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EFFECTS OF NITRIFICATION INHIBITORS ON CHEMICAL COMPOSITION OF PLANTS: A REVIEW

KEYWORDS: retardation of nitrification, form of nitrogen, protein and nitrogenous compounds, organic acids, nitrate, phytotoxicity, cation-anion balance, plant disease, uptake and accumulation of nutrients

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ABSTRACT

The potential of nitrification inhibitors to improve N fertilizer efficiency is well recognized. However, their effects on crop quality have received much less attention. Recent literature pertaining to the effects of nitrification inhibitors on chemical composition of plants is reviewed. The topics examined include how the form of inorganic N and retardation of nitrification affects accumulation and content of protein and nitrogenous compounds, cations and anions, and organic acids. There is ample evidence to suggest that nitrification inhibitors hold promise to improve the quality of crops in situations where accumulation of NO_3^- or organic acids such as oxalic acid is a problem. There is need for future research to examine how

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nitrification retardation affects cation-anion balance in plants under field conditions because experiments conducted under controlled conditions and in the field have at times given divergent results. Since the use of nitrification inhibitors is increasing, investigations to evaluate their use to improve crop quality in addition to quantity should receive priority.

INTRODUCTION

The use of chemicals to retard nitrification in soils holds promise for improving nitrogen efficiency in situations where nitrification results in the loss of N through leaching or denitrification[26,60,80]. However, retardation of nitrification may also influence plant growth and composition in ways not related directly to improved N efficiency. For example, it is possible that the higher ratio of NH_4^+-N:NO_3^--N as a consequence of nitrification inhibition may affect plant metabolism and plant composition by influencing uptake of N, cations and anions, and production of organic acids[7,15,27,36,52,61,94]. Similar to the effects on plant nutrition observed when comparing NH_4^+ and NO_3^- as sole sources of N, these effects will depend on the degree of inhibition, the plant species, and numerous cultural soil and environmental factors[35,93]. However, the effect of nitrification inhibitors on plant composition is not very well understood[15,94]. Use of these materials will likely become more common and it is important to understand their effect on plant composition and crop quality.

We review the literature on the effects of nitrification inhibitors on plant composition with particular reference to NO_3^-, N compounds and protein, cations and anions, and organic acids. The areas of future research that may lead to judicious use of nitrification inhibitors in tailoring desirable plant composition are also examined. It is recognized that there has been relatively less emphasis on nitrification inhibitors research with regard to crop quality; however, it is hoped that our review will stimulate future research in this area.

EFFECT OF AMMONIUM OR NITRATE NUTRITION ON PLANT COMPOSITION

While there is considerable similarity between the results of studies comparing NH_4^+ and those examining the effects of nitrification inhibitors on plant metabolism, they are not directly comparable. When nitrification inhibitors are used, nitrification is not inhibited completely, and there usually is a preponderance of NH_4^+ and NO_3^- in the soil (Table 1).

Plant composition is influenced by the form of N directly or indirectly. First, the form of N directly affects the plant metabolism. Often, plants when grown in a NO_3^--only nutrient medium will show chlorosis (unless pH is adjusted) due to the increase in pH accompanying NO_3^- uptake[22,27]. Indirect effects are usually associated with differential uptake of some cations and anions and organic acids[4,45,47,86]. Recent literature on the effects of the form of N on plant metabolism, growth and

TABLE 1

Effect of Nitrapyrin on Conservation of Ammonium Nitrogen in Soils in the Field.[a]

Nitrapyrin applied	N rate	Ratio of NH_4^+-N treated over untreated	Observation periods	Reference
------ kg/ha	-----		weeks	
0.25-1.0	100	1.5-8.0:1	4-17	Redeman *et al.*[85]
0.5-1.0	100	1.5-4.0:1	6-19	Hughes and Welch[38]
0.5-1.0	200	2.0-15.0:1	7-24	Hughes and Welch[38]
0.5	65	3.0:1	20	Huber *et al.*[32]
0.25-0.50	50-100	1.25-6.0:1	20-21	Kapusta and Varsa[43]
1.0	150	10.0:1	6	Moore[65]

[a] Data adapted from Norris[71].

composition has been comprehensively reviewed by Huber and Watson[35], Lee and Stewart[50], Haynes and Goh[27], and Hageman[22], and briefly by Gasser[15], Kirkby and Hughes[46], Givan[18], and Sahrawat[94].

Nitrogen

It is generally found that plants supplied with NH_4^+ contain higher amounts of total N, free NH_4^+, amides and amino acids[18,22,27,35], and that they detoxify and readily metabolize NH_4^+ to amino acids and amides. However, protein quantity and quality in crops is best expressed when grown in a mixture of NH_4^+ and NO_3^- rather than either alone[22,27].

Reisenauer *et al.*[86] found that ryegrass produced best yields when grown in dilute solution cultures supplying low levels of NH_4^+ and adequate amounts of NO_3^-. At sufficiently low concentration of NH_4^+ the decrease in NO_3^- uptake due to NH_4^+ competition was less than the decline in NH_4^+ uptake. The result was that total N uptake and protein content of the the plants were increased. Many plants grown in high NO_3^- environments accumulate excess NO_3^-[63,114], particularly in plant species which do not possess NO_3^- reductase activity in their roots and if the rate of NO_3^- reduction in leaves is slower than the translocation rate from the roots.[27]

Cations and anions

Wadleigh *et al.*[107] and Wadleigh and Shive[108] initially pointed out that high NH_4^+ relative to NO_3^- in nutrient solution decreased the concentration of bases such as Ca^{2+}, Mg^{2+}, and K^+. Subsequent reports have also confirmed that NH_4^+ nutrition in general decreases the concentration of base cations in plants but enhances those of anions such as phosphate and sulfate, while the opposite effects occur with NO_3^-[15,22,27,35,75].

There appear to be at least two effects of NH_4^+ nutrition on cation and anion composition of plants. First, NH_4^+ uptake lowers the pH of the medium which results in enhanced absorption of anions such as phosphate[24,64,89,99]. Second, cations may compete in ion uptake directly or due to release of H^+ during the uptake of NH_4^+[27]. Rudert and Locascio[101] found that NH_4^+

nutrition suppressed the absorption of Ca^{2+} and Mg^{2+} compared to NO_3^-[22,27,94].

Reisenauer *et al.*[86] studied the comparative efficacy of NH_4^+ and NO_3^- for N nutrition of ryegrass in dilute, controlled composition, flowing nutrient solutions supplying different levels of NO_3^- and NH_4^+. They found that NH_4^+ depressed the uptake of cations, especially Ca^{2+} and K^+, and concluded that NH_4^+, in addition to being phytotoxic, was a comparatively inefficient source of N. Ammonium inefficiency was mainly attributed to detoxification of NH_4^+ which utilizes energy and carbon skeletons within the root.

Organic acids

Production of organic acid anions such as malate and citrate by plants helps to maintain an ionic balance when NO_3^- is readily metabolized to organic compounds. This usually results in higher levels of cations than anions[27,35].

Nitrate nutrition also increases oxalic acid content in plants[10,31,44]. Thus NH_4^+ may be the preferred form of N for plants that accumulate oxalic acid.

Conclusions

The effects of form of inorganic N on plant composition are complex. Hageman[22] has discussed problems associated with comparing the effects of NH_4^+ and NO_3^- on plant growth and metabolism, especially under field conditions. With the available information, it can be generally concluded that NH_4^+ nutrition decreases the content of cations but increases anions in plants while NO_3^- has the opposite effect. Some effects of NH_4^+ nutrition on plant metabolism and composition can be attributed to NH_4^+ toxicity. In some species, NO_3^- nutrition also increases the contents of organic acids which may cause disturbance in the metabolism of nutrients such as Fe. Nitrate nutrition also increases NO_3^- content in plants, especially in those with little NO_3^--reductase activity.

It is generally believed that the best plant composition with regard to protein content and cation-anion balance is neither achieved by NH_4^+ nor by NO_3^- nutrition alone but when both are available. More work is needed to elucidate how the plant composition is affected by the form of N because plant species differ widely in their capacity to utilize and metabolize these forms of N[27,35]. Generalizations across plant species cannot be easily made because the effect of the form of N varies and is further modified by the age of the plant and the growing medium composition, especially pH[35]. As a rough guide, the relative preference of plants for NH_4^+ and NO_3^- forms of N and their relative effects on plant composition[35] are summarized in Tables 2 and 3.

EFFECTS OF RETARDATION OF NITRIFICATION ON PLANT COMPOSITION

While the primary reason for use of nitrification inhibitors is to lower denitrification losses, little attention has

TABLE 2

"Relative Preference" of Plants for Forms of Nitrogen.[a]

Preference for NO_3^--N	Preference for NH_4^+-N
Bush bean	Bermuda grass
Celery	Conifer seedlings
Corn	Corn seedlings
Cotton	Oat seedlings
Grain sorghum	Blueberry
Kale	Mycorrhizas
Onions	Orange trees
Pineapple	Rice
Potatoes	Ryegrass
Squash	Sugar beets (@ pH 7.0)
Sugar beets (@ pH 5.0)	Tea
Tobacco	Wheat (seedling)
Tomato	Wheat (under drought)
Wheat (older)	

[a] From Huber and Watson[35].

TABLE 3

Relative Effects of Form of Nitrogen on Plant Composition.[a]

Constituent	Form of nitrogen: NH_4^+-N	Form of nitrogen: NO_3^--N
Total nitrogen	equal/lower	equal/higher
Protein nitrogen	higher	lower
Amino nitrogen	higher	lower
Amide-N (especially asparagine)	higher	lower
Nitrate-N	lower	higher
Soluble organic nitrogen	higher	lower
Phosphorus	higher	lower
Inorganic cations	lower	higher
Organic acids	lower	higher
Soluble carbohydrate	lower	higher
Total carbohydrate	higher	lower

[a] From Huber and Watson[35].

been paid to the effect of nitrification inhibitors on plant composition[15,94]. This section evaluates available information on the interaction of nitrification inhibitors and crop quality.

Nitrogen

It is generally found that the use of nitrification inhibitors increases the total uptake of N by plants in situations where loss of N due to leaching and denitrification limits plant growth. But in cases where plant metabolism is adversely affected by higher $NH_4^+:NO_3^-$ ratio or where N is not limiting plant growth, retardation of nitrification may either not affect the plant N composition or even may decrease N content[93].

The patented nitrification inhibitor, nitrapyrin [2-chloro-6-(trichloromethyl)pyridine] is the most widely tested chemical for use in improving the efficiency of fertilizer N. In one of the early field studies with nitrapyrin, Swezey and Turner[101] evaluated its effect on growth, yield, and N uptake by cotton, corn and sugar beets. They found that the retardation of nitrification of several NH_4^+ fertilizers and urea resulted in increased leaf N contents and higher uptake of total N by these crops. Similarly, data summarized by Huber et al.[36] showed that the use of nitrification inhibitors improved the total N uptake by several field grown crops (Table 4).

Nishihara and Tsuneyoshi[69,70] found that the retardation of nitrification of urea and ammonium sulfate by several inhibitors

TABLE 4

Effects of Retardation of Nitrification on the Plant Composition of Some Field Grown Crops.[a]

Plant	Tissue	Constituent	Change in plant composition by retarding nitrification
			%
Spinach	Leaf	NO_3^-	-79.0
Wheat	Leaf	NO_3^--N	-50.0
Lettuce	Leaf	NO_3^-	-34.0
Sugar beets	Leaf	Total N	+10.5
Sweet corn	Leaf	Total N	+11.7
Sweet corn	Leaf	Total N	+10.6
Field corn	Grain	Protein	+17.0
Rice	Grain	Protein	+ 8.1

[a] From Huber *et al.*[36]

increased the uptake of N by rice because of increased yield over untreated fertilizer. Weir and Davidson[112] reported that mixing of the nitrification inhibitor AM (2-amino-4-chloro-6-methyl pyrimidine) at the rate of 2 kg/ha with urea (114 kg N/ha) increased the yield and N uptake by Pangola grass forage.

However, Patrick *et al.*[77] found that formulation of nitrapyrin with ammonium sulfate did not affect N composition of rice even though it was effective in retarding nitrification. Similarly, Parish[74] found that retardation of nitrification did not affect the yield or N uptake of sugarcane, but Prasad[79] found that nitrapyrin retarded nitrification and increased the N content of sugarcane leaves (Table 5). Jaiswal *et al.*[39] reported that the application of AM and 2-sulfanilamidothiazole (ST) increased millable cane yield and N uptake by sugarcane grown in a greenhouse pot experiment. Several others have shown that nitrification inhibitor application may not have any effect on yield or N uptake under certain situations[5,11,26,28,29,53,60]. It has been generally reported that with species that prefer NO_3^- such as wheat, retardation of nitrification either decreases or has no effect on N uptake[12,51,68]. Recently Hendrickson *et al.*[28] reported that higher ratio of $NH_4^+:NO_3^-$ due to retardation

TABLE 5

Effect of Nitrapyrin on Nitrogen Content of Sugar Cane Leaves Fertilized with Ammonium Sulfate (AS).[a]

Treatment	Nitrogen in leaves[b] 16 weeks	24 weeks
	%	%
Control	1.43	1.60
AS (103 kg N/ha)	1.67	1.69
AS + nitrapyrin	1.75	1.82
LSD (0.05)	0.10	0.07

[a] Data adapted from Prasad[79].

[b] Top most visible dewlap leaf samples were sampled at 16 and 24 weeks after planting.

of nitrification interfered with the metabolism of potatoes and decreased tuber yield and affected tuber development.

In India, use of nitrification inhibitors such as nitrapyrin and AM increased total N content in rice grain and straw in greenhouse and field studies[82,83], although Narain[66] found no effect on rice grain N content. Sahrawat and Mukerjee[95,96] found that treatment of urea or ammonium sulfate with nitrapyrin and karanjin (a furanoflavonoid from _Pongamia glabra_ seeds) increased yield, N uptake, and rice grain protein concentration (Table 6).

Hendrickson _et al._[28,29] evaluated the effectiveness of nitrapyrin to retard nitrification of anhydrous ammonia (84 to 168 kg N ha^{-1}) applied in early or late fall or just prior to planting corn. Nitrapyrin inhibited nitrification but did not

TABLE 6

Effects of Retardation of Nitrification of Ammonium Sulfate and Urea by Karanjin on the Composition of Rice Grown in Greenhouse Pot Experiments under Submerged Conditions.[a]

Crop particular	Increase by inhibiting nitrification
	%
Total N uptake	36-68
Seed protein	2-14

[a] From Sahrawat[92].

significantly affect yield, % N in the grain, or N uptake by the grain, although the ear leaf % N was occasionally increased (Table 7). They concluded that while the nitrification inhibitors can be effective in retarding nitrification they may not necessarily affect grain yield or plant N composition probably due to diverse soil conditions affecting N loss and the effectiveness of the inhibitor. Others[21,103,105] also have noted no response to application of nitrapyrin or other inhibitors on corn yield and N composition. Chancy and Kamprath[8] found that application of nitrapyrin with urea on sandy soils significantly increased corn grain yields, leaf N concentration, total N

TABLE 7
Effects of Nitrapyrin on Leaf N, Grain N, and N Uptake by Corn Grain.[a]

N application Time	Rate	Nitrapyrin	Ear leaf N	Grain N	N uptake by grain
	----- kg/ha	-----	------ %	--------	kg/ha
Early fall	0	0.0	2.42	1.10	39
	84	0.0	2.82	1.50	75
	84	0.55	2.88	1.47	75
	168	0.0	2.86	1.51	84
	168	0.55	3.13	1.48	83
Spring	84	0.0	2.84	1.42	83
	84	0.55	2.86	1.34	76
	168	0.0	2.90	1.51	86
	168	0.55	2.89	1.48	89
LSD (0.05)			0.20	0.08	10
C.V. (%)			4.90	9.80	9.9

[a] Adapted from Hendrickson *et al.*[29]

accumulation, and fertilizer N recovery in a wet but not in a dry year.

Ashworth et al.[3] injected nitrapyrin, carbon disulfide (CS_2) or trithiocarbonates with aqueous NH_4^+ to retard nitrification of NH_4^+ injected during autumn (November) or spring (February or March) and evaluated their effect on the yield and % N of grass. When applied in autumn, the inhibitors increased both yield and % N of grass after a mild, wet winter. The inhibitors, however, had little effect during the two subsequent winters. Little effect was observed with treatments in spring.

Laboratory work has shown that CS_2 is an effective inhibitor of nitrifiers in soil at relatively low concentration due to its volatility[6,81]. Ashworth et al.[2,3] noted that CS_2 was effective in retarding nitrification and improving N content of crop plants, while Malhi and Nyborg[54] increased the yield and N uptake of barley grain by treatment of fall-applied banded urea or aqua ammonia with CS_2, ammonium trithiocarbonate or potassium trithiocarbonate.

Rodgers and Ashworth[90] showed that the recovery of soil N by wheat was increased by nitrapyrin, dicyandiamide (DCD), or etridiazole (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole) (Table 8). At harvest, grain and dry matter yields were increased by DCD with and without fertilizer N in spring, but there were no consistent increases from etridiazole or nitrapyrin. They suggest that DCD may be more effective because it is more mobile and thus more evenly dispersed throughout the soil profile than the other inhibitors.

TABLE 8

Effects of Nitrification Inhibitors on % N Content and N Uptake of Winter Wheat from Soil Mineralized Nitrogen.[a]

Treatment	Inhibitor rate	N content	N uptake
	kg/ha	%	kg/ha
No inhibitor	0.0	3.42	49.3
Dicyandiamide	5.0	3.90	60.3
Dicyandiamide	20.0	4.67	60.1
Etridiazole	0.5	4.03	51.9
Etridiazole	2.0	4.35	50.1
Nitrapyrin	0.5	4.25	57.8
Nitrapyrin	2.0	4.53	47.1

[a] Data from Rodgers and Ashworth[90].

Studies in the USA have extensively evaluated nitrification inhibitors, especially nitrapyrin, for improving crop production for major agricultural crops under various tillage systems[37,60]. Most of the studies indicate that nitrapyrin and etridiazole retard nitrification under field conditions. However, yield response and increases in N composition of tissue and grain have not consistently been obtained. Responses are influenced by rainfall and other climatic factors, and by soil factors affecting N loss and efficacy of the inhibitors[30,67,72,73,102]. While most of these reports are concerned with the yield response rather than plant tissue or grain composition, it is evident that wherever yield responses are obtained they usually result in higher total N uptake.

Dicyandiamide has been widely tested, especially in West Germany. The literature relating to the effects of DCD on the yield and N composition of different field crops has been recently summarized in the proceedings of a conference[26]. The results obtained to date indicate that although DCD is effective in retarding nitrification in soil, yield responses in the USA have not been consistent. However, results of extensive studies in Germany indicate that the chemical often enhances N recovery by the crop when chemical N fertilizers or organic N sources such as manures are used[1]. Some results pertaining to the effect of DCD on utilization of cattle slurry N by silage corn are shown in Table 9. McGuinn[58] found that DCD did not retard germination of corn when applied in small quantities in direct contact with the seed, but was toxic to plants when applied in large amounts as the sole source of N.

TABLE 9

Effect of Dicyandiamide (DCD) Application on Cattle-Slurry Nitrogen Utilization and Uptake by Silage Maize in Field Experiments.[a]

	Cattle-slurry added		N uptake			
	Total N rate		1979		1980	
Time	1978-79	1979-80	-DCD	+DCD[b]	-DCD	+DCD[b]
	kg/ha					
--	No slurry (check)		82	76	61	57
Aug.	322	407	104	121	71	75
Sept.	237	333	122	123	81	94
Oct./Nov.	366	509	132	144	81	90
March (i)	241	488	112	128	113	126
March (ii)	544	877	151	181	107	117
	LSD (0.05)		11		6	

[a] Data adapted from Amberger[1].

[b] DCD added at the rate of 30 kg/ha.

Protein and organic nitrogen compounds

In general, plants supplied with adequate N in the NH_4^+ form contain higher amounts of total N and N components such as free ammonium, amides, and amino acids than plants supplied with comparable amounts of NO_3^--N[22,27]. The absorbed NH_4^+ apparently is rapidly detoxified to amides and amino acids, mainly in the roots[22]. In general, if yield is increased by use of nitrification inhibitors and protein content usually also increases (see Table 4 and Huber et al.[36], Sahrawat[93,94]). For example, Sahrawat and Mukerjee[95] found that application of karanjin increased rice grain protein by 2 to 14% over treatments using either $(NH_4)_2SO_4$ or urea as the source of N (Table 6). However, Warren et al.[109] found that nitrapyrin did not consistently give higher corn grain protein content (Table 10). As would be expected, where yield response by nitrification inhibitors is not obtained, the plant N protein content is not increased[8,21,28,103-106,111].

Sommer et al.[98] studied the effects of form of N and nitrapyrin on the protein quality and baking quality of winter wheat. They found that albumin and globulin fractions and baking quality of wheat were increased by the use of nitrification inhibitor.

Nitrate

Plants with very low to undetectable nitrate reductase activity accumulate large amounts of NO_3^- when grown exclusively

TABLE 10

Effects of Retarding Nitrification of Fall-Applied Anhydrous Ammonia by Nitrapyrin on Grain Protein Content of Corn.[a]

Treatment	Grain protein[b] Experiment 1	Experiment 2
	------------ %	------------
NH_3	8.0b	8.4a
NH_3 + nitrapyrin	7.6b	9.0b

[a] From Warren *et al.*[109]

[b] Values not followed by the same letter in a column differ significantly at the 5% level of probability.

with NO_3^--N. Also, NO_3^- accumulation can be large in leafy vegetable crops. Wright and Davidson[114] and Maynard *et al.*[57] have extensively reviewed the literature pertaining to the accumulation of NO_3^- in crops with special reference to vegetables and forage crops and the associated health hazards to animals and humans. They concluded that the use of nitrification inhibitors offers an effective practical solution for controlling NO_3^- accumulation in plants.

Extensive evaluation of nitrification inhibitors for a range of crops has clearly established that these chemicals have a potential in checking the problems associated with the tissue accumulation of NO_3^-[22,27,56,62,63]. Typical results pertaining to the effects of nitrification inhibitors on the content of NO_3^- in selected crop plants are shown in Table 4 and Table 11.

Phosphorus

It is generally found in greenhouse studies that retardation of nitrification increases P uptake by plants[14,15,40,49,56,66,68,94,100]. This is generally interpreted in terms of physiological effects and by changes in pH of the system. Koter[49] conducted a two-year pot study with rye fertilized with $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$ or urea with and without nitrapyrin and reported that $(NH_4)_2SO_4$ and urea increased plant P compared to NO_3^- sources. Nitrification inhibitor application also increased plant P and decreased the plant Ca:P in plant tissue. The uptake of P by corn was increased by application of nitrapyrin due to the drop in pH of the soil, which facilitated P uptake[14]. Mathers *et al.*[56] grew winter wheat forage with nitrapyrin added

TABLE 11

Effects of Nitrapyrin and Dicyandiamide (DCD) on the Nitrate and Oxalic Acid Content of Spinach Fertilized with Ammonium Sulfate.[a]

Treatment	NO_3^--N	Total oxalic acid
	%	
Ammonium sulfate	1.15	7.80
Ammonium sulfate + DCD	0.35	2.84
Ammonium sulfate + nitrapyrin	0.22	2.19

[a] Data of Kick and Massen[44].

to control the nitrification of urea, $(NH_4)_2SO_4$ or NH_4NO_3. Nitrapyrin application generally increased P uptake (Table 12).

Field results have not given this effect. Touchton et al.[103] found that nitrapyrin application with urea did not affect the concentration of P in corn ear leaf, grain or stover (see also Rudert and Locascio[91], Maples and Byrd[55], Warren et al.[111], Rhoads and Huffman[87]). The inconsistency in results obtained under controlled conditions and in the field could be explained by the fact that the retardation of nitrification may

TABLE 12

Effect of Nitrapyrin on Nutrient Uptake by Six Cuttings of Wheat Forage Grown in Greenhouse Pots.[a]

Treatment[c]	Nutrient uptake[b]				
	N	P	K	Mg	Ca
	-------------	-------------	mg/pot	-------------	-------------
Check	440	105	660	36	80
Urea	1080	174	1560	69	148
Urea + nitrapyrin	1130	206	1580	68	147
Ammonium sulfate	1050	161	1430	64	149
Ammonium sulfate + nitrapyrin	980	153	1300	57	129
LSD	68	16	96	17	14

[a] Adapted from Mathers et al.[56]

[b] Each pot held 12 kg of air-dried soil.

[c] Urea and ammonium sulfate were applied to give 75 mg/kg. Nitrapyrin was added at a rate of 2% of the applied fertilizer N.

be more complete in the limited volume of potted soil. This may bring about more pronounced changes in soil pH and other associated physiological factors that affect P absorption by plants.

Potassium, calcium and magnesium

The K, Ca, and Mg composition forage crops is very important because these cations influence the quality of forage and the performance of livestock. It has been found that grass tetany and frothy bloat will occur more often in wheat forage from fields moderately or heavily fertilized with N than from unfertilized fields. It is believed that when soil K is high, its uptake is further enhanced in wheat forage by the application of N, while the Ca and Mg concentration of the forage is little affected. This causes an increase in the equivalent K:(Ca+Mg) ratio in the forage. Wheat pastures having a ratio of K:(Ca+Mg) > 2.2 by weight might cause grass tetany[19,20].

In a short-term nutrient culture study in growth chambers with cucumber (*Cucumis sativa* L.), Zawistowska *et al.*[115] found that the absorption of K and Ca was decreased by nitrapyrin or its metabolite 6-chloropicolinic acid (CPA), and CPA was found to be more inhibitory to the uptake of these ions. Relative to untreated controls, K and Ca absorption were restricted in the two-week-old plants during the treatment periods ranging from 30 to 76 hours 17 and 25%, respectively, by nitrapyrin and 36 and 28% by CPA at $5.0x10^{-6}$ *M* concentration. It was suggested that nitrapyrin and CPA affected the uptake of ions by altering the membrane permeability in a manner similar to that proposed for auxin-type compounds.

While controlled studies in greenhouse and growth chambers[49,56,66] indicate that retarding nitrification may reduce the concentration of K, Ca, and Mg in plant tissue (Table 12), results of field studies do not support these findings. For example, Rudert and Locascio[91] reported that the growth and K, Ca, and Mg composition of sweet corn was not affected by nitrapyrin. Warren _et al._[111] found that nitrapyrin did not affect uptake of cations by corn. Others[53,87,103] have obtained similar results.

Kissel _et al._[48] reported that nitrapyrin consistently decreased the Ca and Mg concentration in NH_3-fertilized winter wheat tissue but had little effect on K levels or K:(Ca+Mg). The authors concluded that while the form of N does affect the mineral cation composition of wheat, the effect is not sufficient to exert a significant effect on the wheat or grass tetany problem.

Micronutrients

Spratt[100] pointed out that the maintenance of N in NH_4^+ form by nitrapyrin did not affect the concentration of Zn, Cu, Fe, or Mn in the wheat plants fertilized with ammonium and urea phosphate but Warren _et al._[110] found that Zn concentration in corn was increased by retarding nitrification. Extensive field evaluations in Indiana also indicated that nitrapyrin applied with anhydrous NH_3 increased the corn leaf content of Zn (Table 13), but not the concentration of Cu, Fe, B, Mn, Al, and Ba[111]. Touchton *et al.*[103] found that nitrapyrin application increased only the concentration of Fe in corn leaves.

Cation-anion balance

Thus far there are no studies that have reported how the cation-anion balance in plant tissue changes due to retardation

TABLE 13

Effect of Nitrapyrin Application on Zinc Content of Corn Leaves on a Sandy Loam Soil Fertilized with Anhydrous Ammonia.[a]

Nitrogen fertilizer			
Time of application	N rate	Nitrapyrin rate	Zn content of corn leaves[b]
	----- kg/ha	-----	mg/kg
Fall	0	0.0	29a[c]
	75	0.0	32b
	75	0.5	32b
	150	0.0	41c
	150	0.5	47d
Spring	75	0.0	32b
	75	0.5	36bc
	150	0.0	45cd
	150	0.5	47d

[a] From Warren *et al.*[111]

[b] Leaves opposite the ear harvested at the 50% silk stage. Data are means of five replications for 10 leaves per treatment.

[c] Numbers followed by the same letter in the column for both fall and spring applications do not differ significantly from each other.

of nitrification. Greenhouse and field studies reported have measured cations such as Ca, Mg, and K but among the anions only phosphate has been evaluated. We need crop quality data to evaluate the effects of nitrification inhibitors so that nutritional aspects of inhibitor use can be taken into account in recommendations on their use.

Organic acids

Organic acid anions provide a buffer to maintain an ionic balance in plants. Oxalic acid has been extensively studied because its concentration in feed is important for animal health. Excess of oxalate in forages may adversely affect the health of animals and at times even may lead to their death. Oxalate is also important for human health because excess intake of oxalate may cause deficiencies of Ca, vitamins B1 and B6 and lead to kidney stone problems[27].

Ammonium as a source of N tends to decrease the organic acid content in plants compared to NO_3^-. For example, Jurkowska[41] found that application of DCD with urea or NH_4^+ fertilizers decreased the oxalic acid content in spinach. This was attributed to the fact that plants absorbing NH_4^+ as a result of retardation of nitrification produced smaller amounts of oxalic acid than when NO_3^- was used[42]. Similarly, Kick and Massen[44] found that application of nitrapyrin and DCD decreased the concentration of oxalic acid in spinach fertilized with $(NH_4)_2SO_4$ (Table 11). The retardation of nitrification can also influence the accumulation of organic acids in plants indirectly by affecting the content of Ca, Mg, and K which, in turn, can influence the contents of organic acids such as oxalic, malic and citric[84].

Mathers *et al.*[56] found that aconitic acid concentration in the tissue of winter wheat decreased with nitrapyrin application. Due to differential mobility of urea and nitrapyrin, the retardation of nitrification of urea was less than that of $(NH_4)_2SO_4$ (Table 14). Thus, nitrapyrin was more effective in reducing the aconitic acid content in plants fertilized with $(NH_4)_2SO_4$ than with urea.

TABLE 14

Effect of Nitrapyrin Application on the Uptake of Aconitic Acid from Six Cuttings of Wheat Forage Fertilized with Urea, Ammonium Sulfate or Ammonium Nitrate In a Greenhouse Pot Experiment.[a]

Treatment[b]	Aconitic acid content
	mg/12 kg soil
Urea	1390
Urea + nitrapyrin	1280
Ammonium sulfate	1460
Ammonium sulfate + nitrapyrin	1120
Ammonium nitrate	1450
LSD	83

[a] Data adapted from Mathers *et al.*[56]

[b] Fertilizer N was applied at the rate of 150 ppm of soil and nitrapyrin at the rate of 2% of the applied fertilizer N.

It would appear that the use of nitrification inhibitors can improve the quality of vegetable and forage plants in situations where organic acid accumulation is a problem.

Other effects

In addition to the discussed effects of nitrification inhibitors on chemical composition of crops, there are certain other effects that have relevance to plant growth and its quality and are reported to be associated with retardation of nitrification.

Plant disease. The form of inorganic N (NH_4^+ or NO_3^-) also affects the incidence of plant diseases[35]. Several authors have reported that retardation of nitrification in soil and maintenance of N in the NH_4^+ form helps in reducing the severity of root rot in wheat, verticilium wilt in potatoes, corn stalk rot, and potato scab[9,13,23,33,34,46,78,109,113]. These benefits are a bonus but at times could be important in modifying the effects of N utilization due to differential effects on disease incidence and plant growth. Ultimately, this approach might be fitted into an integrated pest management program.

Phytotoxicity. Studies have indicated that nitrapyrin can be toxic to leguminous plants such as alfalfa and soybean[59,88] and to ryegrass and cotton[76]. Riley and Barber[88] also found that while yields of soybean seedlings were lessened by 8 to 20 mg/kg of nitrapyrin, their morphology was drastically changed with concentrations as low as 1 mg/kg. They suggested that the residual effects of nitrapyrin applied to other crops grown in cropping systems involving legumes in rotation should be considered because of sensitivity of legumes to low concentration of nitrapyrin. However, due to the rapid breakdown of nitrapyrin in soils, this effect is doubtful.

Geronimo *et al.*[16,17] evaluated the phytotoxicity of nitrapyrin and its principal metabolite, CPA to seedlings when applied to soil in concentrations ranging from 1 to 500 mg/kg (Table 15). Plants were grown up to 24 days. Higher rates of nitrapyrin were more phytotoxic to the graminaceous species than was CPA. However, CPA was more toxic than nitrapyrin to the dicotyledonous species tested. In general the dicotyledonous plants were more sensitive than the grasses to both nitrapyrin and CPA. All plant species tested except alfalfa and tomato were tolerant to soil concentrations of nitrapyrin much higher than that used in practice.

PERSPECTIVES

Nitrification inhibitors have been extensively evaluated in agricultural production for improving the efficiency of fertilizer N in situations where loss of N due to leaching or denitrification following nitrification limits N supply for crops. However, the effect of nitrification inhibitors on crop quality has received relatively less attention. Recent research, however, has shown that retardation of nitrification can also affect the quality of crops. The relative shift to NH_4^+ from NO_3^- nutrition affects soil chemistry and plant metabolism leading to

TABLE 15

Comparative Phytotoxicity of Nitrapyrin and 6-chloropicolinic Acid (CPA) to the Seedlings of 9 Crops Grown in a Sandy Clay Loam (pH 6.4).[a]

Crop	Chemical	ED_{50}[b]	HSC[c]
		----- mg/l	----
Alfalfa	Nitrapyrin	16	5
	CPA	5	2
Tomato	Nitrapyrin	35	5
	CPA	9	2
Soybean	Nitrapyrin	31	10
	CPA	7	2
Cotton	Nitrapyrin	62[d]	10
	CPA	16	2
Sugarbeet	Nitrapyrin	44	20
	CPA	11	5
Rice	Nitrapyrin	37	10
	CPA	88	10
Wheat	Nitrapyrin	70	20
	CPA	70	20
Corn	Nitrapyrin	165	20
	CPA	290	50
Sorghum	Nitrapyrin	120	50
	CPA	180	50

[a] From Geronimo _et al._[17] Nitrapyrin and CPA were added to the soil in concentrations ranging from 1 to 500 ppm by weight (ppm w).

[b] Soil concentration required to reduce the fresh weight of plant tops by 50% exprssed as ppm w.

[c] Highest soil concentration causing no significant reduction in fresh weight.

[d] Extrapolated.

differential absorption and accumulation of several plant constituents. There seems to be a striking similarity between the effects of NH_4^+ nutrition, and the effects following [illegible] of nitrification on plant composition. Use of nitrification inhibitors could provide valuable insights into the effects of form of N on plant metabolism and growth, assuming that the inhibitors do not have other side effects such as phytotoxicity or effects on the morphology or physiology of the plants.

Evidence in the literature clearly establishes that the use of nitrification inhibitors holds promise in alleviating the crop quality problems associated in certain situations with the accumulation of high amounts of NO_3^- or organic acids such as oxalic acid. However, their effect on the cation-anion composition of plants is not clearly established mainly because the results from controlled studies and field research have often given divergent trends. Also, it is difficult at times to ascertain whether a particular nutrient is taken up by the plant as a cation or an anion, especially under field conditions. Future research is needed to establish how the use of nitrification inhibitors affect the composition of various agricultural and horticultural crops and to establish how the cation-anion balance and organic acid composition, especially of forage and vegetable crops, are affected by the use of nitrification inhibitors.

In one nutrient culture study, it was found that CPA was more inhibitory than nitrapyrin to the uptake of cations such as

Ca and K at a very low concentration[115]. This only points out the need for more research on the fate of nitrification inhibitors in the soil particularly with reference to metabolites that may be more potent inhibitors of nitrification or have some undesirable traits such as phytotoxicity. These studies will be helpful in explaining some of the unusual results obtained with the nitrification inhibitors in certain soil-plant situations. Such studies have been made with nitrapyrin and can serve as models for other nitrification inhibitors.

Research is also needed to identify plants whose metabolism and quality is adversely affected or not sensibly altered by retardation of nitrification. Results from such studies will be valuable in the judicious use of these important chemicals for improving not only quantity but also quality of crops.

It is suggested that retardation of nitrification may affect plant composition due to one or more of the following factors[75]: (i) pH effects, (ii) ammonia toxicity (especially in poorly-buffered soils, (iii) ion uptake and competitive interactions, (iv) effect on electron-transfer system, and (v) interference with carbohydrate metabolism. At the present time, association of these factors with the use of nitrification inhibitors in relation to plant composition is barely established. However, this provides a good working hypothesis for future research.

There is little doubt that the use of nitrification inhibitors will increase NH_4^+ uptake. With the currently available information, however, it is not possible to precisely assess the effects of nitrification inhibitors on plant composition under field conditions. For fuller exploitation of the potential of nitrification inhibitors to modify crop quality by controlling the form of N supplied to plants, further research in this area merits higher priority. We hope this review will stimulate research to answer some of the unresolved questions pertaining to the use of nitrification inhibitors in relation to crop quality which is as important, if not more so than quantity.

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