 205 427 tonnes (Anonymous, 2004). The use of forcing, where strawberries are transplanted into greenhouses in mid-September and forced into fruiting in December (Oda, 1991), is increasing in Korea. June-bearing cultivars such as Maehyang, Akihime and Nyoho used for forcing have proven difficult to grow due to their susceptibility to anthracnose (Nam *et al*., 1998; Kim & Nam, 1999). Anthracnose is one of the most damaging diseases of strawberries, causing wilting and death of transplants in the nursery field. The pathogen known to be responsible for the disease in Korea is *Colletotrichum gloeosporioides* and was initially reported in 1992 (Kim *et al*., 1992).

The nutritional status of a plant has a major impact on susceptibility to disease, and this has been exploited for the reduction of disease severity (Huber & Watson, 1974; Engelhard, 1989; Huber & McCay-Buis, 1993). In particular, nitrogen, and the form of nitrogen used, can be influential. For example, black root rot complex (*Rhizoctonia fragariae/Pratylenchus penetrans*) of strawberries was suppressed by application of $(NH_4)_2SO_4$ (Elmer & LaMondia, 1999), and *C. phomoides* was controlled using NH_4-N fertilizer (Williams, 1965). However, the severity of fusarium disease of tomato (Duffy & Dèfago, 1999) and anthracnose (*C. gossypii*) of cotton (Huber & Watson, 1974) was elevated with NH_4-N. Similarly, rice blast disease (*Magnaporthe grisea*) is exacerbated in the presence of excess nitrogen (Teng, 1994). In contrast, when calcium was applied, tomato showed increased resistance to fusarium wilt (Woltz *et al*., 1992).

Little is currently known about the influence of plant nutrition on anthracnose of strawberry. The purpose of this study was to investigate the effects of concentration of nitrogen, phosphorus, potassium and calcium on the development of strawberry anthracnose, with the aim of developing practical strategies to manage the disease.

Materials and methods

Plant preparation

Tissue-cultured seedlings of strawberry cv. Nyoho at the three-leaf stage were transplanted into 15 cm (1·6 L) plastic pots containing perlite. Transplants were irrigated for a month with distilled water to leach out nutrients contained in the perlite. This also lowered the tissue nutrient content of the strawberry plants. Following this, plants

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Accepted 13 August 2005

Table 1 Composition of nutrient solutions applied to strawberry plants

Treatment (mM)	Nutrient					
	N	P	K	Ca	Mg	SO_4
N						
0	0	2	14	5	2	5
5	5	2	14	5	2	5
10	10	1	14	5	2	5
15	15	1	14	5	2	5
20	20	1	14	5	2	5
30	30	1	14	5	2	5
P						
0	15	0	15	5	2	5
0·25	15	0·25	14·25	5	2	5
0·5	15	0·5	14·5	5	2	5
0·75	15	0·75	14·75	5	2	5
1	15	1	15	5	2	5
3	15	3	17	5	2	5
K						
0	15	1	0	5	2	2
10	15	1	10	5	2	2
20	15	1	20	5	2	4
30	15	1	30	5	2	6
Ca						
0	15	1	15	0	2	2
1·5	15	1	15	1·5	2	2
3·0	15	1	15	3·0	2	2
4·5	15	1	15	4·5	2	2
6·0	15	1	15	6·0	2	2

The following micronutrients were applied to all pots: $MnCl_2{\cdot}4H_2O$ (1·8 g L^{-1}), H_3BO_3 (2·86 g L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0·22 g L^{-1}), $CuSO_4{\cdot}5H_2O$ (0·08 g L^{-1}), $H_2MoO_4{\cdot}H_2O$ (0·09 g L^{-1}), $Na_2FeEDTA$ (0·79 g L^{-1}).

were watered with the nutrient treatment solution every 2 days for 50 days.

Nutrient solutions

Hoagland nutrient solution (Hoagland & Arnon, 1950) was modified to make treatment solutions containing 0, 5, 10, 15, 20 and 30 mM nitrogen; 0, 0·25, 0·5, 0·75, 1 and 3 mM phosphorus; 0, 10, 20 and 30 mM potassium; or 0, 1·5, 3, 4·5 and 6·0 mM calcium. The composition and chemical sources of micronutrients are shown in Table 1. The pH of all treatment solutions were adjusted to 6·5 using 1 N HCl or KOH. The effect of the form of nitrogen was tested by comparing 5, 10 and 15 mM nitrate and ammonium compounds.

Cultural practice

The experimental design was a completely randomized plot with five replications. Pots were placed 25 cm apart in rows. The experiment was conducted in a glasshouse with a temperature range of 17–18°C at night and 24–28°C during the day. The relative humidity ranged from 40 to 80%, and daylight intensity from 550 to 1200 mol m^{-2} s^{-1} during the experimental period.

Plant analysis

Plant tissue was sampled from shoots after 1 month of nutrient solution treatments. Tissue samples were washed with 0·2 N HCl for 1 min, rinsed with distilled water, dried for 2 days at 70°C, and ground in a Wiley mill to pass through the 1 mm particle size sieve. Total N was determined by a modified Kjeldahl procedure which included a salicylic acid pretreatment to aid in the reduction of NO_3 (Eastin, 1978). Tissue used for P, K and Ca analysis was dry-ashed at 500°C, dehydrated in HCl and finally dissolved in 0·5 N HCl. Analysis of K and Ca was done by atomic absorption spectrometry, and colorimetric analysis was performed for P (Chapman & Pratt, 1961).

Inoculation and disease assessment

Growth characteristics were determined for plants after 1 month of nutrient solution treatments. Inoculum of *C. gloeosporioides* (CGF43) was prepared at 10^6 conidia mL^{-1} and sprayed on the plants. The inoculated plants were incubated in dew chamber at 30°C and 100% RH for 2 days and then moved to a glasshouse held between 24 and 28°C (Gupton & Smith, 1991). After 17 days, the disease severity on each plant was rated. Disease severity was rated as a disease index (DI) on a scale of 0–4, as follows: 0, healthy; 1, < 50% of petioles affected; 2, ≥ 50% of petioles affected; 3, wilted; 4, necrosis formed on entire plants (Ishikawa *et al*., 1989).

Data analysis

Data from nutrient concentrations and different nitrogen source trials were subjected to analysis of variance, and treatment means were compared by Duncan's multiple range test at $P = 0{\cdot}05$. The relationships between nutrient concentrations and dry weight, and nutrient concentrations and disease index, were evaluated using regression analysis (Duffy & Dèfago, 1999; Choi *et al*., 2000). All data analysis was performed using the SAS program (SAS Institute, Inc., USA).

Results and discussion

Dry weight of strawberry plants was greatest when provided with 30 mM nitrogen, 1 mM phosphorus, 10 mM potassium, and 3 mM calcium (Table 2).

Anthracnose symptoms first appeared in the nitrogen treatment approximately 2 weeks after inoculation. A positive correlation (adjusted (Adj) $R^2 = 0{\cdot}96$) was found between nitrogen concentration of treatment solution and anthracnose disease (Fig. 1a). Disease was greatest (DI ~3) at a nitrogen concentration of 30 mM. Anthracnose tended to be more severe in treatments receiving NH_4 than NO_3, but these differences were not significant according to Duncan's multiple range test ($P = 0{\cdot}05$) (Fig. 2). A similar positive relationship (adjusted $R^2 = 0{\cdot}84$) between disease and potassium concentration of treatment solution was shown, with a maximum DI of ~3 at 30 mM potassium

Table 2 Analysis of regression relating concentration of individual nutrients to strawberry plant dry weight

Nutrient	Concentration giving greatest dry weight (mM)	Regression equation	R^2	P
Nitrogen	30	$y = 5{\cdot}08 + 0{\cdot}44x - 0{\cdot}006x^2$	0·9078	0·02
Phosphorus	1	$y = 5{\cdot}49 + 5{\cdot}04x - 1{\cdot}34x^2$	0·8842	0·03
Potassium	10	$y = 5{\cdot}79 + 0{\cdot}23x - 0{\cdot}007x^2$	0·8587	0·37
Calcium	3	$y = 5{\cdot}63 + 2{\cdot}14x - 0{\cdot}33x^2$	0·6526	0·34

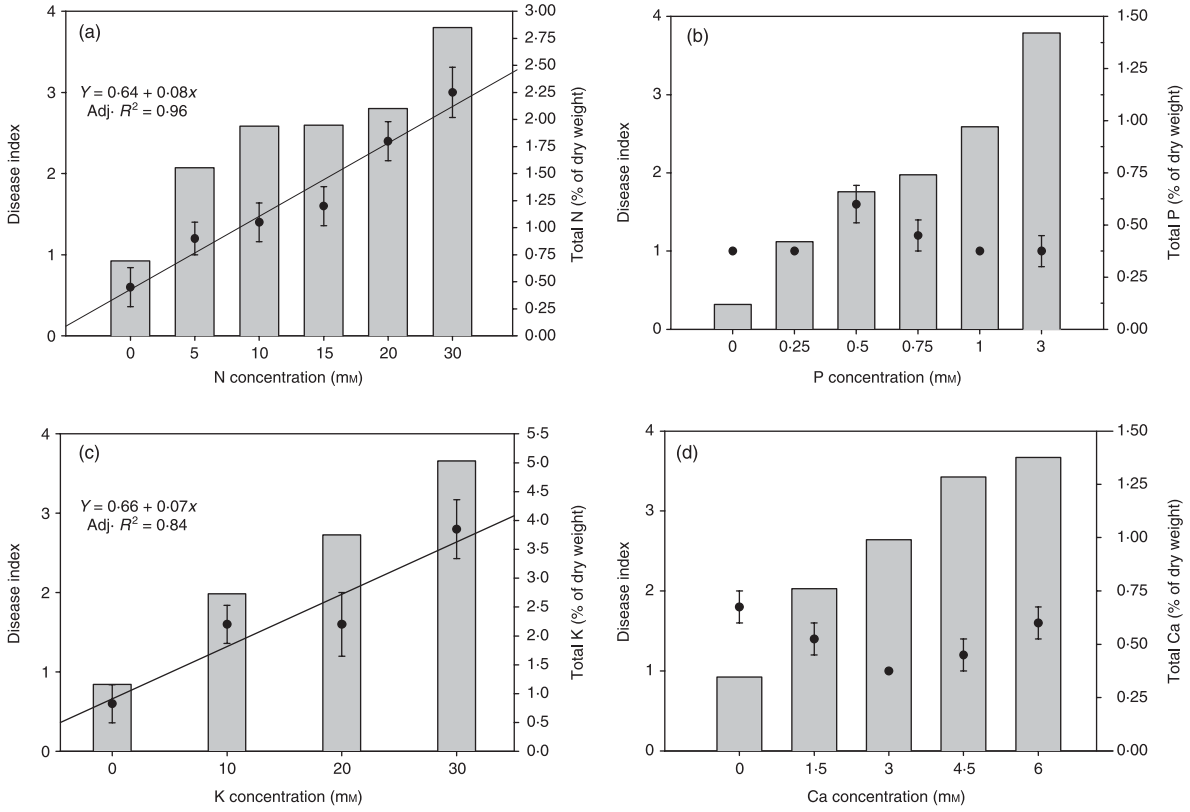

Figure 1 Development of anthracnose (scatter plot with error bars) and nutrient concentration (vertical bars) in strawberry plants treated with different concentrations of nitrogen (a), phosphorus (b), potassium (c) and calcium (d). Disease index: 0, healthy; 1, < 50% petioles affected; 2, ≥ 50% petioles affected; 3, wilted; 4, necrosis formed on entire plants. Adjusted regression coefficients and line derivations in (a) and (c) were significant ($P \leq 0{\cdot}05$). Differences in (b) and (d) were nonsignificant ($P \geq 0{\cdot}62$). Error bars represent ± standard error of mean.

(Fig. 1c). In contrast, neither increasing concentrations of calcium ($P = 0{\cdot}62$) nor those of phosphorus ($P = 0{\cdot}68$) had a patterned effect on disease (Fig. 1b and d). For all treatments, the relative dry matter content of each nutrient in the test plants increased with increasing concentration in the treatment solution (Fig. 1).

Previous studies have shown correlations between nitrogen concentration and severity of anthracnose, as in the case of cotton (Huber & Watson, 1974). Previously, Choi *et al.* (2000) showed that, as tissue nitrogen content decreased, Ca and Mn contents, which have a direct role in phenolic metabolism, increased in the strawberry. Manganese may increase synthesis of host defence products and thereby reduce disease incidence and/or severity (Huber & McCay-Buis, 1993), whilst calcium has been shown to inhibit anthracnose (caused by *C. gloeosporioides* or *C. acutatum*) in apples (Biggs, 1999) and decrease postharvest disease development on strawberry (Cheour *et al.*, 1990). Woltz *et al.* (1992) also found that treatment with $CaCO_3$ (3·0 kg m^{-3}) in tomato reduced fusarium crown rot disease. Calcium may be important for resistance against certain cell-wall-degrading enzymes produced by fungal pathogens (Conway & Sams, 1984; Biggs & Peterson, 1990). However, in this study, no effect was found of calcium content on anthracnose disease in strawberry. As manganese content is increased under low nitrogen conditions in plants, additional work is needed to determine whether manganese levels are related to anthracnose development. Phosphorus supplied as phosphite was effective at reducing phytophthora root and crown rot on tomato and pepper plants but also suppressed plant growth (Förster *et al.*, 1998). In the study reported here,

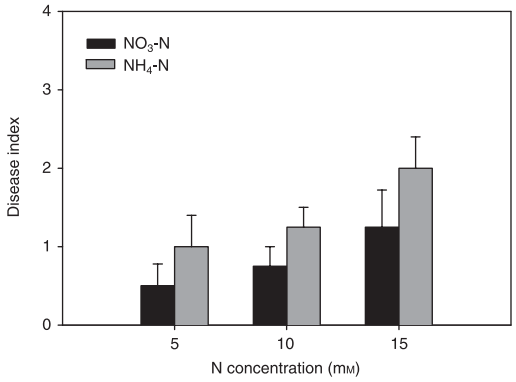

Figure 2 Comparison of anthracnose severity between strawberry plants treated with different nitrogen sources. Disease index: 0, healthy; 1, < 50% petioles affected; 2, ≥ 50% petioles affected; 3, wilted; 4, necrosis formed on entire plants. Vertical bars represent ± standard error of mean.

phosphorus concentration had no influence on anthracnose development.

Strawberry prefers NH_4 to NO_3 nitrogen for optimal growth (Park, 1990). Other studies have shown the use of the former increases cotton anthracnose (Bollenbacher & Fulton, 1971) and fusarium crown rot severity (Woltz *et al*., 1992) more than the use of nitrate. It was found on average that strawberry plants receiving ammonium nitrogen had more disease than those receiving nitrate treatments, but these differences were not significant.

It has been shown that increasing nitrogen and potassium in nutrient solutions resulted in higher dry matter percentages of both elements in strawberry but also increased the severity of anthracnose. To decrease anthracnose in strawberries, it is suggested that a minimum of nitrogen and potassium should be present in any fertilizer used and that this should not exceed 15 and 10 units of nitrogen and potassium, respectively.

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