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Truths or myths, fact or fiction, setting the record straight concerning nitrogen effects on levels of frost hardiness

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ABSTRACT

Approx. 50 papers (found from Scopus) published since 1990 were reviewed to determine whether or not nitrogen additions benefit frost hardiness in some plant species. The results varied according to species, timing of the effect, nitrogen source and plant tissue concentration. The key finding is that in 40% of reported cases nitrogen supply increased frost hardiness, while in 29% of cases nitrogen had no effect on frost hardiness. Together these findings comprise 69%, implying that in the majority of cases nitrogen additions are not deleterious but actually improve frost hardiness, especially in autumn.

1. Background

Nitrogen (N) is an essential element in plant chemistry, but in nature its concentrations may exceed the optimal range due to anthropogenic contaminations (e.g. fertilizers, pollutants). For a long time a consensus has prevailed that, increasing the availability of reactive nitrogen in the environment will impair the development of frost hardiness (Christersson, 1975; Puempel et al., 1975; Aronsson, 1980; Hellergren, 1981; Friedland et al., 1984; Nihlgård, 1985; Stimart et al., 1985; Pietilä et al., 1990; Lähdesmäki, 1990; Lähdesmäki et al., 1990a,b, 1993). This opinion was based mainly on the idea that N may prolong the growing season of plants and thereby delay the cold hardening process, or accelerate dehardening in spring. In some of these studies, however, frost hardiness determinations were not performed, rather the observed injuries under increased N supply were just assumed to be consequences of freezing (Taulavuori et al., 2013, and references therein).

To the best of our knowledge, DeHayes et al. (1989) were the first researchers who documented positive effects of N for plant frost hardiness. Since then more and more publications have indicated that N additions were not always associated with impaired levels of frost hardiness. In addition the number of studies reporting improved frost hardiness attributable to N has increased.

2. Aims and hypotheses

The aim of this paper was to collate the published findings on plant frost hardiness by nitrogen interactions, and identify which if any factors were based on the information provided below. The primary hypothesis (1) is that the number of published positive effects exceeds the number with negative outcomes.

Sheppard and Pfanz (2000) reviewed impacts of pollutants on cold hardiness, and concluded that N pollutants may bring forward spring bud burst and so increase the likelihood of frost damage to newly flushed shoots which are inherently frost sensitive. No experimental evidence was found that N pollutants could reduce cold hardiness via changes in foliar N concentration. The second hypothesis is thus (2) that the incidence of freezing injury depends on the season.

Detrimental effects of N induced predisposition to freezing damage may be indirect through nutrient imbalances arising from depleted base cation status, leached base cations from soil (Sheppard and Pfanz, 2000). Especially, NO_3^- deposition, and nitrification, increase the leaching of base cations through the mobile anion effect (Binkley and Höberg, 1997). Thus it is hypothesized that (3) nitrate underpin most of the reported results from studies where N additions have reduced frost hardiness. (4) In addition, it is hypothesized that some of the freeze-related injuries due to N are not directly related to freezing per se, but rather result from winter desiccation and photo-oxidation (e.g. Taulavuori et al., 2011).

Sheppard and Pfanz (2000) reviewed a case history of red spruce (*Picea rubens*) which naturally grows around 50° N latitudes. It was proposed that this species, which was growing at its range limits, lacked the “safety margin” between hardening capacity and

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minimum temperatures in winter. Some conifer species growing within their tolerance range e.g. Scots pine (*Pinus sylvestris*), at much more northern latitudes (65° N) show remarkable frost tolerance (i.e. LT50 < −100 °C for Scots pine needles during winter time, Taulavuori et al., 1997a,b). Sakai and Larcher (1987, and references therein) also reported that some conifers tolerate extremely low temperatures down to liquid nitrogen (−196 °C) when fully hardy. The difference between these "safety margins" between different latitudes may result from different adaptation mechanisms to annual changes in day lengths, which increase exponentially towards higher latitudes, being 10 h, 13 h, 21 h and 24 h at 50° N, 60° N, 65° N and 66.5° N latitudes, respectively. Logically we assume that the more northern is the species or ecotype, the stronger is the dependence of degree of frost hardening on photoperiod. In other words, frost hardiness in southern species is more controlled by temperature, which explains the low "safety margin". It is thus hypothesized that (5) species and populations from lower latitudes are more sensitive to extra N due to the absence of a safety margin between hardening capacity and minimum temperatures in winter.

Since conifers are generally thought to be most frost hardy among vascular plants (Sakai and Larcher, 1987), it may be hypothesized that (6) there are taxonomic differences in their nitrogen by frost hardiness relationship.

3. Data and the key finding

Our data consists of literature identified in Table 1. The literature is restricted to years 1991 – onwards, since it was about this time that techniques to quantify levels of frost hardiness became available and began to challenge previous thinking. The data consists of 52 case studies, which are classified as: (1) positive (+ve), (2) no effect (0), (3) both positive and negative (+/−), and (4) negative (−ve) effects of N to plant frost hardiness.

Articles were identified from Scopus using the following combinations of key words: (i) plant frost hardiness nitrogen, (ii) plant cold hardiness nitrogen, (iii) plant freezing tolerance nitrogen, and (iv) plant frost hardening nitrogen, in that order. Most of the articles were found using the first combination. Additional screening was made from the abstracts. Most of the experiments were performed in the field. Studies related to cryoprotection, tissue culture or in vitro experiments, for example, were not considered. In addition, there were plenty of inappropriate hits which pulled out liquid nitrogen use in the methodology. Data in Table 1 is organized chronologically based on the year of publication. Information is given for: response, plant genus, nitrogen source and form, nitrogen dose, tissue N concentration in response to nitrogen supply, type of tissue frozen, variables to describe methodology or physiological state, possible interaction or other comment and reference.

Fig. 1 shows the absolute and proportion of cases in each defined class. From the 52 findings, 40% were positive, 27% registered no effect, 6% both positive and negative, and 27% negative effects. Thus, in 67% of the reported studies no negative effects of added N on plant frost hardiness could be shown. So, our first hypothesis was supported by the data: Nitrogen additions are more likely to improve frost hardiness than reduce it.

4. Seasonal timing

Hypothesis 2 also proved correct: N induced freezing injury depends on the season. Interestingly, 5 out of the 13 cases reporting on reduced frost hardiness (Power et al., 1998; Gusta et al., 1999; Fløistad and Kohmann, 2004; Jönsson et al., 2004; Franzaring et al., 2013), concern spring time. This is related to hastened bud break due to higher N concentrations facilitating up-regulation of assimilation leading to an increase in sugar concentrations and consequent acceleration of dehardening, as concluded by Sheppard and Pfanz (2000). The main body of results in Table 1 concern autumn hardiness. There is also a report about reduced frost hardiness in autumn and winter with no effect during April (Sheppard et al., 2008). In olive tree autumn hardiness improved but spring hardiness decreased with nitrogen fertilization (Fernández-Escobar et al., 2011). In *Zoysia* grass the opposite responses were found i.e. decreased frost hardiness in autumn and improved frost hardiness in spring (Pompeiano et al., 2011). These observations indicate that generally the positive effects of added N occur during frost hardening in the autumn, whereas negative effects are possible both in autumn and spring.

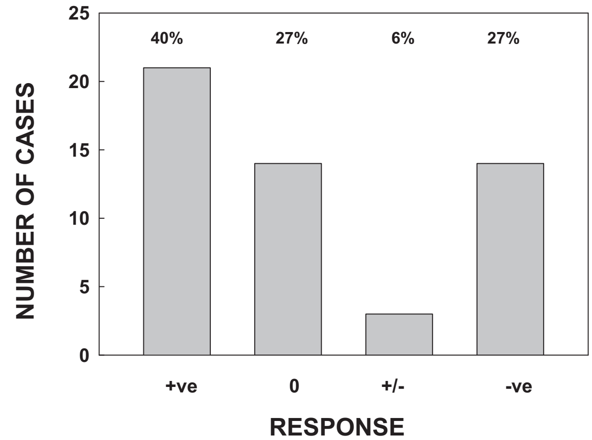

Fig. 1. Total and proportional number of observed cases in each effect classes: +ve = positive effect, 0 = zero effect (no positive and negative effects), +/− = positive and negative effects (both), and −ve = negative effects. The cases are observations collected in Table 1.

5. Nitrogen source

The data are based mainly on observations from fertilization studies. Only 6 cases concern nitrogen pollutants (either experimental supply or ambient) (Clement et al., 1999; Caporn et al., 2000; Shan, 2000; Clement et al., 2001; Sheppard et al., 2008; Franzaring et al., 2013). In these studies, increasing nitrogen supply improved frost hardiness in 2 cases (Clement et al., 1999, 2001) but reduced frost hardiness in 3 cases (Caporn et al., 2000; Shan, 2000; Franzaring et al., 2013).

Sheppard et al. (2008) reported both positive and negative effects. Much (18 articles) of the fertilization in the reviewed papers was supplied as ammonium nitrate sometimes in combination e.g. with PK. The review does not support Hypothesis 3 (i.e. indirect negative effects), since only in 3 cases did ammonium nitrate reduce frost hardiness. Indeed, ammonium nitrate alone increased frost hardiness in 4 cases (Caporn et al., 1994; Taulavuori et al., 1997a,b; Thomas and Ahlers, 1999; Taulavuori et al., 2001), and as NPK in 5 cases (Bigras et al., 1996; Rikala and Repo, 1997; Percival and Barnes, 2005; Fernandez et al., 2007; Hawkins and Stoehr, 2009). The latter results support Hypothesis 3 through improved cation balance provided by P and K.

Urea reduced frost hardiness only in one case (Guak and Fuchigami, 2002), but it increased frost hardiness in many other studies (Zilkah et al., 1996; Jalkanen et al., 1998; Webster and Ebdon, 2005; Li et al., 2012). In addition, in combination with ammonium nitrate, urea also increased frost hardiness two reports on *Quercus ilex* (Andivia et al., 2011, 2012). One special case that needs highlighting is the negative effect of ammonium chloride, where it was suggested that the addition of chloride could have caused the effect, rather than ammonium (Schaberg et al., 2002).