Effect of the waxy endosperm gene on germination, agronomic, and malt quality characteristics in barley (Hordeum vulgare)
by Philip Lyle Bruckner

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
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Abstract:
A series of four waxy-normal endosperm isogenic pairs developed in three barley (Hordeum vulgare) lines were used to evaluate the effect of the waxy endosperm gene on certain germination and agronomic traits during and after kernel maturation as well as on malting quality and speed of imbibition. Plots in two locations were sampled three times per week from shortly after anthesis to harvest maturity to evaluate seed size, alpha-amylase activity, and germination characteristics during kernel maturation. A second set of plots was used to evaluate the effect of the waxy endosperm gene on yield, malt quality, and other agronomic traits at harvest maturity. Speed of imbibition in four Compana isotypes grown in three environments was determined in a 48 hour, 20° C, unaerated steep. Isogenic analysis showed that waxy endosperm isotypes exhibited significantly less dormancy, smaller seeds, and greater alpha-amylase activity during kernel maturation than did normal endosperm isotypes. Waxy and normal endosperm isotypes did not vary significantly in germination behavior, yield, heading date, or in most malting quality characteristics at harvest maturity. Waxy endosperm isotypes were significantly lower in diastatic power, had significantly lower seed weights, and in certain genetic backgrounds were shorter than normal isotypes.

Waxy and normal endosperm isotypes did not differ significantly in speed of imbibition although preliminary information showed that waxy isotypes could reach a 45% moisture level from 9.1 to 14.0 hours sooner than normal Compana isotypes. Naked Compana isotypes imbibed water significantly faster, yielded 17% less, had a 16% smaller seed weight and exhibited significantly less dormancy and a greater germination speed during kernel maturation and at harvest maturity than did covered Compana isotypes.

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ABSTRACT

A series of four waxy-normal endosperm isogenic pairs developed in three barley (Hordeum vulgare) lines were used to evaluate the effect of the waxy endosperm gene on certain germination and agronomic traits during and after kernel maturation as well as on malting quality and speed of imbibition. Plots in two locations were sampled three times per week from shortly after anthesis to harvest maturity to evaluate seed size, alpha-amylase activity, and germination characteristics during kernel maturation. A second set of plots was used to evaluate the effect of the waxy endosperm gene on yield, malt quality, and other agronomic traits at harvest maturity. Speed of imbibition in four Compana isotypes grown in three environments was determined in a 48 hour, 20° C, unaerated steep. Isogenic analysis showed that waxy endosperm isotypes exhibited significantly less dormancy, smaller seeds, and greater alpha-amylase activity during kernel maturation than did normal endosperm isotypes. Waxy and normal endosperm isotypes did not vary significantly in germination behavior, yield, heading date, or in most malting quality characteristics at harvest maturity. Waxy endosperm isotypes were significantly lower in diastatic power, had significantly lower seed weights, and in certain genetic backgrounds were shorter than normal isotypes. Waxy and normal endosperm isotypes did not differ significantly in speed of imbibition although preliminary information showed that waxy isotypes could reach a 45% moisture level from 9.1 to 14.0 hours sooner than normal Compana isotypes. Naked Compana isotypes imbibed water significantly faster, yielded 17% less, had a 16% smaller seed weight and exhibited significantly less dormancy and a greater germination speed during kernel maturation and at harvest maturity than did covered Compana isotypes.

INTRODUCTION

Waxy barley starch is more readily modified by either chemicals or enzymes than is normal barley starch. This and other unique properties of waxy barley starch indicate that waxy barley may become increasingly important in certain industrial processes. Little information has been developed on the effect of the waxy endosperm gene on agronomic, germination, and malt quality characteristics before and after harvest maturity.

The objectives of the following studies were to determine the effect of the waxy endosperm gene on germination, dormancy, seed size, and alpha-amylase activity during kernel maturation and on imbibition and malting quality in the mature grain.

LITERATURE REVIEW

Germination Characteristics of Developing Barley Grain

Isolated barley embryos can germinate at a very early stage of development, even as early as five days after anthesis (Bishop, 1944; Gordon, 1969). The ability of the embryo to germinate at this early stage depends on a critical embryo size (Cameron-Mills and Duffus, 1980). Intact seeds, however, cannot germinate in the early developmental stages. Gordon (1969) reported that only a few intact barley seeds germinated in the first 42 days after anthesis. Wellington (1964) reported no germination in barley seeds until 3-4 weeks after anthesis, while Bilderback (1971) showed no germination up to 20 days after anthesis when the seeds were immediately placed on moist sand. Similar results are reported for wheat (Wellington, 1956a, 1956b; Mitchell et al., 1980).

The seed can, however, germinate at a very early stage if the embryo is exposed to the air by rupturing the pericarp and testa. Gordon (1969) reported a germination rate in barley of 75% ten days after anthesis under these conditions. Wheat can germinate as early as 3-4 weeks after anthesis when the embryo is exposed mechanically (Wellington, 1956b). Drying of the immature grain has a similar effect. Air dried grain germinates at an earlier stage of maturity (10 days after anthesis) and at a faster rate than undried grain

(Mitchell et al., 1980). Wellington reported germination of desiccated immature wheat three weeks after anthesis compared to five weeks after anthesis in undried grain (Wellington, 1956b).

The germination capacity of immature grain develops early after fertilization, probably within the first 10-15 days following anthesis (Gordon, 1969; LaCrox et al., 1976; Gordon et al., 1979; Mitchell et al., 1980; Takahashi, 1980). The germination capacity rises, then decreases rapidly with decreasing moisture content and the induction of dormancy (Takahashi, 1980). High temperatures during seed formation (10-20 days post anthesis) repress dormancy induction while low temperatures enhance the induction of dormancy (Takahashi, 1980). LaCroix et al. (1976) reported a decrease in germination percentage in spring wheat 34-40 days after anthesis. Wellington (1964) reported a 14% and 22% reduction in germination of Domen and Herta barleys respectively, as secondary dormancy was imposed. This dormancy breaks down with increasing maturity and storage.

The pericarp is implicated as having some control over germination in developing barley (Wellington, 1964; Gordon, 1969; Briggs, 1978; Mitchell et al., 1980). Water loss through the pericarp occurs while the embryo and remainder of the grain is still accumulating water. The capacity to germinate first occurs when 50% of the water has been lost from the outer pericarp (Mitchell et al., 1980).

The capacity to germinate at harvest time and during barley

development is a varietal character that is subject to environmental variation. A study of 4 malting and 3 nonmalting barley varieties showed initial germination capacity occurring 25-44 days after anthesis and 50% germination capacity occurring between 30 and 60 days after anthesis (LaBerge et al., 1971). Gordon et al. (1979) showed that a 50% germination potential in wheat occurred 32-38 days after anthesis. Speed of germination increases with increasing maturity in barley (Bilderback, 1971) and wheat (LaCroix et al., 1976).

Alpha-amylase Development in Developing Barley Grain

In barley and wheat at least two types of alpha-amylase exist (Daussant and Mayer, 1980; Kruger, 1980; Gordon, 1980; Sargeant, 1980). Green alpha-amylase, alpha-amylase I, is found in developing seeds. Malt alpha-amylase, alpha-amylase II, is found predominantly in germinating seeds (Daussant and Mayer, 1980; Gordon, 1980). Recent evidence indicates both types of alpha-amylase are present in germinating barley seed, although most of the alpha-amylase activity is due to alpha-amylase II (Daussant and Mayer, 1980).

Green alpha-amylase, located in the pericarp of developing seeds, is independent of the embryo or exogenous gibberellic acid for synthesis (MacGregor et al., 1972; Allison et al., 1974; Daussant and Mayer, 1980; Gordon, 1980; Sargeant, 1980). The aleurone layer and the scutellum synthesize malt alpha-amylase de novo in response to

gibberellins from the embryo or exogenous GA (Daussant and Mayer, 1980). The two types of alpha-amylase have different electrophoretic properties, pH optimums, pH and heat stabilities and starch digesting properties (Daussant and Mayer, 1980).

Alpha-amylases in immature barleys increase rapidly after anthesis, then decline to a low, stable level (Duffus, 1969; LaBerge et al., 1971; MacGregor et al., 1971; MacGregor et al., 1972; Allison et al, 1974; Riggs and Gothard, 1976; Gordon, 1980). Considerable variation in alpha-amylase developmental patterns exists due to environmental and cultivar characteristics (MacGregor et al., 1974; LaCroix et al., 1976; Riggs and Gothard, 1976). LaBerge et al. (1971) in a study of seven barley cultivars, including four malting barleys, showed that alpha-amylase levels increased immediately after anthesis, peaked in the first ten days following anthesis, then declined to a low level by 10-15 days after anthesis. Allison et al. (1974) demonstrated that pericarp anthesis in Goldfield barley peaked about fifteen days after anthesis. Riggs and Gothard (1976) reported peak alpha-amylase activity 10-16 days after anthesis in six barley cultivars although a hulless barley cultivar, Nackta, showed no definite peak. Maximum alpha-amylase activity differed markedly between cultivars although there were no cultivar differences in alpha-amylase activity from 31 days after anthesis to maturity. Conquest barley showed a peak alpha-amylase activity 11 days after ear emergence (two days after anthesis)

when grown in the field while maximum activity of Conquest and Fergus barley grown in a growth chamber occurred 9-10 days and four days after anthesis, respectively (MacGregor et al., 1971; MacGregor et al., 1972). The alpha-amylase activity in Conquest declined to 1/10 its maximum after 28 days and remained constant until maturity. Duffus (1969) reported peak alpha-amylase levels in barley grown in Britain occurred 20-30 days after anthesis and that chlorocholine chloride applied shortly after anthesis inhibited alpha-amylase synthesis. Similar enzyme development patterns and cultivar variations are reported in wheat (LaCroix et al., 1976; Gordon et al., 1979; King and Gale, 1980).

A set of alpha-amylase isozymes which have similar isoelectric points to germination alpha-amylase have been reported in developing immature wheat in addition to the green alpha-amylase normally present (Kruger, 1980). The presence of these isoenzymes indicates that some leakage may occur in the germination control mechanisms and that control over germination may not be 100% effective. Malting barleys, with characteristic strong enzyme systems and short dormancy periods, showed increased alpha-amylase activity during late ripening in response to rain (LaBerge et al., 1971). Riggs and Gothard (1976) reported a strong correlation between peak alpha-amylase activity during barley development and germination energy three weeks after harvest.

The purpose of alpha-amylase in the pericarp during grain development and the reasons for its disappearance during maturity are

unknown (MacGregor et al., 1972; Allison et al., 1974; Riggs and Gothard, 1976; Daussant and Mayer, 1980). MacGregor et al. (1972) and Allison et al. (1974) speculated that green alpha-amylase in the pericarp may hydrolyze pericarp starch early in development to provide energy for growing kernels or produce short chain primers to be used in endosperm starch synthesis. Sargeant (1980) showed that in wheat, green alpha-amylase in the pericarp is not involved in starch synthesis. Disappearance of green alpha-amylase during maturation may be due to inhibition, inactivation, insolubilization, or degradation of enzymatic protein (MacGregor et al., 1972; Riggs and Gothard, 1976; Daussant and Mayer, 1980).

The aleurone synthesizes alpha-amylase in response to gibberellic acid (Gordon, 1980). The ability to respond in this way arises early in development. Gordon (1980) stated that the ability of aleurone layers to respond to GA arises 25-30 days after anthesis, although cultivars vary considerably in this property. The stage of development at which the embryo can synthesize gibberellins remains unclear (Gordon, 1980). Cool ripening environments that slow grain dehydration also slow embryo maturation as well as the aleurone cells' ability to produce germinative alpha-amylase. Marchylo et al. (1980) harvested wheat 25, 32, and 39 days after flowering and found that the embryo scutellum could synthesize alpha-amylase in the absence or presence of GA_3 25 days after flowering. The immature endosperm-aleurone

produced alpha-amylase in response to GA_3 32 days after flowering. At successive developmental stages the tissues synthesized higher levels of alpha-amylase. Bilderback (1971) showed that aleurone layers in Himalaya barley still could not respond to exogenous GA_3 25 days after anthesis. However, dried immature seeds at least 20 days old when incubated in exogenous gibberellic acid, produced large amounts of alpha-amylase. King and Gale (1980) reported no alpha-amylase production in half seeds of wheat after GA_3 incubation until 35-40 days after anthesis. Artificially dried detached half seeds responded to GA_3 as early as 22 days after anthesis. The rate and duration of drying influenced the ability of the immature grain to respond to GA_3 and produce alpha-amylase (King and Gale, 1980).

Barley Starch in the Mature Barley Kernel

Starch, a glucan, forms the food reserve polysaccharide in plants (Greenwood, 1964). Starch, the most significant chemical component of the cereal grain constitutes two-thirds of the dry weight of barley (Harris, 1962). Starch comprises 63-65% of the dry weight of plump, two rowed barley (Harris, 1962; Briggs, 1978). The endosperm stores the majority of the starch in barley, making up 85-89% of this tissue (Briggs, 1978).

Starch is exclusive to plants, being a mixture of two types of glucose polymers, one linear and the other branched (Harris, 1962;

Bewley and Black, 1978; Briggs, 1978; Bohlinski, 1979). Amylose is a straight chain polymer containing D-glucopyranose units linked by alpha(1-4) glucosidic bonds which may be as long as 2000 glucose residues. Amylopectin is a much larger molecule with a highly branched structure in which the alpha(1-4) linked glucopyranose chains are branched with alpha(1-6) linkages every 24-26 glucose residues.

The relative proportion of amylose and amylopectin can be estimated by the iodine binding capacity or iodine affinity test, capitalizing on the fact that amylose binds iodine at constant free iodine concentrations up to a limiting value while amylopectin binds little iodine. Starch, being a mixture of the two polymers, has an intermediate binding capacity. The proportion of amylose is estimated by extrapolation of the linear portion of the amylose and starch binding curves to zero free iodine and using the following relationship (Greenwood, 1964):

$$\% \text{ Amylose} = \frac{\text{Iodine Affinity of starch}}{\text{Iodine Affinity of amylose}} \times 100$$

Enzymes degrade starch (Greenwood, 1964; Bewley and Black, 1978; Briggs, 1978; Bohinski, 1979). The two principle hydrolyzing enzymes are alpha- and beta-amylase. Both attack alpha(1-4) bonds but in different patterns. Alpha-amylase cleaves alpha(1-4) bonds at random points while beta-amylase action removes successive maltose units beginning at the non-reducing terminus of the glucopyranose chain. Neither alpha- nor beta-amylase can remove branch points, with cleavage stopping

two to three glucose units from the alpha(1-6) bond. Thus amylose can theoretically be converted entirely to glucose plus maltose and alpha and beta dextrins by the combined action of alpha- and beta-amylase. The same two enzymes will only partially degrade amylopectin. Even though the theoretical yield of maltose from amylose digested by pure beta-amylase is 100% (Harris, 1962) the actual yield is near 73%. This incomplete conversion of amylose by beta-amylase is probably due to some modification in amylose structure (Greenwood, 1964), possible anomalous alpha(1-6) linkages (Briggs, 1978). Beta-amylase converts only 58% of amylopectin to maltose (Greenwood, 1964).

Starch grains are laid down as insoluble particles. The starch content of the grain during grain development rises in a logarithmic (Harris, 1962) or sigmoidal fashion (Briggs, 1978). Ninety-five percent of the starch is deposited in the initial 11-28 days after spike emergence (MacGregor et al., 1971; Briggs, 1978). Genotype and environmental characteristics control the amount and properties of starch (Harris, 1962). Amylose and amylopectin are not synthesized simultaneously (Harris, 1962; MacGregor et al., 1971; Briggs, 1978). The amylose content of the starch increased from a value of 13.8% after 14 days to 22.5% and remained constant to maturity, indicating that during the early stages of growth, the amylopectin fraction was synthesized at a relatively faster rate than amylose (MacGregor et al., 1971). The proportion of small starch granules increases dramatically with

maturity (MacGregor et al., 1971; Briggs, 1978).

Barley starch granules are generally round and follow a bimodal size distribution pattern. The small and large size granules being 1.7-2.5 um and 22.5-47.5 um in diameter, respectively (Briggs, 1978). Two distinct starch granule sizes were found in wheat, the larger granules being 25 um in diameter and the small granules 5 um in diameter. The small starch granules accounted for 90% of the total number of granules but comprised only 10% of the total starch weight (Bewley and Black, 1978). Small starch granules have a higher gelatinization temperature and amylose content (41.3%) than large granules (26.9%) (Briggs, 1978).

Normal barley starch contains approximately 25% amylose and 75% amylopectin (Bewley and Black, 1978; Eslick, 1979). Various reports on amylose show its range to be from 11-26% of the starch (Harris, 1962; Greenwood, 1964; Briggs, 1978). Genetic mutations in barley and in other cereals have resulted in starches containing almost 100% amylopectin (Banks et al., 1970; Bewley and Black, 1978). In theory, true waxy barleys contain 100% amylopectin (Eslick, 1979) although waxy endosperm barleys do contain some amylose (0-3%) (Banks et al., 1970; Goering et al., 1973; Briggs, 1978). High amylose mutations have also been reported (Greenwood, 1964; Briggs, 1978).

Nonwaxy and waxy barley kernels and pollen grains can be distinguished by iodine staining (Eriksson, 1969). A single recessive gene

on chromosome one that expresses xenia controls the waxy endosperm character (Eslick, 1979). Ericksson (1969) reported the spontaneous mutation rate to the waxy phenotype in barley to range from 5.1×10^{-6} to 15×10^{-6}.

Many properties of waxy endosperm barleys have been studied and documented. A study of two Japanese waxy endosperm barley cultivars showed both to have less than 0.5% amylose, a beta-amylolysis limit of 54% conversion into maltose, and an average chain length of 23 glucose units (Banks, et al., 1970). The average molecular weight of this starch was 300×10^6 and showed a bimodal distribution of large and small starch granules with a range of 22 and 3 um in diameter, respectively. Waxy barley starch gelatinizes a few degrees lower than waxy corn starch.

Waxy endosperm barley starches are more susceptible to enzymatic action possibly because the pure amylopectin starch granules are less resistant to enzymolysis than the amylose/amylopectin hybrid granule found in nonwaxy barley (Goering et al., 1973; Fox and Eslick, 1979). Goering et al. (1973) has shown that waxy barley starch has a 20°C lower pasting temperature than normal barley (75°C vs. 95°C). In corn and sorghum the amylose content of the starch does not influence gelatinization temperature. Goering et al. (1973) also showed waxy Compana barley starch had a similar Brabender cooking curve to that of waxy sorghum starch but had only half the swelling power and one-third the

solubility of waxy sorghum starch, indicating that the amylopectin in waxy Compana was a unique starch. Waxy barley starch malts more rapidly than normal barley starch when measured by maltose production (Fox and Eslick, 1979). Waxy barley flour is higher than normal barley flour in beta-glucans. The starch from waxy short-awn nude Compana grown at one location retained sufficient alpha-amylase-like activity to liquify itself and four times its weight of waxy or normal corn starch (Goering and Eslick, 1976). A new process for production of ultra high maltose syrup from waxy endosperm barleys is based on some of these unique properties of waxy barleys (Goering et al., 1980). It was further suggested that the ease of liquification of waxy barley starch as well as other characteristics make waxy barleys potentially useful in the brewing industry (Goering et al., 1973; Goering and Eslick, 1976).

Sullins and Rooney (1974) demonstrated that waxy sorghum was more rapidly solubilized by alpha-amylase enzymes and by buffered rumen fluid than nonwaxy sorghum and that the waxy sorghum kernel had less peripheral endosperm. Furthermore, waxy sorghum had a peripheral endosperm less dense than nonwaxy sorghum, having large starch granules with a less dense protein matrix more susceptible to enzyme attack (Sullins and Rooney, 1975). They concluded that the properties of the peripheral endosperm were responsible for waxy sorghum starch being more susceptible to enzyme attack.

Yield trials conducted over 43 environments with waxy and normal isogenic pairs have shown no significant yield reduction in the waxy endosperm genotype (Eslick, 1979). Yield comparisons of covered and hulless isogenic pairs grown in replicated yield trials over 149 environments indicate an average yield reduction of 12% for the hulless character (Eslick, 1979). Isogenic comparisons between short awn and long awn isogenic pairs in replicated yield trials over 95 environments have shown no significant differences in yield (Eslick, 1979).

Endosperm Modification and Enzyme Development

The mobilization of cereal endosperm reserves during germination is required to replenish embryo carbohydrate reserves depleted during the first 24-48 hours following imbibition (Bewley and Black, 1978). The embryo controls the mobilization of endosperm reserves. The embryo synthesizes and releases gibberellin to the aleurone layer where production and release of hydrolytic enzymes occurs (Bewley and Black, 1978; Briggs, 1978). Mobilization commences near the scutellum andspreads along the endosperm as the aleurone layer begins enzyme release (Bewley and Black, 1978; Gibbons, 1980).

Hydrolysis of starch grains does not take place if the enclosing cell walls are intact. Wheat cell walls are composed of 15% protein and 75% carbohydrates. Beta-glucans and arabinoxylan make up most of the carbohydrate fraction. Proteinases, arabinoxylanases, and

pentosanases are synthesized in the aleurone layer and released in response to gibberellin (Bewley and Black, 1978). Acid phosphatase, ribonuclease, and endo-beta-glucanase can be synthesized in the absence of gibberellin but their activities are enhanced and their release controlled by gibberellins (Bewley and Black, 1978; Briggs, 1978). Beta-glucanase, arabinoxylanases, and proteinases degrade cell wall constituents and dissolve cell walls. Degradation of the endosperm wall occurs initially in the sub-aleurone layers then progresses to the center of the endosperm. Erosion of starch granules begins only after cell walls have been modified. Dissolution of cell walls renders the enclosed starch accessible to enzymatic attack (Bewley and Black, 1978).

Gibberellins, released by the embryo, induce the aleurone layer to undergo metabolic changes resulting in the release of many enzymes. Much of the work, however, on barley enzyme synthesis has focused on the synthesis of alpha-amylase. Levels of alpha-amylase activity increase during barley germination (Kneen, 1944; Briggs, 1964; Reynolds and MacWilliam, 1966; Briggs, 1968a; Palmer, 1969; Kruger, 1976; Bewley and Black, 1978; Briggs, 1978; MacGregor, 1978; MacGregor and Daussant, 1979; Daussant and Mayer, 1980). Barley kernels synthesize alpha-amylase de novo primarily in the aleurone layer in response to a gibberellin trigger received from the embryo. De novo alpha-amylase synthesis is documented and supported by many facts including

(a) the production of alpha-amylase in isolated barley aleurone layers in response to endogenous GA, (b) subsequent declines and increases in enzyme production as the hormone is removed and reapplied (Briggs 1964; Bewley and Black, 1978), and (c) immediate stoppage of alpha-amylase synthesis by protein synthesis inhibitors (Briggs, 1963, 1964; Bewley and Black, 1978). Definitive proof of de novo synthesis of alpha-amylase was shown by labeling amino acids with ^{18}O (Bewley and Black, 1978) or radioactivity (Briggs, 1964) and subsequently detecting the labeled amino acids in synthesized alpha-amylase.

Several studies have shown synthesized gibberellin is involved in enzyme synthesis. Experiments have shown that excised barley embryos can release gibberellin up to 60 hours in vitro (Briggs, 1978), that the endosperm of degermed grain can be modified in the presence of exogenous GA while little modification occurs without the GA (Briggs, 1968a, 1978), and that the embryo can be removed two to three days after germination begins, with little effect on enzyme level (Briggs, 1978). Up to seven isozymes of alpha-amylase including the two major groups of the enzyme appear in GA treated isolated aleurone layers and during germination (Bewley and Black, 1978; MacGregor, 1978; MacGregor and Daussant, 1979; Daussant and Mayer, 1980).

During the lag phase between gibberellin arrival at the aleurone layer and rapid alpha-amylase synthesis the hormone redirects protein synthesis in the aleurone cells, possibly by enhancing synthesis of RNA

including mRNA for alpha-amylase. Gibberellin may also enhance activation or protection of mRNA in the aleurone cells. There is little firm evidence that GA acts at the translational level of control (Bewley and Black, 1978).

The aleurone layer produces about 85% of the alpha-amylase and the scutellum 15%. Malted barley shows about 7% of the alpha-amylase is located in the embryo with the remaining 93% in the endosperm indicating that about half the alpha-amylase produced in the embryo is released to the endosperm (Briggs, 1964, 1978.). Alpha-amylase accumulates in the aleurone layer before its release (Briggs, 1978). Synthesis of alpha-amylase is probably limited not by the inability of the aleurone layer to respond to gibberellin but by the supply of gibberellin to the aleurone layer (Atanda and Miflin, 1970; Briggs, 1978). The deficiency in gibberellin supply to the aleurone layer is probably due to deficiencies in the transport of gibberellin to the aleurone rather than lack of synthesis at the embryo since endosperm areas distal to the embryo produce disproportionately small amounts of alpha-amylase (Atanda and Miflin, 1970).

High embryo sugar levels may depress levels of alpha-amylase production (Briggs, 1964) or prevent gibberellin production or release from the embryo (Briggs, 1978). Enzyme synthesis may be controlled through a feedback loop in which gibberellin production is controlled by the sugar levels in the embryo while sugar levels are controlled by

enzyme synthesis and ultimately gibberellin production (Briggs, 1978). Proteolysis, removal of starch and calcium ions, and a falling pH contribute to declining enzyme activity (Briggs, 1968a). Externally applied gibberellins can replace or augment embryo produced gibberellin to initiate or increase alpha-amylase formation (Briggs, 1963, 1964; Reynolds and MacWilliam, 1966; Briggs, 1968a; Palmer, 1969; Atanda and Miflin, 1970; Bewley and Black, 1978; Briggs, 1978; MacGregor, 1978; Smith and Briggs, 1978). The amount and rate of alpha-amylase production is also influenced by barley genotype (Atanda and Miflin, 1970; Gothard, 1974; Bewley and Black, 1978; MacGregor, 1978), temperature and water conditions during germination (Kneen, 1944; Briggs, 1968a; MacGregor and Daussant, 1979), oxygen availability (Smith and Briggs, 1978) and mechanical damage (Briggs, 1968b).

Proteinases synthesized de novo in the aleurone layer in response to gibberellin, activate beta-amylase, present in a bound insoluble form in ungerminated barley, by reducing the number of disulfide bridges between the enzyme molecule and insoluble proteins (Bewley and Black, 1978; Briggs, 1978). Beta-amylase is not synthesized de novo. Beta- and alpha-amylases work together in starch breakdown and can completely hydrolyze amylose although amylopectin digestion halts with the production of limit dextrins (Bewley and Black, 1978).

Two debranching enzymes are reported in cereals. R enzyme, found in barley malt, debranches amylopectin and beta-limit dextrins, while

limit dextrinase, reported in Proctor barley and ungerminated oats, attacks alpha- and beta-limit dextrins but does not debranch amylopectin (Bewley and Black, 1978). The aleurone layer synthesizes limit dextrinase de novo at the time of starch breakdown, its activity being enhanced 10-15 fold between the first and fourth days following imbibition (Bewley and Black, 1978).

Starch amolysis produces two major products, alpha- and beta-maltose. Alpha-glucosidase, an enzyme present in the quiescent barley embryo and aleurone layers, hydrolyzes these products (Bewley and Black, 1978; Briggs, 1978). The enzyme activity increases at both sites during the first five days following imbibition. Barley half grains release a de novo-synthesized alpha-amylase from the aleurone layer within 12 hours of the addition of GA (Bewley and Black, 1978). Glucose, the resulting product of alpha-glucosidase activity, is absorbed by the scutellum, converted to sucrose, and transported to the growing embryo (Bewley and Black, 1978).

Germination

The germination process results in growth of the grain embryo, manifested by the growth of the root and an increase in the length of the shoot, with concurrent modification of the contents of the endosperm (Schuster, 1962). Germination begins as the surface layers pick up water which penetrates through the micropyle into the kernel (Briggs,

1978). The grain swells during the first 24 hours and the embryo becomes turgid and increases in size. Germination begins with the emergence of the coleorhiza, primary, and secondary seminal roots from the embryo (Pollock, 1962; Briggs, 1978). At almost the same time the coleoptile breaks through the testa and begins elongation. The coleoptile grows up the dorsal side of the kernel, pressing a groove in the endosperm and emerging from the apex of the kernel or through the lemma along one side (Schuster, 1962; Briggs, 1978). The outside grain coverings probably restrict physically the elongation of the plumule since the elongation of the plumule in dehusked grain during germination is considerably greater than in intact barley kernels (Schuster, 1962). Endogenous reserves support initial growth of the embryo while subsequent growth makes use of products from the endosperm dissolution (Briggs, 1978).

Endosperm breakdown begins immediately below the scutellum in a layer of compressed cells and moves toward the grain apex (Schuster, 1962; Briggs, 1978). After a short period, alteration proceeds faster immediately below the aleurone layer (Briggs, 1978). The asymmetry of endosperm breakdown results from the angle of the scutellar face against the endosperm, the sequence and location in which degradative enzymes are formed, and the property of resistance to enzymatic degradation by residual walls in the nucellar sheaf cells (Briggs, 1978). Endosperm alteration begins with the hydrolytic decomposition of high

molecular weight materials by enzymes present in the barley (Schuster, 1962). The synthesis of certain enzymes follow soon after the beginning of germination (Schuster, 1962). One of these enzymes, alpha-amylase, develops rapidly in germinating grain, and with beta-amylase and certain other enzymes attacks starch granules, causing granule pitting and/or granule erosion and ultimately starch granule breakdown (Schuster, 1962; Briggs, 1978). The degradation products of the enzymes rapidly diffuse through the scutellum and are used for respiration and the energy processes of growth (Schuster, 1962).

Germination begins with water absorption and rehydration of the kernel (Brown, 1975; Bewley and Black, 1978). The kernel requires a minimum of 35% water for active germination (Essery et al., 1954; Kirsop et al., 1967; Brown, 1975; Wainwright and Buckee, 1977). Adequate kernel modification for malting barley requires a further increase in water percentage to 42-45% (Kirsop et al., 1967) or 43-46% (Brookes et al., 1976). Various researchers have concluded that good malting performance is positively correlated with the ability of barley to take up water rapidly (Brookes, et al., 1976; Ulonska and Baumer, 1976; Wainwright and Buckee, 1977).

Various factors affect water imbibition by the barley seed. Large kernels have been reported to initially imbibe water faster than small kernels although small kernels reach the appropriate level for germination more rapidly, and after prolonged steeping have a higher

moisture percentage, than large kernels (Pollock, 1962; Brookes et al., 1976; Davidson et al., 1976). Briggs (1978) reported smaller kernels take up moisture faster and to a higher final level than large kernels. A positive correlation between starch content and time required to reach a predetermined moisture level has been reported (Pollock, 1962; Brookes et al., 1976) as well as a corresponding inverse relationship between nitrogen content and imbibition rate (Brookes et al., 1976). Contradictory reports as to the influence of initial barley moisture content on water uptake exist but it is probable that barley, like other seeds, has a velocity of water uptake inversely proportional to the amount of water previously absorbed (Brookes et al., 1976). Old, dry barley seed absorbs water at a much slower rate than does fresh, moist seed, however. Increasing temperature increases the rate of imbibition (Pollock, 1962; MacLeod, 1967; Brookes et al., 1976; Briggs, 1978). All barleys probably have a similar temperature coefficient of imbibition. A linear inverse relationship exists between temperature and the log of the time required by grain to reach a particular moisture level (Briggs, 1978). Water sensitivity (Brookes et al., 1976), embryo and/or micropyle size (Davidson et al., 1976) and variety (Ulonska and Baumer, 1976) may also influence water uptake.

Upon immersion of barley in water an immediate moisture layer (2-3% fresh weight) forms around the kernel (Kirsop et al., 1967;

Brookes et al., 1976; Davidson et al., 1976; Briggs, 1978). This film of water must be removed for accurate water uptake studies (Briggs, 1978). Drying by blotting fails to remove all water on the external surface and may inflate estimates of water uptake by 1% or more (Davidson et al., 1976). Surface layers hydrate rapidly, within the first two hours in hulled barley and the first 30 seconds in hulless grains (Briggs, 1978). In naked grain the pericarp conducts water so well that the grain interior hydrates at the same rate whether the apex, the base, or the whole grain is immersed in water. During imbibition the moisture content increases rapidly, then at a progressively declining rate until, if germination does not occur, it approximates a limiting value (Briggs, 1978).

Numerous researchers have reported a triphasic pattern of water uptake in germinating seeds (Brookes et al., 1976; Bewley and Black, 1978; Takahashi, 1980). Phase I (imbibition) is purely a physical process, occurring during the first six to ten hours after immersion, characterized by rapid water uptake accounting for 60% of total water uptake. Seed colloids imbibe water previously lost during ripening regardless of dormancy or viability problems. Permeability of the seed integument controls this phase. Phase II (10-20 hours) is the lag or activation phase during which water uptake slows or ceases. Major metabolic processes begin and water uptake resumes with increasing osmotic pressure. Dormant seed does not undergo this phase as control

is exerted by embryo dormancy. Phase III (20+ hours) constitutes a steady linear increase in water uptake that can be correlated to plant metabolism. This phase, an active process, occurs only in viable seed and includes the visible onset of germination.

The surface layers of the barley kernel are the principle barriers to water entry. The testa is semi-permeable and may restrict water entry for the first few hours. The pericarp, however, is the principle barrier to water entry (Brookes et al., 1976). Gaps exist in the pericarp and testa, especially near the embryo, which may allow water entry. The embryo preferentially takes up water (Kirsop et al., 1967; MacLeod, 1967; Brookes et al., 1976). Embryo moisture content may reach 65-70% while the endosperm moisture content is 41%. Most water uptake by the kernel likely occurs in the micropylar region (Pollock, 1962; MacLeod, 1967; Brookes et al., 1976; Briggs, 1978).

A barley imbibition study conducted at 13° C indicated that the axis of the embryo and the scutellum absorb water considerably faster than all other kernel parts except the husk (Reynolds and MacWilliam, 1966). Rapid absorption of water by the embryo for six hours followed by a declining rate of absorption resulted in about 58% moisture after 24 hours in the imbibed embryo. The scutellum lagged behind the embryo in water uptake until about six hours then paralleled the embryo moisture content up to 58% moisture at 24 hours. The endosperm absorbed water more slowly, reaching 35% moisture at 24

hours. Water content of the whole grain was 38.4% after 24 hours. Another study conducted at 25° C showed water content in whole grain increasing from 17.4% to 23.8%, 26.2%, and 30.5% after 2, 4, 6, and 8 hours, respectively (Davidson et al., 1976).

Many factors affect the germination of seed, one of the most important being seed dormancy. Seed dormancy can be defined to include all instances of inhibition of germination, whether they are connected with the properties of the seeds themselves or whether they are determined by environmental conditions. In the broadest sense dormancy is the absence of germination or as a greater or lesser reduction of the germinative capacity of seeds, or finally as the maintenance of the capacity to germinate only within a small range of conditions (Nikolaeva, 1969). Barley seeds sometimes fail to germinate due to primary (intrinsic) dormancy or secondary dormancy, the latter of which is imposed by external factors (Briggs, 1978).

Dormancy varies among samples according to cultivar (Deming and Robertson, 1933; Chang, 1943; Bishop, 1944, 1945, 1946; Essery et al., 1954; Finlay, 1960a; Bell and Lupton, 1962; Carson and Horne, 1962; Pollock, 1962; Wellington, 1964; MacLeod, 1967; Belderok, 1968; Gordon, 1969; Lallukka, 1976; Briggs, 1978; Strand, 1980), weather conditions during growth and harvest (Bishop, 1945, 1946; Wellington, 1964; MacLeod, 1967; Belderok, 1968; Lallukka, 1976; Reiner and Loch, 1976; Briggs, 1978; Derera, 1980; Gordon, 1980; Strand, 1980; Takahashi,

1980), storage and handling procedures (Bishop, 1944, 1945, 1946; MacLeod, 1967; Gordon, 1969; Brookes et al., 1976; Briggs, 1978), and the methods used to test germination (Bishop, 1945, 1946; Essery et al., 1954; Essery et al., 1955; Pollock et al., 1955a, 1955b; Pollock, 1962; Wellington, 1964; Gordon, 1969, 1970; Briggs, 1978; Strand, 1980).

Dormancy expressed as a single number is usually unsatisfactory, since the germination rate, as well as final percentage may change during storage, e.g. a barley lot with 100% germination reached 50% germination within 31 hours immediately after harvest but required only 15.5 hours after three weeks of storage (Gordon, 1969). Thus a discussion of dormancy should include the rate as well as the final germination percentage under a defined set of conditions (Briggs, 1978). Numerous attempts have been made to assign single descriptive numbers to germination tests (Finlay, 1960a, 1960b; Gordon, 1969; Goodchild and Walker, 1971; Bewley and Black, 1978). The best type of presentation appears to be a graph of cumulative germination against time for each set of germination conditions (Briggs, 1978).

A mixture of factors occurring within the grain and its surface layers interacting with environmental factors probably cause barley dormancy. Embryo dormancy in barley is rare (Bishop, 1944; Pollock, 1962; Gordon, 1970; Briggs, 1978) although embryo dormancy has been reported in Hordeum spontaneum (Belderok, 1968). Briggs (1978) reported a study in which intact barley germinated 6% with germination

increasing to 12%, 46%, and 48% as 25%, 50%, and 75% of the endosperm was removed, respectively. Dehusked grain germinated at 12%, 58%, 65%, and 81% under the same conditions. Germination of isolated embryos was 100%, indicating no embryo dormancy and a certain germination inhibiting effect associated with the seed coverings.

The most promising theory on barley dormancy involves the internal level of free oxygen in the embryo (Pollock, 1962; Belderok, 1968; Briggs, 1978). Dormant and nondormant grains respire at about the same rate (Pollock, 1962; Briggs, 1978). The embryos of dormant grains, as shown by experiments with isolated embryos, have a higher free oxygen requirement or require a higher oxygen tension than nondormant embryos. This oxygen requirement of the embryo progressively declines during after-ripening (Pollock, 1962; Briggs, 1978) or the seed coats become more permeable to oxygen. Eventually enough oxygen reaches the embryo for both germination and growth (Belderok, 1968). Respiratory processes in the embryo compete for oxygen with the growth processes (Pollock, 1962; Belderok, 1968). Respiration in dormant seed may require so much oxygen that little may be left for germination (Belderok, 1968). The surface structure of the seed, surface moisture, and microbial populations on the seed limit the oxygen supply to the embryo (Briggs, 1978). The seed surface layers restrict oxygen flow, so dormancy may be broken or reduced by damaging the pericarp mechanically. The surface water film reduces oxygen uptake because of the time taken for oxygen to

dissolve into water, the time to diffuse into the grain, and the lower oxygen content in water as compared to air. Microbial populations on the seed influence seed dormancy by their high consumption of oxygen (Briggs, 1978). The production of gibberellins in plant tissues require aerobic conditions. The embryo requires oxygen in order to form gibberellins or to convert bound gibberellins to free gibberellins (Belderok, 1968).

Pronounced dormancy may be separated into three phases in barley (Belderok, 1968). The first is an immediate post-harvest phase in which practically no grains germinate in the germination test. The second is a transitional phase following the after-ripened state in which dormancy terminates in gradually increasing numbers of grains. In the third phase grains fully germinate at optimum moisture, but cannot do so if excess water is present. This latter behavior demonstrates water sensitivity. Essery et al. (1954) first detected water sensitivity when they found certain barleys would not germinate under normal steeping conditions. These barleys showed normal germinative energy under conditions of the accepted test, but if the amount of water was increased from 4ml to 8ml germination declined (Pollock et al., 1955b). Water sensitive barleys fail to germinate because the embryos fail to respond to the low internal oxygen tension resulting from the presence of an unduly thick water film (MacLeod, 1967). Low germinative energy may also reflect a deficiency of oxygen in the

vicinity of the embryo (Gordon, 1969). Finlay (1960a), however, found no water sensitivity in his study of the genetic and environmental variations in germination behavior in 800 cultivars. In fact, 8ml of water accelerated the germination rate over the use of 4ml of water in the test. Rate of water absorption (Belderok, 1968; Briggs, 1978) and nitrogen content (Bishop, 1946; Belderok, 1968) do not influence dormancy.

The following treatments reportedly break dormancy: drying with heat (Bishop, 1944; Pollock, 1962; Belderok, 1968; Briggs, 1978), gibberellin treatment (MacLeod, 1967; Belderok, 1968; Brookes et al., 1976; Briggs, 1978), removal of seed coat or mechanical damage (Bishop, 1944, 1945; Pollock et al., 1955a, 1955b; Wellington, 1964; Belderok, 1968; Gordon, 1970; Bishop, 1978), freezing, stratification or cold temperatures (Bishop, 1944; Briggs, 1978), germination in oxygen (Bishop, 1944, 1945; Pollock, et al., 1955b; Briggs, 1978) and various chemical treatments (Bishop, 1944, 1945; Pollock et al., 1955b; Pollock, 1962; Brookes et al., 1976; Briggs, 1978). One or two recessive genetic factors reportedly control dormancy in barley (Belderok, 1968).

The water conditions during a germination test also affect the final germination of the barley lot. In addition to conditions favoring water sensitivity and water imbibition as previously discussed, the position of the embryo relative to the water level, water

temperature, and dissolved ions may affect germination. Gordon (1969) reported significant differences in germination in a factorial experiment comparing tap and distilled water.

Temperature also influences germination. Kneen (1944) reported the relative growth rate of wheat and sorghum, as measured by average coleoptile length increased as germination temperature was increased from 14° C to 30° C. Gordon (1969) reported that the germination temperature influenced the expression of total germination under excessive moisture conditions but that it had little effect on the final germination percentage under optimum moisture conditions. The rate of water absorption in barley depends on temperature (Pollock, 1962). Specific temperatures have been noted such as 5° C as minimal, 29° C as optimal, and 38° C as maximal for barley germination (Briggs, 1978). Such figures are misleading since samples of grain vary widely in response to different germination conditions. Within limits mature grain germinates more rapidly at higher temperatures (Briggs, 1978). One study showed rootlet appearance in six days at 5° C, three days at 10.5° C, two days at 15.5° C, and one and three-fourth days at 17.8° C. Dormant grain, however, germinates better at lower temperatures. Most dormant barley will germinate at 2-7° C. As the grain matures, the temperature range over which germination occurs widens (Briggs, 1978). Fixed time or temperature tests may be misleading. Germination of one grain sample showed that germination in five days was best at

12° C (67%). The same seed sample germinated better (95%) in an eleven day test at 5° C. In the longer test germination at 12° C was only 86%.

Mechanical, heat, and pregermination damage reduce germination capacity of barley (Pollock, 1962). Microorganisms influence germination by increasing dormancy or by causing seed or seedling death (Briggs, 1978). Aging of seed leads to a disappearance of dormancy and a slow degeneration of the embryo leading to eventual death (Pollock, 1962). Cultivar characteristics and storage conditions affect barley germinative capacity and life (Pollock, 1962; Briggs, 1978).

Assessment of germination characteristics of grain generally falls into three groups of tests: germination capacity tests, germination energy tests, and special germination tests such as the water sensitivity test (Pollock, 1962). Germination energy tests assess the percent germination of a given seed lot at a particular time under standard conditions (Bishop, 1944; Belderok, 1968; Briggs, 1978; Brookes, 1980). Germination capacity tests determine the viability of the seed lot as the percentage of seed, both dormant and nondormant (Bishop, 1944; Belderok, 1968; Briggs, 1978; Brookes, 1980). The difference between germinative energy and germinative capacity determines the amount of dormancy in the sample (Briggs, 1978). The difference between germination with 4 ml of water and germination with 8 ml of water has been defined as water sensitivity (Essery et al.,

1955; Wainwright and Buckee, 1977; Briggs, 1978).

Many standardized germination methods exist although many have serious limitations (Bishop, 1944, 1945, 1946; Essery et al., 1954, 1955; Pollock et al., 1955a, 1955b; Pollock, 1962; MacLeod, 1967; Belderok, 1968; Gordon, 1969; Wainwright and Buckee, 1977; Briggs, 1978; Gordon et al., 1979). Tests and criteria for germination differ depending on intended use of the seed (Briggs, 1978). Maltsters require rapid germination such as 50% in one to two days and 95-100% in three days and score grains as germinated as soon as the coleorhiza appears. For seed testing purposes the seed must produce a whole, normal, vigorous seedling with an adequate root system, a coleoptile, and at least one seedling leaf (Briggs, 1978). Malting germination tests generally require only three to four days while seed testing germination require 7-14 days (Briggs, 1978). Reliable germination results require replicated subsamples under rigidly standardized conditions of temperature, amount of water, and test duration (Briggs, 1978). Inadequately controlled conditions may lead to serious discrepancies between various germination tests (Essery et al., 1954; Briggs, 1978). Micro-organism infections can cause mold growth and rotting during testing (Briggs, 1978). Most microbial infections can be controlled by seed treatment.

Germinative energy tests are usually conducted at 15-20° C and are about 72 hours in duration although some may take longer (Essery et al.,

1954). Some of the common germinative energy tests include the Schönfeld test, the Aubry method, the Cöldewe or Schönjahn method, and the Buchingor test (Bishop, 1944; Essery et al., 1954; Briggs, 1978). More satisfactory tests can be done in flat dishes, on filter papers, in graded sand or soil that is as uniform as possible to which exactly measured proportions of water are added (Briggs, 1978). No difficulties arise when determining germinative energy in fully mature barley although the precise environment during the test determines germination if some degree of dormancy exists in the barley sample (Pollock, 1962).

Germination capacity tests determine viability directly by forcing dormant seeds to germinate or indirectly by determining viability in some other way (Pollock, 1962; Briggs, 1978). Direct germination capacity tests involve pretreatment to eliminate dormancy. Common methods include steeping in dilute hydrogen peroxide (Thunaeus test) (Pollock, 1962; Wainwright and Buckee, 1977; Briggs, 1978), cutting the grains in half transversely followed by germination (Eckhardt test) (Bishop, 1944; Briggs, 1978), treatment with gibberellic acid (Pollock, 1962), removal of the husk and pericarp with sulfuric acid (Pollock et al., 1955a; Pollock, 1962; Briggs, 1978), mechanical peeling (Bishop, 1944, 1945, 1946; Pollock, 1962; Briggs, 1978), and germination in a solution of potassium nitrate (0.2%) (Briggs, 1978; Gordon et al., 1979). Indirect measurements of viability use chemical substrates to determine the viable proportion of seed. Dead seeds

take up barium chloride and resazurin or indigo carmine stains and these have been used to determine germinative capacity (Briggs, 1978). More successful indirect methods detect living tissues by the ability of their endogenous enzymes and substrates to reduce materials such as dinitrobenzene (Bishop, 1944; Briggs, 1978), salts of selenium (Bishop, 1944, 1945, 1946; Pollock, 1962; Briggs, 1978), tellurium (Briggs, 1978), or tetrazolium salts (Bishop, 1946; Pollock, 1962; MacLeod, 1967; Wainwright and Buckee, 1977; Briggs, 1978) to colored, insoluble substances which stain viable tissues. Tetrazolium tests are the most common, the chemical being reduced to highly insoluble, bright red forazans in viable tissue (Briggs, 1978).

Measurement of seed dormancy may involve determining the length of the dormant period, determining dormancy intensity (% dormant seed) or both (Strand, 1980). The length of dormancy is the number of days between the harvest stage and the time when a given percentage of grain (e.g., 50%, 85%, 98%) is capable of germinating under selected test conditions (Belderok, 1968). Strand (1980) defined the length of the dormant period as the number of storage days at 20° C required to reduce dormancy to an acceptable level. Dormancy length tests generally involve ear-germination tests which begin at harvest ripeness and are conducted at regular intervals until dormancy is gone or germination reaches a predetermined level (Belderok, 1968; Olsson and Mattsson, 1976). Olsson and Mattsson (1976) conducted a study on the

length of the dormancy period in wheat by germinating seeds at 18° C twice per week from full ripening until 50% germination. They found large cultivar differences in the length of the dormancy period. Dormancy intensity can be defined as the percentage of dormant seeds in a sample. Dormancy intensity determination may involve germination at various temperatures with the sensitivity of the test depending on germination temperature (Strand, 1980). Other dormancy intensity tests include hanging grain bundles in a closed room, keeping them moist and evaluating the extent of germination at 7, 10, or 14 days by use of a predetermined scale or alternately by terminating germination after 3-5 days and estimating the amount of germination by an alpha-amylase test (Belderok, 1968). Accurate dormancy determination requires testing at the same maturity stage (Belderok, 1968; Strand, 1980), careful threshing to avoid kernel damage (Strand, 1980), low moisture levels to eliminate water sensitivity (Strand, 1980), careful attention to germination temperatures (Strand, 1980) and standardized storage of lots to be tested (Olsson and Mattsson, 1976; Strand, 1980). Length of wheat dormancy periods increased from 12 to 15 to 50 days as storage temperature decreased from 22° C to 18° C to 2° C, respectively (Olsson and Mattsson, 1976). Noll and Czarnecki (1980) reported accurate dormancy testing could be delayed for up to 4-5 months in wheat by storage at -15° C or by increasing the germination temperature to 30° C. The same varietal ranking for dormancy was

achieved under these conditions as was found immediately after harvest. Results from spike and grain germination tests show that dormancy depends primarily on grain properties while spike type (erect or nodding), density of the head, and the presence of glumes have a minor effect on germination behavior (Belderok, 1968).

Breeding populations can be screened for dormancy in the F_2 or later generations by planting seeds in moist sand in a bench and allowing the seeds to germinate. Upon termination of the germination the seeds are sifted from the sand (Strand, 1980). The germinated seeds are discarded and the dormant seed saved and dried for subsequent planting. Selection pressure is regulated by the dormancy level in the population at the time of the test and the percentage of the population expected to survive the test. The level of dormancy of the population is a function of climatic conditions during ripening, the genetic level of dormancy, storage temperature, and the time from maturity to testing. The test is from 5-10 days in duration at temperatures from 10-20° C. The higher temperatures manifest more dormancy and are applied to low dormancy material or for a low selection pressure toward nondormancy (Strand, 1980).

Germination percentage obtained under specified conditions is the most commonly used measure of germination. It reveals nothing, however, about the rate or uniformity of germination (Bewley and Black, 1978). Another commonly used germination measure is the germination rate which

can be expressed for a single seed, for a population, or a proportion of the population. The mean germination rate of a population may be expressed by Kotowski's coefficient of velocity (C_V) (Bewley and Black, 1978). Various attempts have been made to combine both the germination rate and final germination value into a single numerical value. Czabator's germination value (C) and Timson's germination index have been criticized because similar values may be obtained from different cumulative germination curves (Bewley and Black, 1978). Finlay's germination behavior value (G) (Finlay, 1960a) and Gordon's germination resistance (G.R.) and uniformity factor (U.F.) (Gordon, 1969) provide single numerical values expressing germination behavior. Goodchild and Walker (1971) combined mean germination rate, total germination, and variations in rate into a polygonial regression method or curve fitting while Janssen advocated a method using the average germination time, the standard deviation, and the total accumulated sum of the normal curve (Bewley and Black, 1978).

Barley germination behavior is quite variable and falls into three broad classifications based on a germination behavior value (G) (Finlay, 1960a). The first group (G=460) has fast and even germination and is composed almost entirely of naked barley types although a few exceptional hulled varieties approximated this rate of germination. The majority of barley varieties fall into a medium category (G=300) of

germination. A few barley varieties germinate slowly and unevenly (G=145).

MATERIALS AND METHODS

General Experimental Procedures

In these experiments seeds were germinated between moist blotter papers in plastic germination boxes. The boxes were placed in plexiglass germination chambers with temperature maintained between 20° and 25° C and the relative humidity near 100%. Each box was watered as needed to keep the blotter papers moist. Triplicate samples of one hundred seeds were germinated from each seed lot. All germination tests lasted seven days. Seeds were considered germinated at the first sign of embryo growth. Counts were made after seven days or after specific days of germination as indicated in specific experiments.

Base levels of alpha-amylase, defined in this experiment as that alpha-amylase present in the kernel prior to any germination, were estimated using a gel diffusion-starch digestion technique described by Fox and Eslick (1980). Areas of starch digestion rings were converted to ng dye release/minute/mg sample by calculating a regression line for each plate from three check samples of known alpha-amylase activity and subsequently determining enzyme activity for each sample. Where applicable, alpha-amylase activity was converted to ng dye release/minute/kernel.

Statistical Methods

The statistical analyses utilized varied with the experiment. An F test and/or Duncan's New Multiple Range Test were used to compare mean responses among groups (Steel and Torrie, 1960; Snedecor and Cochran, 1967). Regression and correlation methods were also used where applicable (Snedecor and Cochran, 1967; Sokal and Rohlf, 1969). Paired comparisons were made using a paired t test (Snedecor and Cochran, 1967).

Some of the data violated the analysis of variance assumptions of normality and homogeneity of variance. Such data were transformed and analyzed parametrically or analyzed using nonparametric statistics (Sokal and Rohlf, 1969). Paired nonparametric comparisons were made using the sign test or Wilcoxson's Signed-Rank Test (Steel and Torrie, 1960; Snedecor and Cochran, 1967; Sokal and Rohlf, 1969). Unless different results were obtained by analyzing transformed data or using nonparametric techniques only the parametric analysis of original data is reported.

Experiment 1

The objective of this experiment was to characterize the dormancy intensity and germination behavior of barley. Fifty barley cultivars including 25 two-row and 25 six-row cultivars representing commercial malting and feed barleys were planted 18 April 1978 at

Bozeman, Montana. The barley was damaged by hail on 16 July, during the heading period. Heads from 16 cultivars, presumably representing a wide range of germination behavior, were harvested 30 August, shortly after harvest ripeness (14% moisture).

The heads were air dried one week at 20° C, then threshed and germinated. Germination counts were made after days five and seven.

Speed of germination was defined as the ratio of percent germination on day five to the final germination percent on the seventh day. Residual seed from these cultivars was stored at 20° C for five months, then tested for germination a second time. The difference between final germination percentage February 1979 (Germinative Capacity), and final germination percentage September, 1978 (Germinative Intensity), was defined as the percentage of dormancy that had broken down during five months of storage. Base alpha-amylase activity levels were determined for all cultivars.

Eight malting and eight feed barley cultivars (MBIA, 1981) were equally divided between two- and six-row types (Table 1). An F test was used to compare mean responses among groups. The error mean square was used to test variation within classifications and variation among cultivars. The within classification mean square was used to test variation among classifications, between malt and feed barleys, between two- and six-row barleys, and the interaction among classifications. The data was also analyzed using a logarithmic

Table 1. Experiment 1. Classification of barley cultivars by use and row type (MBIA, 1981).

Cultivar	Principle Use	2 or 6-Row
Betzes	Malt	2
Erbet	Malt	2
Klages	Malt	2
Piroline	Malt	2
Dickson	Malt	6
Larker	Malt	6
Traill	Malt	6
Trophy	Malt	6
Compana	Feed	2
Dekap	Feed	2
Hector	Feed	2
Ingrid	Feed	2
Galt	Feed	6
Steptoe	Feed	6
Trebi	Feed	6
Vantage	Feed	6

transformation although no difference in results was obtained.

Experiment 2

This experiment was conducted to evaluate the effect of the waxy endosperm gene on dormancy, germination, and alpha-amylase activity levels during kernel maturation. Four waxy-normal endosperm near isogenic pairs, developed in the Titan, Betzes, and Compana cultivars, were grown at Bozeman and Huntley, Montana in plots 1.2 meters wide and 35.0 meters long. All isogenics involved in this experiment were developed by backcrossing the waxy endosperm gene seven generations into each cultivar.

Fifty to seventy-five random spikes were taken from each plot every two to three days from shortly after heading until harvest maturity (less than 14% moisture). Twenty-three samples were taken during the period from 27 July to 23 September at Bozeman. Fourteen samples were collected at Huntley (24 July to 22 August). Spikes were weighed, air dried for one week at 20° C in paper bags, re-weighed, threshed, and germinated. Spike moisture was estimated on a fresh weight basis using the following equation:

$$\left(\frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}}\right) \times 100 = \%\ \text{spike moisture}$$

Spike moisture was determined only at Bozeman. Physiological maturity was defined as the point in time at which moisture had

decreased to 40%. Germination counts were made after days three and seven. Germination speed was defined by the following equation:

$$\left(\frac{\%\ \text{germination day 3}}{\%\ \text{germination day 7}} \right) \times 100 = \text{Germination Speed Index}$$

Seed weight for each sample (mg/Kernel), was calculated by counting and weighing two lots of one hundred air dried seeds. Germination capacity was determined after five months of storage. Seeds which did not germinate in the seven day conventional germination test were cut in half lengthwise and placed in tetrazolium chloride (TZ) solution for 30 minutes. Seeds with brightly stained embryos were considered viable. Germination capacity was defined as the sum of percent germination in the conventional germination test plus viable seed as determined by the TZ test. Dormancy of the developing seed was estimated by subtracting the percent germinative intensity, determined one week after sampling, from the percent germinative capacity. Base level alpha-amylase activity, in the ungerminated seed, was determined for each cultivar-date combination.

Data from the 13 September sample grown at Bozeman was omitted from statistical analyses because of considerable sprouting damage. Approximately two inches of rainfall fell shortly before sampling. The heads were harvested wet and improperly dried resulting in dramatically increased alpha-amylase levels and depressed germinative energy and capacity.

Isogenic analyses between waxy and normal endosperm barleys were made by paired comparisons of waxy-nonwaxy lines at each date using a paired t test. Similar comparisons were also made between short awn-nude Compana isotypes and normal (long awn-covered) Compana isotypes and between Washonupana (waxy short awn-nude) and Compana (normal endosperm long awn-covered). Paired t tests were made for each characteristic at each location within each genetic background then across environments, and across genetic backgrounds. The waxy-normal endosperm comparisons across genetic backgrounds were made by averaging waxy and normal endosperm values at each date and subsequently comparing the means (paired by date). Paired t tests across environments were made simply by increasing the number of pairs in the test to include both environments. Isogenic analyses were also performed using the sign and Wilcoxson's Ranked Sign tests.

Graphical presentations of the time courses of various experimental observations were made using a three-sample moving average to smooth and simplify data curves. Bozeman data is presented using the percentage of spike moisture as a maturity index. Huntley data is presented chronologically. The sign of the t statistic presented in tables 4-9 indicates the relative position of isotypes. Normal isotype values were always subtracted from mutant isotype values in the paired t test. Thus a positive t statistic indicates the mutant isotype value is greater than the normal isotype value and vice versa.

Experiment 3

The purpose of this experiment was to determine the effect of the waxy endosperm gene on dormancy, germination, and malt quality characteristics at harvest maturity (less than 14% moisture). Four waxy-normal endosperm isogenic pairs plus three check cultivars, Piroline, Shabet, and Klages were planted on recropped soil at Bozeman, Montana in an effort to maintain protein content within acceptable malting limits. The experiment was planted as a randomized complete block with four replications. Plots were four rows wide with 30 cm between rows. The rows were three meters long and were planted at the rate of 3.25 grams per meter. Heading date for each plot was recorded as the days after 1 January when the first floret of the main tiller reached the flag leaf blade on 50% of the plants. Plant height (cm) was measured from the crown to the top floret at the hard dough stage.

At harvest ripeness 1.48 square meters from the center two rows of each plot were harvested for yield, germination, and malt quality evaluations. Yield, grain weight per unit area, was converted to quintals per hectare. Test weights were determined using the 'Ohaus Test Weight Scale' and converted to Kg/hectoliter. Kernel weights (grams/1000 kernels) were determined by weighing and counting 30 grams of barley seed and then calculating the weight of 1000 kernels. Malt evaluations were made on 350 gram samples, bulked in equal

proportions from each replication, at the USDA-ARS North Central Region Barley and Malt Laboratory at Madison, Wisconsin. Germination behavior was evaluated on 1 October and 3 December of the same harvest year. Daily germination counts were made in the tests. Germination speed was quantified using Finlay's germination behavior index (G) (Finlay, 1960a).

Statistical analyses involved an F test to compare germination and agronomic traits of mutant isotypes, a paired t test to compare malt quality characteristics of waxy and normal endosperm isotypes, and Duncan's Multiple Range Test to compare differences among cultivar means.

Experiment 4

This experiment was conducted to determine the effect of the waxy endosperm gene on the rate of water imbibition. Seed from four Compana isotypes - Washonupana, Shonupana, Wapana, and Compana, grown in three environments was used in the study. Initial moisture content of the seed was 7.24-9.05%. Seed lots from each environment-cultivar combination were immersed in 20°C, unaerated water in three replications and sampled after 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 8.00, 36.00 and 48.00 hours of steep time. At each sampling time, approximately 1000 seeds were removed from the water, immediately blotted to remove surface water, weighed, and then dried in a forced

air oven at 80° C. Final oven dry weights were obtained when no further moisture loss (measured by decreasing weight) occurred. Moisture percentage was calculated on a fresh weight basis. Equations describing water uptake for each isotype and for each environment were developed using regression techniques. These equations are similar to those derived by Shull (1920) for Xanthium seeds. The equation takes the form $Y=m\log_{10}(X+1)+b$ in which Y=the total percentage of moisture in the seed, and X=the hours of steep time, m and b being constants, representing the slope and Y-intercept, respectively.

Seeds from the same isotypes were also tested for germination speed. Seed from all three environments had been stored for a minimum of two years at 20 C, thus reducing dormancy to a minimal level. Germination counts were made daily for seven days. Germination speed was calculated as the germination promptness index (GPI) in which the percentage germination on days one through seven, respectively, are weighed by a factor of seven on day one to one on day seven, and summed.

Data were statistically analyzed using an F test to compare mean responses between waxy and normal Compana isotypes and between short awn-nude and long awn-covered Compana isotypes. Isotype and steep time were fixed effects, and environment was considered a random effect. Mean responses among isotypes were tested using the environment by isotype interaction mean square. Steep time responses were

tested using the environment by steep time interaction mean square. The steep time by isotype interaction was tested using the mean square of the second order interaction. All other F tests involved the residual mean square.

RESULTS AND DISCUSSION

Experiment 1. Characteristics of Malting and Nonmalting Cultivars

Percentage germinative capacity (Tables 2 and 3) varied significantly among cultivars but nonsignificantly between malt and feed types or between two- and six-row barleys. No significant interaction between the malt-feed and two-six row classifications was detected. Significant variation within the six-row classification but not within the two-row classification was observed.

Percentage dormancy (Tables 2 and 3) varied significantly among cultivars, a fact well documented (Deming and Robertson, 1933; Belderok, 1968). Malt barleys had significantly less dormancy than feed barleys. A short dormancy period (LaBerge et al., 1971) and/or a low dormancy intensity (Malting Barley Improvement Association, 1981) are characteristic of malt barleys. Two- and six-row barleys did not vary significantly for dormancy. The interaction between malt-feed and two-six row classifications was also nonsignificant. Significant variation in dormancy existed within all classifications except the malt two-row classification. Significant variation within the feed classifications would be expected. By definition, malt barleys must meet rigid standards for germination while feed barleys include all barleys not meeting malt standards.

Germination speed (Tables 2 and 3) varied significantly among

Table 2. Experiment 1. Mean germination behavior and alpha-amylase activity values for sixteen barley cultivars classified by use and row type.

	Trait			
	Germinative Capacity	Dormancy	Germination Speed	Alpha-amylase Activity
Classification	%	%	$\frac{5 \text{ day } \%}{7 \text{ day } \%} \times 100$	Ring diameter (mm)
Malt	96.8	7.8	96.0	7.9
Feed	88.4	25.5	73.1	7.6
Difference (Malt-Feed)	8.4	-17.7	22.9	0.3
Two-Row	98.5	17.9	81.6	7.6
Six-Row	86.7	15.4	87.5	7.9
Difference (2-row - 6-row)	11.8	2.5	-5.9	-0.3
Malt 2-row	99.1	5.4	97.4	7.6
Malt 6-row	94.5	10.2	94.5	8.1
Feed 2-row	98.0	30.4	65.7	7.5
Feed 6-row	78.8	20.5	80.4	7.8
Overall means	92.6	16.7	84.5	7.75

Table 3. Experiment 1. Analysis of variance for certain germination traits for sixteen barley cultivars classified by use and row type.

Source of Variation	d f	Germinative Capacity M.S.	Dormancy M.S.	Germination Speed M.S.
Cultivars	15	630.7**	807.9**	1084.7**
Among classifications	3	1057.0	1492.8	2543.4*
Malt vs Feed	1	841.7	3763.0*	6291.0*
2 vs 6-row	1	1692.0	77.5	419.5
M vs F x 2 vs 6-row	1	638.0	638.0	916.6
Within classifications	12	524.1**	636.7**	720.8**
Malt 2-row	3	1.0	67.0	18.6
Malt 6-row	3	153.2**	590.5**	38.1
Feed 2-row	3	18.3	1258.3**	1320.8**
Feed 6-row	3	1923.7**	631.3**	1505.7**
Error	32	9.5	29.6	38.3

*, ** Significant at the P=0.05 and P=0.01 probability levels, respectively.

cultivars and between malt and feed types. This agrees with prior research (Finlay, 1960a; Ulonska and Baumer, 1976) reporting wide variation in germination speed. Two- and six-row barleys did not vary significantly for germination speed. Feed barleys had significant variation within classifications, but not the malt barleys.

Levels of base alpha-amylase activity did not vary much among classifications (Table 2) and among cultivars. Statistical analyses were not conducted for alpha-amylase activity due to inadequate replication. Available information supports the finding of Riggs and Gothard (1976). They reported that alpha-amylase levels reached a peak shortly after anthesis, declined to a negligible level by 31 days post anthesis, and then remained stable until maturity. No significant differences were reported among seven barley cultivars for alpha-amylase activity in the period from 31 days after anthesis to maturity.

This experiment confirms that germination behavior varies widely among barley cultivars and between malt and feed barleys. These results also indicate that screening for malt barleys could involve testing for dormancy, germination speed, or both. Row type itself gives no indication of germination behavior.

Experiment 2: Kernel Weight, Germination Characteristics, and Alpha-amylase Development During Maturation

Maturation as measured by moisture percentage

Percentage spike moisture of all barley lines sampled throughout kernel maturation at Bozeman (Figures 1-4) parallels patterns of grain moisture content during kernel development reported for barley (Wellington, 1964; MacGregor et al., 1971) and wheat (Gordon et al., 1979). Isogenic analysis (Table 4) showed spike moisture to be significantly higher for the waxy endosperm lines than for the normal endosperm lines. Waxy endosperm lines averaged one to two percent higher spike moisture than did normal endosperm lines at each sampling date (Figure 1). During kernel maturation Washonupana and Watan had significantly higher spike moisture than Shonupana and Titan, respectively (Table 4). Washonupana had a 2-16% higher spike moisture percentage than Shonupana (Figure 2) while Watan was 0-11% higher in spike moisture percentage than Titan during kernel maturation (Figure 3). No significant differences in spike moisture existed between Wapana and Compana and between Wabet and Betzes during this period.

Paired comparisons among the four Compana isolines showed that the short awn-naked isolines were, in both cases, significantly higher in spike moisture during kernel maturation than the long awn-covered isolines (Table 4). The short awn-naked isolines, Washonupana and Shonupana averaged 2-14% higher in spike moisture than

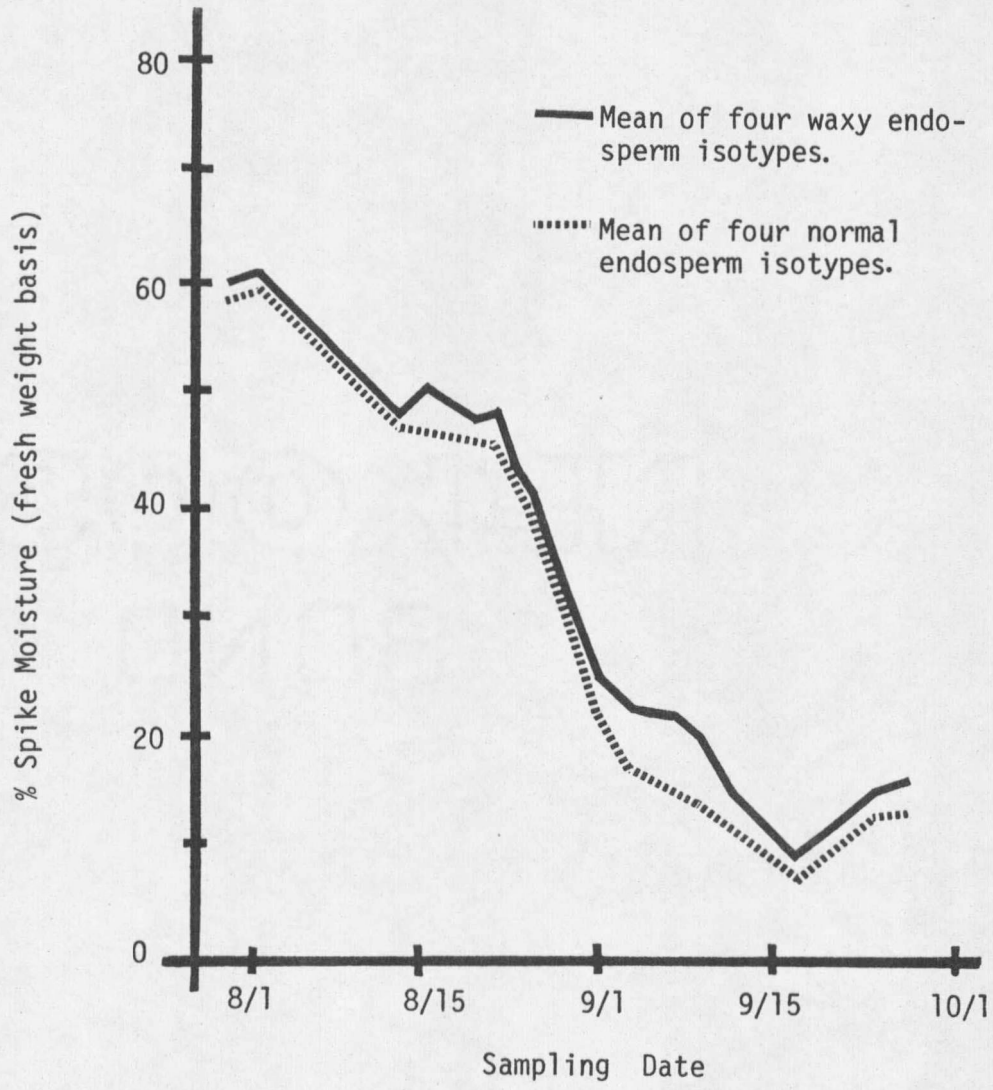

Figure 1. Experiment 2. Average spike moisture of four waxy and four normal endosperm barley isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

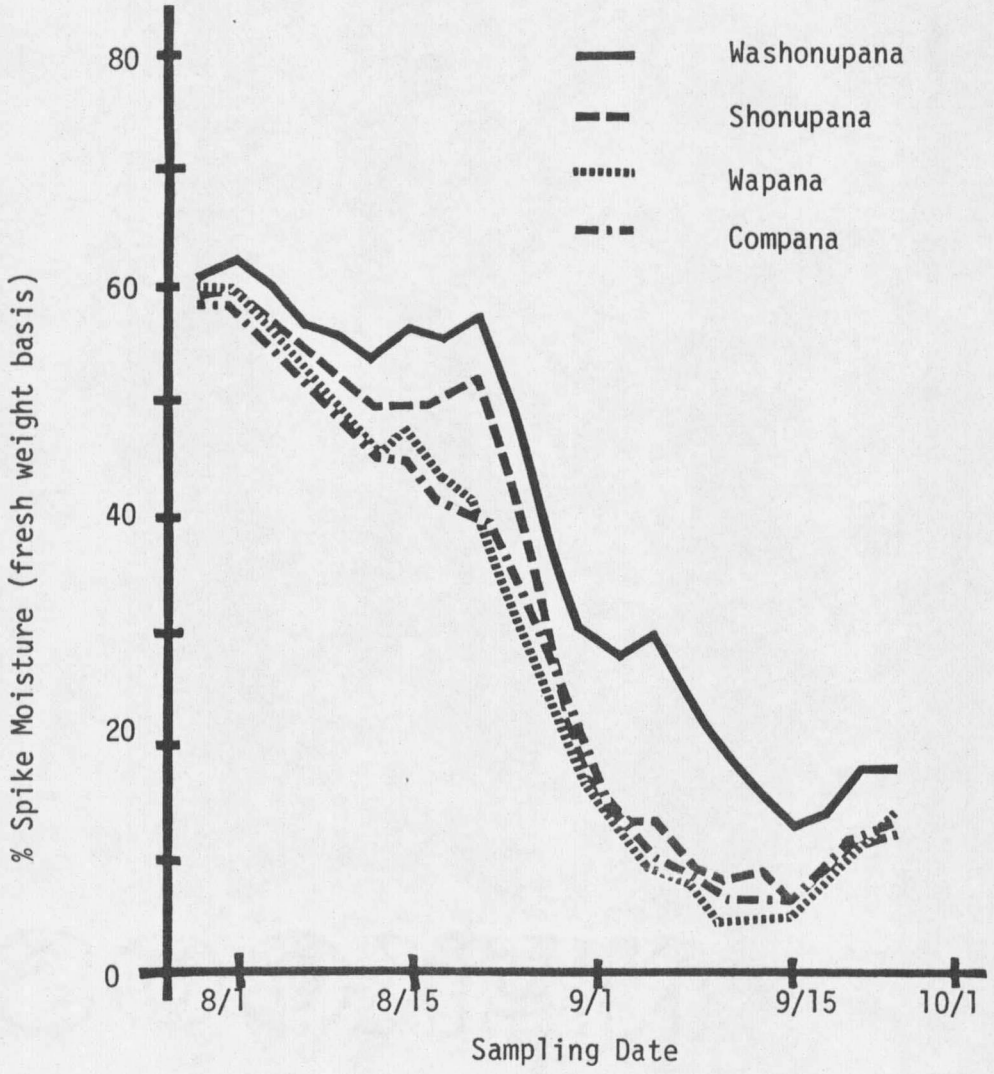

Figure 2. Experiment 2. Spike moisture of four Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

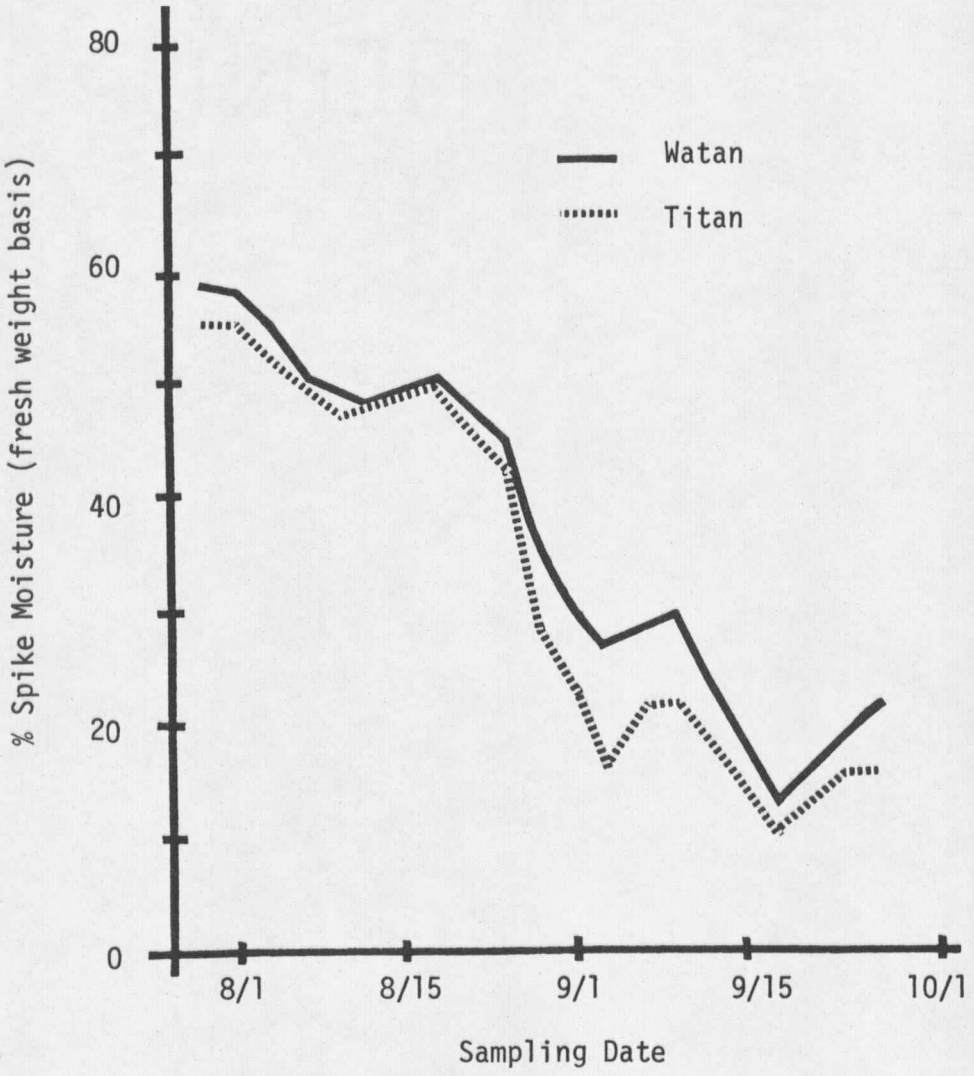

Figure 3. Experiment 2. Spike moisture of two Titan isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

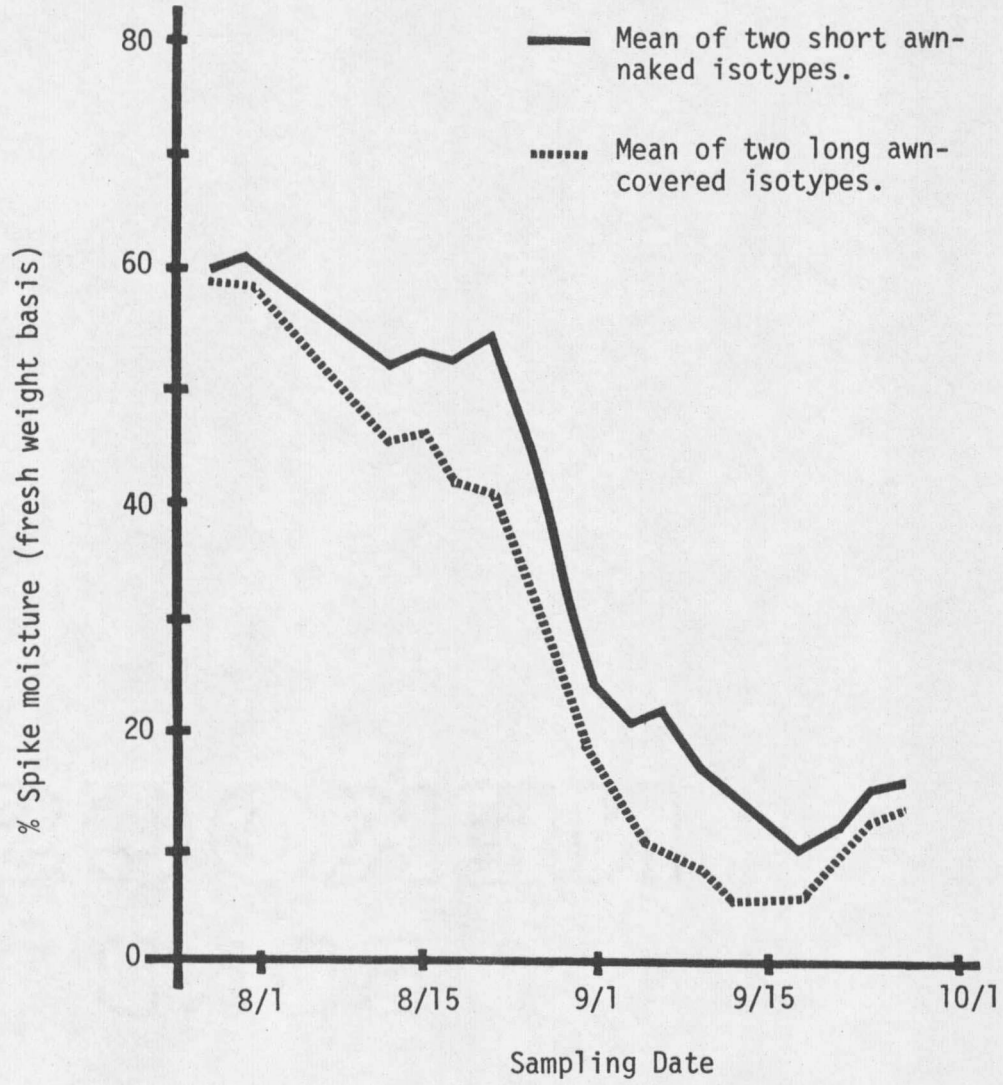

Figure 4. Experiment 2. Average spike moisture of two short awn-naked and two long awn-covered Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

Table 4. Experiment 2. Statistical analysis using a paired t test of percentage spike moisture during kernel maturation for eight barley lines isogenic for waxy and normal endosperm at Bozeman, Montana, 1978.

Comparison		Bozeman 22 df	
Mutant isotype	Normal isotype	Sign of t[+]	S.L.[++]
Washonupana	Shonupana	+	**
Wapana	Compana	-	NS
Watan	Titan	+	**
Wabet	Betzes	+	NS
Waxy (mean)	Normal (mean)	+	**
Washonupana	Wapana	+	**
Shonupana	Compana	+	*
Short awn-naked (mean)	Long awn-covered (mean)	+	**
Washonupana	Compana	+	**

+ Sign of t statistic in paired t test where normal isotype values were subtracted from mutant isotype values.

++ S.L. = Significance level (one tail) NS, *, ** denote nonsignificance and significance at the P=0.05 and P=0.01 probability levels, respectively.

Wapana and Compana during kernel maturation (Figures 2 and 4). Available comparisons did not permit the determination of whether the higher spike moistures in the short awn-naked isolines were due to the naked gene or the short awn gene, or both. Washonupana, carrying the waxy, short awn, and naked genes, maintained significantly higher spike moisture than Compana (Figure 2 and Table 4).

The fact that waxy endosperm and short awn-naked barley isotypes maintained higher levels of spike moisture during kernel maturation than did normal endosperm and long awn-covered isotypes, respectively, indicates that barleys carrying the waxy gene and/or the short awn and naked genes may lag in maturity when compared to barleys not carrying these genes. Defining harvest maturity as the point in time at which moisture in the grain falls below 14%, a one to two day lag in maturity could be expected in waxy endosperm or short awn-naked barleys. Another experiment showed all four Compana isotypes to have statistically identical heading dates (Experiment 3, Table 11). Consequently, it can be assumed that the period from anthesis to harvest maturity is longer in the waxy endosperm and short awn-naked isotypes. This lag in maturity could be due to differences in dry-down rates although spike moisture differences were apparent during the grain filling period (greater than 40% moisture) (Figures 1 and 4).

Kernel weights during maturation

Kernel weights of normal isotypes grown at Bozeman increased rapidly until physiological maturity (40% moisture) and then stabilized (Figure 5). Kernel weights of short awn and/or waxy endosperm isotypes grown at Bozeman continued to increase as moisture content decreased to 30% or less (Figures 5, 7, 10). Growth patterns similar to those of the normal isotypes grown at Bozeman are reported in wheat and barley (MacGregor et al., 1971; Riggs and Gothard, 1976; Gordon et al., 1979). MacGregor et al., (1971) showed that the moisture content of barley samples was about 40% 32 days after anthesis. They further showed that mg dry matter/kernel, mg protein/kernel, fat, ash, fiber, and mg starch/kernel all increased to some point near 32 days after anthesis and then leveled or decreased as the grain approached maturity. Kernel weights of barley grown at Huntley remained relatively stable throughout the period of sampling (Figures 6, 8, 9, 11). The lack of increasing kernel weights during the early sampling dates at Huntley indicate that sampling did not begin until after physiological maturity (40% moisture). This should be considered when interpreting data from Huntley.

Paired statistical comparisons of waxy and normal endosperm isotypes revealed that with the exception of Wabet and Betzes in both environments, and Watan and Titan at Huntley, waxy endosperm

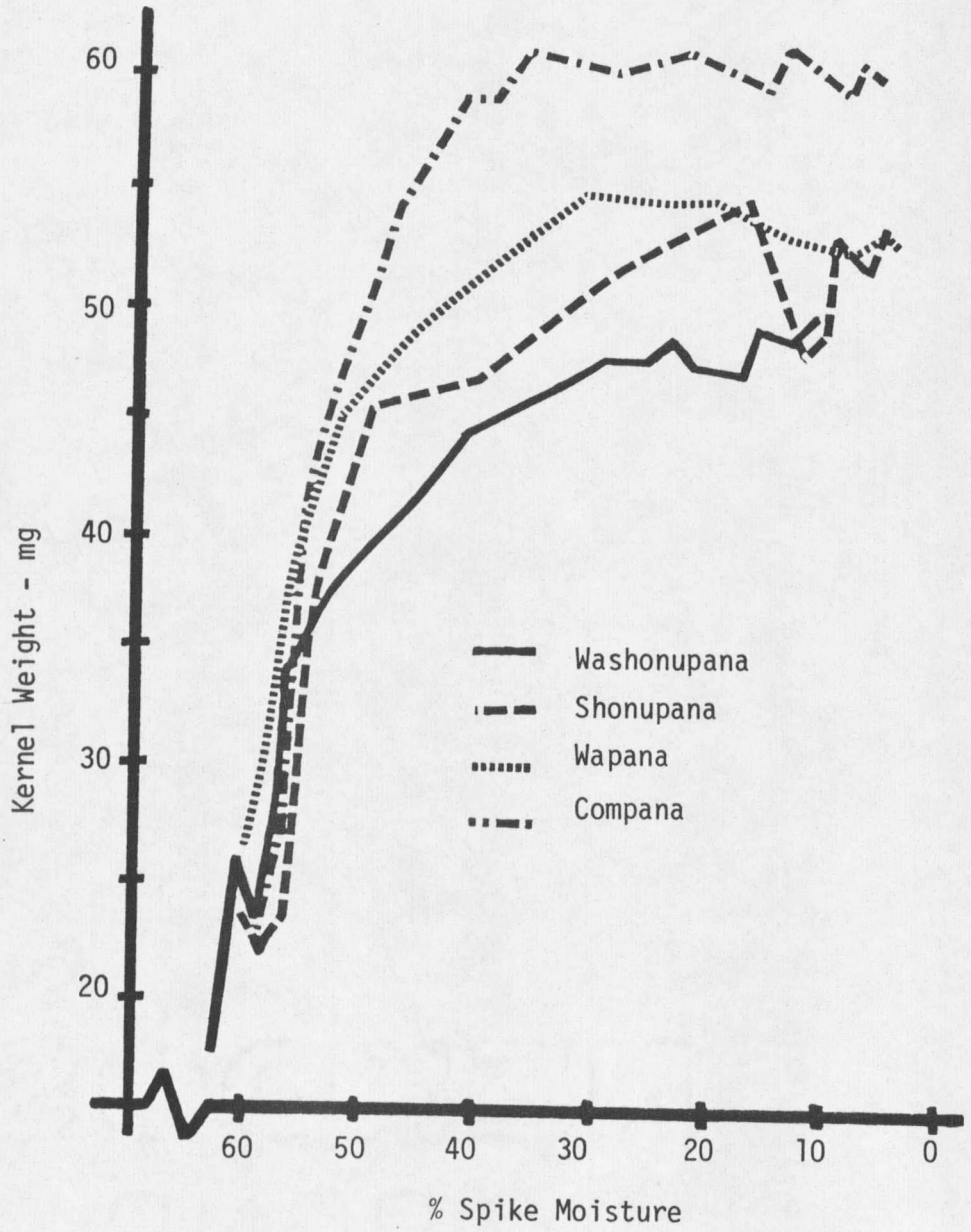

Figure 5. Experiment 2. Kernel weights of four Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

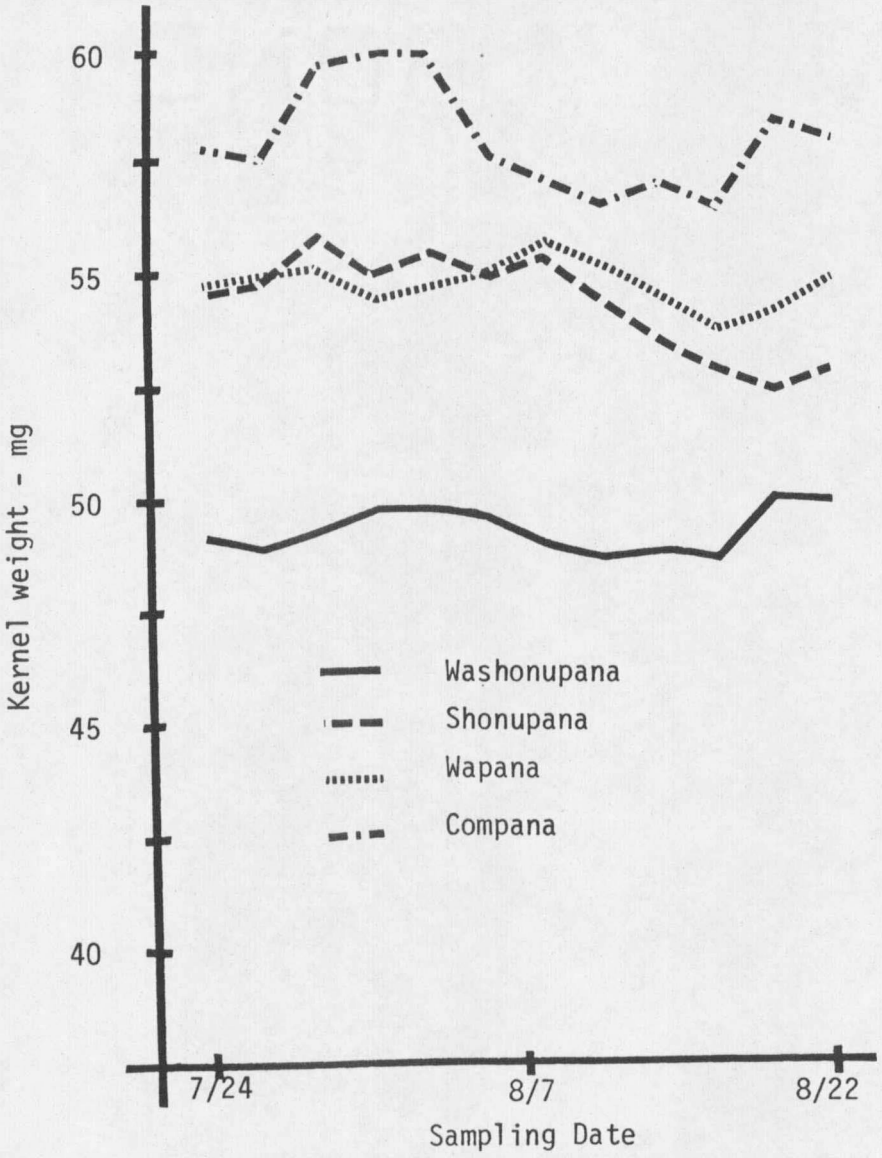

Figure 6. Experiment 2. Kernel weights of four Compana isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

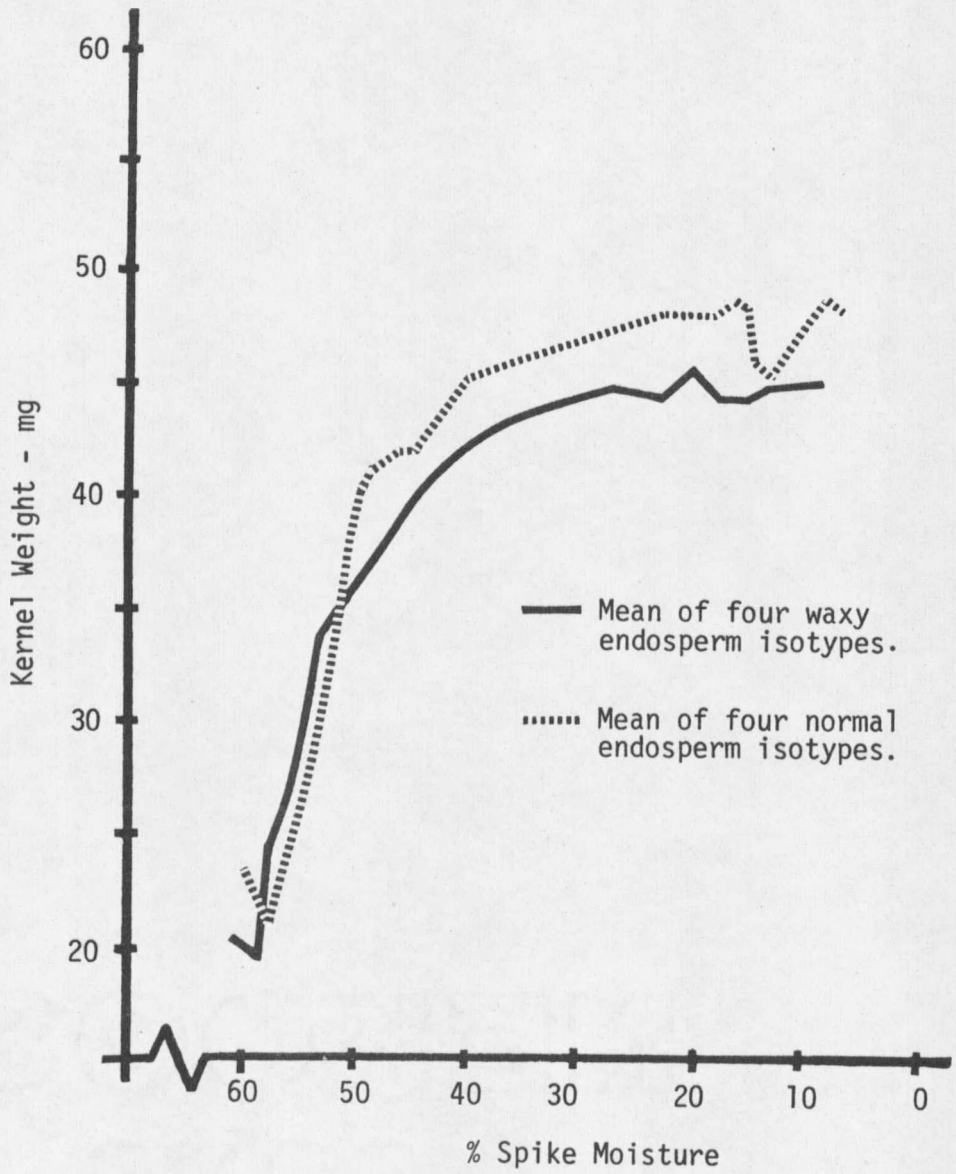

Figure 7. Experiment 2. Average kernel weights of four waxy and four normal endosperm barley isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

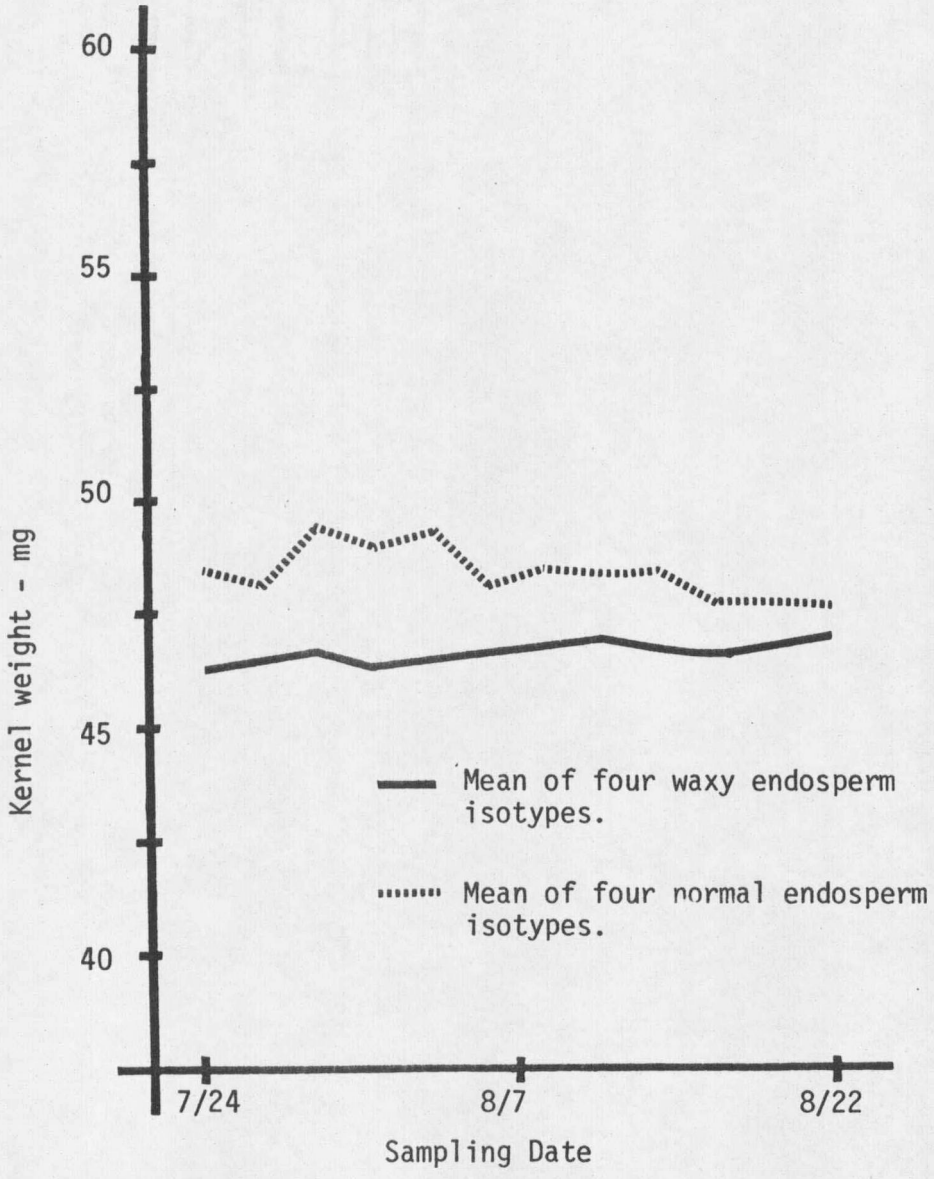

Figure 8. Experiment 2. Average kernel weights of four waxy and four normal endosperm barley isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

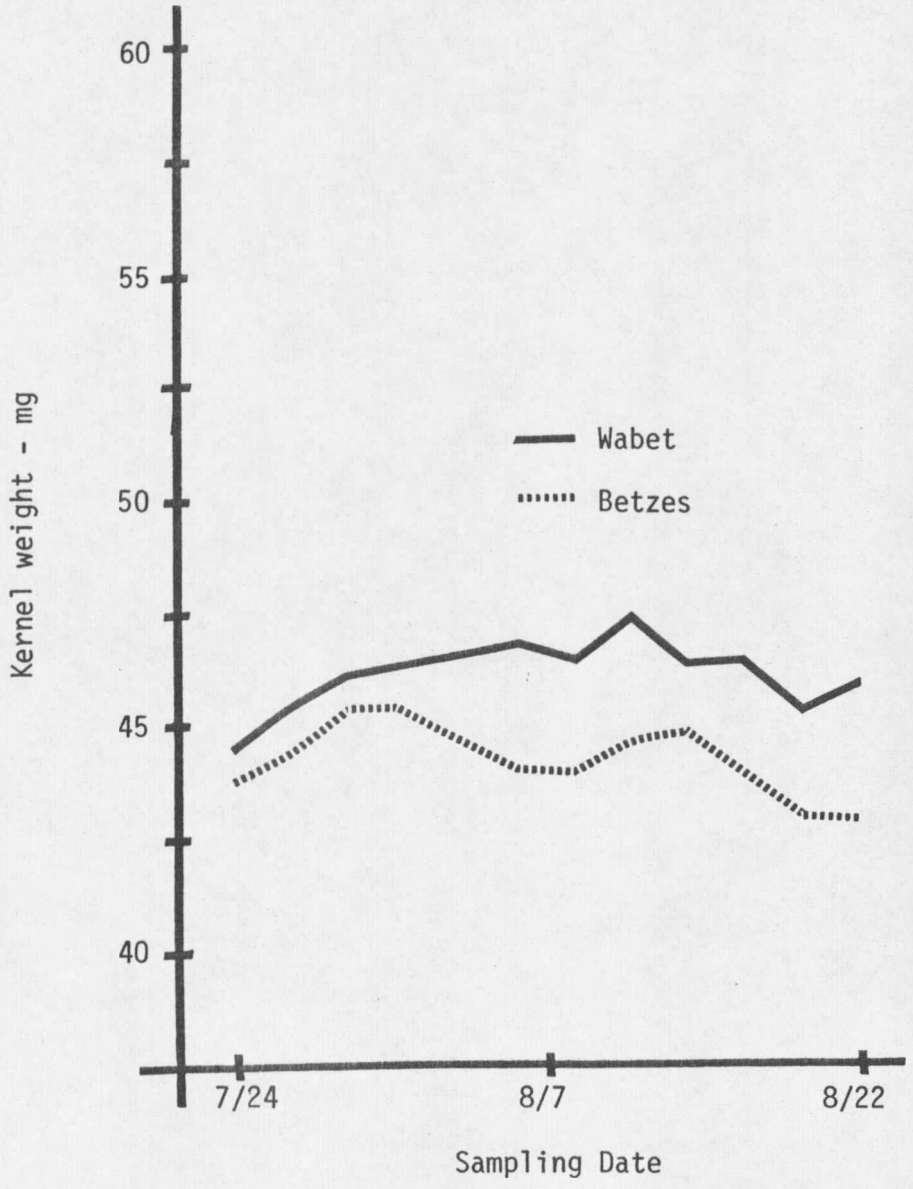

Figure 9. Experiment 2. Kernel weights of two Betzes isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

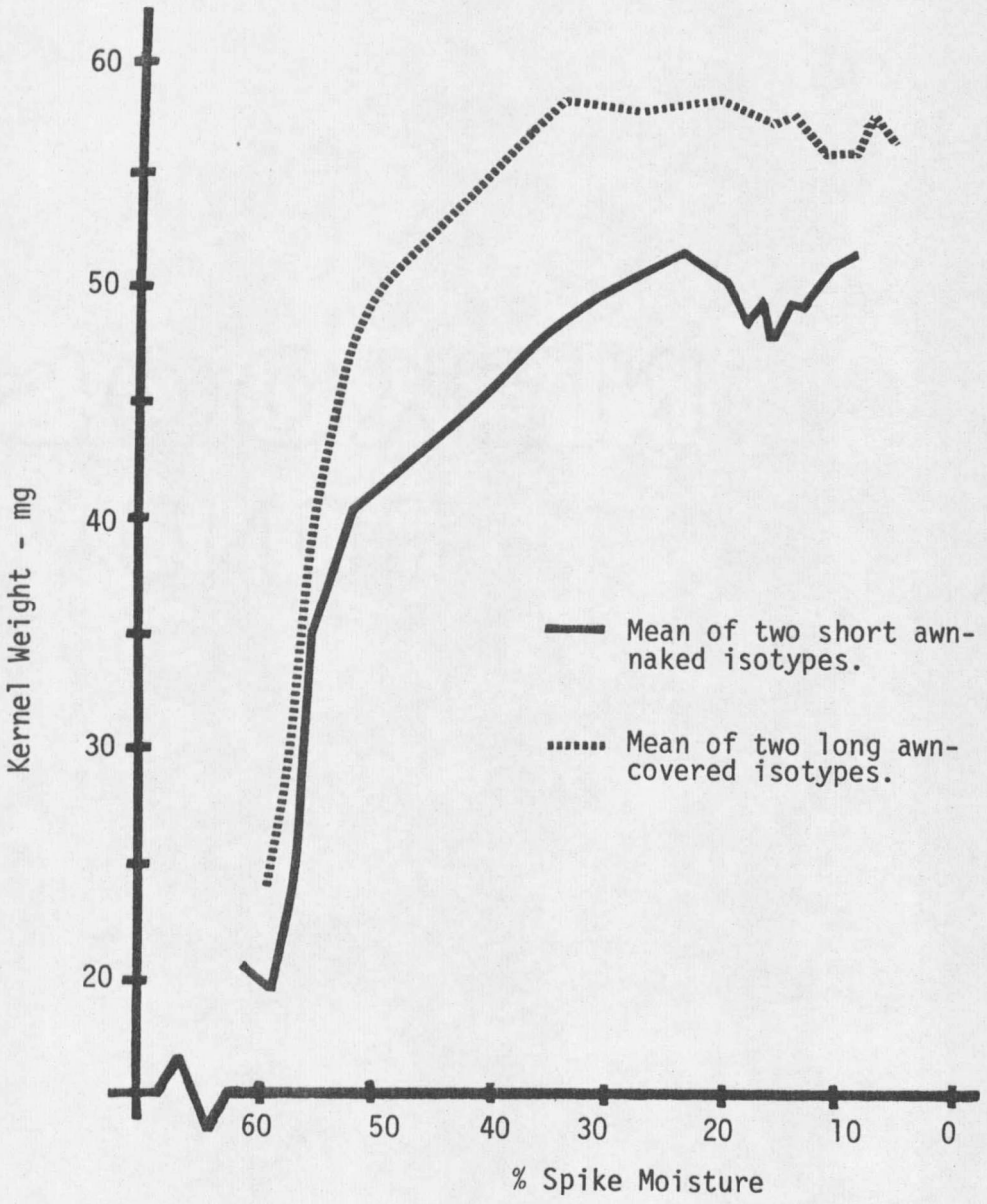

Figure 10. Experiment 2. Average kernel weights of two short awn-naked and two long awn-covered Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

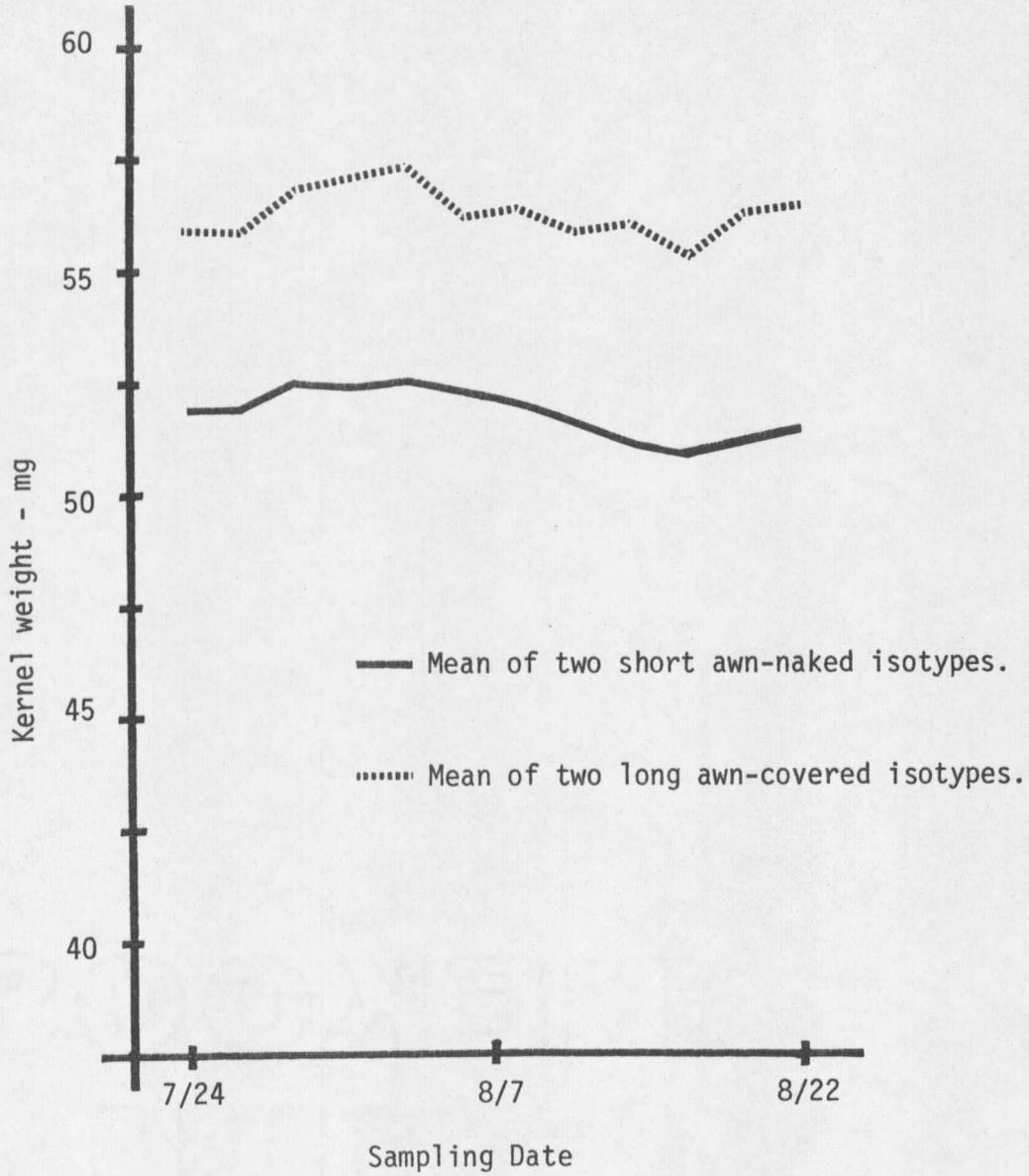

Figure 11. Experiment 2. Average kernel weights of two short awn-naked and two long awn-covered Compana isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

isotypes had significantly smaller kernel weights than normal endosperm isotypes (Table 5). Wabet had lower, though nonsignificant, kernel weights at Bozeman but significantly higher kernel weights than Betzes at Huntley (Figure 9) through kernel maturation. Normal endosperm isotypes had significantly larger kernel weights than waxy endosperm isotypes when analyses were extended over environments except for the Wabet-Betzes comparison (Table 5). Mean kernel weights during maturation were lower in the waxy endosperm lines at Bozeman (Figure 7), Huntley (Figure 8), and across environments. It can be concluded that kernel weights are smaller in waxy than in normal endosperm isotypes at similar maturity stages. The fact that Wabet had a significantly greater kernel weight than Betzes at Huntley cannot be explained. A later experiment showed that Wabet, grown at Bozeman, had a significantly smaller kernel weight than Betzes, and that normal endosperm isotypes, in the same four isogenic pairs, had kernel weights 1.00 - 4.14 mg/kernel greater than the waxy endosperm isotypes at harvest maturity (Experiment 3, Table 11).

Statistical analyses on the Compana isolines showed short awn-naked isotypes had significantly smaller kernel weights than long awn-covered isotypes in each and across both environments (Table 5). Eslick (1979) reported an average yield loss of 12% for naked isotypes in replicated yield trials over 149 environments and that

Table 5. Experiment 2. Statistical analysis using a paired t test of kernel weight during kernel maturation for eight barley lines isogenic for waxy and normal endosperm at Bozeman and Huntley, Montana, 1978.

Comparison		Bozeman 22 df		Huntley 13 df		Across environments 36 df	
Mutant isotype	Normal isotype	sign of t[+]	S.L.[++]	sign of t	S.L.	sign of t	S.L.
Washonupana	Shonupana	-	**	-	**	-	**
Wapana	Compana	-	**	-	**	-	**
Watan	Titan	-	*	-	NS	-	*
Wabet	Betzes	-	NS	+	**	+	NS
Waxy (mean)	Normal (mean)	-	**	-	**	-	**
Washonupana	Wapana	-	**	-	**	-	**
Shonupana	Compana	-	**	-	**	-	**
Short awn-naked (mean)	Long awn-covered (mean)	-	**	-	**	-	**
Washonupana	Compana	-	**	-	**	-	**

[+] Sign of t statistic in paired t test where normal isotype values were subtracted from mutant isotype values.

[++] S.L. = Significance level (one tail) NS, *, ** denote nonsignificance and significance at the P=0.05 and P=0.01 probability levels, respectively.

short awn isotypes have smaller kernels than long awn isotypes. Thus it would be expected that short awn-naked isotypes would have lower kernel weights than long awn-covered isotypes. Long awn-covered isotypes had kernel weights 4-5 mg/kernel higher during kernel maturation than short awn-naked isotypes at Huntley (Figures 6 and 11). At Bozeman, differences in kernel weights between Compana isolines first appeared when spike moisture was in the 50-55% range (Figure 5). During the dry-down period from 50% to 10% spike moisture at Bozeman kernel weights of long awn-covered Compana isotypes remained about 10 mg/kernel greater than in the short awn-naked isotypes (Figure 10).

Germinative capacity during maturation

The patterns of development for germinative capacity (Figures 12 and 13) are similar to those reported in the literature (Wellington, 1964; Gordon, 1970; LaBerge et al., 1971; Gordon et al., 1979; Mitchell et al., 1980). These patterns of development, however, approximate only the later portions of previously reported patterns. Germinative capacity, defined in this experiment as the total percentage of kernels able to germinate in the absence of dormancy, tended to be much higher during the earlier stages of kernel development than had been previously been reported. This discrepancy may have been due to a difference in technique since it was reported that air drying of immature grain increased germinability

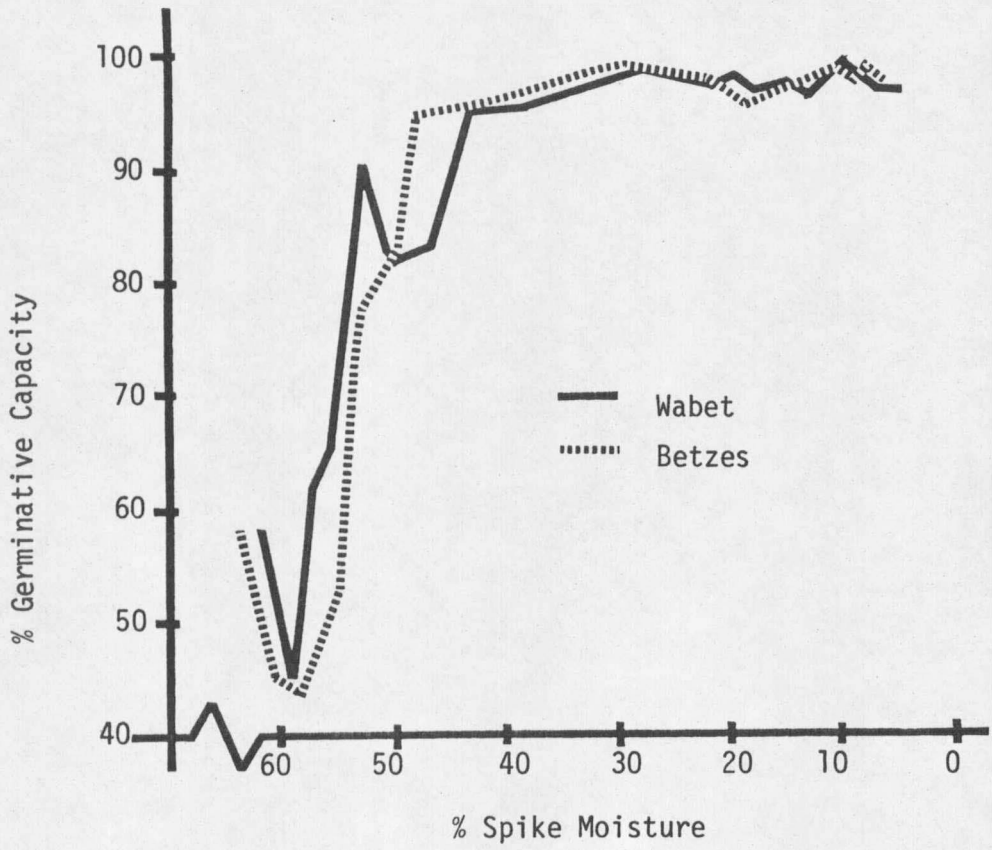

Figure 12. Experiment 2. Germinative capacity of two Betzes isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

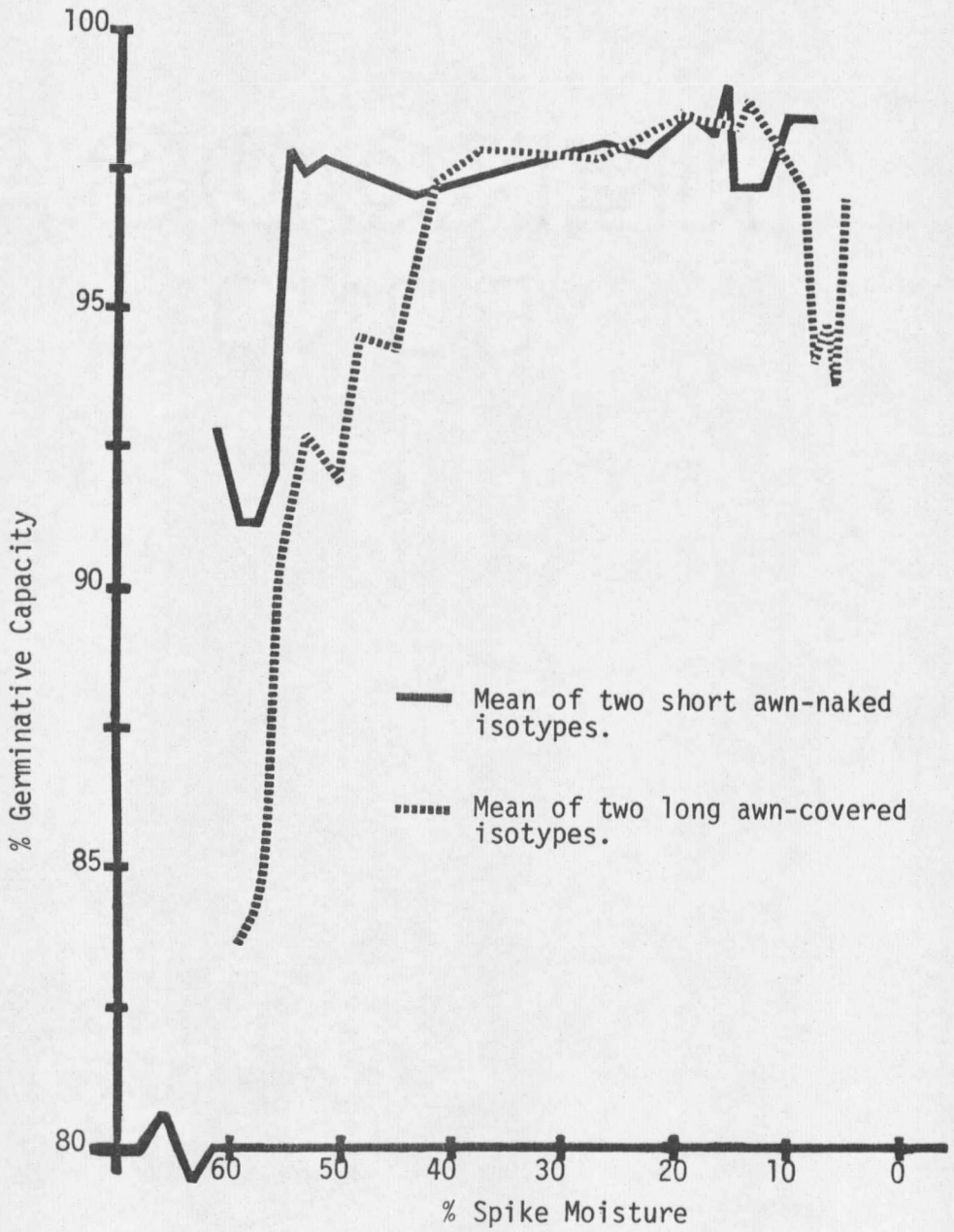

Figure 13. Experiment 2. Average germinative capacity of two short awn-naked and two long awn-covered Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

(Mitchell et al., 1980).

In general, germinative capacity was similar for waxy and normal endosperm barley isotypes. All isogenic comparisons made in the Bozeman environment except for Wabet and Betzes showed no significant differences in patterns of germinative capacity between waxy and normal endosperm isotypes (Table 6). Wabet had a slightly greater germinative capacity at Bozeman (Figure 12). At Huntley all differences between waxy and normal endosperm isogenic pairs were nonsignificant except for Watan and Titan. Watan was significantly lower in germinative capacity than Titan. Across environments and genetic backgrounds all differences between waxy and normal endosperm isotypes were nonsignificant (Table 6).

In all cases short awn-naked Compana isolines were higher in germinative capacity than long awn-covered Compana isolines (Table 6). This relationship was, however, statistically significant only for the Washonupana-Wapana comparison at Bozeman and across environments and for the means of the short awn-naked and long awn-covered isotypes at Bozeman (Figure 13) and across environments. Washonupana had a significantly greater germinative capacity than Compana at Bozeman but not at Huntley.

Dormancy during maturation

The general patterns of dormancy during kernel maturation at Bozeman (Figures 14, 16, 18) showed dormancy increased during the

Table 6. Experiment 2. Statistical analysis using a paired t test of germinative capacity during kernel maturation for eight barley lines isogenic for waxy and normal endosperm at Bozeman and Huntley, Montana, 1978.

Comparison		Bozeman 22 df		Huntley 13 df		Across environments 36 df	
Mutant isotype	Normal isotype	sign of t[+]	S.L.[++]	sign of t	S.L.	sign of t	S.L.
Washonupana	Shonupana	+	NS	+	NS	+	NS
Wapana	Compana	-	NS	+	NS	+	NS
Watan	Titan	-	NS	-	*	-	*
Wabet	Betzes	+	*	-	NS	+	*
Waxy (mean)	Normal (mean)	+	NS	+	NS	+	NS
Washonupana	Wapana	+	*	+	NS	+	*
Shonupana	Compana	+	NS	+	NS	+	NS
Short awn-naked (mean)	Long awn-covered (mean)	+	*	+	NS	+	*
Washonupana	Compana	+	*	+	NS	+	*

[+] Sign of t statistic in paired t test where normal isotype values were subtracted from mutant isotype values.

[++] S.L. = Significance level (one tail) NS, *, ** denote nonsignificance and significance at the P=0.05 and P=0.01 probability levels, respectively.

period shortly after anthesis until physiological maturity (40% moisture), peaked, then decreased steadily with increasing maturity. Considering dormancy patterns during kernel development and maturation to be essentially the inverse of germinative energy developmental patterns, this experiment supports Bishop's interpretation of germinative power of barley at different maturity stages (Bishop, 1944). Bishop's interpretation showed little dormancy at the milk stage, increasing dormancy until near physiological maturity, then a progressive dormancy decline with maturity. This experiment did not support Gordon's interpretation of earlier work to change Bishop's curves of germinative power at different maturity stages (Gordon, 1970). Gordon suggested that germinative energy remained near zero until physiological maturity, then increased rapidly to maturity. Dormancy developmental patterns in the Huntley environment (Figures 15, 17, 19) showed dormancy declined steadily. This is additional evidence that sampling at Huntley began after physiological maturity.

Isogenic analyses of dormancy during kernel maturation (Table 7) showed dormancy was significantly lower in waxy endosperm isotypes than in normal endosperm isotypes when averaged across genetic backgrounds and taken across environments. Waxy endosperm isotypes in the Compana background but not in the Titan and Betzes backgrounds were significantly lower in dormancy in and across both environments than normal endosperm isotypes (Table 7). Major dormancy differences

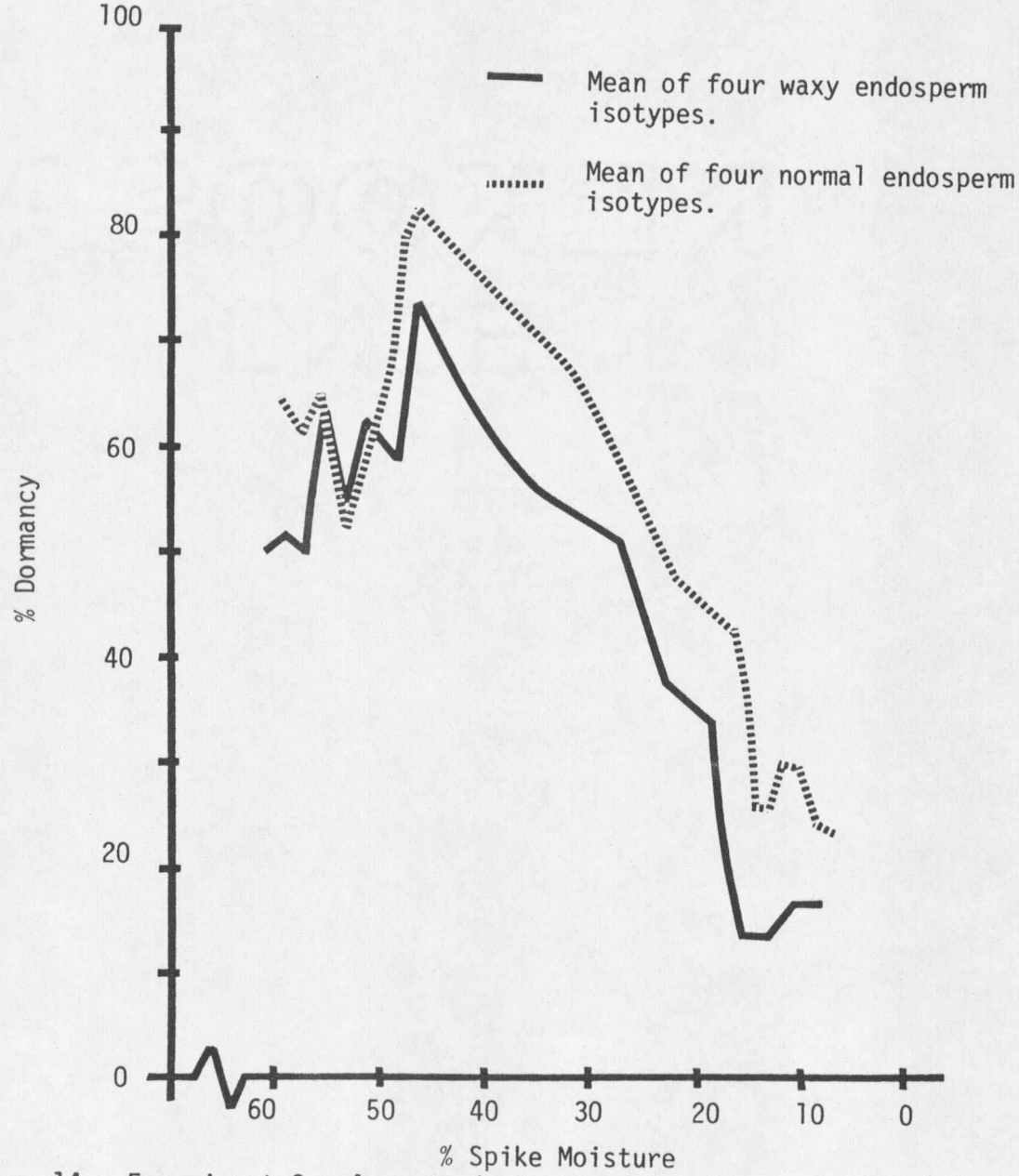

Figure 14. Experiment 2. Average dormancy of four waxy and four normal endosperm barley isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

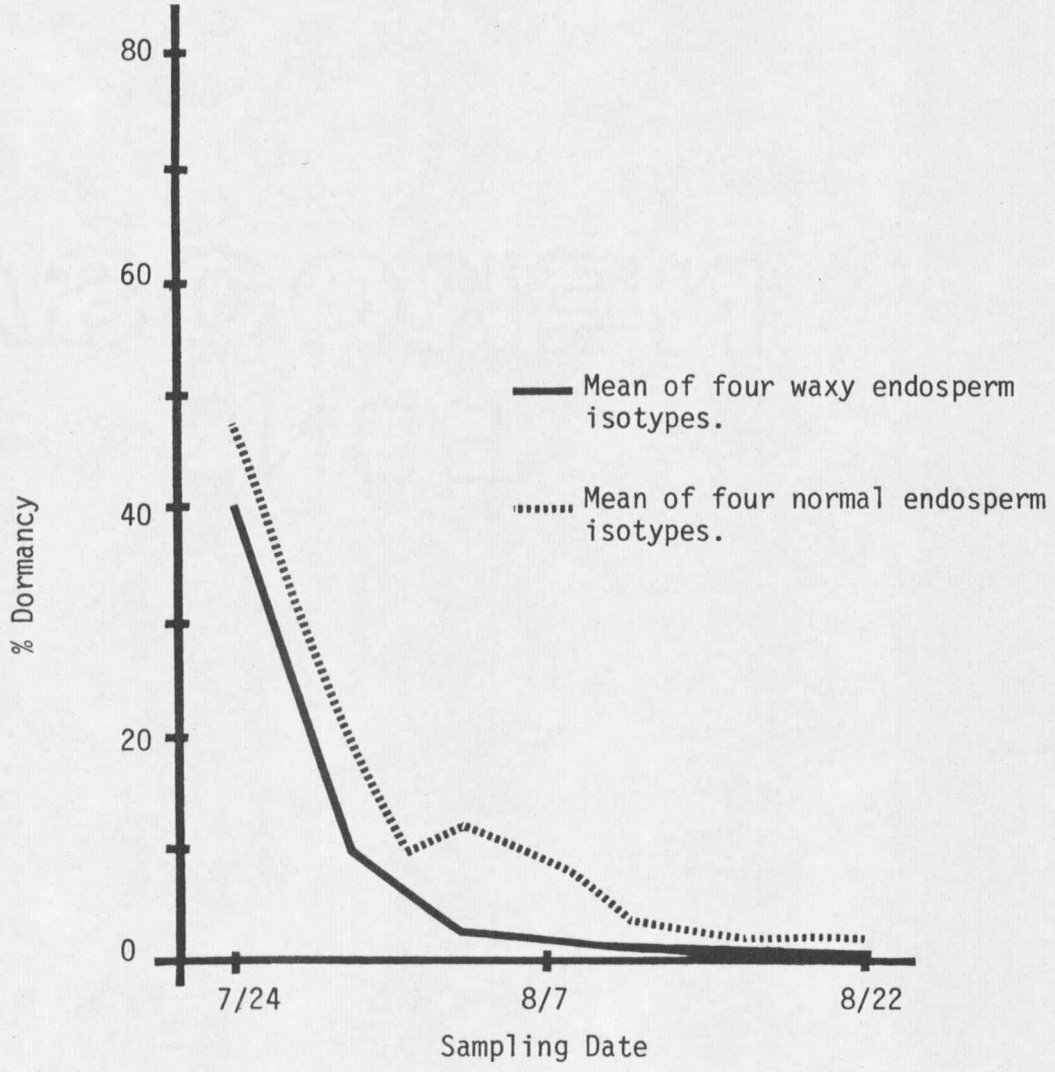

Figure 15. Experiment 2. Average dormancy of four waxy and four normal endosperm barley isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

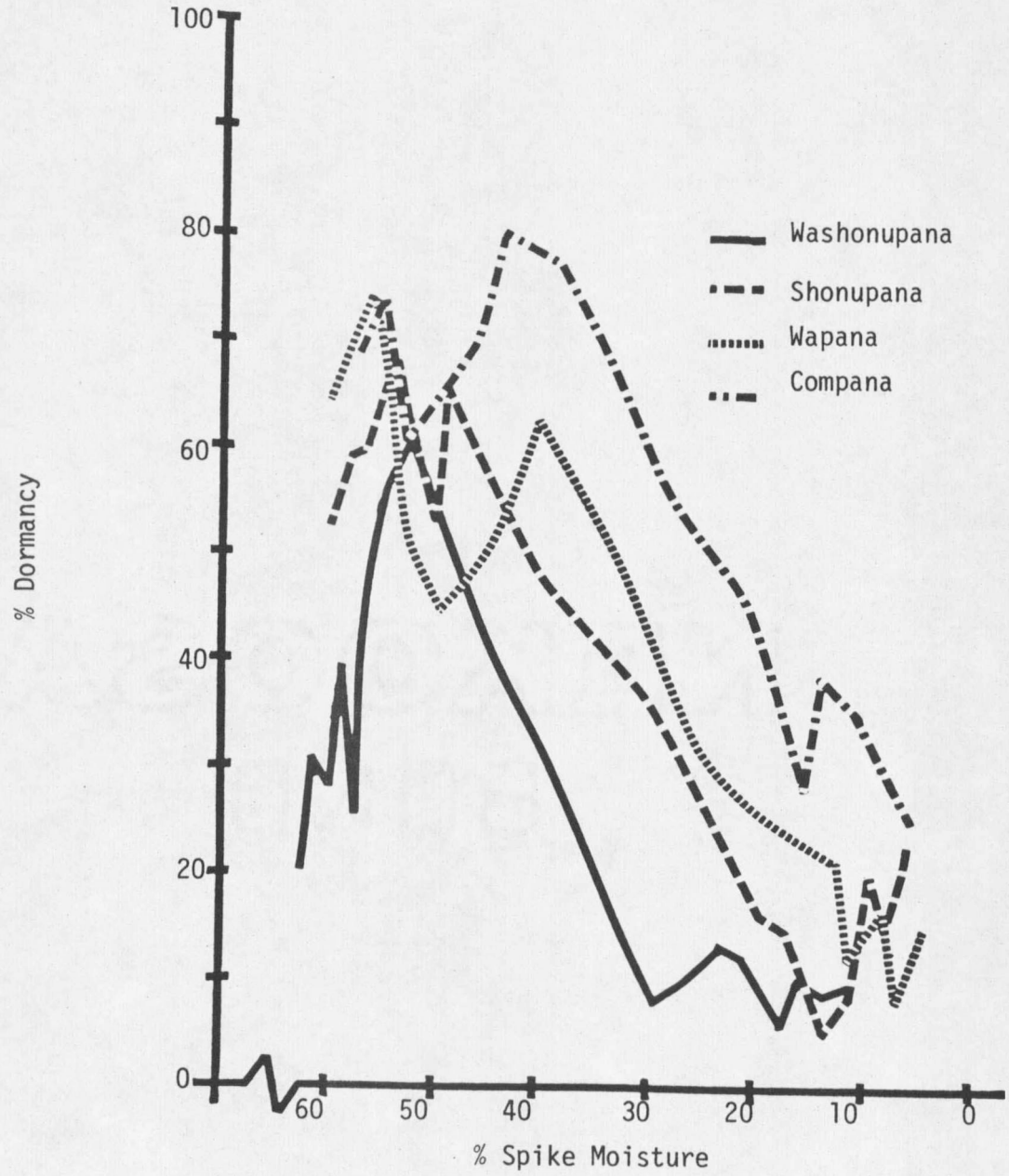

Figure 16. Experiment 2. Dormancy of four Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

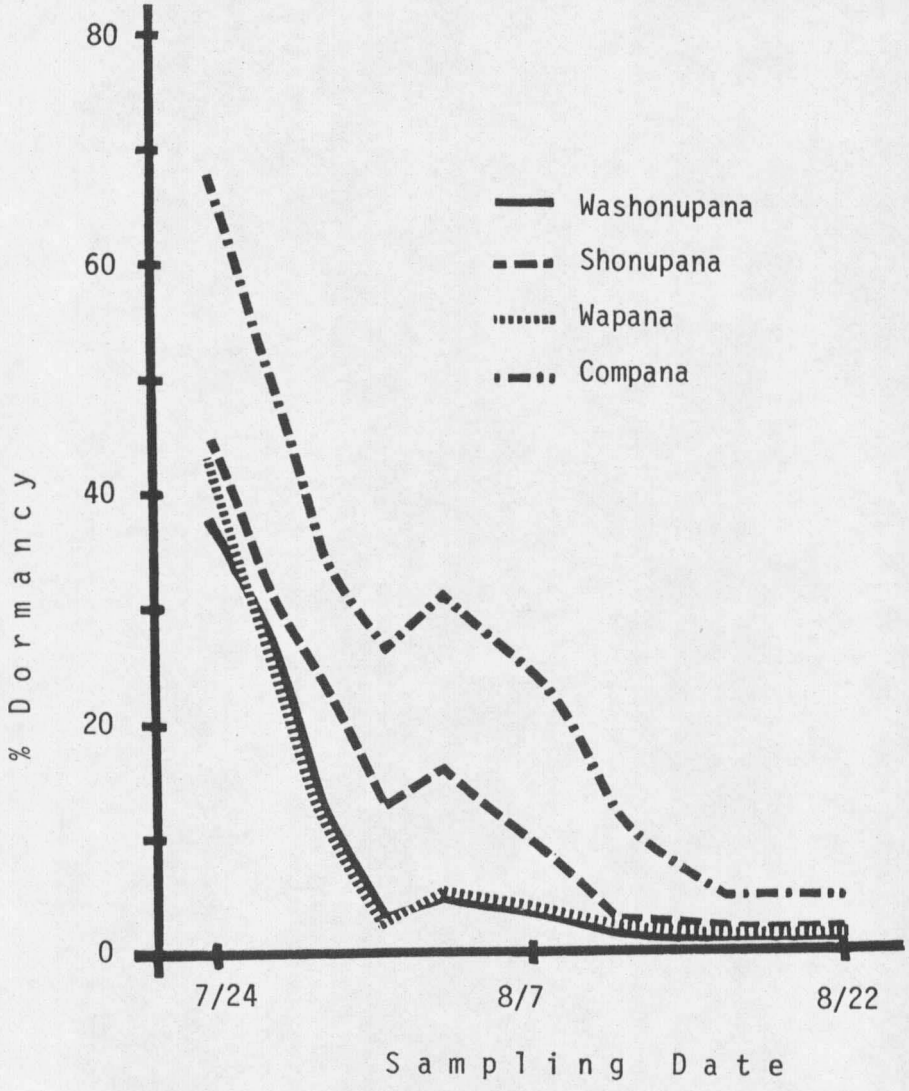

Figure 17. Experiment 2. Dormancy of four Compana isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

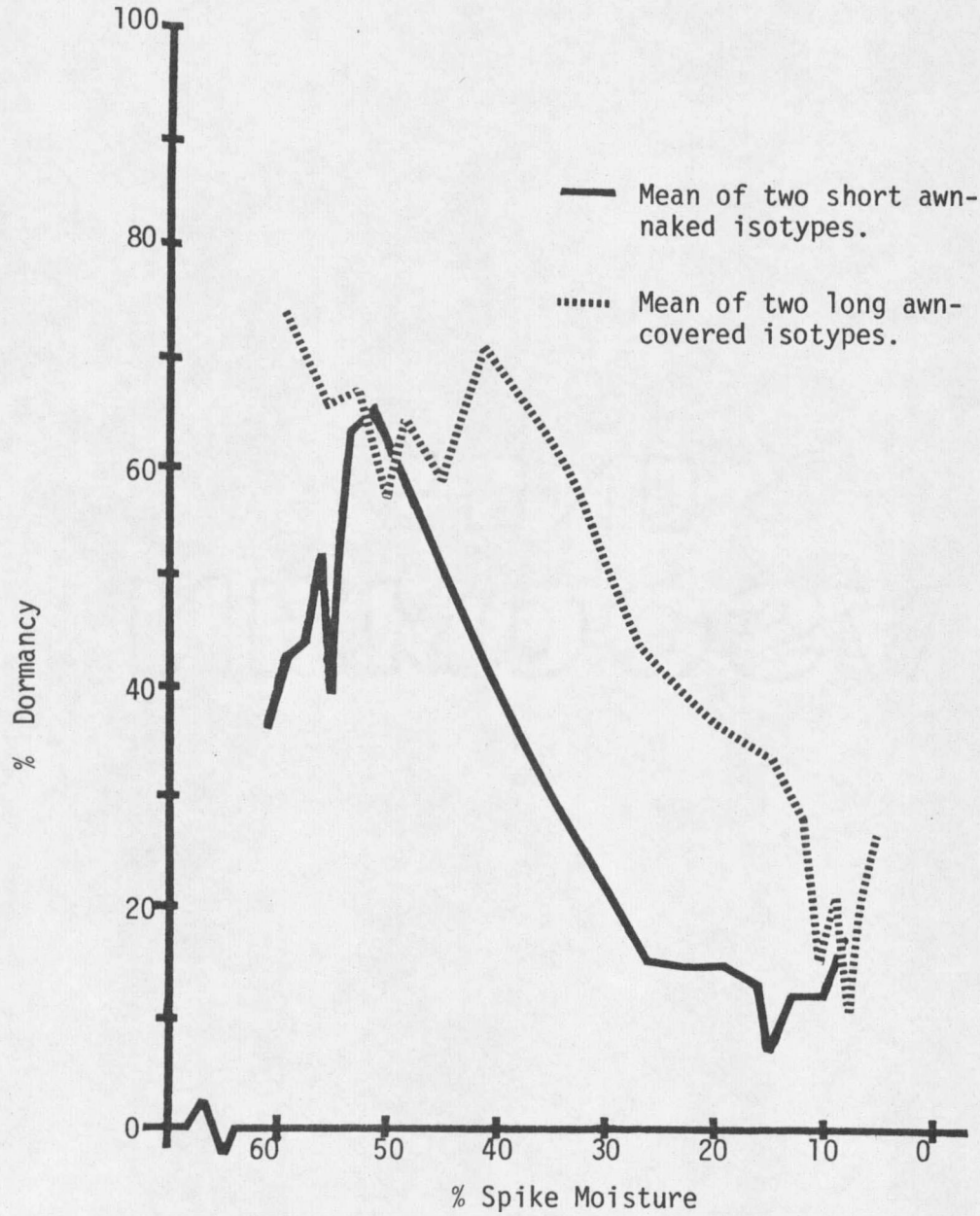

Figure 18. Experiment 2. Average dormancy of two short awn-naked and two long awn-covered Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

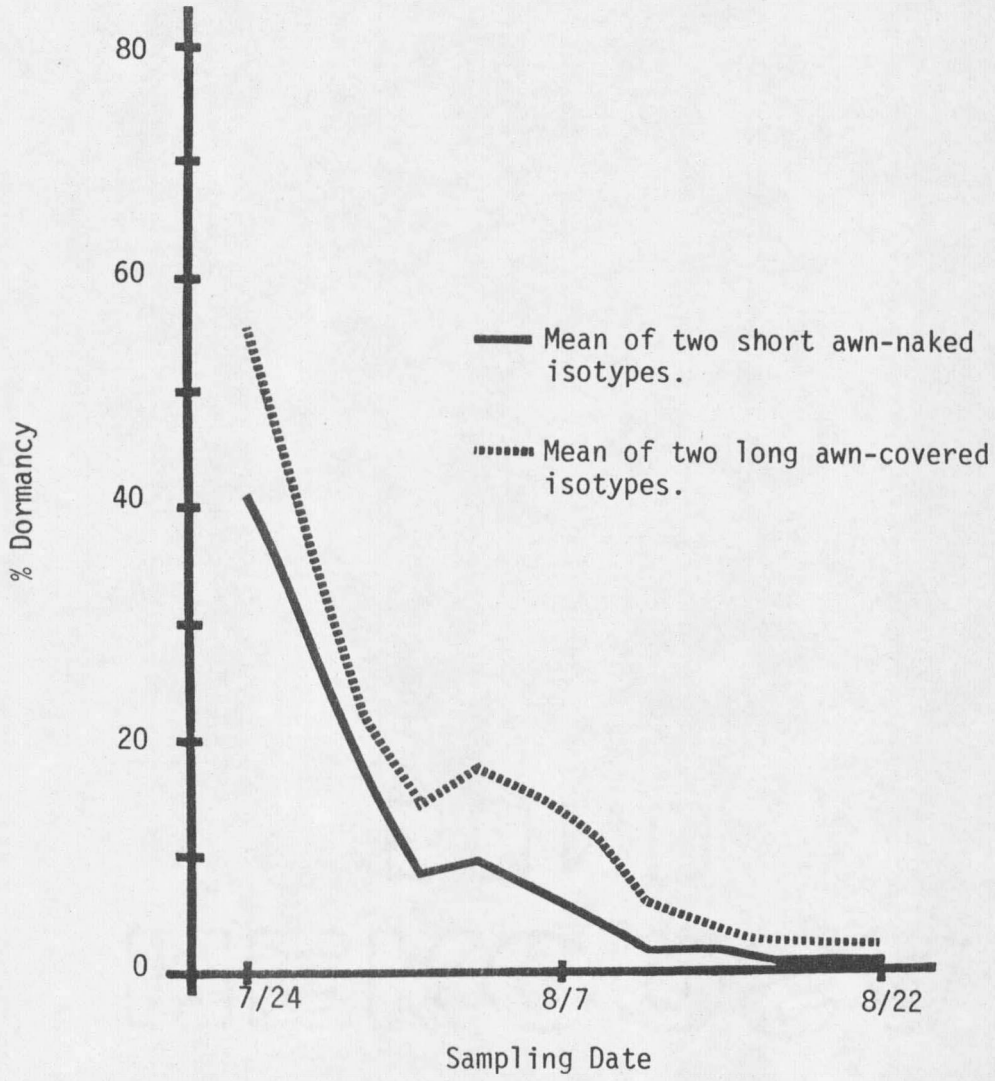

Figure 19. Experiment 2. Average dormancy of two short awn-naked and two long awn-covered Compana isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

Table 7. Experiment 2. Statistical analysis using a paired t test of dormancy during kernel maturation for eight barley lines isogenic for waxy and normal endosperm at Bozeman and Huntley, Montana, 1978.

Comparison		Bozeman 22 df		Huntley 13 df		Across environments 36 df	
Mutant isotype	Normal isotype	sign of t[+]	S.L.[++]	sign of t	S.L.	sign of t	S.L.
Washonupana	Shonupana	-	**	-	*	-	**
Wapana	Compana	-	**	-	**	-	**
Watan	Titan	-	NS	-	NS	-	NS
Wabet	Betzes	+	NS	+	NS	+	NS
Waxy (mean)	Normal (mean)	-	**	-	**	-	**
Washonupana	Wapana	-	**	-	NS	-	**
Shonupana	Compana	-	**	-	**	-	**
Short awn-naked (mean)	Long awn-covered (mean)	-	**	-	**	-	**
Washonupana	Compana	-	**	-	**	-	**

[+] Sign of t statistic in paired t test where normal isotype values were subtracted from mutant isotype values.

[++] S.L. = Significance level (one tail) NS, *, ** denote nonsignificance and significance at the P=0.05 and P=0.01 probability levels, respectively.

between waxy and normal endosperm isotypes were first observed when spike moisture was near 45% at Bozeman (Figures 14 and 16). At Huntley, dormancy differences between waxy and normal endosperm isotypes were apparent throughout sampling but were small at harvest maturity (Figure 15 and 17).

Short awn-naked Compana isotypes exhibited significantly less dormancy in all but one comparison than long awn-covered Compana isotypes (Table 7). This relationship held true across environments and when averaged across isotypes at each environment (Figures 18 and 19). Although the effects of the short awn and naked genes could not be separated in this experiment, it is probable that the lesser dormancy in the short awn-naked isotypes was caused by the naked character. Numerous researchers have reported that the dormancy mechanism in wheat and barley is at least partially controlled by the surface layers of the kernel. Dormancy is a result of the internal level of free oxygen at the embryo (Pollock, 1962; Belderok, 1968; Briggs, 1978) and/or a function of grain dehydration during kernel maturation (Wellington, 1964; Gordon, 1970; Gordon et al., 1979; Mitchell et al., 1980). Whatever the dormancy mechanism, the pericarp in naked isotypes would be a lesser barrier to oxygen uptake and/or grain dessication than the testa-pericarp surface layers in covered barley isotypes.

Germination speed during maturation

Speed of germination of seed harvested at different stages of kernel development generally increased with increasing kernel maturity (Figures 20-26). Repression of germination speed may be considered another form of dormancy (Gordon, 1969). Statistical analyses indicated that germination speed during kernel maturation was strongly influenced by environment and genetic background (Table 8). Germination speed differences between waxy and normal endosperm isotypes were inconsistent. Mean germination speed of waxy endosperm isotypes during kernel maturation was significantly slower at Bozeman and significantly faster at Huntley than the mean germination speed of normal endosperm isotypes (Table 8). Mean germination speed differences between waxy and normal endosperm isotypes were inconsistent at Bozeman (Figure 20) but more consistent at Huntley (Figure 21). Wapana germinated faster than Compana in both environments (Figures 22 and 23). Washonupana germinated significantly faster than Shonupana at Huntley but not Bozeman (Table 8). Titan germinated faster than Watan during kernel maturation at Bozeman (Figure 24) especially within the period when spike moisture dropped from 30% to 10%.

Short awn-naked Compana isotypes, without exception, germinated significantly faster than long awn-covered Compana isotypes (Table 8). Washonupana and Shonupana germinated faster than Wapana and Compana,

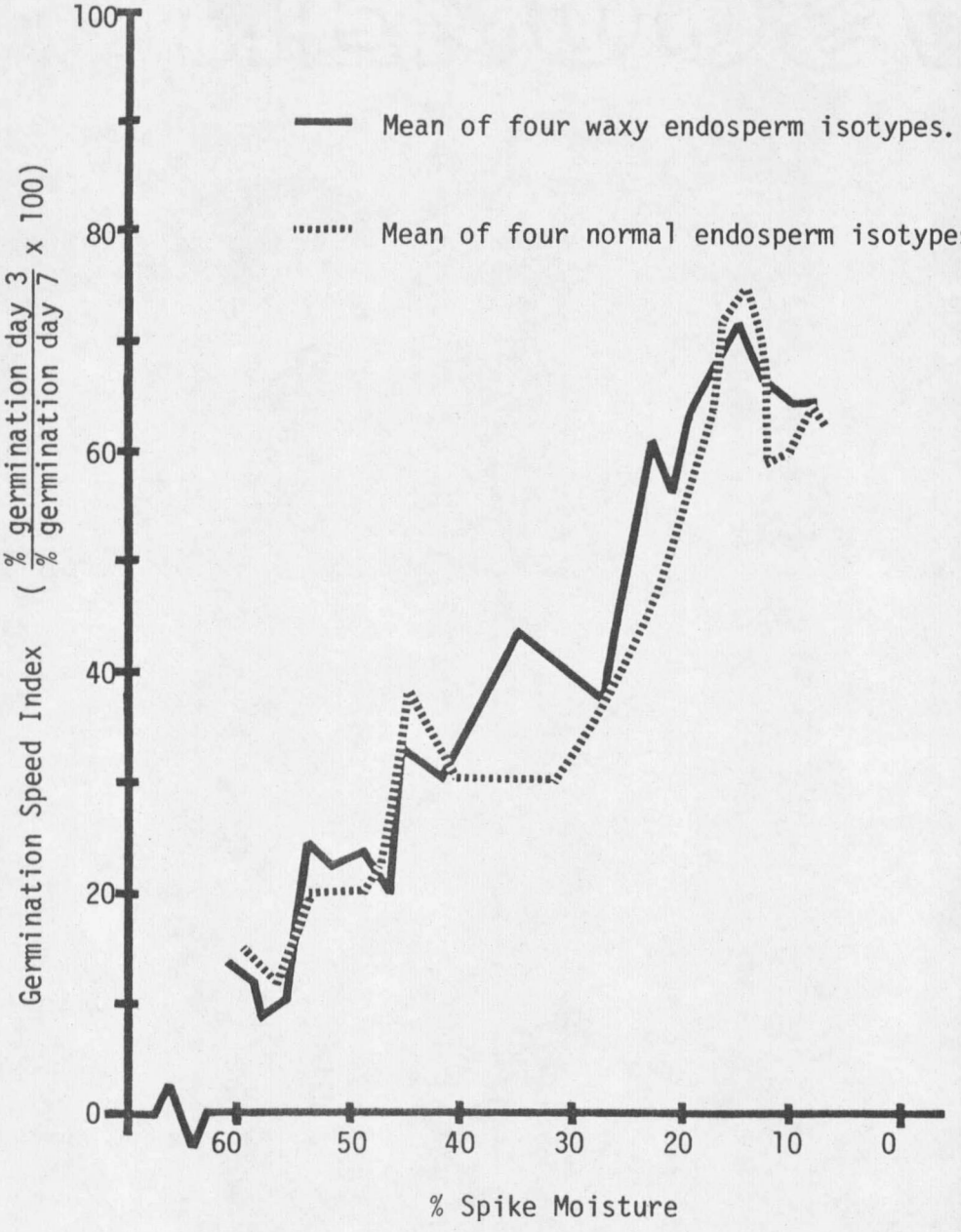

Figure 20. Experiment 2. Average germination speed of four waxy and four normal endosperm barley isotypes sampled 23 times (27 July-23 September 1978) during kernel maturation at Bozeman, Montana.

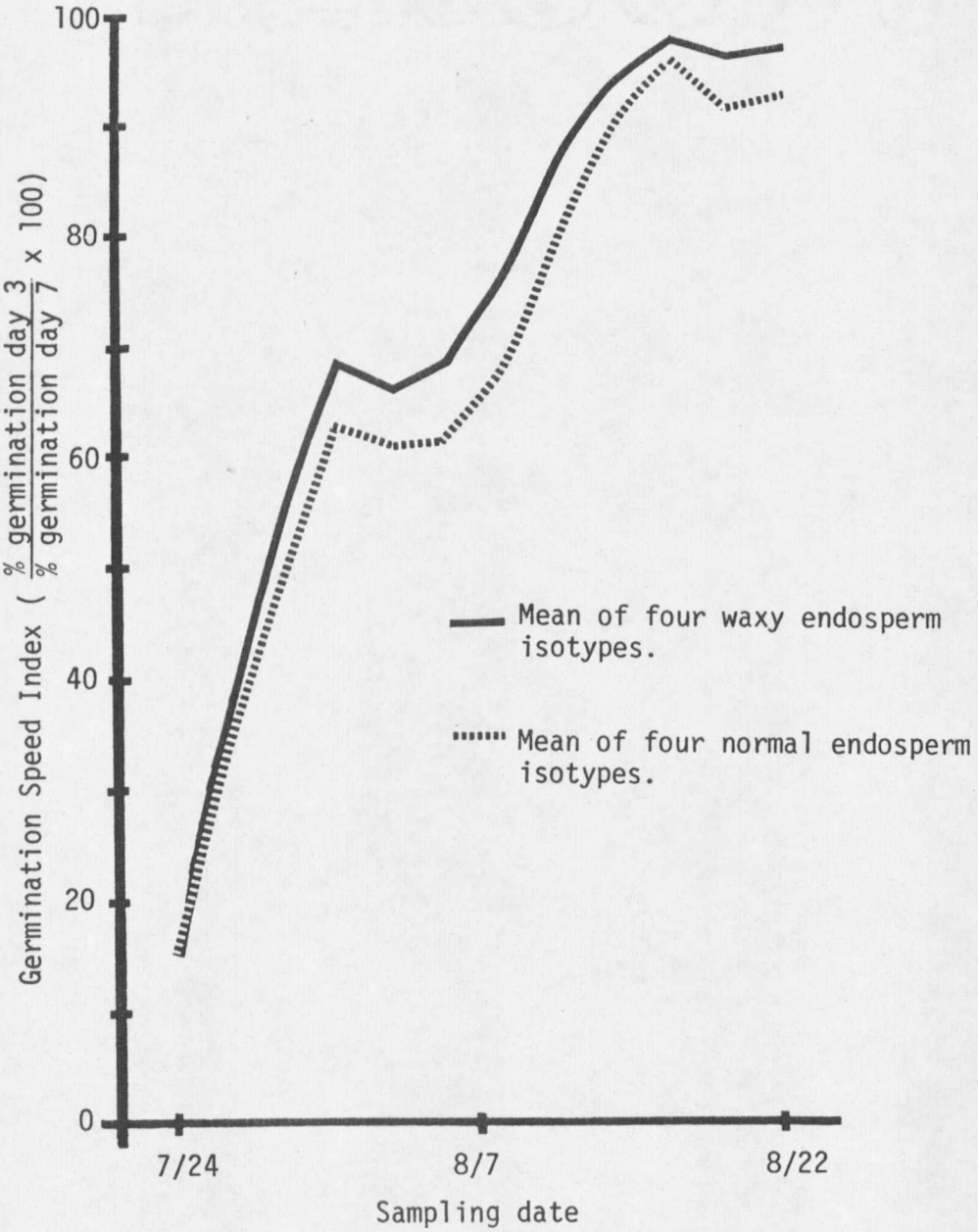

Figure 21. Experiment 2. Average germination speed of four waxy and four normal endosperm barley isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

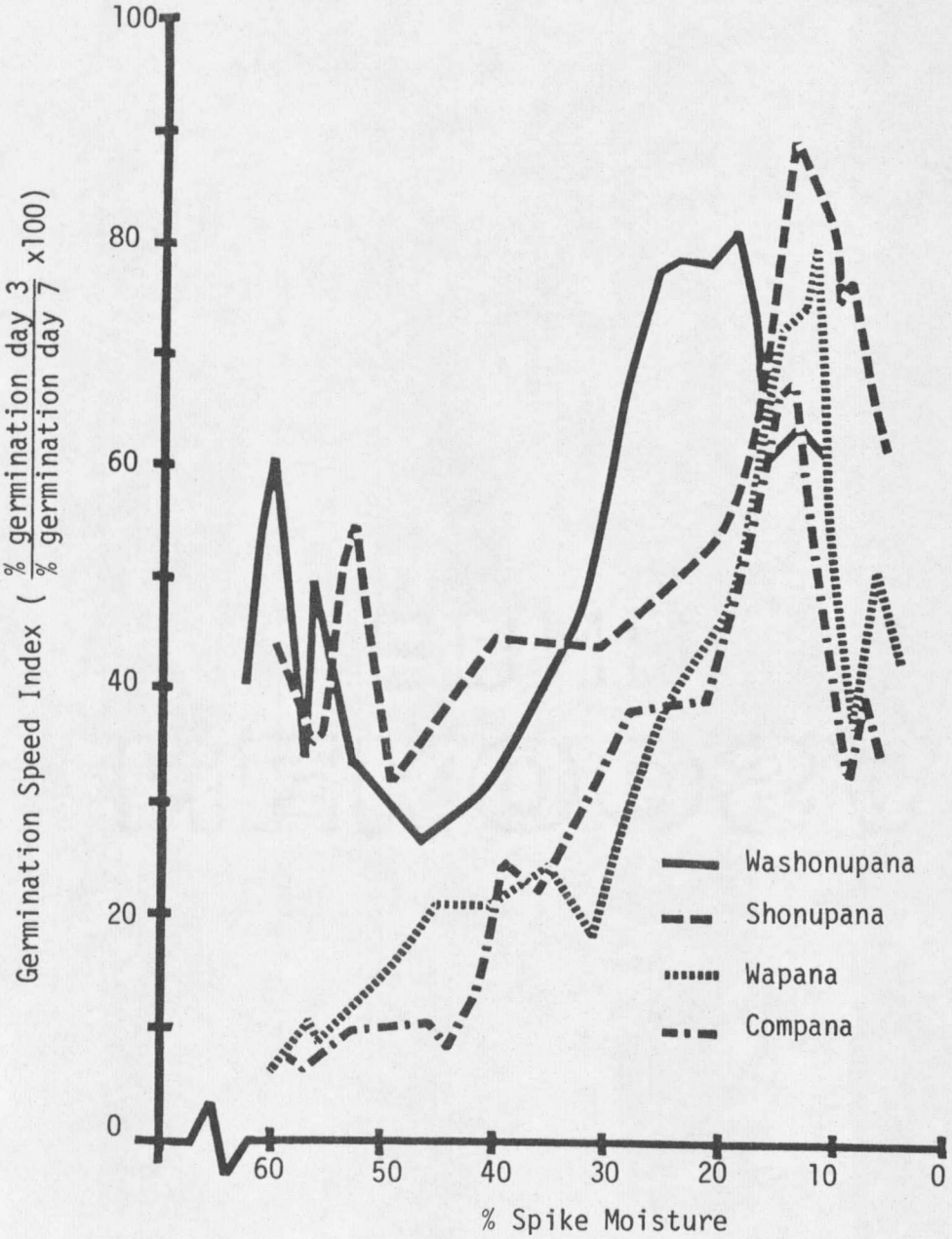

Figure 22. Experiment 2. Germination speed of four Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

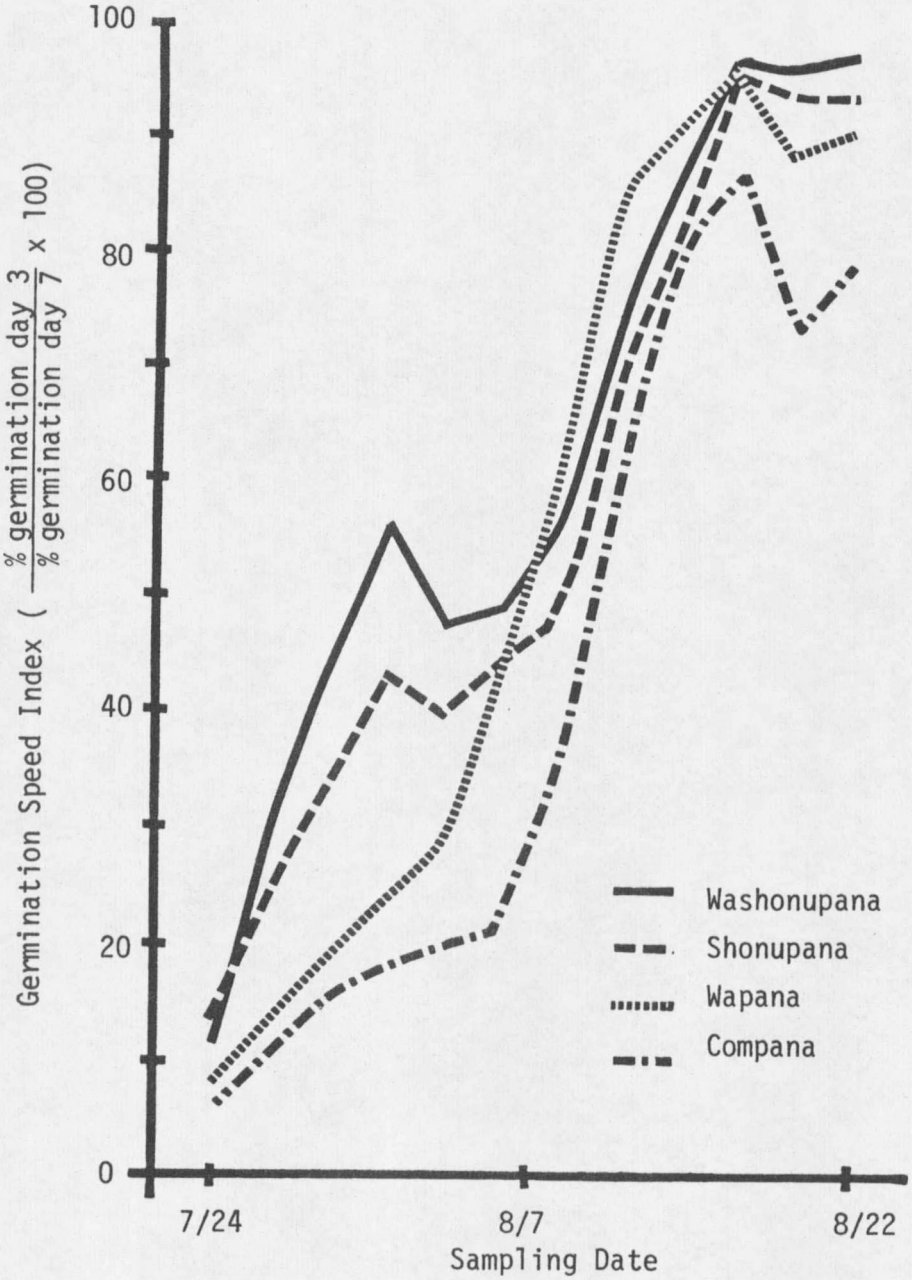

Figure 23. Experiment 2. Germination speed of four Compana isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

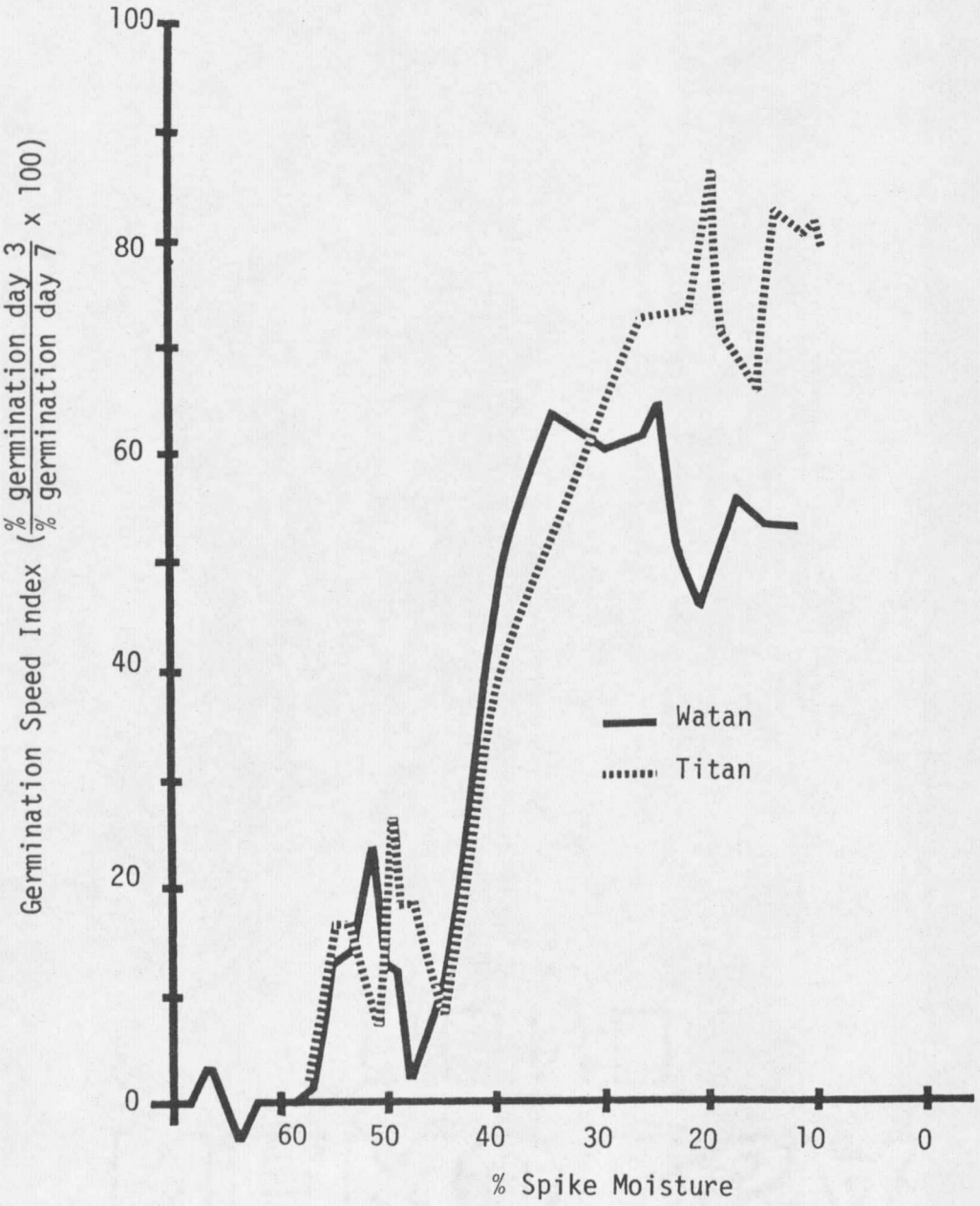

Figure 24. Experiment 2. Germination speed of two Titan isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

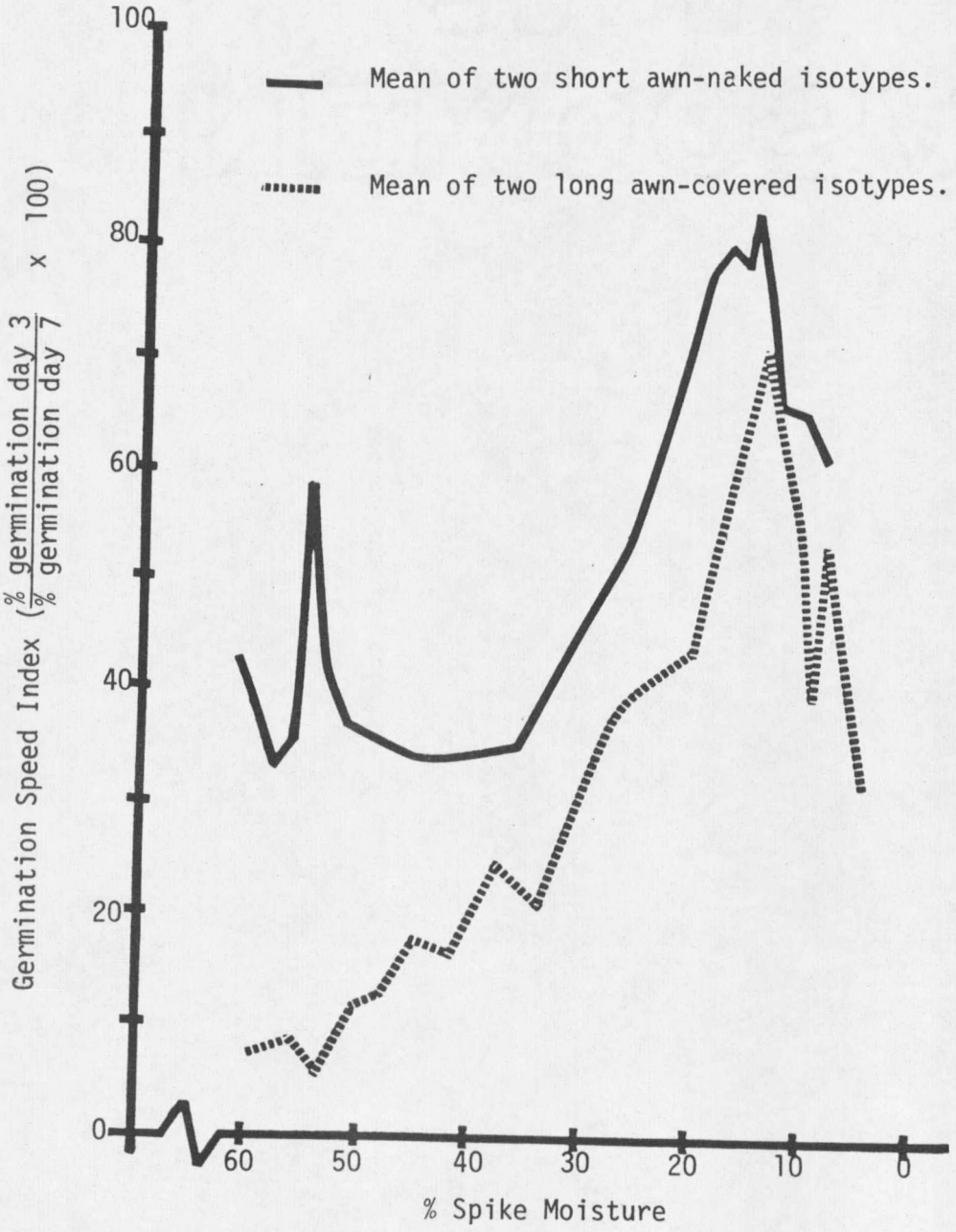

Figure 25. Experiment 2. Average germination speed of two short awn-naked and two long awn-covered Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

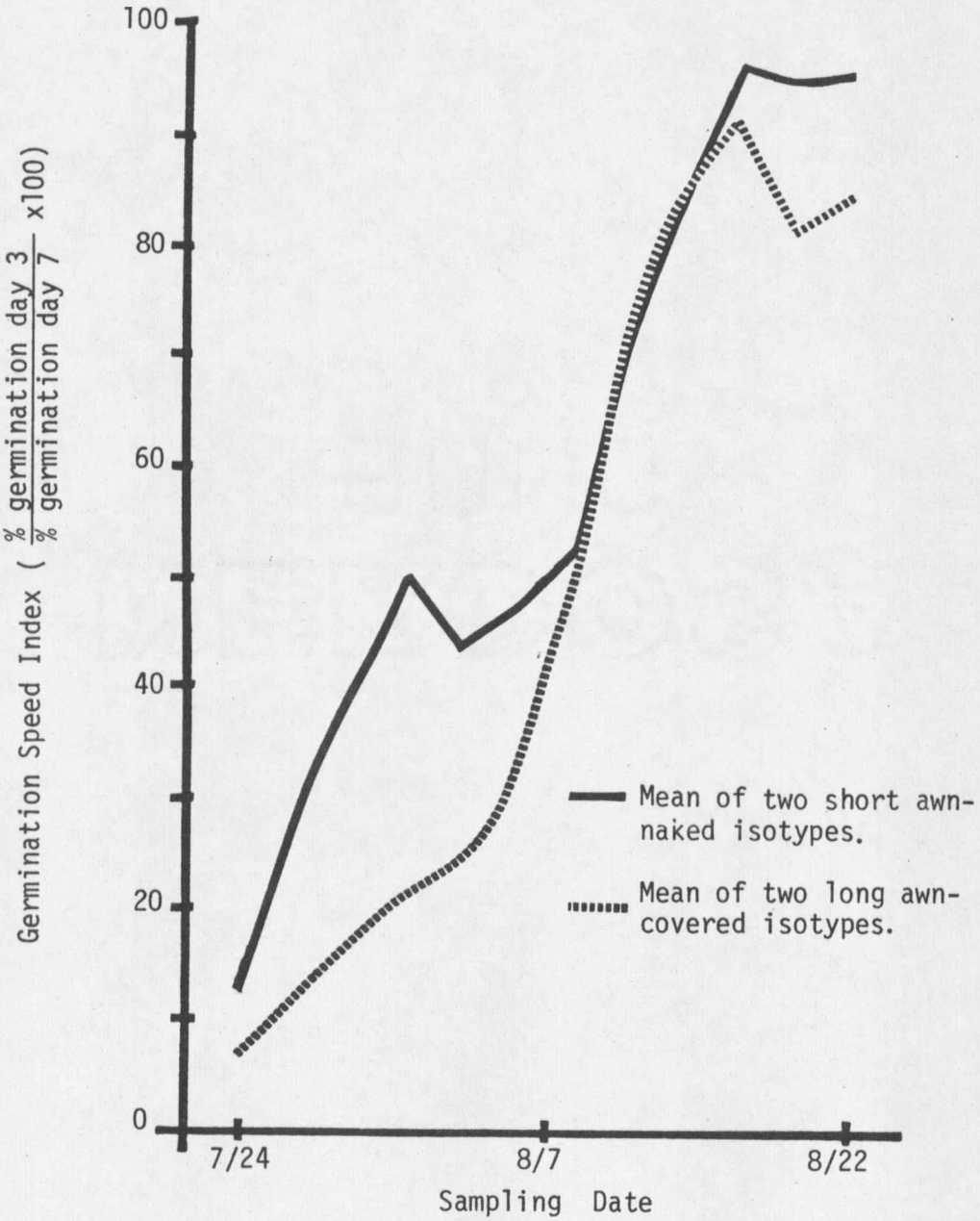

Figure 26. Experiment 2. Average germination speed of two short awn-naked and two long awn-covered Compana isotypes sampled 14 times (24 July - 22 August 1978) during kernel maturation at Huntley, Montana.

Table 8. Experiment 2. Statistical analysis using a paired t test of germination speed during kernel maturation for eight barley lines isogenic for waxy and normal endosperm at Bozeman and Huntley, Montana, 1978.

Comparison		Bozeman 22 df		Huntley 13 df		Across environments 36 df	
Mutant isotype	Normal isotype	sign of t[+]	S.L.[++]	sign of t	S.L.	sign of t	S.L.
Washonupana	Shonupana	-	NS	+	*	+	NS
Wapana	Compana	+	**	+	**	+	**
Watan	Titan	-	**	-	NS	-	**
Wabet	Betzes	+	NS	+	NS	+	NS
Waxy (mean)	Normal (mean)	-	*	+	**	+	NS
Washonupana	Wapana	+	**	+	*	+	**
Shonupana	Compana	+	**	+	**	+	**
Short awn-naked (mean)	Long awn-covered (mean)	+	**	+	**	+	**
Washonupana	Compana	+	**	+	**	+	**

[+] Sign of t statistic in paired t test where normal isotype values were subtracted from mutant isotype values.

[++] S.L. = Significance level (one tail) NS, *, ** denote nonsignificance and significance at the P=0.05 and P=0.01 probability levels, respectively.

respectively, during kernel maturation at both locations (Figures 22 and 23) and when averaged at each location (Figures 25 and 26). Naked barleys may germinate faster than covered barleys because of a faster rate of water imbibition (Briggs, 1978) or, if repression of germination speed is a form of dormancy, by a lesser degree of dormancy in naked barleys as previously discussed. Ulonska and Baumer (1976) in a study of 14 spring barley cultivars grown in two environments reported Nackta, a naked cultivar, had the highest germination speed over six days germination and 11 different steeping times. It would appear that hull type has the strongest influence on speed of germination although endosperm type, genetic background, environment, and their interactions may have a lesser influence.

Alpha-amylase activity during maturation

Patterns of alpha-amylase activity during kernel maturation (Figures 27 and 28) are similar to those reported in the literature in which alpha-amylase activity peaked shortly after anthesis (4 to 30 days post anthesis) then declined to low stable levels until maturity (Duffus, 1969; LaBerge et al., 1971; MacGregor et al., 1971; MacGregor et al., 1972; Allison et al., 1974; Riggs and Gothard, 1976; Gordon, 1980). Sampling in this experiment began at Bozeman shortly after anthesis during the period when alpha-amylase activity was decreasing rapidly. Samples obtained near harvest maturity (10-20% moisture) showed increased alpha-amylase activity in response

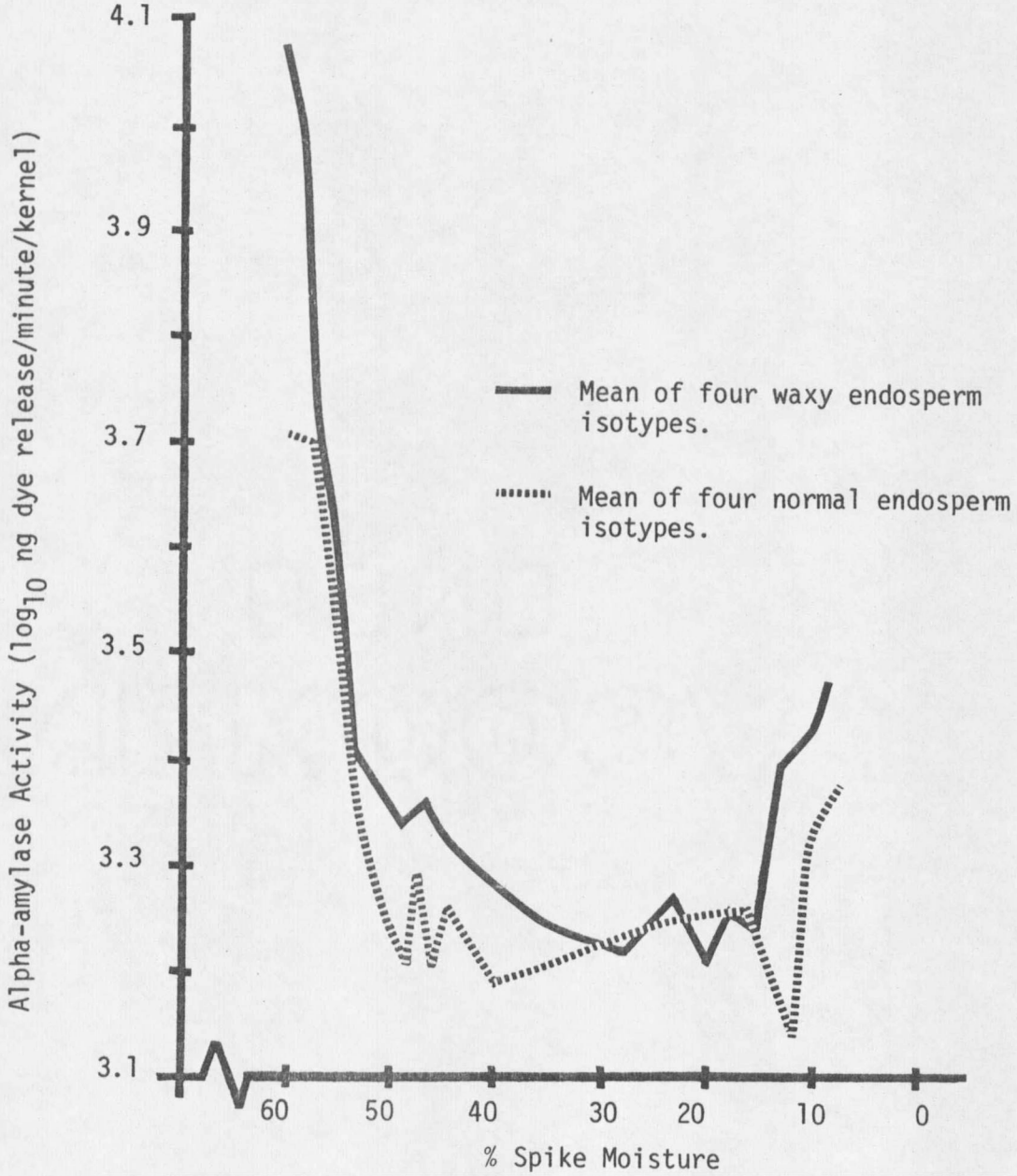

Figure 27. Experiment 2. Average alpha-amylase activity of four waxy and four normal endosperm barley isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

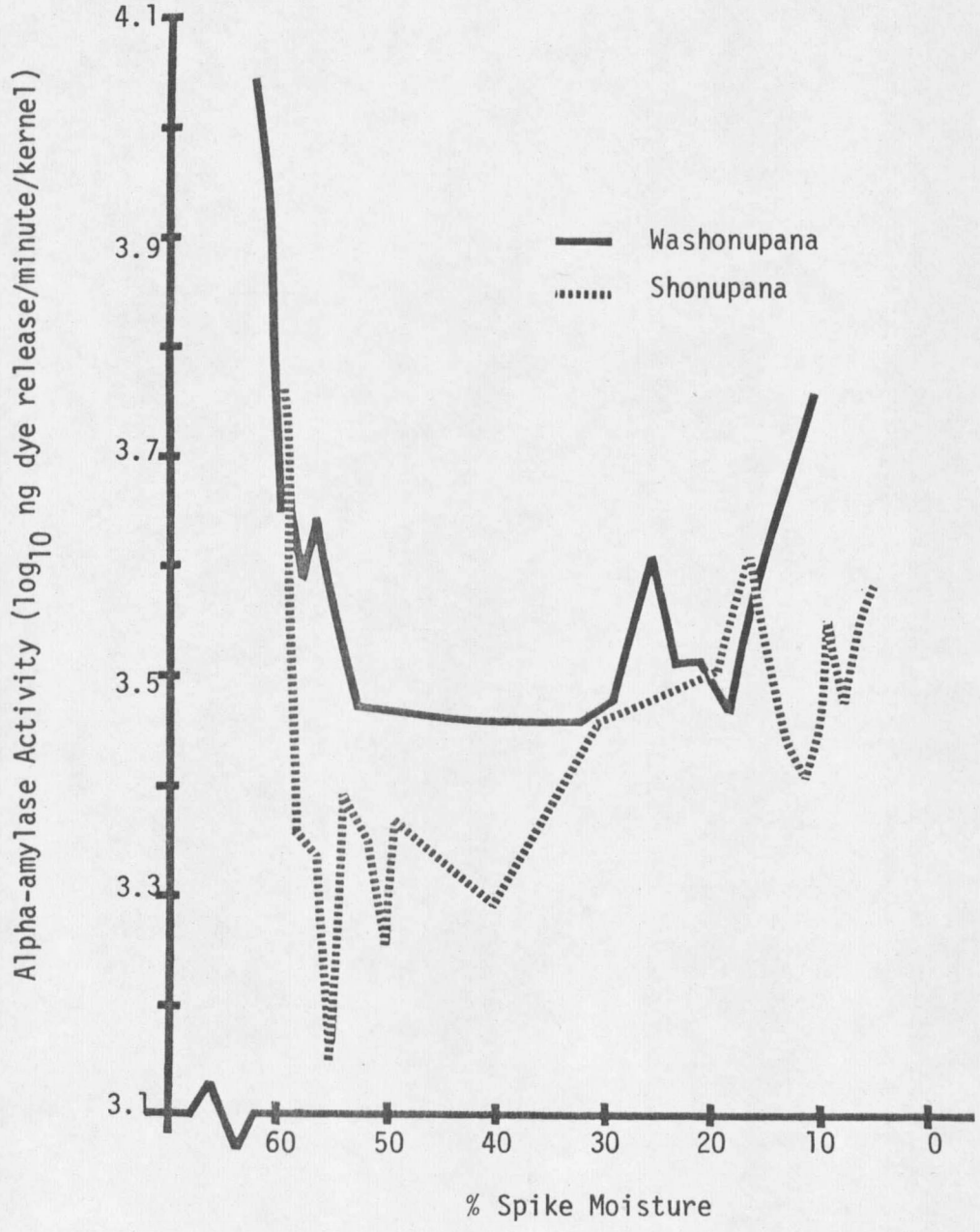

Figure 28. Experiment 2. Alpha-amylase activity of two Compana isotypes sampled 23 times (27 July - 23 September 1978) during kernel maturation at Bozeman, Montana.

to rain similar to that previously reported (LaBerge et al., 1971). Statistical analyses of alpha-amylase activity during kernel maturation showed waxy endosperm isotypes had greater alpha-amylase activity than normal endosperm isotypes, although most often the differences were not significant (Table 9). Mean alpha-amylase activity was greater in waxy endosperm types at Bozeman (Figure 27) but not at Huntley. Washonupana exhibited greater alpha-amylase activity than Shonupana at Bozeman (Figure 28). Goering and Eslick (1976) reported that the same cultivar, Washonupana, grown in one environment, retained sufficient alpha-amylase-like activity on its starch granules to liquify not only itself but four times its weight in either waxy or normal unmodified corn starch when heated in the Brabender amylograph. All alpha-amylase activity differences between waxy and normal endosperm isotypes at Huntley were nonsignificant (Table 9). Kernel maturity at Huntley, as determined by kernel weights and dormancy levels, was advanced well past the stage of physiological maturity when sampling began. Riggs and Gothard (1976) studied alpha-amylase activity in seven barley cultivars and showed that, although significant alpha-amylase activity differences existed between cultivars from anthesis to 31 days post anthesis, all cultivar differences had disappeared by 31 days after anthesis. Thus, we could assume that any alpha-amylase activity differences, if present,

Table 9. Experiment 2. Statistical analysis using a paired t test of alpha-amylase activity during kernel maturation for eight barley lines isogenic for waxy and normal endosperm at Bozeman and Huntley, Montana, 1978.

Comparison		Bozeman 22 df		Huntley 13 df		Across environments 36 df	
Mutant isotype	Normal isotype	sign of t[+]	S.L.[++]	sign of t	S.L.	sign of t	S.L.
Washonupana	Shonupana	+	**	+	NS	+	*
Wapana	Compana	+	NS	+	NS	+	*
Watan	Titan	+	NS	-	NS	+	NS
Wabet	Betzes	+	NS	+	NS	+	*
Waxy (mean)	Normal (mean)	+	**	+	NS	+	**
Washonupana	Wapana	-	NS	-	NS	-	NS
Shonupana	Compana	-	NS	-	NS	-	*
Short awn-naked (mean)	Long awn-covered (mean)	-	NS	-	NS	-	NS
Washonupana	Compana	+	NS	-	NS	+	NS

[+] Sign of t statistic in paired t test where normal isotype values were subtracted from mutant isotype values.

[++] S.L. = Significance level (one tail) NS, *, ** denote nonsignificance and significance at the P=0.05 and P=0.01 probability levels, respectively.

were missed at Huntley because of the maturity stage during sampling. Even though differences in alpha-amylase activity at Huntley were small, waxy endosperm isotypes exhibited significantly greater alpha-amylase activity, with one exception, than normal endosperm isotypes when analyses were extended across both environments (Table 9). Short awn-naked Compana isotypes and long awn-covered Compana isotypes did not vary significantly for alpha-amylase during kernel maturation (Table 9). It would appear hull type has a negligible effect on alpha-amylase activity while endosperm type has a small but often significant effect on alpha-amylase activity.

Summary

The waxy endosperm isotype in the Betzes background, Wabet, had a significantly greater germinative capacity and alpha-amylase activity during kernel maturation than Betzes. Wabet and Betzes did not differ during kernel maturation for spike moisture, kernel weight, dormancy, or germination speed. The waxy and normal endosperm isotypes in the Titan background did not differ for dormancy or for alpha-amylase activity. Watan has significantly higher levels of spike moisture, and was significantly lower in kernel weight, germinative capacity, and germination speed during kernel maturation than Titan. All waxy endosperm isotypes in the Compana background were significantly lower for kernel weight and dormancy, significantly higher in alpha-amylase activity, and did not differ for germinative

capacity during kernel maturation from normal endosperm Compana isotypes. Spike moisture during kernel maturation did not differ between Wapana and Compana but was significantly higher in Washonupana than in Shonupana. Germination speed during kernel maturation was significantly higher in Wapana than in Compana but did not vary between Washonupana and Shonupana.

All short awn-naked isotypes in the Compana background were significantly higher for spike moisture and germination speed, and significantly lower for kernel weight and dormancy during kernel maturation than the long awn-covered Compana isotypes. Washonupana showed a significantly greater germinative capacity than Wapana but did not differ from Wapana for alpha-amylase activity during kernel maturation. Shonupana had less alpha-amylase activity during kernel maturation than Compana but did not differ from Compana for germinative capacity. Washonupana (waxy endosperm-short awn-naked) was significantly higher for spike moisture, germinative capacity, and germination speed, had significantly lower kernel weight and dormancy levels, and did not vary for alpha-amylase activity during kernel maturation from Compana (normal endosperm-long awn-covered).

Experiment 3: Agronomic, Germination, and Malt Quality Traits at Harvest Maturity

The analysis of variance for several agronomic traits at harvest maturity showed that significant cultivar differences existed for

heading date, plant height, yield, test weight, and 1000 kernel weight (Table 10). The cultivar residual mean square, which included all variation not accounted for by endosperm and hull type, was significant for all traits. All waxy endosperm isotypes had a lower test weight and lower kernel weight than their normal isotypes although not a significantly lower kernel weight in the Titan genotype (Table 10). The waxy endosperm isotype of Betzes was earlier and shorter than the normal isotype. The naked isolines in the Compana cultivar yielded less, had significantly higher test weights and significantly lower kernel weights than the covered isolines, as could be expected. Endosperm type by hull type interactions were nonsignificant in the Compana cultivar (Table 10). Duncan's Multiple Range Test (Table 11) further indicated that Washonupana and Wabet were significantly shorter and had a significantly lower kernel weight than Shonupana and Betzes, respectively. The check cultivars fell within the range of the eight waxy-normal endosperm isotypes for all agronomic traits with the exception of heading date. Klages headed significantly later than all other cultivars (Table 11).

The values obtained in this experiment relating to kernel size and yield are similar to those reported earlier by Eslick (1979). He showed that the yield reductions that could be expected by incorporation of the waxy endosperm and hulless genes into a genotype were 3% and 12%, respectively, when compared to normal endosperm and

Table 10. Experiment 3. Analysis of variance for several agronomic traits for eight barley lines isogenic for waxy and normal endosperm and three check cultivars at Bozeman, Montana, 1979.

Source of variation	df	Agronomic trait				
		Heading Date	Plant Height	Yield	Test Weight	1000 Kernel Weight
		M.S.	M.S.	M.S.	M.S.	M.S.
Blocks	3	0.15	10.57	3.78	0.26**	17.29**
Cultivars and isotypes	10	30.54**	61.70**	14.16*	0.73**	170.20**
Compana endosperm type	1	0.56	14.06	5.68	0.15*	26.42**
Compana hull type	1	3.06	5.06	25.78*	3.74**	265.40**
Compana endosperm type x hull type	1	0.06	10.56	0.26	0.02	9.86
Titan endosperm type	1	0.13	0.13	10.15	0.16*	5.66
Betzes endosperm type	1	4.50*	55.13**	1.75	0.14*	23.56*
Cultivar residual	5	59.42**	106.41**	19.60*	0.62**	274.22**
Error	30	1.07	3.87	6.04	0.03	3.27
Total	44					

*, ** Significant at P=0.05, P=0.01 probability levels, respectively.

Table 11. Experiment 3. Mean agronomic responses compared using Duncan's New Multiple Range Test[+] and coefficients of variation for various agronomic traits for eight barley lines isogenic for waxy and normal endosperm and three check cultivars at Bozeman, Montana, 1979.

	Agronomic trait				
	Heading date	Plant height	Yield	Test Weight	1000 kernel Weight
Cultivar or isotype	Julian days	cm	quintals/ hectare	kg/ hectoliter	grams
Washonupana	191.25 de	47.75 d	12.48 b	8.79 a	39.19 c
Shonupana	191.50 cde	51.25 c	13.42 b	9.05 a	43.33 b
Wapana	190.25 ef	50.50 cd	14.77 b	7.89 cd	48.91 a
Compana (derived)	190.75 e	50.75 cd	16.21 ab	8.01 c	49.91 a
Watan	188.75 f	56.25 b	13.29 b	7.69 d	29.93 e
Titan (derived)	189.00 f	56.50 b	15.54 ab	7.97 cd	31.61 e
Wabet	192.50 bcd	55.25 b	14.21 b	7.82 cd	32.66 e
Betzes (derived)	194.00 b	60.50 a	15.14 b	8.09 c	36.10 d
Piroline	192.75 bcd	56.00 b	16.61 ab	8.49 b	39.45 c
Shabet	193.00 bc	57.00 b	19.32 a	7.98 cd	40.36 c
Klages	198.75 a	58.50 ab	14.88 b	8.05 c	40.67 bc
C.V. %	0.55	3.60	16.29	2.22	4.60

[+] Separate letters denote significantly different means (P=0.05).

covered isotypes over many environments. In this experiment, in a single environment, the yield reductions caused by the waxy endosperm and hulless genotypes were 9% and 17%, respectively. The coefficient of variation for yield in this experiment was unexplainably high (Table 11). The average test weight and kernel weight of the waxy endosperm isotypes were 97% and 93%, respectively, of the average test weight and kernel weight of the normal endosperm isotypes. The test weights and kernel weights of the naked Compana isotypes averaged 112% and 84%, respectively of the similar weights of the covered Compana isotypes.

The analysis of variance for germinative energy and germination speed (Table 12) showed that endosperm type had no significant effect on germination characteristics at harvest maturity in all genetic backgrounds. Hull type in the Compana cultivar had a highly significant effect on both germinative energy and germination speed (Table 12). Naked Compana isolines germinated significantly faster than covered Compana isotypes (Table 13). Washonupana and Shonupana had significantly greater germinative energy than Compana (Table 13).

Malting quality of waxy endosperm isotypes, as determined in conventional pilot malts, was generally inferior to malting quality of the normal endosperm isotypes (Table 14). Mean diastatic power and the soluble to total protein ratio was significantly lower for waxy endosperm isotypes than for normal endosperm isotypes. Waxy

Table 12. Experiment 3. Analysis of variance for germinative energy and germination speed for eight barley lines isogenic for waxy and normal endosperm and three check cultivars at Bozeman, Montana, 1979.

Source of variation	d f	Germinative energy M.S.	Germination speed M.S.
Block	3	1.09	7136.7**
Cultivars and isotypes	10	2.69**	13268.0**
Compana endosperm type	1	0.33	306.3
Compana hull type	1	5.88**	101800.0**
Compana endosperm type x hull type	1	0.33	1600.0
Titan endosperm type	1	0.66	10.1
Betzes endosperm type	1	0.91	1596.0
Cultivar residual	5	3.75**	5477.5**
Error	30	0.75	1169.1
Total	44		

*, ** Significant at the P=0.05, P=0.01 probability levels, respectively.

Table 13. Experiment 3. Mean germination responses compared using Duncan's New Multiple Range Test[+] and coefficients of variation for germinative energy and germination speed for eight barley lines isogenic for waxy and normal endosperm and three check cultivars at Bozeman, Montana, 1979.

Cultivar or isotype	Germinative energy %	Germination speed Finlay's G Index
Washonupana	99.85 a	460.75 ab
Shonupana	99.85 a	472.00 a
Wapana	98.92 ab	321.25 fg
Compana	98.35 bc	292.50 g
Watan	98.42 abc	426.75 abc
Titan	99.00 ab	429.00 abc
Wabet	97.92 bc	407.00 bcd
Betzes	97.25 c	378.75 cde
Piroline	98.92 ab	344.50 efg
Shabet	97.90 bc	358.50 def
Klages	98.00 bc	420.00 abc
C.V. %	0.89	8.72

[+] Separate letters denote significantly different means (P=0.05).

Table 14. Experiment 3. Mean responses and statistical analysis using a paired t test for several malt quality characteristics in conventional pilot malts for four waxy and four normal endosperm barley isotypes and three check cultivars at Bozeman, Montana, 1979.

	Barley Protein	Kernel Weight	Plump Barley	Malt Extract	Fine-course diff.	Wort Protein	Protein ratio Sol/total	Diastatic Power	Alpha-amylase
	%	mg	%	%	%	%	%	degrees	20°units
Waxy endosperm isotypes $\bar{X}$	10.98	36.33	66.55	80.40	3.53	3.82	34.28	96.30	30.78
Normal endosperm isotypes $\bar{X}$	11.15	37.73	69.13	81.28	3.58	3.90	35.63	122.80	29.23
T statistic (3 df)	-2.05	-2.76	-0.63	-2.88	-0.23	-0.85	-3.58	-7.23	2.21
p-value for H_0: Waxy=normal	0.13	0.07	0.57	0.06	0.83	0.46	0.04	0.01	0.12
Piroline	11.10	35.80	86.90	79.60	3.30	3.34	31.90	133.00	26.00
Shabet	10.80	39.00	68.80	80.70	3.50	3.80	37.00	119.00	25.30
Klages	10.90	39.00	71.00	82.80	2.70	4.53	42.90	173.00	41.80

endosperm isotypes produced more, although not significantly more, alpha-amylase during malting than normal isotypes. In all malting characteristics, both waxy and normal endosperm isotypes were much inferior to Klages, the current malt barley standard in the area.

The fact that waxy endosperm barley starch is more readily modified by either enzymes or chemicals than normal barley starch (Goering et al., 1980) had led to the hypothesis that waxy endosperm barleys should be superior to normal endosperm barleys for malting. This hypothesis was further supported by experiments which showed waxy endosperm barleys have less dormancy than and in some cases germinate faster than normal endosperm barleys, at least during kernel maturation. Apparently waxy endosperm barleys are not superior to normal endosperm barley in malting quality as determined in conventional malting tests. Perhaps modified malting techniques are necessary to exploit the unique starch properties in waxy endosperm barleys.

Experiment 4: Water Imbibition in Compana Isotypes

The general patterns of water uptake by four Compana isotypes in a 48 hour, 20° C, unaerated steep are similar to those previously reported (Kirsop et al., 1966; Reynolds and MacWilliam, 1967; Brookes et al., 1976). Initial water uptake was rapid followed by a gradually decreasing rate (Figure 29). The analysis of variance for

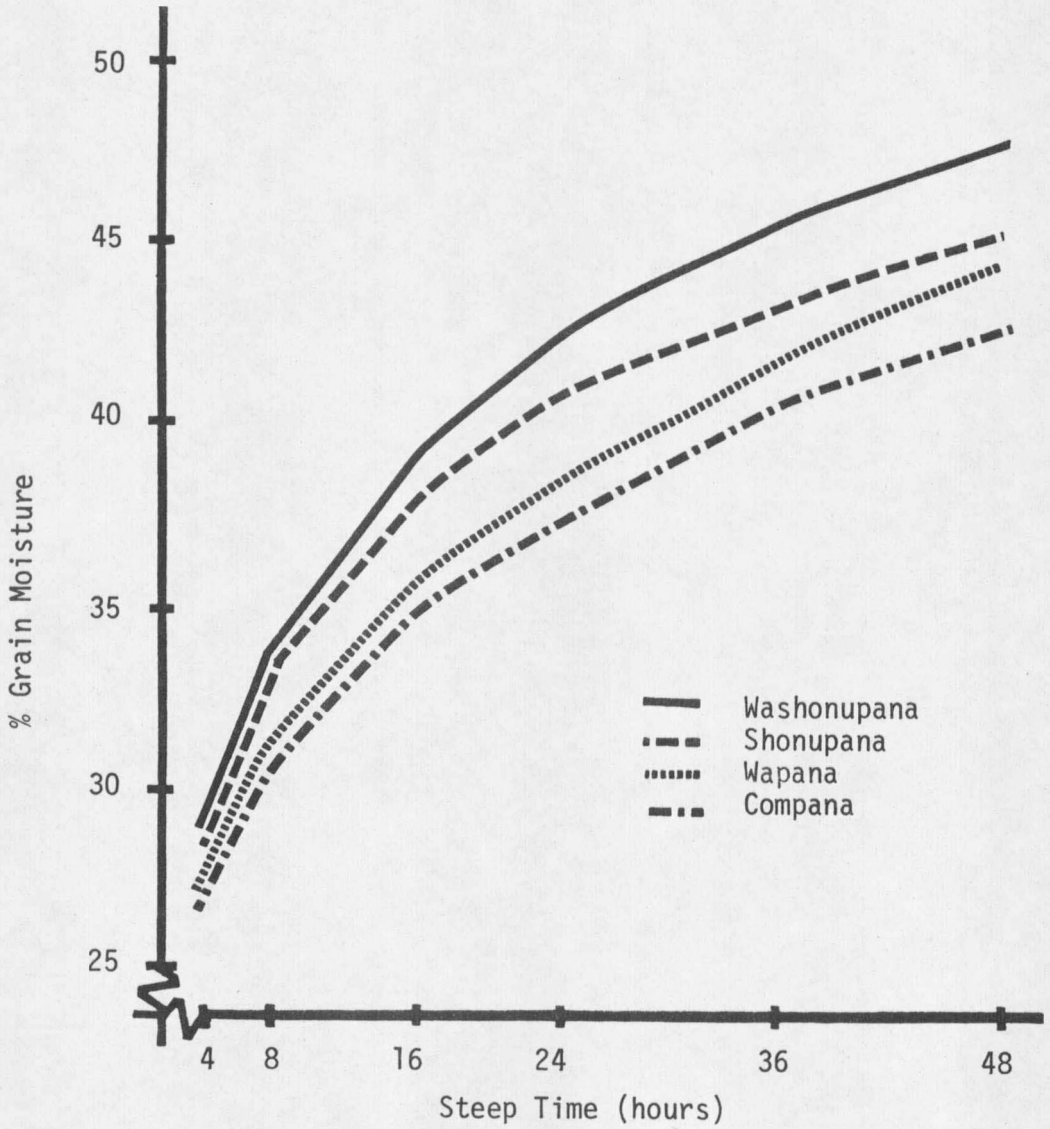

Figure 29. Experiment 4. Patterns of water uptake during a 48 hour, 20° C, unaerated steep for seed from four Compana isotypes grown in three environments.

the water uptake of four Compana isotypes grown in three environments revealed that water uptake was significantly influenced by the environment in which the grain was grown, by isotype, hull type, and steep time (Table 15). Waxy endosperm isotypes took up water faster than normal endosperm isotypes during the steep but not by a significant amount. Naked Compana isotypes imbibed water significantly faster than the covered Compana isotypes (Table 15 and Figure 29). The hull type by endosperm type interaction was nonsignificant.

The isotype by steep time interactions were significant because of the differential response of the four Compana isotypes early in the steep as compared to later in the steep, or because of experimental error. Briggs (1978) reported initial water uptake was much faster in naked than in covered barley. Davidson et al., (1976) reported that blotting the seed dry failed to remove all the water held by the surface layers of the kernel and consequently overestimated water uptake by about 1%. Since initial moisture differentials were small, this interaction may be significant because of experimental techniques which removed more of water from the surface layers of the naked barley than from the covered barley.

Significant environmental interactions may be explained by variability in the metabolic activity or viability of the seed since the germinative energy and germination speed did vary significantly between environments (Table 17). This variability could be due to

Table 15. Experiment 4. Analysis of variance for kernel moisture percentage in a 48 hour, 20° C, unaerated steep for four Compana isotypes grown in three environments.

Source of variation	d f	M.S.
Replication	2	27.20**
Environment	2	20.17**
Isotype	3	52.69*
Hull type	1	123.50**
Endosperm type	1	33.78
Hull type x endosperm type	1	0.74
Environment x isotype	6	5.80**
Environment x hull type	2	8.24**
Environment x endosperm type	2	0.62
Environment x (hull type x endosperm type)	2	8.54**
Steep time	10	3739.51**
Environment x steep time	20	1.26**
Isotype x steep time	30	16.85**
Hull type x steep time	10	44.27**
Endosperm type x steep time	10	5.58**
(Hull type x endosperm type) x steep time	10	0.71
Environment x isotype x steep time	60	0.45**
Residual	262	0.26

*, ** Significant at the P=0.05, P=0.01 probability levels, respectively.

seed age or possibly differential kernel damage in seed lots from one or more environments. Naked barley kernels are more sensitive to embryo damage during threshing than most covered barley kernels.

Regression equations, fitted to cumulative water uptake curves for each of the four Compana isotypes, were used to summarize water imbibition (Figure 30). Similar equations were used to describe water uptake patterns in Xanthium seeds (Shull, 1920). Times required to reach given moisture percentages, were calculated from the regression equation of each isotype (Table 16). Waxy endosperm and naked Compana isotypes, at least under the conditions of this steep, reached the moisture conditions necessary for germination and for satisfactory modification during malting before normal endosperm and covered Compana isotypes. Minimum water contents in the kernel required for active germination and satisfactory modification during malting are near 35% and 45%, respectively (Brown, 1975; Brookes et al., 1976). This being the case, Washonupana, Shonupana, Wapana, and Compana would require 9.4, 10.9, 13.0, and 14.8 hours, respectively, to reach moisture levels necessary for germination and 32.2, 41.3, 58.4, and 72.4 hours, respectively to reach 45% moisture, near optimum for satisfactory modification during malting (Table 16). Of course, water uptake is a function of temperature and other steep conditions so would vary somewhat under a different set of steep conditions. Assuming the maltster requires a covered barley type,

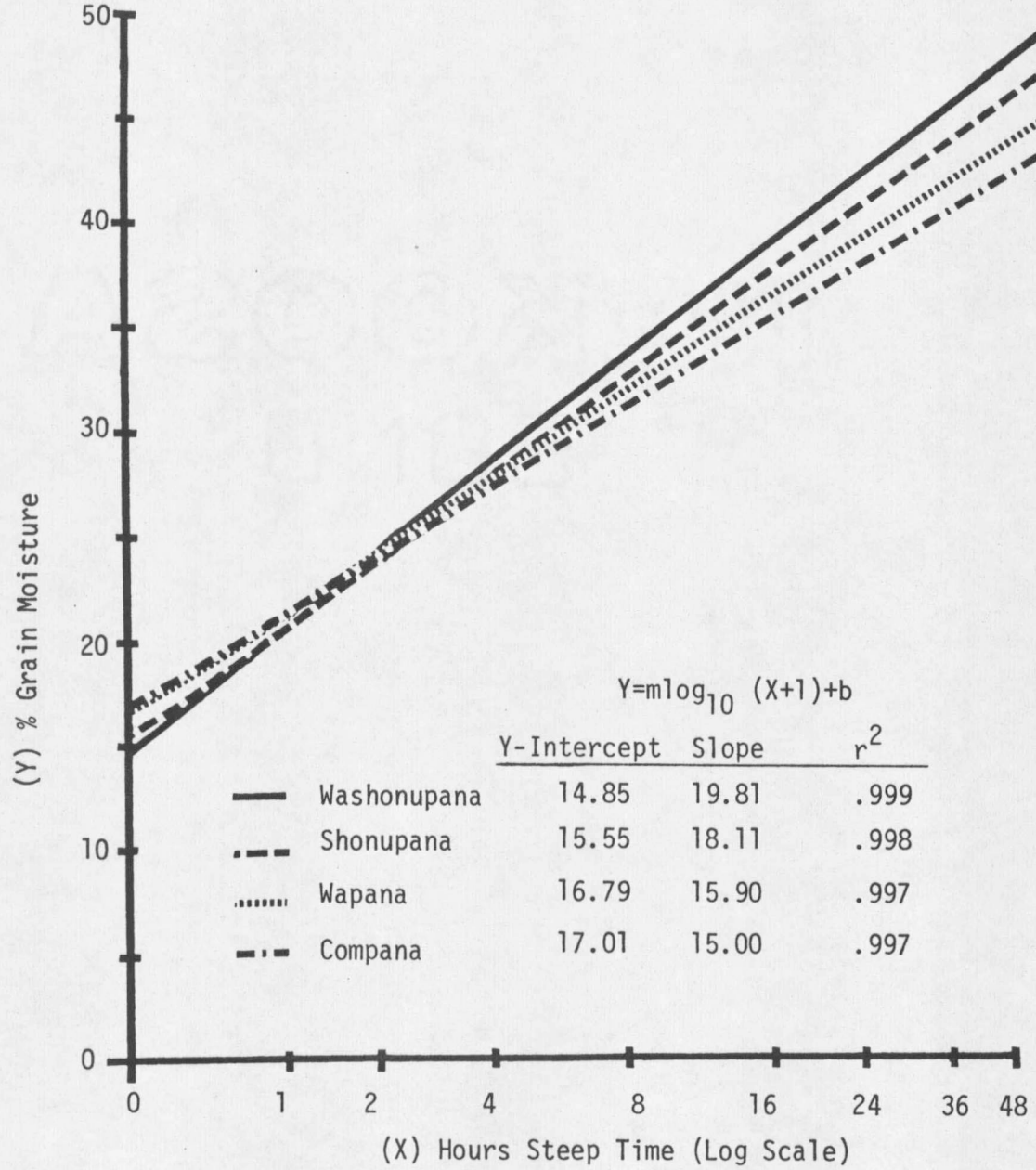

Figure 30. Experiment 4. Regression equations describing the water uptake during a 48 hour, 20° C, unaerated steep of seed from four Compana isotypes grown in three environments.

Table 16. Experiment 4. Rate of water uptake by four Compana isotypes in a 20° C unaerated steep. Hours to reach indicated moisture percentages.

Isotype	Moisture Percent			
	25	35	40	45
Washonupana	2.2	9.4	17.6	32.2
Shonupana	2.3	10.9	21.4	41.3
Wapana	2.3	13.0	27.8	58.4
Compana	2.4	14.8	33.1	72.4

this experiment indicates that steep time could be shortened substantially (14 hours at 20° C in the Compana cultivar) by using waxy endosperm isotypes for malting instead of normal endosperm isotypes. Uniform moisture distribution within the kernel is also important.

The analysis of variance for germinative energy and germination speed of four Compana isotypes grown in three environments revealed significant effects on germination behavior were caused by the growing environment (Table 17). Significant environment by isotype interactions were apparent although the effect of isotype on germination behavior was nonsignificant. Neither endosperm type nor hull type significantly influenced germination behavior. Naked Compana isotypes did, however, germinate faster than covered Compana isotypes. This was nonsignificant because the mean square of the enviornment by isotype interaction, which was used to test the effect of hull type on germination speed, was so large (Table 17).

Rates of water uptake do vary between waxy and normal endosperm and between naked and covered isotypes. The difference in rates of water uptake between naked and covered isotypes may explain why naked barleys germinate faster than covered barleys. The fact that waxy barleys take water up faster, although not by a significant amount, than normal barleys may be an important consideration in the future of waxy barleys as malting barleys.

Table 17. Experiment 4. Analysis of variance for germinative energy and germination speed for four Compana isotypes grown in three environments.

Source of variation	d f	Germinative energy M.S.	Germination speed M.S.
Environment	2	267.6**	14246.2**
Isotype	3	31.2	1692.3
Hull type	1	9.4	3396.9
Endosperm type	1	84.0	1666.0
Hull type x endosperm type	1	0.0	14.1
Environment x isotype	6	30.3**	1354.0**
Environment x hull type	2	15.5*	733.1*
Environment x endosperm type	2	75.1**	3160.4**
Environment x (hull type x endosperm type)	2	0.4	168.6
Residual	24	4.5	215.4

*, ** Significant at the P=0.05 and P=0.01 probability levels, respectively.

CONCLUSIONS

Considerable variation in germination behavior does exist among barley genotypes. Generally, malt barleys germinated faster and exhibited less dormancy or at least a shorter dormancy period than barleys used for feed. Screening barleys for malt potential could involve germination speed tests and/or dormancy tests, although such tests are time consuming and laborious.

Waxy endosperm barley isotypes differ from normal endosperm barley isotypes in certain germination and agronomic traits during the period of kernel development and maturation and at harvest maturity. During kernel maturation waxy endosperm isotypes exhibited significantly less dormancy, a smaller seed size, greater alpha-amylase activity, and maintained a higher percentage of moisture in the spike than normal endosperm isotypes. The higher moisture content of waxy endosperm isotypes may be evidence that the period from anthesis to harvest maturity is longer in waxy types than in normal types. In specific genetic backgrounds waxy endosperm isotypes germinated faster than normal endosperm isotypes. By harvest maturity differences between waxy and normal endosperm isotypes in dormancy and germination speed were no longer apparent. Waxy endosperm isotypes were not significantly different from normal endosperm isotypes for yield or date of heading. Waxy endosperm isotypes in specific genetic

backgrounds were shorter than normal endosperm isotypes. The test weights and kernel weights of waxy endosperm isotypes were significantly lower than similar weights in normal endosperm isotypes. In a 48 hour, 20° C, unaerated steep waxy endosperm isotypes took up water faster, but not significantly faster, than normal endosperm isotypes. In the Compana genetic background, waxy endosperm isotypes reached 45% moisture, near optimum for satisfactory modification during malting, from 9.1-14.0 hours quicker than in normal endosperm isotypes. Despite the apparent advantages for malting possessed by waxy endosperm barleys, namely more rapid starch modification by enzymes or chemicals, less dormancy, and faster uptake of water, waxy endosperm isotypes were inferior to normal endosperm isotypes for malting quality as determined by conventional pilot malts. Normal barley isotypes were significantly higher in diastatic power and had a higher soluble protein to total protein ratio.

Short awn-naked and long awn-covered Compana isotypes also differed during kernel maturation and at harvest maturity. Naked Compana isotypes had smaller seeds, less dormancy, germinated faster, and maintained a higher spike moisture percentage throughout kernel maturation than did covered Compana isotypes. Hull type had no effect on heading date or plant height but significantly influenced yield, test weight, seed weight, germinative energy and germination speed at maturity. Naked Compana isotypes yielded 17% less than covered Compana isotypes. Seed size in the naked isotypes was

16% less than in the covered isotypes. Short awn-naked Compana isotypes germinated faster and had less dormancy than long awn-covered Compana isotypes at harvest maturity. In a 48 hour, 20° C, unaerated steep naked Compana isotypes imbibed water significantly faster than covered Compana isotypes.

REFERENCES

Allison, M. J., R. P. Ellis, and J. S. Swanston. 1974. Tissue distribution of alpha-amylase and phosphorylase in developing barley grain. Journ. Inst. Brewing 80:488-491.

Atanda, O. A., and J. Miflin. 1970. Factors affecting the production of alpha-amylase in barley grains. Journ. Inst. Brewing 76:51-55.

Banks, W., C. T. Greenwood, and J. T. Walker. 1970. Studies on the starches of barley genotypes: the waxy starch. Starch 22:149-152.

Belderok, B. 1968. Seed dormancy problems in cereals. Field Crop Abstracts 21:203-211.

Bell, G. D. H., and F. G. H. Lupton. 1962. The breeding of barley varieties. p. 57-58. In A. H. Cook (ed.) Barley and Malt; Biology, Biochemistry, Technology. Academic Press, New York and London.

Bewley, J. D., and M. Black. 1978. Physiology and Biochemistry of Seeds in Relation to Germination. Volume 1. Development, Germination, and Growth. Springer-Verlag, Berlin and New York.

Bilderback, D. E. 1971. Amylases in developing barley seeds. Plant Physiol. 48:331-334.

Bishop, L. R. 1944. Mamorandum on barley germination. Journ. Inst. Brewing 50:166-185.

Bishop, L. R. 1945. Second memorandum on barley germination. Journ. Inst. Brewing 51:215-224.

Bishop, L. R. 1946. Third memorandum on barley germination. Journ. Inst. Brewing 52:273-282.

Bohinski, R. C. 1979. Modern Concepts in Biochemistry. Third edition. Allyn and Bacon, Inc., Boston. p. 306-308.

Briggs, D. E. 1963. Biochemistry of barley germination. Action of gibberellic acid on barley endosperm. Journ. Inst. Brewing 69:13-19.

Briggs, D. E. 1964. Origin and distribution of alpha-amylase in malt. Journ. Inst. Brewing 70:14-24.

Briggs, D. E. 1968a. Alpha-amylase in germinating, decorticated barley. I. Alpha-amylase, conditions of growth, and grain constituents. Phytochemistry 7:513-529.

Briggs, D. E. 1968b. Alpha-amylase in germinating decorticated barley. II. Effects of physically damaging the grain. Phytochemistry 7:531-538.

Briggs, D. E. 1978. Barley. Chapman and Hall, Ltd, London. p. 1-291.

Brookes, P. A. 1980. The significance of pre-harvest sprouting of barley in malting and brewing. Cereal Res. Communications 8(1):29-38.

Brookes, P. A., D. A. Lovett, and I. C. MacWilliam. 1976. The steeping of barley. A review of the metabolic consequences of water uptake, and their practical implications. Journ. Inst. Brewing 82:14-26.

Brown, P. L. 1975. Wheat and barley - germination, morphology, and stages of development. Montana Cooperative Extension Service. Bulletin 1119. Montana State University. Bozeman, Montana.

Cameron-Mills, V., and C. M. Duffus. 1980. The influence of nutrition on embryo development and germination. Cereal Res. Communications 8(1):143-149.

Carson, G. P., and F. R. Horne. 1962. The identification of barley varieties. p. 148-150. In A. H. Cook (ed.) Barley and Malt; Biology, Biochemistry, Technology. Academic Press, New York and London.

Chang, S. C. 1943. Length of dormancy in cereal crops and its relationship to after-harvest sprouting. Agron. J. 35:482-490.

Daussant, J., and C. Mayer. 1980. Immunochemistry of cereal alpha-amylases in studies related to seed maturation and germination. Cereal Res. Communications 8(1):49-60.

Davidson, D., M. A. Eastman, and J. E. Thomas. 1976. Water uptake during germination of barley. Plant Sci. Letters 6:223-230.

Deming, G. W., and D. W. Robertson. 1933. Dormancy in small grain seeds. Colorado Agric. Exp. Station Tech. Bull. 5.

Derera, N. F. 1980. The audit of sprouting. Cereal Res. Communications 8(1):15-22.

Duffus, C. M. 1969. Alpha-amylase activity in the developing barley grain and its dependence on gibberellic acid. Phytochemistry 8:1205-1209.

Eriksson, G. 1969. The waxy character. Hereditas 63:180-204.

Eslick, R. F. 1979. Barley breeding for quality at Montana State University. p. 2-25. In Proceedings of Joint Barley Utilization Seminar. Feb. 26-March 6, 1979. Seweon, Korea. Korea Science and Engineering Foundation.

Essery, R. E., B. H. Kirsop, and J. R. A. Pollock. 1954. Studies in barley and malt. I. Effects of water on germination tests. Journ. Inst. Brewing 60:473-481.

Essery, R. E., B. H. Kirsop, and J. R. A. Pollock. 1955. Studies in barley and malt. II. Tests for germination and water-sensitivity. Journ. Inst. Brewing 61:25-28.

Finlay, K. W. 1960a. Genetic study of barley germination behavior. I. Genetic and environmental variation. Journ. Inst. Brewing 66:51-57.

Finley, K. W. 1960b. Genetic study of barley germination behavior. II. General and specific combining ability. Journ. Inst. Brewing 66:58-64.

Fox, G., and R. F. Eslick. 1979. Effects of several genes on speed of maltose production in germinating barley. p. 128. 1979 Agronomy Abstracts. ASA, Madison, Wisconsin.

Fox, G., and R. F. Eslick. 1980. Rapid determination of cereal alpha-amylase using amylose azure blue-dye starch suspended in an agar medium. p. 124. 1980 Agronomy Abstracts. ASA, Madison, Wisconsin.

Gibbons, G. C. 1980. Immunohistochemical determination of the transport pathways of alpha-amylase in germinating barley seeds. Cereal Res. Communications 8(1):87-96.

Goering, K. J., B. W. DeHaas, D. W. Chapman, R. F. Eslick, and R. E. Gramera. 1980. New process for production of ultra high maltose syrup from special genetically derived barley. Starch 32:349-352.

Goering, K. J., and R. F. Eslick. 1976. Barley starch. VI. A self-liquefying waxy barley starch. Cereal Chemistry 53:174-180.

Goering, K. J., R. Eslick, and B. W. DeHaas. 1973. Barley starch. V. A comparison of the properties of waxy Compana barley starch with the starches of its parents. Cereal Chemistry 50:322-328.

Goodchild, N. A., and M. G. Walker. 1971. A method of measuring seed germination in physiological studies. Ann. Bot. 35:615-621.

Gordon, A. G. 1969. Some observations on the germination energy tests for cereals. Proc. Assoc. Official Seed Anal. 59:58-72.

Gordon, A. G. 1970. Pre-germination in barley. Journ. Inst. Brewing 76:140-143.

Gordon, I. L. 1980. Germinability, dormancy, and grain development. Cereal Res. Communications 8(1):115-129.

Gordon, I. L., L. N. Balaam, and N. F. Derera. 1979. Selection against sprouting damage in wheat. II. Harvest ripeness, grain maturity, and germinability. Aust. J. Agric. Res. 30: 1-17.

Gothard, P. G. 1974. Screening of barley varieties for alpha-amylase content. Journ. Inst. Brewing 80:387-390.

Greenwood, C. T. 1964. Structure, properties, and amylolytic degradation of starch. Food Technology 18:732-738.

Haber, A., and R. P. Runyon. 1973. General Statistics. Second edition. Addison-Wesley Publishing Company, Reading, Mass. 401 p.

Harris, G. 1962. The structural chemistry of barley and malt. p. 431-582. In A. H. Cook (ed.) Barley and Malt; Biology, Biochemistry, Technology. Academic Press, New York and London.

King, R. W., and M. D. Gale. 1980. Pre-harvest assessment of potential alpha-amylase production. Cereal Res. Communications 8(1):157-165.

Kirsop, B. H., T. Reynolds, and C. M. Griffiths. 1967. The distribution of water in germinating barley. Journ. Inst. Brewing 73:182-186.

Kneen, E. 1944. A comparative study of the development of amylases in germinating cereals. Cereal Chemistry 21:304-314.

Kruger, J. E. 1976. Biochemistry of pre-harvest sprouting in cereals and practical applications in plant breeding. Cereal Res. Communications 4(2):187-194.

Kruger, J. E. 1980. Progress in the chemistry of some quality-affecting enzymes resulting from pre-harvest sprout damage. Cereal Res. Communications 8(1):39-47.

LaBerge, D. E., A. W. MacGregor, and W. O. S. Meredith. 1971. Changes in alpha- and beta-amylase activities during the maturation of different barley cultivars. Can. J. Plant Sci. 51: 469-477.

LaCroix, L. J., P. Waikakul, and G. M. Young. 1976. Seasonal and varietal variation in dormancy of some spring wheats. Cereal Res. Communications 4(2):139-146.

Lallukka, U. 1976. The effect of the temperature during the period prior to ripening on sprouting in the ear in rye and wheat varieties grown in Finland. Cereal Res. Communications 4(2):93-96.

MacGregor, A. W. 1978. Changes in alpha-amylase enzymes during germination. Journ. Am. Soc. Brewing Chemists 36:1-5.

MacGregor, A. W., and J. Daussant. 1979. Evolution of alpha-amylase components during initial stages of barley germination with and without prior steeping. Cereal Chemistry 56:541-545.

MacGregor, A. W., A. G. Gordon, W. O. S. Meredith, and L. LaCroix. 1972. Site of alpha-amylase in developing barley kernels. Journ. Inst. Brewing 78:174-179.

MacGregor, A. W., D. E. LaBerge, and W. O. S. Meredith. 1971. Changes in barley kernels during growth and maturation. Cereal Chemistry 48:255-269.

MacGregor, A. W., R. G. Thompson, and W. O. S. Meredith. 1974. Alpha-amylase from immature barley: purification and properties. Journ. Inst. Brewing 80:181-187.

MacLeod, A. M. 1967. The physiology of malting - a review. Journ. Inst. Brewing 73:146-162.

Marchylo, B. A., L. J. LaCroix, and J. E. Kruger. 1980. The synthesis of alpha-amylase in specific tissues of the immature wheat kernel. Cereal Res. Communications 8(1):61-68.

Malting Barley Improvement Association. 1981. Know your malting barleys. MBIA. Milwaukee, Wisconsin.

Mitchell, B., M. Black, and J. Chapman. 1980. Drying and the onset of germinability in developing wheat grains. Cereal Res. Communications 8(1):151-156.

Nikolaeva, M. G. 1969. Physiology of Deep Dormancy in Seeds. Z. Shapiro (translator). M. Kohn and H. Mills (eds.) Israel program for scientific translations, Jerusalem. 220 p.

Noll, J. S., and E. Czarnecki. 1980. Methods of extending the testing period for harvest-time dormancy in wheat. Cereal Res. Communications 8(1):233-238.

Olsson, G., and B. Mattsson. 1976. Seed dormancy in wheat under different weather conditions. Cereal Res. Communications 4(2):181-185.

Palmer, G. H. 1969. Increased endosperm modification of abraded barley grain after gibberellic acid treatment. Journ. Inst. Brewing 75:536-541.

Pollock, J. R. A. 1962. The nature of the malting process. p. 303-398. In A. H. Cook (ed.) Barley and Malt; Biology, Biochemistry, Technology. Academic Press, New York and London.

Pollock, J. R. A., R. E. Essery, and B. H. Kirsop. 1955a. Studies in barley and malt. III. New and convenient germinative capacity test. Journ. Inst. Brewing 61:259-300.

Pollock, J. R. A., B. H. Kirsop, and R. E. Essery. 1955b. Studies in barley and malt. IV. Experiments with dormant barley. Journ. Inst. Brewing 61:301-307.

Reiner, L., and V. Loch. 1976. Forecasting dormancy in barley - ten years experience. Cereal Res. Communications 4(2):107-110.

Reynolds, T., and I. C. MacWilliam. 1966. Water uptake and enzymic activity during steeping of barley. Journ. Inst. Brewing 72:166-170.

Riggs, T. J., and P. G. Gothard. 1976. The development of barley grain: comparisons between cultivars for growth rate and alpha-amylase activity. Journ. Agric. Sci., Camb. 86:603-608.

Sargeant, J. G. 1980. Alpha-amylase isoenzymes and starch degradation. Cereal Res. Communications 8(1):77-86.

Schuster, K. 1962. Malting technology. p. 281-286. In A. H. Cook (ed.) Barley and Malt; Biology, Biochemistry, Technology. Academic Press, New York and London.

Shull, C. A. 1920. Temperature and rate of moisture uptake in seeds. Bot. Gaz. 69:361-390.

Smith, M. T., and D. E. Briggs. 1978. The mechanisms by which tumbling and abrasion alter enzyme formation in malting grain. Journ. Inst. Brewing 85:160-167.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th edition. Iowa State Univ. Press, Ames, Iowa. 593 p.

Sokal, R. R., and F. T. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, San Francisco. p. 175-252, 299-403.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York. 841 p.

Strand, E. 1980. A seed dormancy index for selection of cultivars of cereals resistant to pre-harvest sprouting. Cereal Res. Communications 8(1):219-223.

Sullins, R. D., and L. W. Rooney. 1974. Microscopic evaluation of the digestibility of sorghum lines that differ in endosperm characteristics. Cereal Chemistry 51:134-142.

Sullins, R. D., and L. W. Rooney. 1975. Light and scanning electron microscopic studies of waxy and nonwaxy endosperm sorghum varieties. Cereal Chemistry 52:361-366.

Takahashi, N. 1980. Effect of environmental factors during seed formation on pre-harvest sprouting. Cereal Res. Communications 8(1):175-183.

Ulonska, E., and M. Baumer. 1976. Investigation of the value of water uptake and germination for the estimation of malting quality in barley. p. 579-593. In Barley Geretics III. Proc. 3rd Int. Barley Genetics Symposium. Publ. Verlag Karl Thiemig, Munchen.

Wainwright, T., and G. K. Buckee. 1977. Barley and malt analysis - a review. Journ. Inst. Brewing 83:325-347.

Wellington, P. S. 1956a. Studies on the germination of cereals. 1. The germination of wheat grains in the ear during development, ripening, and after-ripening. Ann. Bot. 20:105-120.

Wellington, P. S. 1956b. Studies on the germination of cereals. 2. Factors determining the germination behavior of wheat grains during maturation. Ann. Bot. 20:481-500.

Wellington, P. S. 1964. Studies on the germination of cereals. 5. The dormancy of barley grains during ripening. Ann. Bot. 28:113-126.