

ABSTRACT

A real need exists to increase N fertilizer use efficiency by crops and to determine what soil and plant factors related to available N may limit crop productivity in given environments. Two forms of available N,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , may be supplied to a crop by choosing fertilizer N forms and nitrification inhibitors. The potential to supply a crop with two N forms,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  and to increase crop yields and the efficiency of N use by the crop was studied in field experiments with corn. Yield increases from 807 to 2421 kg/ha (12 to 36 bushels/acre) were obtained from treatments that increased the amount of available N in  $\text{NH}_4^+$  form. Nitrogen use efficiency by the crop was increased 18% by these treatments which can be utilized in farm practices.

INTERPRETIVE SUMMARY

The potential to supply a crop with both N forms,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and increase crop yields and the efficiency of N use by the crop, compared with the same amount of available N in either form alone, has been studied only in a few field experiments. The conditions where these phenomena are most likely to occur are those crop and soil environments that produce high yields and photosynthate supply and reduced N levels in leaves become limiting for grain yields.

The fertility treatments and crop yields with different varieties will provide information on how to improve production practices and crop quality, and the data will lead to knowledge about soil nutrient supply interactions with crop varieties. This research has the potential to increase the efficiency of N fertilizer use by crops and reduce the impact of escalating costs of agricultural chemicals on farm income. This research has the potential to explore and identify the factors which currently limit crop productivity in soils in a given environment.

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Net income per acre increases when farmers can apply management practices that improve soil productivity and increase corn yields per acre to maximum economic levels. For example, if a farmer produces 140 bu/ac (average yield for U.S. is 110 bu/ac), his net income will be approximately \$80 per acre. If a farmer can produce 300 bu/ac, his net income will be about \$520 per acre.

The potential to supply a crop with both N forms,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and increase crop yields and the efficiency of N use by the crop, compared with the same amount of available N in either form alone, has been studied only in a few field experiments. The conditions where these phenomena are most likely to occur are those crop and soil environments that produce high yields and photosynthate supply and reduced N levels in leaves become limiting for grain yields. In this paper we summarize evidence from the literature to suggest that about equal amounts of these two N forms will increase yields and N use efficiency by crops.

A real need exists to increase N fertilizer use efficiency by crops and to explore what soil and plant factors related to available N limit crop productivity in given environments. In a normal sequence of events, fertilizer N is applied to soil and  $\text{NH}_4$ -N forms are rapidly nitrified to  $\text{NO}_3$ -N, which is then the form of N mainly used by plants. This  $\text{NO}_3$  form also leaches readily and it may be displaced from the root zone. Studies with nitrification inhibitors have been mainly designed to keep more fertilizer N in the slowly leachable  $\text{NH}_4^+$  form, and thus conserve N for the crop. Crop residues and farmyard manure, as N sources for crops, also conserve N to some degree by holding N in non-leachable forms.

The relative effectiveness for corn growth of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  sources of N has been studied mainly in solution cultures and short-term greenhouse trials. Cox and Reisenauer (1973) found that wheat yield was increased 50% by adding  $\text{NH}_4^+$  to cultures supplying the maximum utilizable concentration of  $\text{NO}_3^-$ . They suggested this yield increase may be related to more efficient utilization of the plant's limited energy supply. Schrader et al. (1972) observed that growth of corn was most rapid when both N forms were supplied. More N was absorbed and assimilated when both N forms were provided and uptake rates were similar. When both forms of N were absorbed,  $\text{NH}_4^+$  was used preferentially for synthesis of amino acids and proteins. Tomato yields were highest in nutrient culture when the N supply was an equal mixture of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  forms.

The relative physiological capability for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake may change between vegetative and ear development growth in corn. The extent of this alteration may be influenced by source-sink relationships during ear growth, i.e., the grain is a sink for reduced N and zein plus glutelin are the main storage proteins in the endosperm. In solution-grown sweet

corn,  $\text{NO}_3^-$  uptake was slightly greater than  $\text{NH}_4^+$  uptake, from equal concentrations of each form, prior to silking, but  $\text{NO}_3^-$  uptake decreased about three times compared to  $\text{NH}_4^+$  uptake during ear development. The major transport form of N to the developing endosperm is glutamine, which is also the major product formed in roots absorbing  $\text{NH}_4^+$ . In other experiments with corn where  $\text{NO}_3^-$  was the main source of N, the progressive loss of reduced N from the stover showed that the rate of supply of newly reduced N from  $\text{NO}_3^-$  was insufficient to compensate for or to delay the remobilization of vegetative N during ear growth. Under these conditions stalk  $\text{NO}_3^-$  remained nearly the same. Below et al. (1981) assumed that  $\text{NO}_3^-$  has to be transported to the leaves to be reduced. Their data show that stalk  $\text{NO}_3^-$  acts as a storage reserve; however, utilization of this reserve is slow. Thus, the physiological response of corn to the two N forms in soil under conditions of equal availability, and subsequent effects on grain yield are not well characterized.

The effectiveness with which N is used by corn (*Zea mays* L.) is important because of increasing costs of manufacture and distribution of N fertilizer. In the past 30 years the increase in corn yields has not been proportional to increased N use (Messmer et al., 1984). The isolation of genotypes that are better able to utilize N fertilizer to produce higher grain yields per unit area would improve this relationship. Differences in N utilization among corn genotypes have been shown, not only in differential responses to N fertilizer, but also in differences in absorption and in utilization of absorbed N (Schrader et al., 1972; Beauchamp et al., 1976; Chevalier and Schrader, 1977; Moll and Kamprath, 1977; Tsai et al., 1978; Pollmer et al., 1979; Reed et al., 1980; Below et al., 1981; Moll et al., 1982; Swank et al., 1982; Messmer et al., 1984; Pan et al., 1984; Tsai et al., 1984). The potential for developing and finding superior, N-efficient hybrids appears to exist.

The metabolism of carbon and nitrogen are closely linked and with the increased use of fertilizer N being closely associated with higher corn yields, it is logical that both C and N metabolism should be considered in attempts to identify factors that limit productivity. Shading, plant density, and canopy studies indicate effects on photosynthesis as well as on nitrate reduction and assimilation. For rice, high yields are possible only under high levels of N supply and plant characteristics that confer high yielding abilities are often associated with responsiveness to N. Nitrogen has two major roles, 1) the establishment of the yield capacity and 2) the establishment and maintenance of photosynthetic capacity (Yoshida, 1972). Other work showed 90% of the N lost from the leaves during normal senescence was from the chloroplasts (Morita, 1980). Wittenbach et al. (1980) showed the loss of N from leaves of field-grown soybeans was concurrent with changes in chloroplast structure and loss of photosynthetic activity during the grain-filling period. In cereals, Mengel and Kirkby (1982) note that an adequate N supply during early growth stages was important in determining the number of ears per unit area. Tollenaar (1977) has suggested that sink sizes may frequently limit crop yields.

For corn, little information is available on the physiological processes that determine ear and kernel initiation. Krantz and Chandler (1954) showed that increasing soil N level from deficient to sufficient increased grain weight 3 or 4 times more than stover weight. A decrease

in leaf N of corn during the grain-filling period was concurrent with the loss of chlorophyll (Christensen et al., 1981). A third role for N as a factor limiting corn yields is indicated by work showing that protein accumulation in the kernel, especially zein, could be a factor in regulating kernel development (Tsai et al., 1978, 1984).

Efficiency in uptake and utilization of N in the production of grain requires that those processes associated with absorption, translocation, assimilation, and redistribution of N operate effectively. The relative contribution of these processes to genotypic differences in N use efficiency is unknown and may vary among genetic populations and in different environments, including N supply and the form of N, whether  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . The physiological response of corn to the two N forms in soil under conditions of equal availability, and subsequent effects on grain yield are not well characterized. Vegetative growth of solution-grown corn was increased by the simultaneous presence of both N forms in the root environment compared with either form alone (Schrader et al., 1972). Solution-grown sweet corn exhibited a greater affinity for  $\text{NH}_4^+$  over  $\text{NO}_3^-$  during ear development (Mills and McElhannon, 1982). Nitrogen uptake during ear development contributes significantly to the N translocated to the grain (Friedrich and Schrader, 1979; Moll et al., 1982). Swank et al. (1982) suggest it is the continued input of N into the plant that is responsible for the maintenance of leaf duration and continued photosynthetic activity. The availability of current photosynthate and reduced N ensures the longer duration of grain fill. The end result was a higher yield per plant.

The supply of reduced N to the ear during reproductive growth is important in the establishment and maintenance of a viable sink (Tsai et al., 1978, 1980, 1984). However, a balance between N in the vegetative plant parts and the N supply to the developing sink (kernels) must be achieved to allow maximum productivity. Extensive accumulation of reduced N in plant tissues could indicate a potential for maximum photosynthate production and sink development. Sinclair and de Wit (1976) concluded that the improvement of photosynthetic capabilities during seed development in soybeans without an increase in N assimilation at some point in the life cycle would be useless because maintenance of higher photosynthetic rates would be at the expense of seed development. In simulation model studies they found an enhanced rate of N assimilation greatly increased grain yield. Christensen et al (1981) concluded that the loss of N from corn leaves to the stalk and ear is a major cause of senescence. A long period of grain-fill could be facilitated by a large N supply in leaves at the start of rapid grain filling. A large N supply in leaves should allow a more appropriate redistribution of N to support ear development and leave more N in the leaves to maintain the photosynthetic apparatus (Messmer et al., 1984).

Pan et al. (1984) found that with  $\text{NO}_3^-$  as the N source for corn, grain yields for the five genotypes differing in prolificacy (capacity to produce more than one ear) indicate that the reproductive sink capacity of the prolific genotypes was under utilized and resulted in smaller yields than the nonprolific genotypes. The inability to absorb  $\text{NO}_3^-$  during ear development appeared to be a critical factor in limiting yields in the prolific genotypes. Grain yields were increased when these prolific genotypes were grown with the high supply of urea-supplied N rather than

$\text{NO}_3^-$ . Thus,  $\text{NH}_4^+$  was a more utilizable source of N in prolific plants during grain fill.

The relative physiological capability for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake may change between vegetative and reproductive growth in corn, and the extent of this alteration may be influenced by source-sink relationships during reproductive growth. With solution-grown sweet corn,  $\text{NO}_3^-$  uptake was slightly greater than  $\text{NH}_4^+$  uptake (50-50 ratio of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) prior to silking, but dramatically declined during ear development. In contrast, they observed that  $\text{NH}_4^+$  uptake rates increased during ear development and were nearly three times greater than  $\text{NO}_3^-$  uptake rates 3 weeks after silking (Mills and McElhannon, 1982).

The differential response to N source may be energy related. With rice, uptake of  $\text{NO}_3^-$  decreased considerably more than  $\text{NH}_4^+$  uptake as the energy status of the plants was changed from high to low (Mengel and Viro, 1978). No evidence is available concerning the relative responses to energy status during reproductive growth nor to the possible effects of plant density on these processes. Very few, if any, critical studies have been made of the relative effectiveness of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  for plant growth through the grain filling period or to maturity, with soil as the growth medium. Whereas, plant utilization of  $\text{NO}_3^-$  has been investigated extensively, the conditions associated with beneficial assimilation of  $\text{NH}_4^+$  and of the mixed forms are largely unknown and call for further investigation in both laboratory and field.

Results with a number of plant species subjected to  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nutrition have shown that each ion produces a different physiological response within the plant (Cox and Reisenauer, 1973; Richter et al., 1975; Haynes and Goh, 1978). Weight gains by adding  $\text{NH}_4^+$  to an all  $\text{NO}_3^-$  system have been observed in 14 crop species (Haynes and Goh, 1978; Tsai et al., 1978; Ganmore-Neumann and Kafkafi, 1980; Mengel and Kirkby, 1982; Mills and McElhannon, 1982; Pan et al., 1984). Why  $\text{NH}_4^+$ -N has this growth promoting effect is not known. However, the reduction of  $\text{NO}_3^-$  to  $\text{NH}_3$  requires energy and it may be reasoned that by supplying  $\text{NH}_4^+$ , energy is conserved and diverted to other metabolic processes including ion uptake and growth (Viets and Hageman, 1971; Cox and Reisenauer, 1973; Tsai et al., 1978; Pan et al., 1984). Three reports show that  $\text{NH}_4^+$  stimulates the activity of ribulosediphosphate carboxylase compared with  $\text{NO}_3^-$  in a marine alga (Paasche, 1971), in chloroplasts (De Benedetti et al., 1976), and in corn (Tsai et al., 1978).

Uribe and Lüttge (1984) discussed how the plant cell derives and uses energy, and how this use of energy is related to the movement of solutes across cell membranes. A pair of cell membranes, the plasmalemma and tonoplast, are the sites of solute transport systems driven by adenosine triphosphate (ATP). These membranes also serve as barriers to the diffusional movement of solutes out of the cell. The cell contains enzyme systems that conserve energy released in glycolysis and respiration, or energy captured in photosynthesis, in the form of ATP. Adenosine triphosphate is a major source of metabolic energy. Large amounts of energy are released from ATP in hydrolysis (by ATPase), when the molecule loses one atom each of hydrogen and inorganic phosphate to become adenosine diphosphate (ADP). A question central to solute transport is the means by which the free energy derived from the hydrolysis of ATP is used to drive this process.

The free energy obtained from hydrolysis of ATP by membrane-localized enzymes may thus be used to establish a proton gradient. Proton-translocating ATPases of this type are now known to have a widespread distribution in nature, which suggests a central role for the electrochemical proton gradient in processes of energy conversion. Thus, the use of the free energy of ATP hydrolysis in generating a transmembrane electrochemical proton gradient provides a general mechanism for energy-driven solute movement across plant cell membranes (Uribe and Lüttge, 1984).

The existence of ATPase localized in the plasma membrane has been shown for corn leaf mesophyll cells (Perlin and Spanswick 1981) and the roots of barley (Nagahashi et al. 1978) and corn (Dupont et al. 1981). The tonoplast exhibits ATPase activity in a variety of higher plant tissues, including corn roots (Dupont et al. 1982), red beet roots (Walker and Leigh, 1981, and Kalanchoe (Aoki and Nishida, 1984).

The function of the proton pumping ATPase provides the link from the utilization of the chemical bond energy conserved in photosynthesis and respiration to the establishment of a high-energy state common to the entire membrane. The ability to utilize metabolically generated energy in achieving the transport of solutes across semipermeable membranes is a prime requisite for the survival of a plant. The capacity to control the process is crucial so that cellular solute composition and concentration is maintained at levels that are optimal for the function of enzyme systems. These requirements of solute transport, as related to metabolic activity must be met while cell water potentials are also regulated, to provide the cell with an adequate supply of water (Uribe and Lüttge, 1984).

The properties of ATPase activity of the tonoplast of red beet cells shows an interesting possible connection with the metabolism and utilization of N forms by corn or other crops. The ATPase had a specific requirement for  $Mg^{2+}$  and in the presence of  $Mg^{2+}$  it was stimulated by salts of monovalent cations (Walker and Leigh, 1981). The degree of stimulation was influenced mainly by the anion and the order of effectiveness of the anions tested was  $Cl^- > HCO_3^- > Br^- > malate > acetate > SO_4^{2-}$ . For any given series of anions the magnitude of the stimulation (at 50 mM) was influenced by the accompanying cation ( $NH_4^+ > Na^+ > K^+$ ). In eight experiments with chloride salts the stimulation produced by  $NH_4Cl$  was always greater than  $NaCl$  or  $KCl$ .

This ATPase was inhibited by  $KNO_3$  and by N, N'-dicyclohexylcarbodiimide, diethylstilbestrol, and mersalyl, an -SH group poison (Walker and Leigh, 1981). For example, the relative ATPase activity (with  $MgSO_4$ ) was 1.00, 1.89 with  $KCl$ , 2.18 with  $NH_4Cl$ , 1.45 with  $KNO_2$ , and 0.28 with  $KNO_3$ . The cause of inhibition with  $KNO_3$  is not known. However, stimulation of ATPases by specific ions is often taken as evidence that the ATPase is directly involved in the membrane transport of those ions. Butz and Jackson (1977) proposed that a transmembrane nitrate reductase (NR) tetramer functions as a carrier for  $NO_3^-$  transport across root cells. An ATPase is visualized to be closely associated with the NR tetramer and this ATPase is inhibited by ADP. The connection between these cellular reactions and the utilization of  $NH_4^+$  and  $NO_3^-$  forms of N by crops in grain production is not known at present, but the evidence suggests a relationship that needs to be investigated.

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