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REVIEW & INTERPRETATION

Overwinter Low-Temperature Responses of Cereals: Analyses and Simulation

D. B. Fowler,* B. M. Byrns, and K. J. Greer

ABSTRACT

Winter survival of cereals is dependent on complex, environmentally induced responses that affect just about every measurable morphological, physiological, and biochemical characteristic of the plant. Simulation models offer a valuable means for the integration of knowledge accumulated from detailed physiological, agronomic, genetic, and genomics studies, thereby improving our understanding of complicated plant responses. A well-designed model also provides an effective extension and teaching tool and the opportunity for systematic investigation of production risks, cause-and-effect processes, genetic theories, and adjustments needed to mitigate the possible effects of climate change. Earlier, we developed and deployed a Winter Cereal Survival Model based on a series of equations that described acclimation, vernalization, dehardening, and damage due to low temperature (LT) stress. A modular design has permitted modification and allowed for interfacing with other simulation models. Recent advances in our understanding of this agronomically important character have provided us with the opportunity to develop a more robust winter survival simulation model with a wider geographic application that now also considers cultivar acclimation threshold induction temperature (T_i), respiration stress, photoperiod, and other developmental factors. The model has been field validated and provides the opportunity for the simulation of a wide range of species and overwinter environments.

D.B. Fowler and B.M. Byrns, Dep. of Plant Sciences, Univ. of Saskatchewan, Saskatoon, Canada; and K.J. Greer, WesternAg Group of Companies, Saskatoon, SK, Canada. Received 8 Mar. 2013. *Corresponding author (Brian.Fowler@usask.ca).

Abbreviations: $DL_{critical}$, critical day length; FLN, number of leaves produced on the main stem before transition to the reproductive stage; GDD, growing degree days; LT, low temperature; LT_{50}, temperature at which 50% of a population is killed in an artificial freeze test; MFLN, minimum final leaf number; $PP_{strength}$, strength of photoperiod genes; T_c, daily mean crown temperature; T_i, threshold induction temperature; V_{req}, vernalization requirement; VR, vernalization saturation in the revised model; VRT, vegetative to reproductive transition.

BACKGROUND

Autumn establishment and winter survival provides winter annual cereals with a competitive advantage over spring sown crops. This difference is the primary factor determining the geographical distribution and commercial potential of winter compared with spring habit cereals. Winter survival, however, is a much more complicated phenomenon than is suggested by the genetically simple 3 spring to 1 winter segregation ratio normally observed for spring by winter growth habit crosses (Brule-Babel and Fowler, 1988). It is determined by complex, environmentally driven responses that produce a myriad of changes that affect just about every measurable morphological, physiological, and biochemical characteristic of the plant (Levitt, 1980). These responses involve genotype by environment interactions that are complex and not clearly understood, making it difficult to design plant

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breeding and agronomic strategies to optimize production potential, control input costs, and minimize cropping risks. Genomics studies (Laudencia-Chingcuanco and Fowler, 2012; see for recent reviews: Collins et al., 2008; Kosova et al., 2008; Fowler, 2008a; Galiba et al., 2009; and Winfield et al., 2010) designed to identify the biological mechanisms governing low-temperature (LT) adaptation have confirmed the complexity of the pathways and regulatory mechanisms involved further emphasizing the need for a systems approach that will integrate the results of research into this important crop stress.

The genetic systems responsible for cold acclimation in cereals are developmentally regulated and induced by exposure to LT (Fowler et al., 1996a, 1996b). For these reasons, full expression is only revealed under genotypically dependent optimum combinations of time and environmental cues (Fowler and Limin, 2004). There is an inverse relationship between temperature and acclimation rate and the most rapid changes in LT tolerance occur during the initial stages of acclimation when plants are grown at constant temperatures in the acclimation range. Once fully acclimated, cereals can maintain a high level of cold hardiness provided crown temperatures remain near or below freezing (Andrews et al., 1974a, 1974b; Gusta and Fowler, 1976a). At this stage, acquired LT tolerance is rapidly lost (Salmon, 1933) once acclimated plants are exposed to temperatures above the acclimation threshold. However, the process of LT acclimation can be reinitiated by exposing plants that are still in the vegetative stage to inducing temperatures (Mahfoozi et al., 2001a).

Low temperature acclimation and vernalization are induced by temperatures that fall in a similar range, and the LT requirement for cold hardening is also the cue that results in the conversion of winter cereals to a spring form. This conversion occurs under long days after fulfillment of vernalization requirements (V_{req}, vernalization saturation), which has often been cited as the primary reason for an overwinter decline in LT tolerance observed in cereals maintained below their T_i (Andrews, 1960; Tsenov, 1973; Tumanov et al., 1976; Roberts, 1979; Fowler and Limin, 2004; Prasil et al., 2004). An adequate energy supply is important in maximizing plant genetic potential, but it will not prevent this decline in the LT tolerance of cereals once vernalization saturation has been achieved. The critical observations are that, irrespective of each genotype's T_i, transition from the vegetative to the reproductive growth stage (VRT) is a critical switch that initiates the downregulation of LT tolerance genes and the higher the crown temperatures, the more rapid the rate of decline in cold hardiness levels (Fowler and Limin, 2004).

Genetic and physiological analyses at the whole plant level have shown that duration and rate of gene expression determine the degree of LT tolerance in cereals (Fowler et al., 1996a, 1996b; 1999; Mahfoozi et al., 2001a). In this system, duration of LT tolerance is determined by the rate of phenological development and the time to VRT, which in turn is a function of (i) V_{req}, (ii) photoperiod requirements, (iii) leaf number, (iv) length of plastochron (Limin and Fowler, 2002), and (v) LTs that delay reproductive transition in plants that have reached the stage of competence to flower (Mahfoozi et al., 2001b; Fowler and Limin, 2004). Plant development toward flowering progressively reduces the plant's ability to LT acclimate and the duration of time in early developmental stages determines the degree to which the LT tolerance genetic potential is expressed. Related studies have also shown that the mechanism regulating the level of expression of LT induced genes is associated with a gene(s) integrated into the developmental pathway and the rate of acclimation is determined by (i) acclimation temperature and (ii) LT tolerance genetic potential (Fowler et al., 1999; Fowler and Limin, 2004).

Both spring and winter habit genotypes can cold acclimate after reproductive transition and before heading, demonstrating that the VRT does not act as an off switch for LT tolerance genes (Fowler et al., 1996b). However, plants have only a limited ability to cold acclimate during this period and they reach their maximum level of LT tolerance very quickly once they are exposed to temperatures in the acclimation range, indicating that a short, rapid LT response mechanism is functional up to the time of jointing and/or heading.

The LT tolerance of winter cereal crowns is reduced by prolonged exposure to near-lethal temperatures, and death of the critical crown tissue will result if the soil temperature falls below the plant's minimum survival temperature (Pomeroy and Fowler, 1973). Consequently, because cold hardiness expression, maintenance of LT tolerance, and degree of LT injury are directly related to the stage of phenological development and sequence of temperature changes to which the plant is exposed, it has been difficult to separate cause and effect adjustments to LT and other environmental cues that signal seasonal changes (Fowler et al., 1999).

Simulation models offer a valuable means for the integration of our understanding of these complicated plant response functions in a so-called *systems approach*. In addition to providing a framework for the integration of the knowledge accumulated from detailed physiological, genetic, and genomics studies, a well-designed simulation model provides an effective extension and teaching tool and facilitates the investigation of production risks, cause-and-effect processes, genetic theories, and the potential effects of climate change. Earlier, we developed (Fowler et al., 1999) and deployed (Fowler and Greer, 2003) a winter survival algorithm for the CERES wheat (*Triticum aestivum* L.) model (Ritchie, 1991). This algorithm was a stand-alone winter survival model that was based on a series of equations describing LT acclimation, dehardening, and damage due to LT stress. The basic assumptions

behind the model were consistent with our understanding of LT gene regulation at that time.

Our laboratory and collaborating groups have continued research efforts aimed at expanding our understanding of the complex system that determines winter survival of cereals with the objective of developing a more robust model with a wider geographic application. The resources for this project included LT response research data for cereals that have been accumulated from over 40 yr of field and controlled environment studies, >100 published papers on LT tolerance, plus related reports from our laboratory, input from collaborating groups, and a literature review. Historical records of winter cereal field soil temperatures at a depth of 2 to 5 cm were available for 129 site years, mainly located western Canada (www.wheatworkers.ca/FowlerSite/winter_cereals/WWModel.php, verified 11 Aug. 2014). In addition to measures of soil temperatures, overwinter LT_{50} data (temperature at which 50% of the population is killed in a controlled freeze test) for plants from 11 site-years (a total of 20 field trials) has allowed for field validation of the model. This large database, which included field, genetic, genomic, and physiological observations, and a total of 1990 LT_{50} measurements has allowed us to quantify plant LT responses under both controlled and field conditions. It has also allowed us to use both mechanistic and empirical approaches to build a winter survival model that includes the roles of acclimation T_i, acclimation, vernalization, photoperiod, other developmental factors, dehardening, respiration stress, and damage due to LT stress. The revised model was developed using the Stella modeling and simulation software package from isee Systems, Inc. (Lebanon, NH).

Low-Temperature Tolerance Genetic Potential (LT_{50}^c)

To survive LT stress, cereals have evolved adaptive mechanisms that are temperature regulated. Levels of acclimation, maintenance of LT tolerance, and degree of injury are directly related to the exposure temperature and the genetic potential of the plant. Damage to the crown tissue of the plant is the main cause of winterkill in most climates (Chen et al., 1983). For this reason, soil temperature at crown depth (Table 1) is critical to the acclimation process and winter survival.

While large genetic differences are recognized, the general pattern of overwinter LT response is similar for genotypes both within and among cereal species (Salmon, 1933; Dantuma and Andrews, 1960; Gusta and Fowler, 1976a,b, 1977). This allows the genetic differences in LT tolerance potential to be adjusted for in the model through the use of genetic coefficients (LT_{50}^c). Differences in LT tolerance genetic potential can often be detected in non-acclimated plants (Fowler and Carles, 1979; Brule-Babel and Fowler, 1989); however, it is poorly expressed at this stage and difficult to accurately quantify. Greatest cultivar differences have been observed when plant LT tolerance is at its maximum (Dantuma and Andrews, 1960; Marshall, 1969; Tumanov et al., 1976; Brule-Babel and Fowler, 1989). Therefore, cultivar LT_{50}'s at full acclimation in controlled freeze tests that have been field validated in western Canada (Fowler, 1992) have been used as the LT_{50}^c for LT tolerance in the winter survival model.

Table 1. Winter survival model input parameters.

Model input parameter	Definition and default value
LT_{50}^c	genetic potential of genotype (–24°C for 'Norstar')
DD_{min}	minimum growing degree-days GDD† to the vegetative-to-reproductive transition in spring habit and fully vernalized winter habit genotypes grown under long days (370 for spring Norstar)
$DL_{critical}$	critical day length (13.5 h)
$PP_{strength}$	strength of photoperiod genes (1 for spring Norstar, 0 for 'Sisler' spring barley)
V_{req}	vernalization requirement (49 calendar days for Norstar)
Latitude	geographic latitude
Longitude	geographic longitude
Seed date	optimum date of seeding, day of year
T_c	mean crown temperatures for each day of simulation

† GDD, growing degree days.

Duration of LT stress influences the lethal temperature. However, cultivar rankings from short and long duration controlled-freeze tests suggest that most genotypes respond in a similar manner to changes in duration of LT stress (Thomas et al., 1988). The model has been calibrated using a LT_{50}^c of –24°C for the fully acclimated winter wheat 'Norstar' (Grant, 1980) determined using the controlled-freeze procedure outlined by Limin and Fowler (1988) where the LT stress is removed as soon as the plants are exposed to a predetermined minimum temperature (Fowler and Carles, 1979). Consequently, for effective use of the model, LT_{50}^c of all genotypes must be adjusted to reflect their potential relative to a LT_{50}^c of –24°C for Norstar.

The winter survival model can be used in the simulation of overwintering for a wide range of plant species. Examples of LT_{50} values for cereal genotypes with a range of LT tolerance can be found in the literature (Fowler, 1992; Fowler et al., 1996b) and in the variety drop-down menu at www.wheatworkers.ca/FowlerSite/winter_cereals/WWModel.php (verified 11 Aug. 2014). Although there is an overlap among species, cultivars of rye (*Secale cereale* L.) have the best LT tolerance of the cereals. Rye is followed by cultivars of common wheat and triticale (×Triticosecale Wittmack), then durum wheat (*Triticum turgidum* L. var. durum) and barley (*Hordeum vulgare* L.), and finally oat (*Avena sativa* L.; Fowler and Carles, 1979). The LT_{50}^c values during the establishment year can be determined

from published LT tolerance ratings of cultivars representing other cereal relatives and forage grass species (Fowler and Gusta, 1977a; Dvorak and Fowler, 1978; Limin and Fowler, 1981, 1982, 1984, 1987, 1988; Limin et al., 1985). The $LT_{50}{}^{c}$ values can also be modified to accommodate the effects of management practices on LT gene expression (Fowler, 1992; Fowler and Greer, 2003).

Threshold Induction Temperature and Low Temperature Acclimation

In the original model (Fowler et al., 1999), a T_i of approximately 10°C (Olien, 1967; Alden and Hermann, 1971) was utilized for the initiation of cold acclimation in wheat and its relatives. An inverse relationship between temperature and acclimation rate for temperatures between 10°C and freezing had been established and preliminary studies suggested that the relationship between temperature and acclimation rate broke down when plants were acclimated at –5°C (Gusta et al., 1982). In the absence of measured responses, we assumed that acclimation rates at nonlethal below-freezing temperatures were similar to those at 0°C and acclimation was allowed to occur until the VRT.

Changes in LT tolerance are difficult to quantify during the initial stages of acclimation (Brule-Babel and Fowler, 1989) and we do not have a clear picture of when and how the acclimation mechanisms are activated. Differences in T_i have been reported for cereal genotypes acclimated under field conditions (Fowler and Carles, 1979) and the expression of some LT regulated genes (Campoli et al., 2009; Galiba et al., 2009) has been observed in plants grown at temperatures warmer than those normally considered in the induction range. Data from recent studies (Fowler, 2008b) has shown >11°C difference in the LT gene T_i of hardy rye compared with tender barley cultivars, emphasizing the large differences in adaptation potential available within and among cereal species. These studies also established there is an inverse relationship between temperature and acclimation rate for temperatures between the T_i and approximately 0°C. Faster rates of initial acclimation are a function of the plant's T_i. Consequently, from a practical standpoint, genotypes with a warmer T_i have a longer time to prepare for LT extremes and are also in a better position than more tender relatives to cope with unexpected frosts early in the growing season.

Differences in T_i have been shown to be closely related to the differences in LT tolerance at full acclimation (Fowler, 2008b). Consequently, because the genotypic differences in LT tolerance are largest and can be determined more simply with greater accuracy and precision in fully acclimated plants ($LT_{50}{}^{c}$), this close relationship ($r^2 = 0.88$, $P < 0.001$) has been utilized to estimate the T_i in the revised model using the equation:

$$T_i = 3.72135 - 0.401124 \times LT_{50}{}^{c} \quad [1]$$

where T_i is the maximum temperature at which acclimation will occur. The daily change in LT_{50} due to acclimation is then determined using the equation

$$\frac{\Delta LT_{50}}{\Delta t} = 0.014(T_i - T_c)(LT_{50} - LT_{50}^{c}) \quad [2]$$

This relationship was established using published data (number of data points = 60) from field (Fowler and Gusta, 1977a; Fowler and Carles, 1979) and controlled environment (Gusta and Fowler, 1979; Tyler et al., 1981; Gusta et al., 1982; Chen et al., 1983; Brule-Babel and Fowler, 1989) experiments, and the results of more recent studies (Fowler and Limin, 2004; Mahfoozi et al., 2001a, 2001b; Limin and Fowler, 2002), some of which have not been published. Variation in crown temperature explained 93% of the variability in LT_{50} when the data from all the studies was utilized.

Vegetative Reproductive Transition Factor

As outlined earlier, transition from the vegetative to the reproductive growth stage is the critical switch that initiates the downregulation of LT tolerance genes. The VRT is determined by (i) V_{req}, (ii) photoperiod requirements, and (iii) minimum final leaf number (MFLN). All three of these requirements must be met before the plant will enter the reproductive stage. Low temperatures also act to delay the VRT in plants that have reached the stage of competence to flower (Mahfoozi et al., 2001b; Fowler and Limin, 2004). Vernalization and MFLN are essentially stop-start decisions with short transitions. In contrast, the delay in transition to the reproductive phase at temperatures near freezing and a photoperiod requirement act more like caution flags that slow the transition until environmental conditions are deemed favorable. All spring and winter habit cultivars have a MFLN requirement that must be met before they will go reproductive. The vernalization and photoperiod genes both act to increase the length of the vegetative growth phase, primarily through mechanisms that increase the number of leaves produced on the main stem before transition to the reproductive stage (FLN), which in turn allows the opportunity for greater accumulation and longer retention of LT tolerance (Mahfoozi et al., 2000, 2001b).

In the model, an estimate of the progress to VRT is calculated as

$$VRT_{progress} = MIN(MFLN_{progress}, VERN_{progress}, PP_{progress}) \quad [3]$$

where MFLN, vernalization (VERN), and photoperiod (PP) can take values between 0 and 1. The transition is considered complete once VRT progress is equal to 1 and the plant is

exposed to warm temperatures. To facilitate a smooth transition between acclimation and dehardening effects, a VRT transition factor (VRT_{factor}) is calculated as follows:

$$VRT_{factor} = \frac{1}{1+e^{80(VRT_{progress}-0.9)}} \quad [4]$$

This function depends on an estimation of VRT and lessens the burden of pinpointing the VRT precisely. It is discussed in more detail in the dehardening section.

Final Leaf Number and Length of the Plastochron

Plant development and time to VRT in fully vernalized genotypes grown under long days is dependent on highly heritable, temperature-dependent genotypic differences that determine the FLN and length of the plastochron (the interval between successive leaf appearances). The length of the plastochron is dependent on both time and temperature (Bauer et al., 1984; McMaster, 1997). For example, a greater number of days are required to produce a leaf at 4°C than at 20°C. This makes accumulated thermal units, such as growing degree days (GDD), a better measurement of development and time to the VRT than calendar days (Klepper et al., 1982; Frank and Bauer, 1995; McMaster, 1997).

Although there are exceptions, spring habit cultivars grown under long days are the fastest developing. When grown under warm (high) temperatures and long days, the FLN for true spring wheat genotypes normally ranges from between six or seven (Levy and Peterson, 1972; Hay and Kirby, 1991) and 11 or 12 (Limin and Fowler, 2002) leaves. As a result, time to the VRT in spring habit genotypes grown in long days can be described by the product of the FLN multiplied by the GDD required to produce each leaf (Klepper et al., 1982; Frank and Bauer, 1995; McMaster, 1997). When grown under conditions favorable for acclimation, spring habit genotypes with a low FLN and a short plastochron have a limited ability to express their full LT tolerance potential because they quickly reach the VRT. Conversely, because growth rate is slow under conditions for LT acclimation, the additional GDD required for spring habit genotypes with a higher FLN and/or a longer plastochron have a greater time to accumulate LT tolerance. This increase in LT tolerance is dependent on shifting the VRT point and is brought about by one critical alteration of plant development—extension of the vegetative growth phase.

The shoot apex in cereals can have as many as five or six leaf primordia when the first leaf emerges under field conditions (Hay and Kirby, 1991; Brooking, 1996), making it difficult to establish the GDD to VRT with certainty. A more accurate estimate of when the transition results in a downregulation of the LT tolerance genes can be arrived at by determining the GDD required to reach the lowest estimated LT_{50} in cultivar acclimation curves (Limin and Fowler 2002; Fowler and Limin, 2004). In the model, the coefficients required to determine progress towards reproductive transition for spring habit genotypes are 340 GDD for genotypes (e.g., 'Manitou' spring wheat) with a low FLN and a short plastochron and 370 GDD for those (spring Norstar) with a high FLN and a long plastochron (Table 1). Growing degree days to the VRT in spring habit and fully vernalized winter habit genotypes grown under long days is then calculated using the following equations:

$$DD_{fln} = (0.9773DD_{min} - 321.7587)(T_c - 2) + DD_{min} \quad [5]$$

and

$$MFLN_{progress} = DD/DD_{fln} \quad [6]$$

The default for the minimum GDD (DD_{min}) to vegetative to reproductive transition is set at 370; T_c is the daily mean crown temperature.

Vernalization

A V_{req} delays the VRT in the autumn by increasing the number of leaves the plant produces before going reproductive. In the original model (Fowler et al., 1999), the number of days at temperatures between 0 and 10°C was used to determine progress toward vernalization saturation. In a related review, Porter and Gawith (1999) summarized the results from 11 studies reported in the literature and concluded that the base temperature for vernalization was –1.3 with a maximum of 15.7°C. As noted earlier, an estimate of when vernalization saturation results in a downregulation of the LT tolerance genes can be arrived at by determining the days required to reach the lowest estimated LT_{50} in cultivar acclimation curves. Results from these studies (Limin and Fowler, 2002; Fowler and Limin, 2004) indicated that vernalization progressed at the same rate for temperatures between 2 and 10°C, which was the range considered. Based on these observations, the equation that determines progress to vernalization saturation in the revised model (VR) generates the maximum rate when T_c is between –1.3 and 10°C. The rate decreases to 0 as T_c approaches 15.7°C:

$$VR = \begin{cases} 1, T_{min} \le T_c \le T_{opt} \\ \dfrac{2(T_c - T_{min})^{\partial}(T_{opt} - T_{min})^{\partial} - (T_c - T_{min})^{2\partial}}{(T_{opt} - T_{min})^{2\partial}}, T_{opt} < T_c \le T_{max} \\ 0 \text{ otherwise} \end{cases} \quad [7a]$$

where

$$\partial = \frac{\ln(2)}{\ln\left[\dfrac{T_{max} - T_{min}}{T_{opt} - T_{min}}\right]} \quad [7b]$$

and T_{min} = –1.3°C, T_{opt} = 10°C, and T_{max} = 15.7°C. Vernalization saturation is considered complete when $VR/V_{req} = 1$, where V_{req} is an input parameter specifying the number of days under optimal temperatures required to complete vernalization for the current cultivar (Table 1).

Photoperiod

A photoperiod response in cereals normally serves to delay the VRT until the lengthening days of spring signal the start of conditions favorable for growth and development. A long day (or short night) requirement can also slow plant development in the autumn, thereby preventing the VRT before the onset of subzero soil temperatures. Photoperiod sensitivity of cereal genotypes can range from mild to extreme. When grown under short days, genotypes with a strong photoperiod requirement, like 'Dicktoo' barley and spring Norstar, have demonstrated exceptional LT tolerance in the absence of a V_{req} (Mahfoozi et al., 2000; Limin and Fowler, 2006). In winter-habit genotypes, photoperiod sensitivity influences LT tolerance gene expression even before vernalization saturation (Mahfoozi et al., 2001b), implying that vernalization is progressive and that plant development can be influenced by photoperiod during the vernalization process.

Cereal plants are expected to flower when a critical daylength has been exceeded, but this value has been difficult to establish. Limited reports suggest the critical daylength for winter wheat is 12.25 h at North Platte, NE, and 13.25 h at Sidney, MT (Heurer et al., 1978). As noted above, there are genetic differences in daylength sensitivity in cereals. Consequently, it is reasonable to expect similar variability exists among genotypes for critical daylength. Data accumulated from our field and laboratory studies (unpublished data, 2014) indicate that the critical daylength affecting the VRT as it relates to LT tolerance gene expression is approximately 13.5 h for the winter wheat cultivar Norstar (Table 1). In northern latitudes with long, cold winters and a rapid transition from winter to spring, like western Canada, this daylength usually coincides with the advent of warm spring temperatures. Consequently, in these climates, photoperiod sensitivity is expected to have a larger impact on genotypes that do not have a V_{req} by preventing the VRT in the early autumn. A strong photoperiod requirement is assumed to be of greater importance in warmer climates that experience >0°C temperatures that allow for periods of active growth during the winter. The scarcity of data for these climatic conditions has been a limitation to our current efforts and is identified as a critical area requiring future attention.

A strong photoperiod by temperature interaction (Fig. 1) has been reported and the dependence on temperature is especially important in winter cereals because photoperiod activity is reduced or prevented by LT (Rahman and Wilson, 1978; Yan and Wallace, 1996). Controlled environment studies in our laboratory (unpublished data, 2014) have shown that there is a base temperature between 2 and 6°C that allows activity of the photoperiod genes, and expression increases logistically as temperature increases to approximately 20°C in cereals. Although the plants may have received the signal, transition to the reproductive phase is normally delayed at temperatures near freezing (Mahfoozi et al., 2001b; Fowler and Limin, 2004). As temperatures increase, the photoperiod requirement becomes stronger, further slowing progress to the reproductive stage.

The model calculates the daylength starting at the date of seeding (optimum seeding date is assumed—see later discussion for the effect of suboptimal seeding dates) using an algorithm (http://williams.best.vwh.net/sunrise_sunset_algorithm.htm, verified 11 Aug. 2014) developed by the United States Navy. The civil solar zenith value of 96 degrees is used. The effects of temperature and daylength on the timing of the VRT are then estimated by the following equation:

$$PP_{progress} = \left[3.5 - \frac{3.5}{1 + e^{0.50419(DL - DL_{critical}) - 0.32077(T_c - 13.2420)}}\right] \times \frac{1}{180 \times PP_{strength}} \quad [8]$$

This function has two parameters, critical daylength ($DL_{critical}$) and photoperiod strength ($PP_{strength}$) that adjust for genetic differences in photoperiod sensitivity. The $DL_{critical}$ is the daylength at which photoperiod response progresses most rapidly (default value = 13.5 h). The $PP_{strength}$ has a default value of 1, which was derived from the photoperiod response of spring Norstar. At this default value, the $PP_{progess}$ function ranges from 0 to 0.019, and its output represents the daily percentage of progress towards photoperiod requirement satisfaction. Once accumulated to a value of 1 (100%), the photoperiod requirement is considered satisfied. A delay in transition from the vegetative to the reproductive stages due to photoperiod sensitivity becomes meaningful any time the temperature is >4°C (Fig. 1). At short days, the time to transition is extended. The response surface generated by the photoperiod function is based on a regression analysis of Norstar controlled environment data (unpublished data, 2014).

Dehardening

Plant growth and rate of dehardening are both temperature dependent (Salmon, 1933; Laude, 1937; Pomeroy et al., 1975; Gusta and Fowler, 1976a). Temperatures above the acclimation threshold result in a loss of cold hardiness at all stages of plant development. Plants that are still in the vegetative stage have the ability to reacclimate, while those that have reached the VRT will gradually lose their

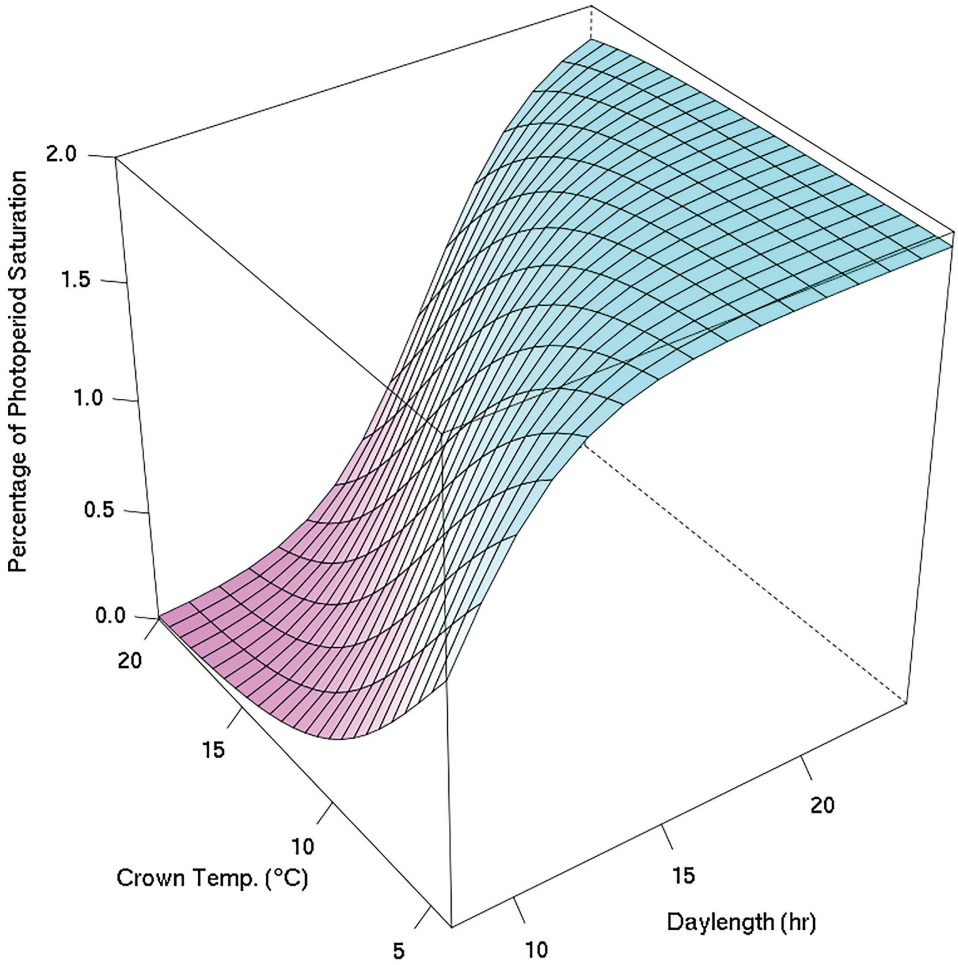

Figure 1. Daily percentage of progress towards photoperiod saturation and the response surface that models the temperature by photoperiod interaction described in Eq. [8].

LT tolerance when stored at temperatures above −4°C (Mahfoozi et al., 2001a).

The database (Gusta and Fowler, 1976a,b; Pomeroy et al., 1975; Fowler and Gusta, 1977b) available for construction of the original model was limited for mean daily temperatures warmer than 18°C (Fowler et al., 1999). In the absence of critical studies, we assumed that dehardening rates at temperatures above 18°C were similar to those at 18°C, and the equation used for determining the rate of dehardening was:

$$0.0002(T_c + 4)^3 (LT_{50} - LT_{50i}) \quad [9]$$

In subsequent field studies, this function was found to overestimate the rate of dehardening at warmer temperatures, causing sharp increases in LT_{50} values in more moderate spring environments. The revised dehardening rate equation is:

$$\frac{\Delta LT_{50}}{\Delta t} = \frac{5.05}{1 + e^{4.35-0.28T_c}} \quad [10]$$

Dehardening using this equation is active whenever the crown temperature is above the acclimation threshold temperature. If the crown temperature is below the acclimation threshold temperature and above −4°C, the equation is multiplied by one minus the VRT factor (VRT_{factor}; Eq. [4]). This permits dehardening to occur simultaneously with acclimation at temperatures between −4°C and the acclimation threshold. At the same time, the rate of dehardening is gradually increased from 0 initially to the maximum as development progresses towards the reproductive transition. This change allows dehardening to start near the predicted VRT and removes the rigid on-and-off behavior of acclimation or dehardening at the reproductive transition that was used in the earlier version of the model.

Respiration

Soil can remain unfrozen during the winter months under deep, persistent snow cover. Under these conditions, winter wheat plants have been shown to rapidly lose their LT tolerance (Bergjord et al., 2008). The primary cause for this loss of LT tolerance is thought to be an accumulation of metabolites to toxic levels from anaerobic respiration. An algorithm developed by Bergjord et al. (2008)

to simulate this overwinter loss in LT tolerance has been included in the revised winter survival model. Their simulation required inputs of soil temperature at a 2 cm depth and the depth of snow cover.

Snow cover on winter cereal fields is generally quite variable and difficult to measure, but a deep, persistent snow cover is required to maintain unfrozen soil near 0°C for any length of time in most winter environments. In the revised winter survival model, it is assumed that a deep snow cover is required to maintain soil temperatures near 0°C for a minimum of 10 d, after which respiration damage will start to occur as long as the soil temperature remains near 0°C. An algorithm that measures crown temperature ranges and their standard deviation is used to determine conditions of snow cover that will create this environment. Low temperature tolerance loss due to respiration under these conditions is calculated as follows:

$$\textbf{IF } (T_m < 1.5 \textbf{ AND } T_m > -1 \textbf{ AND } T_{std} < 0.75)$$

$$\textbf{THEN } \frac{\Delta LT_{50}}{\Delta t} = \frac{0.54(e^{0.84+0.051T_c} - 2)}{1.85} \quad [11]$$

$$\textbf{ELSE } \frac{\Delta LT_{50}}{\Delta t} = 0$$

where T_m = crown temperature mean of last 5 d, T_{std} = standard deviation of T_c for last 5 d.

Winter Kill

The procedure used to estimate winter kill in the earlier version of the model (Fowler et al., 1999) has been retained in the revised model. It recognizes that both temperature and exposure time play an important role in determining degree of injury (Hill and Salmon, 1927; Anderson and Kiesselbach, 1934; Olien, 1967; Pomeroy et al., 1975; Gusta and Fowler, 1977; Gusta et al., 1982; Fowler and Limin, 1997). The $LT_{50}{}^c$ for the model is determined using a series of test temperatures where the LT stress is removed as soon as the crown tissue is exposed to a predetermined minimum temperature (Fowler and Carles, 1979). Immediate damage and a reduction in cold hardiness occurs when winter cereal plants are exposed to crown temperatures that are 2 to 3°C warmer than their minimum survival temperature (Pomeroy and Fowler, 1973) and LT tolerance is reduced by prolonged exposure to near-lethal temperatures. The relationship between duration of LT stress and LT_{50} (Fowler et al., 1999) was determined using data (number of data points = 15) from both field and controlled environment trials (Pomeroy et al., 1975; Gusta and Fowler, 1977; Gusta et al., 1982; C.J. Andrews, personal communication, 1993). Variation in duration of LT stress explained 96% of the variability in LT_{50} in these studies.

The LT_{50} estimates are updated daily in the model to provide a current record of the plant's ability to tolerate LT. When the minimum daily adjusted LT_{50} ($LT_{50}{}^M$) is colder than –3°C and $\frac{LT_{50}^M}{2} > T_C$, a loss of LT tolerance due to cold stress is calculated using the relationship described in Eq. [12] from Fowler et al. (1999):

$$\frac{\Delta LT_{50}}{\Delta t} = \frac{LT_{50}^M - T_c}{e^{0.654(LT_{50M} - T_c) - 3.47}} \quad [12]$$

The LT^M_{50} is equal to the cultivar LT tolerance genetic coefficient ($LT_{50}{}^c$) when full acclimation is achieved. Death of the plant is assumed to occur if the average daily soil temperature at crown depth falls below the daily adjusted LT_{50}. Observations from simulation studies have demonstrated that small differences in cultivar genetic potential ($LT_{50}{}^c$) and/or exposure temperature translate into large differences in LT_{50} when the cumulative effects of LT stress enter the critical range for winter survival.

Field Validation

The revised winter survival model has been validated against the same data set used to validate the original version of the model (Fowler et al., 1999). This data was collected from field trials in 1995–1996 and 1996–1997 at Saskatoon, SK, Canada. Norstar winter wheat ($LT_{50}{}^c$ = –24; Fowler, 1992) was seeded on conventional summer fallow (high stress environment) and direct seeded into standing stubble (low stress environment) at the recommended seeding dates in both years. Plants were collected and LT_{50} values were determined during fall acclimation in 1995 and spring dehardening in 1996 and 1997. Weather stations recorded 2 cm soil temperatures (crown depth) at two locations (Probes 1 and 2) within each environment starting 1 September (Day 0) in both years. Patchy winterkill was observed at the low-stress site in the spring of 1996. The model predicted no damage at the Probe 1 and six winterkill events at the Probe 2 locations in December when the soil temperature was 1 to 2°C warmer for Probe 1 compared with Probe 2 at this site. Model simulations closely followed the measured LT_{50} values during acclimation ($r^2 = 0.89$★★★) and the complete winterkill observed at the high stress location was accurately predicted in both years.

Between 2003 and 2013, autumn to spring LT_{50} data was collected from an additional 12 trials where cultivars were grown using recommended management practices. When the complete data set (129 LT_{50} measurements) was utilized, model simulations were found to provide a good prediction ($r^2 = 0.90$, $P < 0.001$) of the measured LT_{50}'s. Good model performance was also indicated by a

Willmott's Index of Agreement equal to 0.97, a RMSE of 2.43 degrees (observed LT_{50} range = 30.5 degrees), and a Nash-Sutcliffe Model Efficiency of 0.88.

Application

Low-temperature adaptation and winter survival in cereal species is determined by a complex environmentally induced genetic system. Parts of this system have been studied in detail using physiological, agronomic, genetic, and genomic tools; but much of this work has been conducted in controlled environments under uniform conditions that consider only a small part of the total response mechanism. Simulation models provide a framework for the integration of knowledge acquired from this research in a systems approach that allows for the exploration of a wider range of responses and complex interactions. They also serve to identify knowledge gaps that require further investigation—if you can't model it, you have an incomplete understanding of the system. As examples, our efforts have identified (i) a critical shortfall in objective measures of LT response (e.g., LT_{50}) for genotypes with a wide range of winter survival potential when acclimated under field conditions, (ii) a clear need for more meteorological (especially soil temperatures at crown depth) and overwinter field LT_{50} data for regions with warm to moderately warm winter climates, and (iii) a better understanding of the role photoperiod plays in regulating LT gene expression.

We have attempted to combine information from the database available in the literature with our observations on LT responses to construct a simulation model that will improve our understanding of mechanisms involved in winter survival of cereals and their relatives. In this process, we have utilized newly acquired information and the input of collaborating groups to revise and expanded our earlier Winter Cereal Survival Model (Fowler et al., 1999). The model now takes into account the roles played by acclimation T_i, acclimation, vernalization, photoperiod, other developmental factors, dehardening, respiration stress, and damage due to LT stress. There are a large number of additional factors that influence LT responses, which we have not attempted to incorporate directly into this version of the model. A few of the agronomic management related variables have been accounted for by the addition of a Management Impact Calculator, where the cultivar LT tolerance genetic potential (LT_{50}^{c}) can be adjusted based on winter survival differences observed in field trials conducted in western Canada (Fowler, 1992). These adjustments include the influence of seeding date or plant age, seeding depth, and the application of phosphorous fertilizer (Fowler and Greer, 2003).

A fully interactive model that complies with known LT responses provides the opportunity for evaluation of the genetic potential of a wide range of species and the identification of critical stress periods in different overwinter environments. Model input parameters (Table 1) can be adjusted to determine the optimum combinations of genetics and agronomy on a regional basis. An opportunity to evaluate what-if scenarios provides a valuable tool in developing plant breeding strategies and crop management programs designed to optimize production potential, control input costs, minimize cropping risks, and evaluate cause-and-effect processes, genetic theories, and the adjustments needed to mitigate the possible effects of climate change. As an example, our interactive web based version of the winter survival model (www.wheatworkers.ca/FowlerSite/winter_cereals/WWModel.php, verified 11 Aug. 2014) has a variety menu that offers the choice of a wide range of cereal species and cultivars and a management impact calculator that allows for the exploration of complex genotype × environment × management interactions. The data files contain soil temperature records for sample years and locations that can be expanded when new data becomes available. It has been an effective out-reach and teaching tool for use by farmers, extension workers, researchers, and others interested in acquiring an understanding of LT stress in cereals. When combined with a large database that can be quickly and easily supplemented, this creates a tool that allows the systematic investigation and improvement of our understanding of this important crop stress by users throughout the world.

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