



REVIEW PAPER

Post-head-emergence frost in wheat and barley: defining the problem, assessing the damage, and identifying resistance

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Abstract

Radiant frost is a significant production constraint to wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), particularly in regions where spring-habit cereals are grown through winter, maturing in spring. However, damage to winter-habit cereals in reproductive stages is also reported. Crops are particularly susceptible to frost once awns or spikes emerge from the protection of the flag leaf sheath. Post-head-emergence frost (PHEF) is a problem distinct from other cold-mediated production constraints. To date, useful increased PHEF resistance in cereals has not been identified. Given the renewed interest in reproductive frost damage in cereals, it is timely to review the problem. Here we update the extent and impacts of PHEF and document current management options to combat this challenge. We clarify terminology useful for discussing PHEF in relation to chilling and other freezing stresses. We discuss problems characterizing radiant frost, the environmental conditions leading to PHEF damage, and the effects of frost at different growth stages. PHEF resistant cultivars would be highly desirable, to both reduce the incidence of direct frost damage and to allow the timing of crop maturity to be managed to maximize yield potential. A framework of potential adaptation mechanisms is outlined. Clarification of these critical issues will sharpen research focus, improving opportunities to identify genetic sources for improved PHEF resistance.

Key words: Barley, frost; reproductive frost; spring radiant frost; wheat.

Introduction

Post-head-emergence frost (PHEF) damage of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) is a problem of economic significance internationally. Frost susceptibility generally increases with plant maturity. In particular, crop sensitivity to frost is increased after the awns or spikes start to emerge from the auricle of the flag leaf [Zadoks decimal growth stage 49, (Zadoks *et al.*, 1974)], (Livingston and Swinbank, 1950; Single, 1964; Afanasev, 1966; Paulsen and

Heyne, 1983; Frederiks *et al.*, 2011a, b). Radiant frost occurs when still cold air, clear skies and a dry atmosphere combine, allowing rapid radiation of heat to the night sky (Foley, 1945; Hocevar and Martsolf, 1971; Willcocks and Stone, 2000). Such radiant frosts are a particular problem for autumn-sown spring-habit crops, grown through winter, that develop to susceptible post-heading stages in spring. The term 'spring radiant frost' has been used to describe these events.

Abbreviation: Post-head-emergence frost (PHEF).

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PHEF damage to cereals during spring radiant frosts is a complex process and is distinct from a number of other cold-mediated production constraints. For example, although many elite winter habit cereals show significant freezing tolerance in vegetative stages (Fowler and Carles, 1979), both winter- and spring-habit types suffer severe damage at more moderate temperatures during the reproductive stages (Livingston and Swinbank, 1950; Chatter and Schlehber, 1953; Paulsen and Heyne, 1983; Fuller *et al.*, 2007). In winter-habit wheat, the transition from the vegetative to reproductive growth stages is crucial to the regulation of low-temperature tolerance (Mahfoozi *et al.*, 2001). Vegetative freezing tolerance is well defined in the literature (Fowler and Carles, 1979; Fowler, 2008; Galiba *et al.*, 2009; Rizza *et al.*, 2011). However, relatively few studies of PHEF resistance have been published (Single, 1984, 1991; Woodruff *et al.*, 1997). As a result, PHEF is less widely understood and can potentially be confused with other cold-mediated production constraints.

Research over several decades has not identified PHEF resistance in wheat or barley at levels useful for breeding (Frederiks *et al.*, 2005, 2011a; Fuller *et al.*, 2007; Frederiks, 2010), despite periodic reports of initially encouraging results (Fletcher, 1983; Maes *et al.*, 2001; Reinheimer *et al.*, 2004). Identifying PHEF resistance presents researchers with a number of significant challenges, which have limited the rate of progress. Developing methods for screening PHEF resistance has proved difficult, as outlined in a recent review (Frederiks *et al.*, 2012). Similarly, ice nucleation and propagation in plants is complex, particularly under radiant frost conditions. In Frederiks *et al.* (2012) ice nucleation and issues with artificial freezing tests are also discussed. With an increasing recent focus on reproductive frost resistance in cereals (Reinheimer *et al.*, 2004; Fuller *et al.*, 2007; Rebeck *et al.*, 2007a, b; Chen *et al.*, 2009a, b; Zinn *et al.*, 2010; Frederiks *et al.*, 2011a; Gusta and Wisniewski, 2013; Wisniewski *et al.*, 2014), it is important to clearly define the phenomenon of PHEF, the extent of plant damage, and how it relates to other cold stresses.

In this review, we define the characteristics of spring radiant PHEF. We update the current understanding of the causes and impacts of spring radiant frost damage at different growth stages. Finally, strategies to manage PHEF risk and to identify sources of resistance are discussed. Many of the examples discussed here are from Australian cropping systems. However, the discussion is relevant to PHEF research internationally.

Defining the problem

PHEF crop losses typically arise in two ways: firstly, crop loss due to direct frost damage; secondly, lost yield resulting from late planting to delay heading until after the main period of frost risk has passed. The relative importance of direct frost damage and lost yield potential varies with climate and cropping systems.

Geographical extent and impact of PHEF damage

Economically significant PHEF damage typically affects spring-habit cereals but damage to winter-habit cereals has

been periodically reported (Livingston and Swinbank, 1950; Chatter and Schlehber, 1953; Paulsen and Heyne, 1983, Fuller *et al.*, 2007). PHEF crop damage is most important in regions with humid subtropical, Mediterranean, or maritime temperate climates. The problem is particularly common where, due to hot dry summers, spring habit cereals are grown through the winter and spring. Paradoxically, crops grown in warmer subtropical climates can be at greater risk of PHEF injury than those growing at higher latitudes, due to faster development from the relatively resistant vegetative stages to the more susceptible reproductive stages (Single, 1964, 1991; Frederiks *et al.*, 2011a, b). In subtropical regions, temperatures at night can fall to damaging levels despite mild daytime temperatures ideal for rapid plant growth (Single, 1984). In Mediterranean regions, more frequent rainfall and cloud cover may lead to a lower frequency of damaging night time temperatures, while lower day time temperatures can slow crop progression to susceptible growth stages. However, damaging conditions still occur, particularly during drier seasons.

Direct losses resulting from irregular frost damage of heading wheat and barley are significant internationally and have been widely described in Australia (Farrer 1900; Single, 1961, 1991; Woodruff, 1992; Frederiks, 2010; Frederiks *et al.*, 2011a, 2012). Even under optimal management where delayed sowing is used to postpone spike emergence past the main, mid-winter frost risk period, late spring frosts can result in significant crop losses. For example, ~50% of the total wheat crop in the Australian state of New South Wales was lost due to heavy frost damage occurring in early October 1965 (Boer *et al.*, 1993). Similarly, frosts in 2005 are estimated to have resulted in a 700,000 tonne loss in wheat production in the state of Western Australia (Department of Agriculture and Food, WA Government; GRDC 2012). Losses in particular districts may be more severe, with losses in excess of 85% recorded (Paulsen and Heyne, 1983). In affected areas, individual grain-growers risk crop failure.

Winter-habit cereals grown in temperate cropping regions can also suffer PHEF damage when an unusually early break in spring results in crops heading early, leaving them vulnerable to late spring radiant frosts. PHEF damage to winter-habit cereals has been periodically reported (Livingston and Swinbank, 1950; Chatter and Schlehber, 1953; Paulsen and Heyne, 1983). More recent examples include Canada, the mid-west of the USA and eastern USA in 2004, 2006 and 2007, respectively (Gu *et al.*, 2008).

Current management options and limitations

Frost escape by manipulating heading time is the main method currently available to minimize PHEF risk. Wheat and barley are planted late to delay heading past the main frost risk period, often leading to grain filling when warmer temperatures and drought conditions prevail. Heat and drought during grain filling and ripening can dramatically reduce crop yield potential. In Australia, for example, yield declines of as much as 16% have been reported for each week that anthesis is delayed past the optimum time (Kohn and Storrier, 1970; McDonald *et al.*, 1983; Woodruff and Tonks, 1983; French

and Schultz, 1984; Kerr *et al.*, 1992; Woodruff, 1992). Long-term yield is maximized when heading time is managed to balance the effects of frost risk with those of terminal heat and/or drought (Woodruff, 1981, 1992; Passioura, 2012).

The example of subtropical Australia illustrates the challenges faced by grain-growers as they balance the many variables in their production system. Growers need to manage variety choice at planting to manage frost risk. Depending on the timing of rain for planting, a quicker- or slower-maturing variety will optimize yield potential while maintaining an acceptable frost risk. Variety choice also depends on agronomic and economic considerations, such as diseases, pests, nutrients, water availability, grain yield and quality of varieties (Woodruff and Tonks, 1983; Woodruff, 2000a, b). Computer programs integrating many of these factors have been developed to aid decision-making (Woodruff, 1992; Wheatman™ Version 6.1, 2000). Unfortunately, despite best efforts to match planting opportunities with varieties, often the decision concerning which variety to plant can be dictated by the availability of commercial seed and the timing of rain events.

Post-head-emergence frost in relation to other cold stresses

A useful framework for classifying freezing injury in plants is provided by Gusta *et al.* (2003). Modifying this framework, plant cold-mediated production constraints may be grouped into four categories:

- (a) Chilling sensitive—suffering damage at temperatures above 0°C.
- (b) Freezing sensitive—damaged at temperatures close to the melting point with little potential to supercool before freezing (for example 0 to -2°C).
- (c) Supercooling but freezing sensitive—able to supercool to temperatures several degrees less than 0°C (for example at crop temperatures of -4 to -7°C), but suffering damage when tissue freezing occurs (Gusta and Wisniewski, 2013; Wisniewski *et al.*, 2014).
- (d) Freezing tolerant—able to tolerate extracellular ice formation within tissues. Freezing tolerance often requires a period of cold acclimation and the level of tolerance achieved following acclimation varies (Gusta *et al.*, 2003; Gusta and Wisniewski, 2013; Wisniewski *et al.*, 2014). For example, cold acclimated winter habit wheat and barley varieties can survive temperatures of -21°C and -15°C respectively during vegetative growth stages (Fowler and Carles, 1979). Freezing tolerance and cold acclimation develops over periods of days or even months while radiative frost damage typically occurs when night temperatures drop to damaging levels over several hours (Gusta *et al.*, 2003).

Using this scheme, examples of category (a) would be cereals such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and rice (*Oryza sativa* L.) that can be damaged by temperatures >0°C (Taylor *et al.*, 1974; Miedema, 1982; Kang and Saltveit, 2002; Bhosale *et al.*, 2007). Many broadleaved horticultural crops such as cucumber and tomatoes are injured or killed at freezing temperatures close to zero, and so fall

into category (b) (Gusta *et al.*, 2003). Wheat and barley, post head-emergence, supercool to temperatures below 0°C and avoid damage, but then suffer severe damage once freezing occurs (Single, 1964), placing them in category (c) (Single and Marcellos, 1974; Fuller *et al.*, 2007, 2009).

In reality classification is more complex, as some plants can fit into more than one of the above categories, depending on the developmental stage and the type of stress. In wheat, which is capable of supercooling and is classed as category (c) at heading, prolonged low daytime temperatures >0°C during pollen development and meiosis, can result in chill-induced sterility or category (a) damage (Qian *et al.*, 1986; Săulescu and Braun 2001; Cantrill *et al.*, 2007). Similarly, over-wintering winter-habit wheat would fit into category (d) in terms of tolerance during the vegetative period.

Chilling damage is distinct from frost damage

Prolonged chilling temperatures (>0°C), particularly under low light conditions, can result in pollen sterility. Chill-induced pollen sterility is regarded as a problem of economic significance in subtropical Asia (Subedi *et al.*, 1998) and useful genotypic variation is available to allow breeding selections (Qian *et al.*, 1986). However, improved adaptation to pollen chilling has received little breeding effort (Săulescu and Braun, 2001). Given that chilling damage can occur at mild temperatures (<10°C), it is somewhat counterintuitive that chilling damage is not a greater problem. Chill-induced pollen damage is rarely of economic significance either in Australia (Single, 1984) or in a number of other regions internationally where PHEF is a problem (Săulescu and Braun, 2001). In subtropical or Mediterranean climates, chilling conditions are typically relieved daily, even mid-winter. This diurnal variation in temperature may explain the low incidence of chilling damage observed.

Some have suggested that pollen development is the growth stage most sensitive to freezing temperatures in cereals (Powell *et al.*, 2012). However, empirical studies suggest that the loss of the physical protection of the flag leaf sheath is more important than the stage of pollen development. Full sensitivity to freezing temperatures is not achieved until after awn-emergence, with both female and male floral structures usually equally affected following freezing damage (Livingston and Swinbank, 1950; Single, 1964; Frederiks *et al.*, 2011a, b). There is little to suggest involvement of chill-induced pollen sterility in PHEF freezing injury or, by extension, that genotypic differences in chilling injury confer differences in PHEF resistance. Researchers need to be mindful of the possibility of chill-induced injury so as not to confuse this damage with true frost or freezing injury when screening for PHEF resistance. It is important that researchers carefully inspect trials for pre-existing PHEF damage and unrelated sterility before frost events (Frederiks *et al.*, 2012).

Characterizing the frost environment

Conditions leading to PHEF damage

Radiant frosts are the common cause of PHEF damage, occurring when still cold air, clear skies, and a dry atmosphere

combine to allow rapid radiation of heat to the night sky (Foley, 1945; Hocevar and Martsolf, 1971; Willcocks and Stone, 2000). Meteorologists report ‘ground frosts’ when grass temperatures fall to less than 0°C (Foley, 1945). Post-head-emergence wheat and barley crops can resist damage from mild ground frosts (Single and Marcellos, 1974; Single, 1991). However, as air temperatures within the crop canopy fall below -3.5°C (Woodruff *et al.*, 1997), the actual plant minimum temperature can reach approximately -5°C. At crop canopy air temperatures of -3.5 to -4.5°C and below, post-head-emergence wheat and barley crops can suffer damage (Frederiks *et al.*, 2004a; Frederiks, 2010; Frederiks *et al.*, 2011a). PHEF damage typically results from injury due to ice formation in the tissues (Fuller *et al.*, 2007; Frederiks, 2010).

The physical freezing of crop tissues during PHEF is mediated by factors such as plant species, plant development stage (Single, 1964), the presence of ice-nucleators (Lindow, 1983; Wisniewski *et al.*, 2014), the extent of supercooling (Gusta and Wisniewski, 2013; Wisniewski *et al.*, 2014) and temperature. The temperature experienced by a crop can vary widely in a given region due to the interactions of topographical, meteorological, environmental, plant physiological and canopy architectural factors (Marcellos and Single, 1975; Woodruff *et al.*, 1997; Gusta and Wisniewski, 2013).

Spatial temperature variation during radiant frost

Temperature measurements can be used as a guide to determine when crops should be assessed for PHEF damage and as an indicator of the intensity or damaging potential of frost events. Damage increases rapidly once a critical plant minimum temperature is reached (Woodruff *et al.*, 1997; Frederiks *et al.*, 2011a, 2012). However, a number of factors need to be considered when determining the most informative temperature measurement.

In field screening studies, canopy air minimum temperatures and plant minimum temperatures are typically more useful than Stevenson screen minimum temperatures for characterizing frost events. Canopy air temperature can be measured using fine thermocouples, thermistors or thermometers near the crop canopy and exposed to the night sky. The temperature of the plant itself can be measured using fine thermistors or thermocouples directly attached to the exposed flag leaves (Frederiks *et al.*, 2012). At mild sub-zero air temperatures, the minimum temperature of crop plants is typically ~1 to 3°C colder than canopy air temperatures measured by exposed canopy thermometers (Fig. 1). The Stevenson screen air temperatures most commonly quoted by meteorologists can be poorly correlated with plant temperatures during radiant frosts (Hayman *et al.*, 2007; Frederiks *et al.*, 2011a). The screen protects the instruments from radiant heat loss so that the temperature recorded inside the screen drops more slowly than the exposed crop. As there is little wind during a radiant frost event, temperature differences can develop between the Stevenson screen, the air near the crop canopy, and the plant itself. During a radiant frost event, Stevenson screen temperatures tend to be higher than those of the air near the crop canopy, which are higher than that of the plant.

Measurements of plant minimum temperature give the best indication of likely plant damage, are precise, and are useful for research purposes.

The low levels of air movement during radiant frosts allow temperature gradients to develop. Plant and air temperature within the crop canopy can vary by several degrees during a radiant frost (Fig. 1; Marcellos and Single, 1975). The coldest conditions are typically observed near the top of the canopy where temperatures can be 2°C colder than temperatures measured mid-canopy, due to rapid radiative heat loss from the upper canopy. Temperatures measured low in the canopy are usually milder. Soil temperatures within the crop can be several degrees warmer than air or plant temperatures. In exposed areas, where meteorology stations are predominately located, a temperature inversion often occurs where the coldest temperatures are observed at or near ground level (Foley, 1945) rather than at the canopy level as occurs for crops once the crop canopy has closed (Marcellos and Single, 1975; Woodruff *et al.*, 1997). Differences in crop canopy structure (Gusta and Wisniewski, 2013) can affect the dynamics of air movement and heat fluxes in the canopy and around the exposed spikes. Crops with a more open canopy may allow better drainage of cold air from above the canopy near the spikes, reducing the risk of damage. Similarly, an open canopy or wide row spacing, leaving exposed soil between crop rows, may increase radiation reaching the soil resulting in increased soil temperature during the day. This may allow increased re-radiation of heat from the soil to the crop canopy at night. Residual stubble may also affect heat flux within the crop canopy by intercepting radiation during the day and by impeding re-radiation of heat from the soil at night (Woodruff 2000a, Rebbeck *et al.*, 2007a). Soil moisture also has an effect on the soil heat sink. Strategies aimed at reducing canopy density and increasing heat storage and radiance of the soil have been proposed (Rebbeck *et al.*, 2007a, b) however, frost escape by reduced canopy density may also lead to decreased yield potential by reducing grain number per unit area. Similarly, removing stubble to increase soil heat storage and re-radiation may be counterproductive when viewed on a farming system level, particularly under zero-till cropping systems, due to potential reductions in soil moisture infiltration and retention.

The crop temperature can vary widely due to differences in topography (Kelleher *et al.*, 2001), micro-environment (Marcellos and Single, 1975) and recording method (Hayman *et al.*, 2007). For example, small differences in topography can cause significant temperature variation by impeding or facilitating the drainage of cold air.

The temperature at which damage occurs can also vary under experimental conditions. For example, in freezing chambers, wheat heads routinely supercool to temperatures lower than -5°C without freezing (Fuller *et al.*, 2009), and supercooling to temperatures as low as -15°C has been observed (Fuller *et al.*, 2007). Misting plants with water reduces deep supercooling but does not alleviate the problem (Fuller *et al.*, 2009, Frederiks *et al.*, 2012). In contrast, under field frost conditions, where damage can occur at plant minimum temperatures of approximately -4°C, universal severe damage is

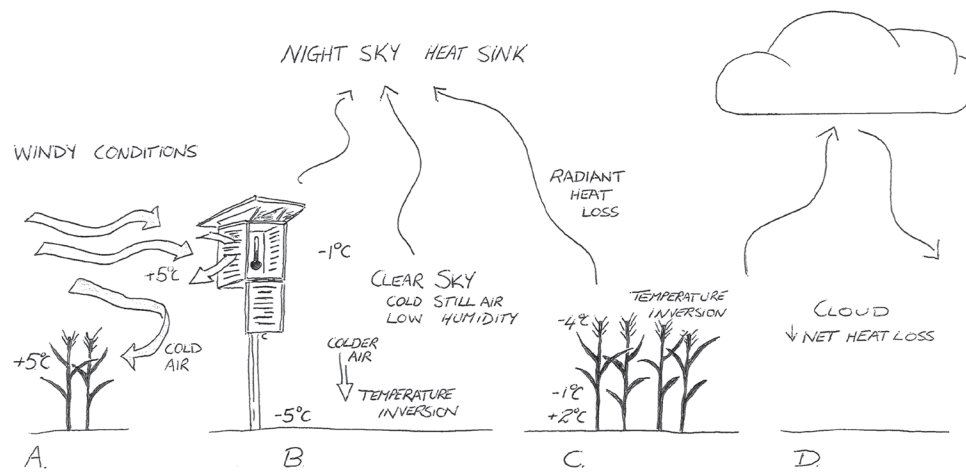


Fig. 1. Schematic of relative temperatures during conditions which are: (A) clear and windy, (B) clear and still in an open area, (C) clear and still in a cropping area, and (D) cloudy. Cold windy days draw the temperature down, however the wind causes air mixing and prevents a radiant frost from developing. If clear skies and cold, low humidity air combine under still conditions, heat can be lost rapidly to the night sky resulting in a radiant frost. Temperature inversions can occur with the grass temperature significantly colder than the Stevenson screen temperature. During a frost the screen prevents radiant heat loss and, with little air movement, the screen temperature may poorly reflect the temperature of the crop. In a crop, the temperature inversion during a radiant frost can occur towards the top of the canopy. Temperature variation in the order of several degrees can be observed vertically within the canopy (Marcellos and Single 1975). Cloud cover reduces the net radiant heat loss and thus the likelihood of radiant frost.

typically observed once the temperature of the wheat heads fall lower than approximately -6°C (Frederiks, 2010). The reason for this discrepancy is not fully understood. However, it indicates that results from artificial frost chambers should be interpreted with caution and be validated by field experimentation (Frederiks *et al.*, 2012).

Temporal characteristics of PHEF events

Often in the days prior to a spring radiant frost, cold, low humidity and windy conditions draw the temperature down which, if followed by clear, calm night-time conditions, can result in a radiant frost. Natural radiant frost events are unpredictable in timing, frequency and intensity. Before, during and after frost events, dramatic and rapid changes in temperature can be observed. Fig. 2 shows the temperature variation during a typical frost event on 16 June 2001 at a test site in southern Queensland, Australia (Kingsthorpe, 27.51°S , 152.10°E , ~ 480 m a.s.l.). In general terms, maximum temperature is reached in the early afternoon. In the period from 4 pm to 8 pm, a rapid temperature drop can be observed (Fig. 2), followed by a more linear rate of cooling after ~ 7 pm to 8 pm. During damaging frosts, cooling typically occurs at a rate of approximately 0.7 to 1°C per hour from 8 pm until 6 am (Fig. 2). However, this rate of nocturnal cooling is not necessarily constant. During any given frost, periods of more or less rapid cooling can be interspersed with periods of warming or periods of little change. Gentle, local air movements and sparse but sporadic cloud cover contribute to this variation. The transitory minimum temperature is typically reached at or just before dawn, and in the case of Fig. 2, at $\sim 6:45$ am. After dawn, in this particular example, the temperature rose rapidly (7°C per hour) for about 2.5 hours, with further temperature rises occurring at a decreasing rate.

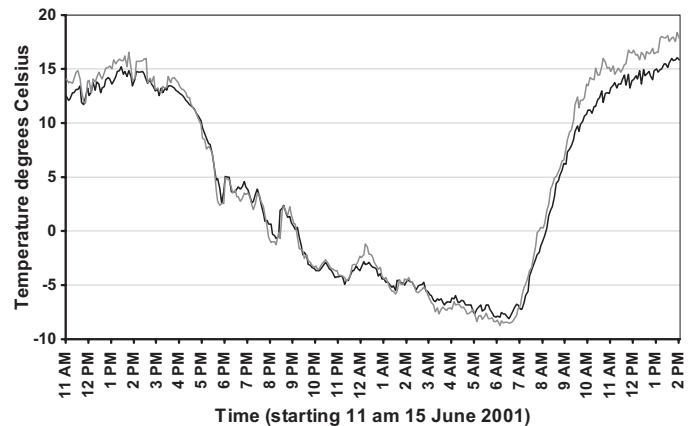


Fig. 2. Typical plant minimum temperature traces recorded every 5 min by two independent probes spaced 10–15 m apart within the crop canopy of the trial during a frost event on 16 June 2001 at the Kingsthorpe field site in Queensland Australia (Frederiks, 2010).

Classifying and assessing damage

Frost damage varies with growth stage and species

Symptoms of frost damage vary with growth stage and species. Frost susceptibility in cereals generally increases with plant maturity (Single, 1964, 1984, 1991). In particular, plants become more susceptible post head-emergence. This is why PHEF is of particular concern. For example, in Australia, severe damage to crops prior to heading is not common, and when it does occur, there is typically time for later-formed tillers to partially compensate, enabling reasonable recovery of yield (Single, 1991; Woodruff *et al.*, 1997). There is good evidence to indicate that post-head-emergence wheat is less resistant than barley (White, 2000; Frederiks 2010; Frederiks, *et al.*, 2011a). Wheat can be affected when the canopy air temperature reaches -3.5°C , with damage increasing rapidly as

the temperature falls further (Woodruff *et al.*, 1997). Barley crops can suffer significant damage at crop canopy temperatures of about -4 to -5°C (Frederiks, 2010; Frederiks *et al.*, 2011a). Understanding the physiological basis of this difference in susceptibility to PHEF between wheat and barley may be helpful in gaining additional adaptation in wheat. Oat crops, like barley, are generally considered more resistant than wheat (White, 2000), while recent work on triticale suggests that it is less resistant than wheat (Tshewang, 2011). Hence the ranking for PHEF adaptation in key temperate cereals is barley/oats > wheat > triticale. Although cereal crops are most susceptible to radiant frosts post-head-emergence, damage can occur at earlier stages under more severe conditions.

Young crops

In vegetative stages, cold-acclimated winter-habit wheat and barley can develop improved levels of freezing tolerance (Fowler and Carles, 1979; Gusta *et al.*, 2003; Fowler, 2012). There is considerable variation for vegetative frost tolerance in winter-habit and, to a lesser extent, spring-habit wheats (Fowler *et al.*, 1999a, b; Limin and Fowler, 2006; Frederiks, 2010).

For spring-habit cereals growing through winter, leaf scorching of young crops can occur. Leaves go limp, with affected areas appearing water-soaked, sometimes leading to scorching. The scorched tissues gradually desiccate and die (Fig. 3). Damage may be confined to leaf tips, or alternatively, the large horizontal leaves may be scorched at their point of maximum sky exposure, since exposed horizontal leaves tend to lose heat faster than erect leaves (Gusta and Wisniewski, 2013). Young crops will usually regrow from damage at this stage (Afanasiev, 1966; Woodruff *et al.*, 1997). More severe frosts can result in crop failure and such damage has been reported for wheat crops at canopy air temperatures $<-7^{\circ}\text{C}$ (Woodruff *et al.*, 1997).

Advanced crops prior to head-emergence

As crops enter the reproductive phase and as head development proceeds, susceptibility to frost increases. With heads and awns fully enclosed by the flag leaf sheath, significant damage to stems and developing heads can occur at canopy air temperatures $<-6^{\circ}\text{C}$ in wheat (Woodruff *et al.*, 1997). Damage to stems is usually observed adjacent to the nodes. At milder sub-zero temperatures when stems remain undamaged, damage to the developing ear inside the leaf sheath can still occur. If the head emerges after such a frost event, this damage often presents as a bleached section with incomplete ear structure and aborted florets, as seen in Fig. 4 (Woodruff *et al.*, 1997). When heads are nearly fully formed, but still completely enclosed in the flag leaf sheath, prolonged periods of low temperatures $>0^{\circ}\text{C}$ during pollen development and meiosis (particularly associated with low light intensity) can result in chill-induced pollen sterility, as discussed previously.

Advanced crops (heads emerging or emerged; PHEF damage)

Cereals generally reach maximum susceptibility to radiant frost during and after awn and head emergence from the



Fig. 3. Vegetative frost damage can occur even in subtropical cropping regions. This forage oat trial at Wellcamp in Southern Queensland, Australia, was severely frosted in the vegetative stages during a frost in June 2010.



Fig. 4. Frost damage to a developing wheat ear prior to head-emergence results in individual florets failing to form. This symptom distinguishes damage prior to head-emergence from PHEF damage, which can kill fully formed individual florets (see Fig. 6).

auricle of the flag leaf (Frederiks *et al.*, 2011b). Stem and whole-head damaging frosts occur most commonly at this stage, leading to severe yield reduction or total crop loss. The crop is also susceptible to milder temperatures than those required for economic damage prior to head emergence. For

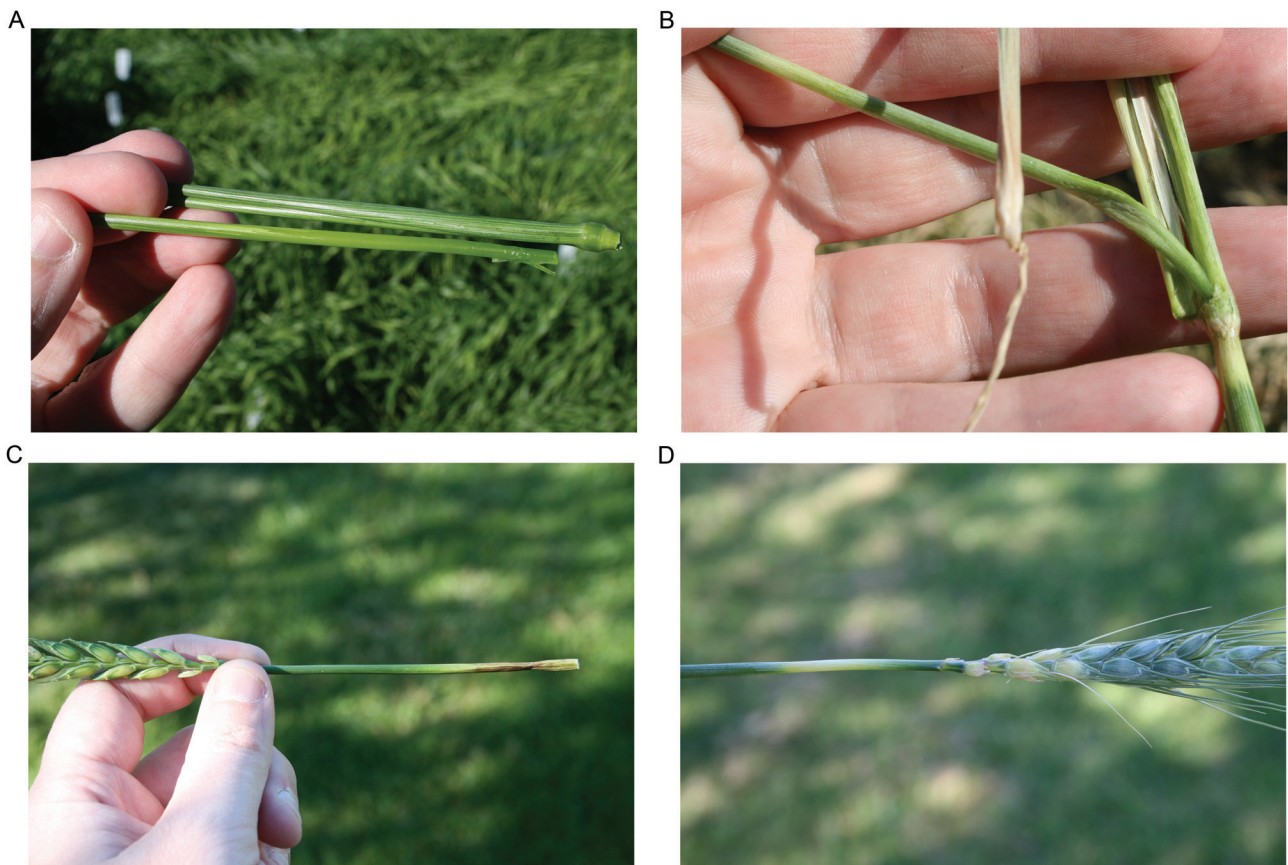


Fig. 5. Wheat stem showing severe frost damage. (A) Stem frost damage on the morning of 25 June 2013 at Wellcamp, southern Queensland, Australia. Note the lower peduncle appears water-soaked and dark green, typical of stem freezing. (B, C) Elongating stems will often collapse around the lower 30mm of peduncle. (D) Less severe frosts can result in a bleached collar that may not result in complete loss of stem conductance or grain yield.

example, post head-emergence crops can be damaged at canopy air temperatures of about -3.5 to -4.5°C for wheat and about -4 to -5°C for barley. Damage to the peduncle is often the most noticeable within 30mm of the top node (Fig. 5; Woodruff *et al.*, 1997), where tissue becomes water-soaked and dark green in colour (Fig. 5A), later shrivelling and drying out (Fig. 5B, C). This stem damage may sever the vascular connection between the head and the rest of the plant causing the head to die (Afanasiev, 1966). When damage is less severe, a range of symptoms may be observed depending on temperature and fine differences in developmental stages. Stem conductance may be reduced but not cut completely, leading to shrunken grains and reduced grain weight. Less severe stem damage may result in chlorotic bands (Fig. 5D) on the peduncle, even though stem function and yield are largely retained (Shroyer *et al.*, 1995). Stem damage may be concurrent with damage to individual floral structures, however this is not always the case (Single and Oliin, 1967). When stem function is not lost, floret damage may still result in individual grains within the head being damaged. This is most commonly observed in barley at plant temperatures of about -6.5°C (Fig. 6; Frederiks *et al.*, 2011a). The cause of this damage may be ice formation in the spikelet but without the ice front crossing through the spikelet nodes into the vascular tissue of the rachis or peduncle (Single, 1984; Woodruff *et al.*, 1997). This damage to fully formed florets (Fig. 6) can be distinguished from partial head damage occurring prior to

head-emergence (Fig. 4), as in the latter case damaged areas lack fully formed florets.

PHEF-damaged heads may appear outwardly normal for some time after damage, so close examination and dissection of florets is often required to identify frost damage (Fig. 7). Damaged stigmas lose their feathery appearance and become 'clumped'. Anthers can appear deformed and eventually discolour. As damaged heads desiccate, anthers can extrude from gaping glumes to give dead heads the superficial appearance of anthesis. Frost damage during grain fill is also difficult to assess. It is generally believed that the attachment between the grain and the ear is constricted. Regardless of precisely where the damage occurs, damage to the supporting stem or grain itself will have a similar final result. Damage usually shows as discoloured grains, which may be shrunken, water-soaked or hollow in cross section and when squashed, exude straw-coloured, rather than milky dough. These grains subsequently dry back to form shrivelled grain (Fig. 8). If harvested, this shrivelled grain can lead to down-grading due to excessive screenings (Woodruff *et al.*, 1997; Cromey *et al.*, 1998).

Defining and identifying resistance

Given the limitations of current frost risk management strategies, genetic PHEF resistance would greatly enhance grower options. This section outlines some strategies for identifying PHEF resistance.



Fig. 6. Frosted barley head showing individual floret damage. When frosted before grain fill, individual damaged florets appear translucent. Note that florets are fully formed prior to frost damage versus Fig. 4, which shows florets damaged before they were fully formed.



Fig. 7. Wheat head showing severe frost damage. Stigmas have lost their normal 'feathery' appearance, now appearing 'clumped'. Anthers are bleached and bowed. Orange spray paint on the upper glumes enabled the phenological stage on the day of the frost event to be later determined when the culm was harvested to assess frost damage as described by Frederiks *et al.* (2012).

Defining frost adaptation

To clarify the physiological basis of adaptation to PHEF in cereals, it is helpful to use a framework outlined by Levitt (1972) for describing drought adaptation. Using such a framework, frost adaptation can be assessed in terms of frost escape and frost resistance. The latter can be further partitioned into freezing avoidance and freezing tolerance (Fig. 9). With freezing avoidance, the plant faces the challenge of temperatures lower than 0°C but avoids ice formation, for example by deep supercooling. This is similar to category (c) in the plant cold response framework discussed above. By contrast, freezing tolerance describes the ability of tissues to freeze without causing death. This is comparable to freezing tolerance in the vegetative stages discussed in category (d) of the plant cold response framework above.

Current management strategies employ frost escape by manipulating planting date and cultivar selection so that susceptible heading stages occur outside the main frost risk period. Wheat and barley are highly adaptable in phenology and may be selected for extreme differences in heading dates. For example in subtropical northern Australia, significant progress has been made in balancing yield potential with frost risk by optimizing variety sowing times (Woodruff 1992). However, genetic PHEF resistance would greatly enhance the

capacity of grain-growers to produce crops that head earlier, targeting yield potential, while maintaining an acceptable frost risk.

Finding PHEF resistance

Resistance to stem and whole head PHEF damage would provide a significant advantage but is yet to be identified, despite research for over a century (Farrer, 1900; Single, 1984, 1991; Frederiks, 2010; Frederiks *et al.*, 2011a). Variation in individual floret damage has been reported among barley genotypes following frosting at, or before, anthesis (Reinhiemer *et al.*, 2004; Frederiks *et al.*, 2011a). However, resistance that offers an improvement over current elite cultivars has yet to be identified (Frederiks *et al.*, 2011a, 2012). The failure to identify resistance after years of testing suggests that PHEF resistance may be rare. However, the number of genotypes rigorously evaluated remains relatively small (Frederiks *et al.*, 2012). A novel strategy has been proposed to identify likely candidate genotypes from germplasm collections using a focused identification of germplasm (FIGS, Mackay *et al.*, 2004) approach based on environmental factors at collection sites, followed by field screening using a multiple step

screening approach (Frederiks *et al.*, 2012). However, field screening of large numbers of genotypes for PHEF still remains a difficult task.

Expanding our understanding of the potential physiological basis of resistance will enhance our capacity to identify mechanisms of resistance and better direct the search for



Fig. 8. Frost damage during grain fill results in shrunken shrivelled grains. Frosted grains dry back to shrivelled potentially harvestable grains. Frosted grain may initially appear relatively normal before desiccating and ‘pinching’. The final size of the desiccated grain depends on the stage of grain fill when the frost occurs. Before fully dry, 7–14 days after a frost, grain may appear discoloured, shrunken, water soaked or hollow.

germplasm with the desired mechanisms. As described above, PHEF resistance may involve either freezing avoidance or freezing tolerance (Fig. 9). Developing new cultivars that can supercool (i.e. avoid freezing) to lower temperatures is one potential strategy to improve resistance. For example, if crops could avoid freezing at temperatures $\sim 2^{\circ}\text{C}$ lower than the limit of current cultivars, the incidence of frost damage could be dramatically reduced in Australia (Woodruff 2000a; b). How are heading wheat and barley plants able to deep supercool to temperatures as low as -10°C to -15°C without freezing in artificial freezing chambers (Frederiks *et al.*, 2004b; Fuller *et al.*, 2007)? In a recent review on plant cold hardiness, Gusta and Wisniewski (2013) also asked why some plants can supercool to -20°C , while others can supercool to -40°C . What features at the cell or organ level explain such differences? Supercooling remains poorly understood despite the widespread prevalence of this phenomenon in many plant species (Gusta and Wisniewski, 2013). Improved insight into the mechanisms of supercooling is critical to advancing our knowledge of PHEF resistance.

Similarly, the causes of ice nucleation and freezing in the field are not well understood. For example, what is the initial site of nucleation under field radiant frost conditions? Is external ice initiating freezing? Can the propagation of ice from the site of nucleation be slowed or prevented? Tissue structures, for example both the nodes and crowns of cereals, can affect the direction and rate of ice propagation (Single and Marcellos, 1981; Pearce and Fuller, 2001; Wisniewski *et al.*, 2009). As previously highlighted, nodal resistance to ice transmission may prevent the spread of ice between the floret and stem when individual barley florets are killed without damage to the supporting stem. Infrared thermal imaging has been demonstrated as one technique to better understand the site of nucleation and the spread of ice within plants under controlled conditions (Wisniewski *et al.*, 1997; Pearce and Fuller, 2001; Hacker and Neuner, 2008) and during radiant frosts in the field (Frederiks *et al.*, 2009; Fuller *et al.*, 2009; Frederiks, 2010). Infrared thermal imaging under field radiant frosts has great potential to improve our understanding of the physical freezing process and physiology underpinning PHEF damage. With a better understanding of field frosts, it should be possible to postulate (i) mechanisms by which plants might be adapted to minimize damage, and (ii) improvements to freezing chambers to better reflect the

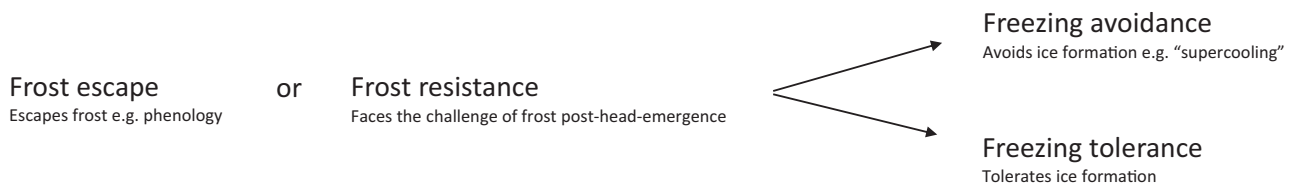


Fig. 9. Defining frost adaptation (as described by Levitt, 1972) in terms of frost escape, freezing avoidance and freezing tolerance provides a useful framework to discuss the physiological basis of potential adaptation mechanisms. Using this framework, frost escape can be viewed as the evasion of frost by crop phenology. For example, employing shorter or longer season varieties such that heading occurs at a time outside the main frost risk period. Cereals may survive frost through one of two frost resistance strategies: freezing avoidance and freezing tolerance. In the case of freezing avoidance, the plant faces the challenge of frost but avoids ice formation by, for example, supercooling. Alternatively, the ability of tissues to freeze but survive would be described as freezing tolerance.

damage observed in the field (Frederiks *et al.*, 2012). Infrared thermal imaging has been used for a number of years to study freezing of plants in controlled environments (Le Grice *et al.*, 1993; Pearce and Fuller 2001). However, the sensitivity, accuracy and spatial resolution of the infrared cameras limited the interpretation of results from earlier studies (Wisniewski *et al.*, 1997). A recent review by Wisniewski *et al.* (2014) provides a more detailed update on ice nucleation and propagation in plants.

Another strategy to develop PHEF resistance is to activate a freezing tolerance response in the reproductive tissues (Fig. 9). For example, could the freezing tolerance in vegetative tissue of certain acclimated winter habit cereals be maintained or reactivated to also protect later reproductive tissues from spring radiant frost? If so, activation of this response in reproductive tissues would be worth investigating. The response would likely need to be independent of the known cold acclimation requirements for the vegetative response, since daytime maximum temperatures are sufficient to cause de-acclimation in many areas where PHEF damage is a problem (see Fig. 2, mid-winter daytime maximum temperatures of ~15°C). One recent study by Al-Issawi *et al.* (2013) investigated whether cold acclimation could be induced in reproductive wheat tissues. In these tissues, typical acclimating temperatures of 4°C induced only a modest up-regulation of the *Cbf14* gene, implicated in vegetative cold acclimation, and then only at early stages of ear development. Attempts at inducing acclimation against frost in this study were largely unsuccessful.

Thus, improved resistance could potentially be achieved by extending freezing avoidance to lower temperatures (supercooling), by slowing or preventing the spread of ice through plant tissues, and/or by activating freezing tolerance mechanisms in reproductive tissues. However, it is currently difficult to predict which avenue is most likely to succeed. We suggest fundamental work should continue to improve the understanding of (i) the phenomenon of radiant frost, (ii) the anatomy of frost damage in cereals, and (iii) mechanisms of resistance (both avoidance and tolerance).

Conclusions

PHEF damage is a significant limitation to wheat and barley yields globally. Wheat and barley become more susceptible to frost after head-emergence. Even post-head-emergence, wheat and barley can supercool, without damage, to several degrees below 0°C. PHEF damage is the result of freezing injury at lower temperatures when the capacity to supercool is exceeded. With improved PHEF resistance, significant economic and crop security gains could be achieved by reducing direct damage of cereals post-head-emergence and improving yield potential by optimizing flowering time. Yield gains have been made through better management of frost risk, particularly by optimizing the combination of planting dates and varieties. However, these practices are essentially frost escape strategies, and improvements in true genetic PHEF resistance have been limited. Chilling damage

and over-winter vegetative freezing tolerance are different issues and should not be confused with PHEF. While genetic variation in response to both of these challenges has been reported, this provides little promise of improving PHEF resistance. Currently, only a small proportion of the genetic diversity of cereals in international germplasm collections has been explored in the search for resistance. We believe that screening of diverse germplasm should be pursued (Frederiks *et al.*, 2012), along with research to increase our understanding of potential resistance mechanisms.

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