Effects of root temperature during the grain fill stage on nutrient uptake and reproductive development in soybeans

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Effects of root temperature during the grain fill stage on nutrient uptake and reproductive development in soybeans

by

Brooks Edward Engelhardt

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology

Major Professor: Russell E. Mullen

Iowa State University

Ames, Iowa

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Iowa State University

This is to certify that the Master's thesis of

Brooks Edward Engelhardt

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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Root temperature can influence soybean growth and development. A greenhouse study was conducted to evaluate root temperature effects on soybeans during grain fill. Soybean plants were grown in 7.5-liter plastic buckets containing a 2:1 mixture of silica sand and perlite and placed in water baths at early R5, to maintain root temperature treatments of 18, 27 and 36°C. Treatments also included two levels of nutrient solution, zero and complete. Root temperature did not influence stem and root dry weight, reproductive components, grain nutrient concentration, nor germination and seed vigor. However these response variables were significantly affected by nutrient solution. Root dry weights from plants receiving fertilizer were reduced by 22%. Conversely stem dry weights in fertilized plants increased by 11%. Pod retention, number of seeds per plant, seeds per pod and grain yield of fertilized plants increased, by an estimated 38, 45, 11, and 24%, respectively. The number of large seeds per plant and per gram of fertilized plants increased by 30 and 15%, respectively. Grain nutrient concentrations of most elements significantly increased when plants received fertilizer during seed fill. Grain macro- and micronutrient concentration from fertilized plants increased by an estimated average of 27 and 38%, respectively. Grain N, P, K, Ca, and Mg concentration from fertilized plants increased by an estimated 15, 36, 19, 62, and 5%, respectively. Additionally grain Mn and B from the same plants increased by an estimated 23 and 41%, respectively. However, plants fertilized during seed fill had an estimated 18% reduction in grain Fe concentration, and no change in grain Cu.
concentration. Fertilizing maternal plants during seed fill resulted in a significant increase of progeny germination rates, but had no influence on seedling vigor. Average germination rates were 67 and 61% from fertilized and non-fertilized plants, respectively. We found no evidence that root temperatures of 18 to 36°C during grain fill influenced soybean seed formation and development, nutrient concentration, germination and vigor. However, maternal plant nutrient stress during this stage can affect yield determinants, dry weights, seed nutrient concentration and germination rates.
GENERAL INTRODUCTION

Investigations by researchers have been conducted on soybean plant responses to various soil temperatures. Areas investigated include soil temperature effects on soybean plant growth, root and shoot development, carbon and nitrogen assimilation and partitioning, nodulation, nitrogen fixation, and nutrient uptake. However, little research has been done to test the response of soybean seed development and yield to root temperature stress.

It is well documented that temperature affects plant growth (e.g. Payne and Gregory, 1988). Numerous studies have been conducted on the effects of air temperature on vegetative and reproductive growth of plants (e.g. Stutte and Weiland, 1980; El Mourid et al., 1986). However, the growth of aerial plant parts is dependent upon the growth, development, and function of the root system. According to Killham (1994, p. 29), small changes in root zone temperatures can effect root growth and the uptake of nutrients. This in turn can affect the growth and development of aerial plant parts. Gaining an understanding of how plants respond to changes in root temperature is a necessary component when studying the vegetative and reproductive development of plants.

When different temperatures are imposed on plants for research purposes, whole plants are often exposed to various temperatures. This makes it difficult to discern if observable plant responses are a result of air temperature effects on aerial plant parts or roots (Hagan, 1952). Therefore it is useful to study plant responses to differential root and shoot temperatures.
A great deal of experimental work has been conducted to evaluate the effects of root temperatures on soybeans during the vegetative stages of development. However, there is very little work evaluating the effects of root temperature on soybean grain yields. Therefore, to gain an understanding of how soil temperatures may influence soybeans during the reproductive stages of development the literature review in this paper will report on a broad range of experimental work that evaluates plant responses to root temperature.

**Thesis organization**

This thesis is organized in a general format that includes an abstract, general introduction, literature review, materials and methods, results, discussion, general conclusion, appendix, and sources cited section.
LITERATURE REVIEW

Root temperature effects on nitrogen fixation

Stoyanova (1996) conducted a root temperature experiment in 1991 and 1992 in which soybean seedlings, inoculated with the nitrogen fixing bacteria *Bradyrhizobium japonicum*, were subjected to three root temperature treatments of 18, 25, and 28°C, for 53 days. Stoyanova (1996) found significant increases in total plant dry weights, and dry weights of leaves and pods as root temperatures increased. There were no significant differences among stem dry and root dry weights.

One factor to consider in Stoyanova's (1996) experiment is that all soybeans undergoing root temperature treatments did not receive mineral nitrogen during the experiment, but relied on nitrogen fixation as a source of nitrogen. Stoyanova (1996) found that low root zone temperatures significantly affected nodulation, nitrogen fixation and total plant nitrogen content. Nodule number and mass, acetylene reduction assay, and total plant nitrogen content decreased as root temperatures were lowered from 28 to 18°C. Because factors associated with nitrogen fixation were affected by root temperature, plants in the lower root temperature treatments essentially had less nitrogen for growth than plants undergoing the higher root temperature treatment. Therefore, it is difficult to ascertain if plant growth responses in Stoyanova's (1996) experiment were due to changes in soil temperature or to the direct effect of root temperature on nitrogen fixation.
Legros and Smith (1994) studied the effects of root temperature on nitrogen fixing- and mineral nitrogen supplied-soybeans. They found that low root zone temperatures had a greater effect in limiting growth and nitrogen assimilation in nitrogen fixing soybeans than in ammonium nitrate supplied soybeans. Munevar and Wollum II (1981) evaluated the effects of high root temperatures on nitrogen fixing- and nitrogen supplied-soybean plants. They found that growth and nitrogen accumulation of nitrogen fixing soybeans were adversely affected when growing in a root temperature of 34°C as compared to plants in the 28°C root zone temperature. Conversely, nitrogen supplied soybean plants receiving the 34°C treatment had greater amounts of top growth dry weight than plants with the 28°C root temperature. Nitrogen accumulation in aerial plant parts of N supplied soybeans remained unaffected by the 28 and 34°C root temperatures. However, dry weight and nitrogen content of top growth for all plants, regardless of nitrogen source was reduced by the 40°C root temperature treatment.

Findings from other studies also indicate that measurements associated with nitrogen fixation in soybeans decrease in sub- and supra-optimal root temperatures (Kuo and Boersma, 1971; Lindemann and Ham 1979; Munevar and Wollum II 1982; Sinclair and Weisz, 1985; Walsh and Layzell, 1986). Therefore, because root temperatures can influence soybean nitrogen fixation, it may be difficult to discern if root temperature alone or the direct effects of root temperature on nitrogen fixation influence soybean growth and development. Therefore, the primary focus of this
literature review will be on the response of non-nodulated soybeans to root temperature.

**Root temperature effects on soybean plant growth**

Root temperature is a factor affecting plant development (Earley and Cartter, 1945; Richards et al., 1952; Osmond and Raper, 1981; Cruz et al., 1993; Lee and Takakura, 1995). There are implications that root temperature has an effect on root system development (Kaspar and Bland, 1992), hydraulic conductivity (Radin 1990), nutrient uptake (Neilsen, 1974), hormonal balances (Atkin et al., 1973), stem elongation Hood and Mills (1994), leaf growth (Pardossi et al., 1994), photosynthesis (Sawada et al., 1987), transpiration (McWilliam et al., 1982), carbon and nitrogen partitioning (Rufty et al., 1981), dry matter production (Monteith, 1979), root to shoot ratios, and reproductive development (Payne and Gregory, 1988).

Earley and Cartter (1945) conducted greenhouse experiments between 1938 and 1940 in which they measured the amount of dry weight produced in roots and shoots of soybean plants grown in various root temperatures. They found that temperature of the root zone indeed affected the amount of dry matter produced by soybean plants. Dry weight of top growth generally increased across a root zone temperature range of 2 to 32°C, with a decrease in dry weight at 32°C and higher. Root temperature did not significantly influence root growth, but Earley and Cartter (1945) reported soybean root dry weights generally increased with increasing root temperatures up to 27°C. In addition to dry weight, plant height also increased as a function of increasing root temperatures and height decreased at root temperatures.
higher than 27°C (Earley and Cartter, 1945).

An experiment conducted by Pushkala and Yagarajara (1988) on soybean growth as affected by soil temperature generated similar results to Earley and Cartter's (1945) experiment described above. However, in addition to measuring soil temperature effects on plant height and weight, Pushkala and Yagarajara (1988) also quantified soil temperature effects on leaf water potential and leaf area. In short, they discovered that soil temperature effects on soybean growth was hardly noticeable the first 26 days after planting, but were notable 33 days after planting, and most soybean growth measurements reached a maximum at 28.6°C soil temperature. The exception was that maximum root weight was achieved at 30.9°C. Pushkala and Yagarajara (1988) stated that root temperature did not have a significant affect on leaf temperature, diffusive resistance, and transpiration.

**Illuminance and root temperature interaction**

Pushkala and Yagarajara (1988) reported that soil temperatures clearly had a substantial effect on soybean growth. However, they speculated that the results might have differed had there been higher levels of illuminance. Earley and Cartter (1945) however, observed an interaction between the effects of root temperature and illuminance on plant dry matter production. They found that with higher illuminance, the magnitude of dry matter increases in top growth were greater across rising root temperature treatments than under low illuminance. Earley and Cartter (1945) also indicated that the optimum root temperature for top growth was lower under reduced illuminance. Additionally there were larger reductions in plant...
dry weight production at root temperatures of 2 and 7°C under higher illuminance relative to plants growing under low illuminance at the same temperatures.

Results from an experiment conducted by Rufty et al. (1981) also suggested that an interaction between root temperature and illuminance can occur. Rufty et al. (1981) found that roots of soybean plants treated with low illuminance were sensitive to rising root temperatures in that they were smaller at root temperatures of 24 and 30°C as compared with roots at 18°C. When aerial CO₂ concentration was increased under low illuminance, root growth sensitivity decreased in response to changes in root temperatures. However, adverse effects to root growth were still observed at root temperatures of 30°C.

In a root temperature study conducted by Stone and Taylor (1983), soybean dry weight production from plants grown in a greenhouse at root temperatures between 21 and 29°C greatly varied when the experiment was repeated across time. However, in contrast to the results reported by Earley and Cartter (1945), and Rufty et al. (1981), Stone and Taylor (1983) did not observe a significant relationship between plant biomass production and illuminance as affected by changes in solar angle.

**Interactions of cool root temperature and illuminance**

Earley and Cartter (1945) found that soybeans grown under high illuminance and cool root temperatures of 2 and 7°C produced less dry matter in plant tops than those grown under low illuminance at the same root temperatures. Earley and Cartter (1945) observed that plants receiving root temperature treatments of 2 and
7°C wilted on sunny days. The researchers concluded that plants growing in the 2 and 7°C root temperatures suffered a greater degree of moisture stress under high illuminance than plants in the same root temperatures under low illuminance. The results indicated that soybeans growing under high illuminance had a higher moisture requirement than those growing under low illuminance, and that soybeans growing in cool root temperatures had a decreased ability to take up water. Decreased water uptake in low root temperatures is possibly due to an increase in the viscosity of water (Nielsen and Humphries, 1966) and protoplasm (Richards et al., 1952); and a decrease in the hydraulic conductivity of membranes in the roots (McWilliam et al., 1982).

McWilliam et al. (1982) found results similar to Earley and Cartter's (1945) in that cotton and bean (Phaseolus vulgaris L.) seedlings were more vulnerable to water stress when grown with low root temperatures under light rather than in darkness. Reducing root temperatures to 5°C in the light caused significant reductions in leaf water potential for both cotton and beans. Severe wilting was observed in cotton. When whole seedlings (roots and shoots) were chilled to 5°C in the dark, rates of reduction in water potential were less than those in the light, and leaf water potentials for cotton seedlings remained higher than those growing under light with chilled roots. No visible signs of wilting were observed among seedlings chilled in the dark. McWilliam et al. (1982) also found that rates of stomatal closure were reduced when seedling root temperatures were reduced to 5°C.

Pardossi et al. (1994) also found that water uptake by roots was reduced in
Phaseolus vulgaris when root temperatures were reduced from 25 to 10°C. They implied that rates of stomatal closure were reduced as a result of root cooling and resulted in plants undergoing moisture stress and wilting. However, five to eight hours after the onset of root cooling, leaf stomata closed, transpiration rates were reduced and the plants essentially recovered from the water stress. In spite of the apparent recovery from cool root temperature induced water stress, leaf relative growth rates and leaf turgor remained significantly lower than the control plants.

The effect of light on the plant is that of a stimulus for opening stomata (Sharkey and Rashke, 1981) which allows for transpiration to occur (Salisbury and Ross, 1992), thus leading to a net loss of water from the plant. McWilliam et al. (1982), applied abscisic acid, a stress hormone triggering stomatal closure (Salisbury and Ross, 1992), to leaves and roots of Phaseolus vulgaris chilled to 5°C and found that plants suffered much less water stress and displayed no visible signs of wilting. Therefore, reduction in water uptake and reduced rates of stomatal closure are most likely the chief causes of water stress in plants under going cool root temperature stress while exposed to light.

**Root temperature effects on photosynthesis**

Cool root temperatures can have an indirect effect on photosynthesis by reducing plant growth, which in turn will reduce the amount of photosynthetic surface area. Osmond and Raper (1981) found that when tobacco plants received cool root temperature treatments of 16°C, dry weight increases were significantly lower than plants growing in 24 and 32°C root temperatures. The cool root
temperature treated plants suffered water stress and wilted during the first week of the treatment period. However, by the end of the second week, the plants seemed to acclimatize to the cool root temperatures. Plants began growing new roots and leaf water potentials improved to the point that they were no longer different than water potentials for plants growing under 24 and 32°C root temperatures. In spite of the apparent recovery from cool root temperature stress, the tobacco plants growing in the low root temperatures produced significantly less dry weight during the 38-day treatment period. Osmond and Raper (1981) attributed reduced dry weight production of plants growing in the 16°C root temperature to water stress during the period of acclimation to cool root temperatures. The water stress resulted in smaller leaves, 57 and 49% of the leaf area index for the plants growing in the 24 and 32°C root temperatures, respectively. The reduction in leaf area resulted in a reduced amount of photosynthetic surface area.

Osmond and Raper (1981) did not report whether CO₂ exchange rates for tobacco plants were significantly affected by root temperature during the first two weeks of exposure to low root temperatures. However, they did observe that CO₂ exchange rates were not significantly different once the plants had acclimatized to the root temperature treatments. On the other hand, Choi et al. (1995), McWilliam et al. (1982), and Sawada et al. (1987) observed that photosynthesis was affected by root temperature stress. Choi et al. (1995) found that photosynthesis in cucumber plants was reduced when grown in low root temperatures. Choi et al. (1995) speculated that the photosynthetic reduction may have been attributed to an
increased resistance in stomata. Sawada et al. (1987) found that photosynthetic rates were reduced by 59% when rooted soybean leaves were exposed to a root temperature of 6°C. McWilliam et al. (1982) had similar findings in which photosynthetic rates in cotton seedlings were reduced by 55% when roots were cooled to 5°C.

In the Sawada et al. (1987) experiment, dry weight increases of roots on rooted soybean leaves, were reduced when root temperature was lowered from 20 to 6°C, conversely, leaf dry weights increased by 170%. The increase in leaf dry weight was mainly due to starch and sucrose accumulation. Sawada et al. (1987) observed that the increased leaf sucrose content resulted in reduced rates of photosynthesis. However, the increase in leaf starch content apparently did not affect photosynthetic rates. Sawada et al. (1987) also observed that roots of the rooted soybean leaves accumulated carbohydrates in the 6°C root temperature, but did not utilize them for growth thus indicating that low root temperatures can alter assimilate utilization. Although McWilliam et al. (1982) and Sawada et al. (1987) reported that photosynthetic rates were affected by root temperature stress, they did not indicate if plants undergoing root temperature treatments acclimatized to different root zone environments.

Root temperature effects on nitrogen and carbohydrates

Root temperature stress can influence carbohydrate utilization, storage, partitioning and assimilation. In an experiment conducted by McCoy et al. (1990) root-carbon immobilization, which was defined in the study as carbon used for
storage or growth, increased when soybean seedlings were grown under water stress at a root temperature of 10°C relative to plant roots growing at 25°C. However, under no water stress, root carbon immobilization was reduced at a 10°C root temperature relative to plants growing at 25°C. Carbon immobilization rates in source leaves however, decreased at the lower root temperature under water stress, but decreased at the higher root temperature under no water stress. Additionally, McCoy et al. (1990) reported that rates of carbon partitioning in plants with 25°C root temperatures were dependent upon the size of carbon pools, which is defined as mobile carbon, in the organs exporting carbon. However, this relationship was not observed in the plants growing in the 10°C root zone treatment. McCoy et al. (1990) observed that carbon pool sizes were, in general, larger in plants with cool root temperature treatments. However, increased sizes of carbon pools could be due to reduced respiration, as this has been found to occur when root temperatures are lowered (Walsh and Layzell, 1986).

Rufty et al. (1981) studied root temperature effects on soybean growth and whole plant response. They reported that varied amounts of root growth in response to different temperature treatments were the result of altered carbohydrate usage. According to Rufty et al. (1981) root metabolism is extremely sensitive to changing root temperatures. As a consequence of low root zone temperatures, production of metabolic energy in young root tissue was reduced, thus altering root carbohydrate utilization.

Walsh and Layzell (1986) reported that root respiration was affected by root
temperature when experiments were conducted with nodulated soybeans grown at two root temperatures. They found that when root temperatures were lowered to 15°C, there was a 45% decrease in root respiration rates as compared with soybeans grown in 25°C root temperatures. Rates of photosynthesis, based on leaf area, did not significantly respond to root temperature. Therefore, the decrease in root respiration resulted in an excess amount of carbon accumulation, which was comparable to a 24% increase in net photosynthesis. Dark respiration rates also were reduced in the shoots of cool root treated plants. Therefore, more carbon was made available for plants growing in the 18°C root temperatures as compared with soybeans in 25°C the root temperature. Mature leaves, roots and nodules of the plants growing in the 18°C root temperatures accumulated greater amounts of dry weight than those growing in 25°C. However, the plants growing in the 18°C root temperature produced less dry matter in new leaves than plants with 25°C root temperatures. The reduced production of new leaf dry matter is because rates of nitrogen fixation in the treated plants (18°C root temperature) were reduced which resulted in nitrogen accumulation in roots and mature leaves at levels similar to the same organs of plants growing at the 25°C root temperature. However, due to the decreased availability of nitrogen in the 18°C plants, new leaf tissue only received half the amount of nitrogen as new leaves in the plants growing at a 25°C root temperature.

Rufty et al. (1981) reported that soybean root carbohydrate utilization changed as root temperatures increased because carbohydrate partitioning was not
equal among different functions of the root. The researchers concluded that maintenance respiration increased with higher root temperatures, which resulted in reduced root growth. Maintenance respiration is a dominant sink within the root thus limiting the amount of carbohydrates available for growth (Rufty et al., 1981).

At high root temperatures nitrate absorption, which needs a continuous energy supply, is limited due to sink dominance of root respiration (Rufty et al., 1981). Transport of the nitrogen out of the root is affected differently at high root temperatures than at low temperatures. As root temperatures increased in the Rufty et al. (1981) experiment, greater proportions of absorbed nitrogen was translocated out of the roots in to the shoot. However, because root nitrogen absorption decreased with increasing root temperatures, less nitrogen was available to plant tops. Limited supplies of available nitrogen to growing points on the shoot can limit new leaf growth, which in turn limits CO₂ fixation and reduces overall plant growth (Rufty et al., 1981).

**Root temperature effects on nutrient uptake**

Root temperature can affect nutrient uptake, assimilation, and translocation (Neilsen, 1974). Little work has been done to study the influence of root temperature on soybean nutrient uptake. However, nutrient uptake by other crops has been studied under various root temperatures.

The general trend reported in the literature is that the uptake of most nutrients will increase with rising root temperatures and decline after temperatures are above a particular optimum range. The response of nutrient uptake to root
temperature will vary based on the type of nutrient. For example Del Valle and
Harmon (1968) found that a root temperature of 24°C was optimum for maximum
production of dry weight and complete nutrient uptake in collards (*Brassica oleracea* var. *acephala*). However, individual nutrient uptake patterns varied with soil
temperature. For example, uptake of K and Mg peaked at 35°C, Ca at 24°C, and P
at 30°C (Del Valle and Harmon, 1968). Nielsen and Cunningham (1964) found that
Ca and Mg concentration in Italian ryegrass (*Lolium multiflorum* Lam) increased with
higher soil temperatures. To a lesser degree soil temperature influenced uptake of

It has also been reported that rising soil temperatures may result in increased
uptake of specific nutrients and limited uptake of others. Nielsen et al. (1960), found
that concentrations of N and P, and contents of K, and Mg in oat (*Avena sativa* L.)
top growth increased in warmer soil temperatures, but Ca content decreased.
Conversely in an experiment conducted by Choi et al. (1995), Ca$^{2+}$ concentration in
cucumber (*Cucumis sativus* L.) xylem sap increased as soil temperatures increased
from 12 to 22°C. However, NH$_4^+$, Mg$^{2+}$, and SO$_4^{2-}$ concentrations in xylem sap
decreased with increasing root temperatures of 12 to 32°C (Choi et al., 1995).

According to Horrocks and Yang (1983), contrasting reports in the literature
regarding nutrient uptake as influenced by root temperature might be due to the
different ages of plant tissue at the time nutritional status is evaluated. Horrocks
and Yang (1983) found that soil temperatures of 20 and 25°C did not influence
sorghum (*Sorghum bicolor* L.) biomass production; however, soil temperature
effects on plant nutrient composition varied based on the growth stage of the plant. At the eight-leaf stage of development N, K, Mg, and Cu concentrations were significantly greater in plants growing under a root temperature of 25°C than plants growing under the 20°C root temperatures. However, at the twelve-leaf stage, opposite results occurred. Concentrations of N, K, Mg, and Cu were significantly lower in the plants growing under the 25°C root temperatures (Horrocks and Yang, 1983). They also found that as plants increased in age, changes in plant nutrient levels varied depending on root temperature. For example, in a root temperature of 20°C, there were no changes in plant nitrogen concentration as plants matured from the eight-leaf stage to the twelve-leaf stage. However, the nitrogen concentration of plants growing in the 25°C treatment was reduced by 49% over the same period (Horrocks and Yang, 1983). A similar trend was observed for Fe, Mn and Zn (Horrocks and Yang, 1983).

It is possible that an interaction between soil temperature and fertility may influence plant growth and nutrient uptake. Moraghan (1985) found that when chelated Fe concentrations in a calcareous soil were increased, dry weight production of soybean plants was reduced at a soil temperature of 15°C. However, at a soil temperature of 24°C, there was no reduction in plant dry weights when the soil concentration of Fe chelate was increased. Additionally, Moraghan (1985) indicated that mottling in old leaves and the severity of chlorosis in young leaves were greater at the higher concentration of chelated Fe and a soil temperature of 15°C. However, when plants growing under 15°C soil temperature and elevated
levels of Fe chelate received a foliar application of MnSO$_4$, plant dry weights increased and the nutrient deficiency symptoms of chlorotic and mottled leaves disappeared. The findings by Moraghan (1985) indicate that an interaction between soil temperature and Fe chelate may influence soybean Mn deficiency and dry weight production.

In another experiment Moraghan (1987) found that P and Mn concentrations in plant tops of Fe-ineffective soybeans were significantly affected by soil temperature. However, Moraghan (1987) indicated that soil temperature affects on concentration of Fe were small. In spite of this, the Fe-ineffective soybean varieties were severely affected by Fe deficiency in the warmer soil temperature. Moraghan (1987) concluded that the Fe deficiency was possibly due to increased plant growth rates, associated with the warmer soil temperatures, diluting Fe in the new growth, and a higher vegetative P concentration which may interfere with movement of Fe within the plant.

**Root temperature effects on root growth**

While it has been reported in the literature that root temperature can influence plant nutrient uptake, reasons for this are often not stated. However, the direct effects of root temperature on root growth may be the primary reason nutrient uptake becomes limited when root temperatures are outside an optimal range.

Root distribution and morphology (Nielsen, 1974), rate of extension (Bland, 1993; Stone and Taylor, 1983) and rooting depth (Stone et al., 1983) are influenced by soil temperature. Stone and Taylor (1983) found that both taproot and lateral
root extension rates of soybeans were significantly affected by soil temperature. As soil temperatures increased so did rates of root extension. Bland (1993) found similar results when comparing the rates of cotton and soybean root extension with rates of soil warming. Bland (1993) found that the downward growth rate of cotton roots was slower than the downward rate of soil warming. Similarly he found that soybean roots penetrated through soils behind 20 and 18°C isotherms in fast and slow warming treatments, respectively. Bland (1993) indicated that if the downward growth rate of roots exceeded the downward movement of a certain soil temperature isotherm, the roots eventually would extend into colder soils, which would slow root growth rates. Therefore, if root growth is limited due to suboptimal soil temperatures, nutrient uptake may be reduced because the root system would be restricted to a smaller volume of soil, thus having a limited area for nutrient absorption. When plant roots absorb nutrients at rates faster than they can be replaced via mass flow and diffusion, the root system must extend into new soil regions to meet the plant's nutrient demands (Nissen, 1996). However, if nutrient concentrations are high enough, roots may be able to provide the aerial plant parts with adequate amounts of nutrients (Marschner, 1986, p.445). For example, P uptake significantly increased with soil temperature (Moraghan, 1987; Raeini-Sarjaz and Barthakur, 1995). However, Nielsen and Cunningham (1964) reported that soil temperature had only a small effect on P uptake by Italian ryegrass. They indicated that this might have been due to high levels of P fertilizer added to the soil. Therefore, because the uptake of P is primarily by root contact (White, 1981) and
root branching is decreased in cool soil temperatures (Nielsen and Cunningham, 1964), sufficient quantities of this nutrient may be absorbed when the soil has an abundant supply of it.

**Soil temperature effects on root respiration**

Nutrient uptake may also be influenced by the direct effect of soil temperature on root respiration. In response to increases in root temperature root respiration rates have increased (Gur et al., 1972; Szaniawski and Kielkiewicz, 1982; Walsh and Layzell, 1986). Additionally the uptake of some ions is an energy consuming process (Nissen, 1996; Pitman, 1976; Salisbury and Ross, 1992 p.155-154) and can account for a substantial amount of energy produced from respiration. For example, Veen (1980) found that when maize (*Zea mays* L.) plants were grown in a normal environment, approximately 50% of the respiration in maize roots was attributed to ion uptake. Additionally ion absorption and transport accounted for approximately 20% of whole plant respiration. Veen (1980) also found that as nutrient uptake increased, due to an increased supply of ions, root respiration also increased. The increase in respiration therefore, necessitates more respirable substrate (Salisbury and Ross, 1992, p. 284-285). Additionally, when there is a root temperature increase, soluble carbohydrate levels in roots can decrease (Kafkafi, 1990). Rufty et al. (1981) speculated that lower levels of root carbohydrates, associated with increased root respiration at higher soil temperatures, may be the cause for decreased nitrate uptake in warmer root temperatures. They contended that respiration associated with root maintenance, which is known to increase with root
temperature, is a dominant sink over nitrate uptake and root growth. Therefore, if carbohydrate supply to the root were limited, nitrate uptake would be reduced. Rufty et al. (1981) found that the root-temperature-induced reduction in $\text{NO}_3^-$ uptake resulted in reduced leaf growth, which lead to a decrease in $\text{CO}_2$ assimilation. The researchers concluded that these results suggested that an interdependent relationship, governed by nitrogen and carbohydrate flux existed between the root system and aerial plant parts.

**Influence of root zone temperature and feedback on nutrient uptake**

Root temperature can play a feedback role in regulating nutrient uptake (Klock et al., 1997). If changes in root temperature alter relative growth rates and plant biomass production, the influences of root temperature on plant nutrient concentration may not be evident. The requirement for plant nutrients may increase or decrease based on the amount of vegetative matter supported by the root system (Bowen, 1991). Shoot-to-root ratios influenced by root temperatures can become an important component in influencing rates of nutrient uptake. For instance, Engels et al. (1992) found that when maize seedlings were exposed to cool root temperatures, root growth and nutrient uptake decreased. However, three days after the onset of treatments, seedlings in the cool root temperatures, with their shoot apical meristems above the zone of cooling, responded with an increase in vegetative shoot growth but no significant change in root growth. This resulted in greater shoot-to-root ratios in the cooler root temperatures, which resulted in a greater shoot requirement of nutrients per unit of fresh root weight. Therefore, rates
of nutrient uptake, based on fresh weight of the root system, increased in the lower root temperatures. In a study by Cumbus and Nye (1982) the effects of root temperature on nitrate inflow, which are rates of nitrate uptake based on root length, were small across a root temperature range of 10 to 30°C. However, they indicated that nitrate inflow increased at a root temperature of 35°C. Cumbus and Nye (1982) also indicated that total plant biomass production was not affected by the root temperature treatments. They stated that the dry weights of plants across all temperature treatments were comparable because plants were not harvested until the uptake of nitrate had almost stopped. Plant growth rates, dry weights of roots, and root-to-shoot ratios however, were affected by root temperature. Plants growing in the 25 and 30°C root temperatures had the largest root-to-shoot ratios, as well as the fastest rates of growth and nitrate uptake compared with plants growing in the 10 and 35°C root temperatures. However, Cumbus and Nye (1982) indicated that rates of nitrate inflow, were not affected by root temperature in the 10 to 30°C treatments. Nitrate inflow rates, however, were higher in the 35°C treatment. Cumbus and Nye (1982) concluded that the larger shoot-to-root ratios, which occurred in the 10 and 35°C treatments, placed such a large demand for nitrogen on the smaller root systems, that nitrate inflow was sustained at 10°C and increased at 35°C. Clarkson et al. (1986) discovered similar results when *Lolium perenne* L. plants were subjected to various root temperatures ranging from 3 to 25 °C. Clarkson et al. (1986) found that plant growth rates, root-to-shoot ratios and specific absorption rates of N, P, and K, which is a rate of nutrient uptake per unit of root
fresh weight, increased with rising root temperatures during the first nine days of the experiment. During the last five days of the experiment, root-to-shoot ratios stabilized and specific absorption rates were much less responsive to root temperature. Clarkson et al. (1986) suggested that the reduced effect of root temperature on specific absorption rates were due to root-to-shoot ratios resulting from the root temperature treatments. For example, in the warmer root temperatures, the larger root systems essentially had a lower nutrient uptake requirement to sustain top growth. But in the lower root temperatures, the smaller root systems supported a larger proportion of top growth, and therefore had a greater requirement for nutrient uptake.

**Root temperature effects on reproductive development**

Mack and Ivarson (1972) conducted an experiment in which field grown soybean plants were subjected to soil temperature treatments beginning at the third day after emergence and continuing through maturity. They found that plant height, shoot dry weights, pods per plant and grain yields increased with soil temperature and concluded that there was a relationship among grain yields, vegetative production, and soil temperatures. However, because soil temperature treatments were in effect throughout the growing season, it was possible that the grain yield response may have partly been due to the effects of soil temperature on root and shoot growth. Production of plant biomass was lower in the cooler soil temperatures, so it was possible that leaf area index and therefore canopy photosynthesis would have been lower in these treatments (Campbell et al., 1990),
thus contributing to lower grain yields (Sharma et al., 1982; Wells et al., 1982).

Duke et al. (1979) found that grain yield reductions of approximately 46% occurred when soybean root temperatures were reduced from 20 to 13°C 42 days after onset of seed imbibition with harvest occurring 21 days later. The authors did not provide a harvest growth stage. Additionally there was a reduction in the number of pods and seeds produced by plants in the cooler root temperature, but average weight per seed was unaffected by the change in root temperature. The lower root temperature also reduced other plant parameters such as fresh weight of roots and shoots as well as rates of root respiration, by 77%, and photosynthesis by almost 50%.

In addition to low root temperatures reducing soybean yields, Hijaz et al. (1984) found that high root temperatures can also reduce grain production. Hijaz et al. (1984) grew two cultivars of potted soybeans in a greenhouse. To achieve low soil temperatures, pots were covered with aluminum. Pots left uncovered had higher root temperatures. Soil temperatures fluctuated throughout the plant’s life cycle, so Hijaz et al. (1984) reported that total daily soil temperature accumulation for the season was 2255 °C for the cool treatment and 2522 °C for the warm treatment. Grain yield in the cool root temperature treatment was increased by 15% over the warm soil temperature treatment. Additionally, production of plant dry matter and individual seed weight in the cultivar Gieso was significantly increased by the cool soil temperature, but the other cultivar, Altona, had no significant response in either of these parameters. The cool soil temperature also increased pod
production by Gieso but had no effect on Altona.

Summary

One of the principle functions of the root system is to provide the plant with water and nutrients (Killham, 1994, p. 38). Therefore, if conditions in the root zone are not optimal for the plant's root system, the plant as a whole may not achieve its genetic potential. It is evident in the literature that root zone temperatures can influence the growth, dry weight production and metabolism of many different plant species including soybeans. However, most root temperature work has been conducted on plants during seedling and vegetative stages of development. Little work has been done to investigate the effects of soil temperature on reproductive development, and no studies have focused on soil temperature treatments applied only during reproductive growth.
MATERIALS AND METHODS

On August 13, 1997, non-inoculated Harosoy Dt2, maturity group II, semi-determinate soybeans were planted in 7.54 liter plastic buckets. The buckets had no drainage holes so a porous, plastic disk was placed one inch above the bottom of each bucket to allow for accumulation of gravitational water. This water was siphoned out of each bucket every time plants were watered. Three seeds were planted in each bucket that contained a mixture of two-thirds silica sand and one-third perlite. After V-1 (Fehr and Caviness, 1977) the seedlings were thinned to one plant per bucket. The soybeans were grown in a south facing green house in Ames, Iowa. The supplemental light source was high intensity sodium lamps, with the day length set at sixteen hours until V-6 (Fehr and Caviness, 1977) at which time day length was reduced to 15 hours to induce flowering. At R6, day length was reduced to 14 hours to hasten maturity. On a daily basis soybean plants were given a complete nutrient solution consisting of 0.5988 grams per liter of a commercial fertilizer (Miracle Gro® Excel™, 21-5-20 All Purpose Fertilizer) supplemented with 1.01 mM MgSO₄⋅7H₂O, 0.71 mM MgCl₂⋅6H₂O, 2.13 mM CaCl₂⋅2H₂O, 0.01uM NiCl₂⋅6H₂O, 0.17 uM CoSO₄⋅7H₂O (Limsande and Edwards, 1988). The nutrient solution was adjusted to a pH of 6.7 prior to giving to plants. To reduce nutrient salt accumulation, all buckets were flushed with two liters of distilled water at a of pH 6.8 every two-weeks prior to root temperature treatments and weekly there after. When plants reached R1 (Fehr and Caviness, 1977) flowers were pulled off the plants until they had completed vegetative growth. This helped insure more uniform flowering
along the length of the main stem. By allowing the plants to complete vegetative growth prior to the temperature treatments, the possibility of temperature stress effects on vegetative growth would be eliminated giving the opportunity to look solely at the effects of root temperature stress on soybean seed fill.

The experiment was a split-plot design blocked on a north-south gradient. At early R5, approximately 35 days after R1 and developing seeds were three to five mm in length (Fehr and Caviness, 1977), root temperature treatments were imposed. On a daily basis, half of the plants in each temperature treatment were given 800 milliliters of nutrient solution, where as the other half were given 800 milliliters of distilled water. Root temperature treatments were 18, 27, and 36°C with air temperatures of 25°C during the day and 22°C at night. The standard errors of the mean root temperatures for the treatment period were ±0.1°C for the 18 and 36°C treatments, ±0.2°C for the 27°C treatment. The standard errors of the mean high and low air temperatures during the treatment period were ±0.1 and 0.2°C, respectively. The seven year average bare soil temperatures at a 10 cm depth during the month of August in Ames, IA from 1950-1956, were 20.6 and 26.9°C at 0700 and 1900, respectively (Elford and Shaw, 1960).

Root temperatures were achieved and regulated by a water bath system (Figure 1). There were three 135-liter water baths per temperature treatment. The three water baths were connected to a water pump and 30.2-liter tank. The water pump, with a 680.4-liter per hour pumping capacity, provided continuous water circulation through all baths and tank within each treatment. Depending on the
Figure 1. Diagram of the root temperature system for the 18°C root temperature treatment. The 27 and 36° root temperature systems had a heating element in the 30.2-liter tank rather than the cold water line as illustrated. All three Omega CN132 Autotune Temperature Micro Controllers, one per temperature, were contained in the control panel.
treatment, the circulating water was heated or chilled at the 30.2-liter tank. The tanks associated with the 27°C and 36°C treatments were heated with a 150 watt submersible heating element regulated by an Omega CN132 Autotune Temperature Micro Controller, 1 32-1 DIN size. Water flowing from a greenhouse cold water line into the 30.2-liter tank cooled water circulating through the 18°C water bath system. A solenoid valve regulated by a refrigeration thermostat controlled the amount of cold water entering the 18°C system.

The treatment period was 36 days and was terminated at R6. Immediately following treatment termination, plants were left in the pots to dry down. After plants were air dried, pods were hand harvested and counted, stems were cut at the soil surface and oven dried at 59°C for 54 hours and subsequently weighed. Roots from 60% of the plants were harvested after air drying and separated from sand and perlite by sifting through a fine gauged screen. The presence or absence of nodules was recorded. Root weights were based on air dry weights.

The seeds from each plant were hand-shelled and counted, placed in an oven at 35°C for a period of 48 hours, and subsequently weighed. Seeds were then separated by size using a 5.56 mm screen. After screening, a three-gram sample of seed was randomly selected from each plant with a boerner divider. The three-gram seed samples were dried for an additional 48 hours at 60°C then ground in a coffee grinder and submitted for nutrient analysis.

The remaining seeds from each plant were used for germination and vigor tests. Three reps of 25 seeds from each plant were randomly selected with the
boerner divider for the standard germination test, and three reps of 25 for the accelerated aging test. The standard germination test was conducted according to the procedure described in the *Journal of Seed Technology* (AOSA 1993). The seeds were placed in germination chambers at 25°C. The substrata for germination was KIMPAK® (Custom made Hydro-crepe™ by the Kimberly-Clark Corporation) which would fall under the AOSA category of TC (AOSA, 1993). After the AOSA specified germination period of five and eight days (AOSA, 1998) seedlings were counted and judged to be normal or abnormal according to the guidelines set forth in *Seedling Evaluation Handbook* (AOSA, 1992). The radicals and cotyledons of all normal seedlings were removed and discarded after counting. The seedlings were then dried for 48 hours at 60°C and subsequently weighed.

The Accelerated Aging test was conducted according to the guidelines described in the *Seed Vigor Testing Handbook* (AOSA 1993). Twenty-five seeds from each plant were placed on 10.4 x 10.4 x 3.0 cm screens, which were placed in 11.3 x 11.3 x 4.6 cm clear plastic, germination boxes with lids. Forty milliliters of water was added to each germination box for the Accelerated Aging test. The germination boxes with water and seeds were placed in an oven at 41°C for 72 hours. After which the standard germination test and seedling evaluation was conducted as described above.

The F-test, from the SAS mixed model procedure (PROC MIXED) for a split-plot, randomized complete block design was used to evaluate the main effects and interactions of treatments (Littell et al., 1996). Block, root temperature, and fertilizer
were classified as fixed effects for the model statement, with the block by root
temperature interaction being the random effect (Littell et al., 1996).

Differences among treatments were further evaluated by estimating contrasts
of main effects with the ESTIMATE statement in SAS. Treatment least squares
means and their standard errors were calculated by SAS with the LSMEANS
statement. Least squares means and their standard errors will be used for the
results and discussion in this paper.
RESULTS

Root temperature effects

Root temperature did not significantly influence stem and root dry weight, reproductive structures, grain nutrient concentration, nor percent germination and seedling weight. The estimated temperature treatment means for all response variables are given in Table 1. The estimated overall means for stem and root dry weight was 13.5 and 7.1 grams per plant across all treatment combinations, respectively. In this experiment nodule formation was not influenced by root temperature or nutrient solution (data not shown).

Reproductive structures also remained unaffected by the root temperature treatments. The estimated average number of pods and seeds produced per plant regardless of treatment was 106 and 229 respectively. The estimated total grain yield averaged 37.0 grams per plant, which would have been equivalent to 3641.6 kg ha\(^{-1}\).

Root temperatures did not affect seed size. All plants in the experiment produced an average of 229 and 34 large and small seeds per plant, respectively. The estimated yield contribution of small seeds to total grain yield was approximately 0.5 grams per plant.

Grain nutrient concentration was not influenced by root temperature. Grain nitrogen concentration ranged from 4.5 to 6.3% with an estimated mean of 5.3% across all treatment combinations. Other grain macro nutrient concentrations had
Table 1. Least squares means with standard error from the root temperature treatments.

<table>
<thead>
<tr>
<th>Response variables</th>
<th>18°C (n=18)</th>
<th>27°C (n=17)</th>
<th>36°C (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem dry weight&lt;sup&gt;2&lt;/sup&gt;</td>
<td>13.2 ± 0.65</td>
<td>12.3 ± 0.51</td>
<td>13.3 ± 0.58</td>
</tr>
<tr>
<td>Roots&lt;sup&gt;3&lt;/sup&gt; (n=14)</td>
<td>7.2 ± 0.98</td>
<td>7.2 ± 1.07</td>
<td>9.1 ± 0.81</td>
</tr>
<tr>
<td>Number of pods per plant</td>
<td>113.1 ± 5.90</td>
<td>109.6 ± 4.56</td>
<td>117.0 ± 5.37</td>
</tr>
<tr>
<td>Number of seeds per plant</td>
<td>268.1 ± 14.50</td>
<td>249.5 ± 11.21</td>
<td>267.8 ± 12.89</td>
</tr>
<tr>
<td>Number of seeds per pod</td>
<td>2.0 ± 0.04</td>
<td>1.9 ± 0.03</td>
<td>2.0 ± 0.04</td>
</tr>
<tr>
<td>Number of large seeds per plant</td>
<td>205.3 ± 14.23</td>
<td>212.2 ± 11.00</td>
<td>227.3 ± 12.65</td>
</tr>
<tr>
<td>Large seed yield&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.0 ± 2.2</td>
<td>33.0 ± 1.71</td>
<td>35.3 ± 1.97</td>
</tr>
<tr>
<td>Number of large seeds per gram</td>
<td>6.9 ± 0.20</td>
<td>6.6 ± 0.16</td>
<td>6.5 ± 0.18</td>
</tr>
<tr>
<td>Number of small seeds per plant</td>
<td>62.8 ± 9.27</td>
<td>37.3 ± 7.17</td>
<td>40.5 ± 8.24</td>
</tr>
<tr>
<td>Small seed yield&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.6 ± 0.72</td>
<td>2.5 ± 0.56</td>
<td>2.9 ± 0.64</td>
</tr>
<tr>
<td>Number of small seeds per gram</td>
<td>15.2 ± 2.23</td>
<td>20.0 ± 1.74</td>
<td>16.3 ± 1.99</td>
</tr>
<tr>
<td>N&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5.7 ± 0.11</td>
<td>5.7 ± 0.09</td>
<td>5.5 ± 0.10</td>
</tr>
<tr>
<td>P&lt;sup&gt;5&lt;/sup&gt;</td>
<td>6202.7 ± 232.88</td>
<td>6092.6 ± 180.04</td>
<td>6228.2 ± 206.95</td>
</tr>
<tr>
<td>K&lt;sup&gt;5&lt;/sup&gt;</td>
<td>24 278.0 ± 424.27</td>
<td>23 878.0 ± 327.99</td>
<td>23 956.0 ± 377.02</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1399.8 ± 74.93</td>
<td>1416.2 ± 57.93</td>
<td>1461.3 ± 66.59</td>
</tr>
<tr>
<td>Mg&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2599.0 ± 63.02</td>
<td>2533.7 ± 50.45</td>
<td>2450.1 ± 56.78</td>
</tr>
<tr>
<td>Mn&lt;sup&gt;5&lt;/sup&gt;</td>
<td>21.9 ± 0.75</td>
<td>23.2 ± 0.64</td>
<td>22.2 ± 0.69</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;5&lt;/sup&gt;</td>
<td>61.2 ± 2.15</td>
<td>57.7 ± 1.76</td>
<td>54.1 ± 1.95</td>
</tr>
<tr>
<td>B&lt;sup&gt;5&lt;/sup&gt;</td>
<td>18.4 ± 0.93</td>
<td>18.8 ± 0.76</td>
<td>16.8 ± 0.84</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;5&lt;/sup&gt;</td>
<td>12.0 ± 0.52</td>
<td>11.7 ± 0.44</td>
<td>11.7 ± 0.48</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;5&lt;/sup&gt;</td>
<td>54.1 ± 1.63</td>
<td>55.2 ± 1.26</td>
<td>53.4 ± 1.45</td>
</tr>
<tr>
<td>Na&lt;sup&gt;5&lt;/sup&gt;</td>
<td>12.3 ± 2.86</td>
<td>11.8 ± 2.21</td>
<td>16.8 ± 2.54</td>
</tr>
<tr>
<td>Germination rate</td>
<td>0.6 ± 0.03</td>
<td>0.7 ± 0.03</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td>Seedling dry weight</td>
<td>0.5 ± 0.04</td>
<td>0.6 ± 0.04</td>
<td>0.6 ± 0.05</td>
</tr>
</tbody>
</table>

<sup>1</sup> Least squares means plus or minus the standard error of the mean.
<sup>2</sup> Oven dried weight, grams per plant.
<sup>3</sup> Air dried weight, grams per plant.
<sup>4</sup> Grain percent nitrogen.
<sup>5</sup> Grain nutrient concentration in ug g<sup>-1</sup>.

Estimated means of 5322, 21917, 1073, and 2446 ug g<sup>-1</sup> for P, K, Ca, and Mg, respectively. Grain Mn, Fe, and B concentrations had estimated averages of 21, 58, 17 ug g<sup>-1</sup>, respectively.

While the main effects of root temperature had no influence on grain nutrient concentration, there was a significant root temperature by nutrient solution
interaction on grain Mn and B concentration. Plants receiving nutrient solution during the grain fill period yielded seeds with significantly higher concentrations of Mn and B. Grain Mn concentration increased by an estimated 4.1 and 3.4 $\mu$g g$^{-1}$ when plants received nutrient solution in the 18 and 27 °C root temperatures, respectively. However, when plants in the 36°C root temperature treatment received nutrient solution, grain Mn content increased by an estimated increase of 6.3 ppm (Figure 2). Grain B concentration, however, had a greater increase when

![Graph](image)

Figure 2. Estimated treatment means of grain Mn concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
plants received fertilizer in the cooler root temperature treatment. By providing plants with a complete nutrient solution during the grain fill period, grain B concentration increased by 8.8 µg g⁻¹ when maternal plants were grown in the 18°C root temperature. A smaller grain B increase of 4.0 and 5.4 µg g⁻¹ was observed in the 27 and 36°C root temperatures, respectively (Figure 3). No additional root temperature by fertility interactions were observed in other response variables.

Figure 3. Estimated treatment means of grain B concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
**Nutrient treatment effects**

Nutrient stress during the grain fill period had a significant effect on most response variables measured in this experiment. Plants receiving nutrient solution during the treatment period generally increased in dry weight yields, retention of reproductive structures, and grain nutrient concentration than plants not receiving fertilizer during the grain fill stage.

Stem dry weights were reduced by 10.6% when plants did not receive nutrient solution. However, when plants received nutrient solution during the grain fill stage of development, there was a 22% decrease in root dry weights (Table 2).

Table 2. Effect of fertilizer treatments imposed during the grain-fill stage on soybean plant dry weights and yield determinants.

<table>
<thead>
<tr>
<th>Response variables (n=26)</th>
<th>fertilized means(^1)</th>
<th>non fertilized means(^1)</th>
<th>% change(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem dry weight(^3)</td>
<td>13.6 ± 0.50</td>
<td>12.3 ± 0.52</td>
<td>10.6(^*)</td>
</tr>
<tr>
<td>Roots(^4) (n=14)</td>
<td>6.9 ± 0.67</td>
<td>8.8 ± 0.61</td>
<td>-21.6(^*)</td>
</tr>
<tr>
<td>Number of pods per plant</td>
<td>131.3 ± 4.50</td>
<td>95.2 ± 4.76</td>
<td>37.9(^**)</td>
</tr>
<tr>
<td>Number of seeds per pod</td>
<td>2.1 ± 0.03</td>
<td>1.9 ± 0.03</td>
<td>10.5(^**)</td>
</tr>
<tr>
<td>Number of seeds per plant</td>
<td>309.7 ± 11.07</td>
<td>213.9 ± 11.54</td>
<td>44.8(^**)</td>
</tr>
<tr>
<td>Number of large seeds per plant</td>
<td>243.0 ± 10.87</td>
<td>186.9 ± 11.33</td>
<td>30.0(^**)</td>
</tr>
<tr>
<td>Large seed yield(^3)</td>
<td>34.9 ± 1.69</td>
<td>30.8 ± 1.76</td>
<td>13.3(^*)</td>
</tr>
<tr>
<td>Number of large seeds per gram</td>
<td>7.1 ± 7.01</td>
<td>6.2 ± 0.16</td>
<td>14.5(^**)</td>
</tr>
<tr>
<td>Number of small seeds per plant</td>
<td>66.7 ± 7.08</td>
<td>27.0 ± 7.38</td>
<td>147.0(^**)</td>
</tr>
<tr>
<td>Small seed yield(^3)</td>
<td>5.1 ± 0.55</td>
<td>1.5 ± 0.57</td>
<td>240.0(^**)</td>
</tr>
<tr>
<td>Number of small seeds per gram</td>
<td>11.5 ± 1.67</td>
<td>22.8 ± 1.76</td>
<td>-49.6(^**)</td>
</tr>
</tbody>
</table>

\(^*\), \(^**\) Differences in means significant at the 0.05 and 0.01 levels of probability, respectively.
\(n\) Number of observations per nutrient solution treatment.
\(^1\) Least squares means plus or minus the standard error of the mean.
\(^2\) Percent change = (fertilized non-fertilized\(^1\)) x 100 - 100.
\(^3\) Oven dried weight, grams per plant.
\(^4\) Air dried weight, grams per plant.
Supplying plants with nutrient solution during the grain fill period also influenced yield components. Pod retention was significantly improved when plants received nutrient solution. Fertilized plants averaged 131 pods per plant, but those not receiving nutrient solution retained an average of 95 pods per plant. Additionally, the number of seeds produced per pod was increased by 11 percent in the fertilized plants. Likewise, a grain yield improvement occurred when plants received fertilizer during the grain fill period. Fertilized plants produced 40.0 grams of seed per plant whereas plants undergoing nutrient stress only produced 32.3 grams of seed per plant. The number of large seeds per plant and per gram in the fertilized plants increased by 30.0 and 14.5%, respectively. The number of small seeds per plant in fertilized treatment increased by 147% but the number of small seeds per gram decreased by 49.6%.

The grain nutrient analysis revealed that the concentration of most elements in seed harvested from fertilized plants was significantly greater than seed nutrients from non-fertilized plants (Table 3). The average increase in the estimated mean, grain macro nutrient concentration was 27%. Grain N, P, and K increased by 10.6, 36.2 and 18.5%, respectively when plants received fertilizer during the seed fill stage of development. Grain Ca and Mg concentration had estimated increases of 61.9 and 5.0%, respectively when plants received nutrient solution.

There were significant increases in grain micronutrients as well. Grain Mn, B, and Zn increased by 22.9, 40.9 and 13.2% respectively. However, grain Fe concentration was reduced by 17.9% when plants were given nutrient solution.
during late the grain fill period. Grain macro- and micronutrient concentration of
seeds harvested from the fertilized plants in this experiment are comparable to
soybean nutrient levels reported by Hanway (1976).

Providing maternal soybean plants with nutrient solution during the grain fill
period increased seed germination rates 9.8%. Seedling dry weights however, were
not influenced by the fertilizer treatments applied to the maternal plants (Table 4).

Table 3. Effect of fertilizer treatments imposed during the seed fill stage on grain
nutrient concentration.

<table>
<thead>
<tr>
<th>Grain nutrient (n=26)</th>
<th>fertilized means¹</th>
<th>non fertilized means¹</th>
<th>% change²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N³</td>
<td>6.0 ± 0.08</td>
<td>5.2 ± 0.09</td>
<td>15.4**</td>
</tr>
<tr>
<td>P⁴</td>
<td>7119.8 ± 177.81</td>
<td>5229.2 ± 185.40</td>
<td>36.2**</td>
</tr>
<tr>
<td>K⁴</td>
<td>26 073.0 ± 323.93</td>
<td>22 002.0 ± 337.77</td>
<td>18.5**</td>
</tr>
<tr>
<td>Ca⁴</td>
<td>1762.9 ± 57.21</td>
<td>1088.7 ± 59.65</td>
<td>61.9**</td>
</tr>
<tr>
<td>Mg⁴</td>
<td>2589.6 ± 46.85</td>
<td>2465.6 ± 48.67</td>
<td>5.0*</td>
</tr>
<tr>
<td>Mn⁴</td>
<td>24.7 ± 0.52</td>
<td>20.1 ± 0.54</td>
<td>22.9**</td>
</tr>
<tr>
<td>Fe⁴</td>
<td>52.0 ± 1.57</td>
<td>63.3 ± 1.63</td>
<td>-17.9**</td>
</tr>
<tr>
<td>B⁴</td>
<td>21.0 ± 0.68</td>
<td>14.9 ± 0.70</td>
<td>40.9**</td>
</tr>
<tr>
<td>Cu⁴</td>
<td>12.1 ± 0.36</td>
<td>11.5 ± 0.38</td>
<td>5.2</td>
</tr>
<tr>
<td>Zn⁴</td>
<td>57.6 ± 1.24</td>
<td>50.9 ± 1.30</td>
<td>13.2**</td>
</tr>
<tr>
<td>Na⁴</td>
<td>19.8 ± 2.18</td>
<td>7.5 ± 2.28</td>
<td>164.0**</td>
</tr>
</tbody>
</table>

¹, ** Differences in means significant at the 0.05 and 0.01 levels of probability, respectively.
² Number of observations per nutrient solution treatment.
³ least squares means plus or minus the standard error of the mean.
⁴ percent change = (fertilized non-fertilized¹) x 100 – 100.
⁵ Grain percent nitrogen.
⁶ Grain nutrient concentration in ug g⁻¹.
Table 4. Effect of fertilizer treatment on the germination and vigor of seeds produced from plants receiving nutrient solution during the grain fill stage.

<table>
<thead>
<tr>
<th>Response variables (n=26)</th>
<th>Fertilized means</th>
<th>Non fertilized means</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination rate³</td>
<td>67.0 ± 1.96</td>
<td>61.0 ± 1.93</td>
<td>9.8**</td>
</tr>
<tr>
<td>Seedling weight⁴</td>
<td>0.5 ± 0.03</td>
<td>0.6 ± 0.03</td>
<td>-11.7</td>
</tr>
</tbody>
</table>

** Differences in means significant at the 0.01 level of probability.

n Number of observations per nutrient solution treatment.

¹ least squares means plus or minus the standard error of the mean.

² percent change = (fertilized non-fertilized¹) x 100 – 100.

³ percent germination.

⁴ grams per 25 seeds.
DISCUSSION

Root temperature

The main effects of root temperature did not influence soybean stem and root dry weight, reproductive components, grain nutrient concentration, germination and vigor of progeny during this experiment. It is probable that the timing of treatment induction may be the primary reason why root temperatures did not influence the parameters measured in this experiment. The plants grown in this experiment had completed most of their vegetative (Bernard, 1972; Ritchie et al., 1996) and root development (Hicks, 1978; Kaspar, 1985) near the onset of treatments and therefore were at a stage of development where root temperatures from 18 to 36°C would have little influence.

Previous research has shown that root temperature can influence soybean dry weight production (Earley and Cartter, 1945). However, in this experiment root temperatures had no effect on stem and root dry weight. This most likely is due to the timing of treatment onset and the fact that the soybeans grown in this experiment were semi-determinate. Stems of semi-determinate soybeans stop growing after a time of flower production (Bernard, 1972), and stems and leaves begin translocating assimilates to developing seeds not long after R5.5 (Hanway and Weber, 1971; Ritchie et al., 1996). Therefore, root temperature treatments imposed at early seed development were applied too late in the life cycle of the plants to significantly influence stem dry weight production.
Root dry weights also were not affected by the temperature treatments in this experiment. While results from previous experiments provide evidence that root temperature can influence root growth (e.g. Kaspar and Bland, 1992), these effects are often observed for plants that received treatments beginning in the vegetative stages of development (Earley and Carter, 1945; Pushkala and Yagarajarao, 1988). It is also reported that soybean root growth can continue well into the grain fill stage (Mitchell and Russell, 1971; Kaspar et al., 1978). Therefore, the rooting growth during grain fill stage could be sensitive to sub- or supraoptimal soil temperatures. However, in this experiment it is possible that the main limiting factor for root development was root growth being confined to 7.5-liter containers and plants possibly would have achieved near maximum levels of root dry weight at a growth stage prior to or near R5.

The timing of root temperature treatments in other experiments reported in the literature occurred when soybean plants were in the seedling or vegetative stages of growth. During vegetative growth, roots and shoots are actively growing and carbon and nitrogen partitioning between shoot and root can be affected by root temperatures, which in turn can alter plant development (e.g. Rufty et al., 1981). However, in this experiment, root temperatures were not initiated until beginning seed fill, at which time the developing fruit became the dominant sink, with little competition for assimilate from vegetative structures (Egli and Leggett, 1973). Therefore, because root temperature did not influence nutrient uptake as measured
by grain nutrient concentration, root or vegetative systems in this experiment, reproductive development remained unaffected as well.

The fact that root dry weight was not affected by root temperature in this experiment may be the primary reason why there were no root temperature effects on yield components, progeny germination, and nutrient uptake as measured by grain nutrient concentration. Because root and stem dry weights were unaffected by root temperatures, it is possible that shoot-to-root ratios remained unaltered from the temperature treatments.

Shoot-to-root ratios can be an important component in influencing rates of nutrient uptake and can diminish or nullify the effects of root temperature on nutrient uptake. Results from previous experiments conducted on other plant species indicate that sensitivity of nutrient uptake to root temperature is low or non-existent, but is more a function of shoot demand (Cumbus and Nye, 1982; Clarkson et al., 1986; Engels et al., 1992). Because shoot-to-root ratios in this study were essentially established prior to treatment onset, the nutrient demand of aerial plant parts may have had more influence on nutrient uptake than root temperatures ranging from 18 to 36°C.

In contrasts to the results of this experiment, Duke et al. (1979) found that the fresh weight of soybean roots, vegetative and reproductive structures were reduced when root temperature was lowered from 20 to 13°C. Additionally, Duke et al. (1979) stated that rates of photosynthesis, based on leaf area, were reduced by almost 50% when plants were moved to the cooler root temperature treatment.
Therefore, the lower fresh weight yields of pods and seeds, in their 13°C root temperature treatment, may have been the result of a reduction in photosynthetic source capacity. Root temperature effects on photosynthesis can be a secondary response as a result of the influence root temperature has on leaf growth (Osmond and Raper, 1981). Another possible reason for the contradictory findings between the two experiments is that the cool root temperature treatment in the Duke et al. (1979) experiment was 13°C, as compared with 18°C in this study. A root temperature of 13°C may be more limiting to soybean root functions than minimum root temperatures in the present study.

Root temperature by fertility interaction on grain Mn and B

The significant root temperature by fertility interaction on grain Mn and B concentration indicates that in a nutrient stressed environment the plant’s ability to absorb Mn and B may decrease at high and cool root temperatures, respectively. Although the main effects of root temperature did not affect grain Mn and B concentration, the magnitude of the fertility effect on the grain concentration of these nutrients was greatest at a root temperature of 36 and 18°C, respectively.

Published results regarding root temperature by fertility interactions on plant growth and nutrient uptake are limited. Moraghan et al. (1986) observed significant interactions of two levels of soil temperature and FeEDDHA on soybean dry weight and Mn concentration during the vegetative stages of development. When the root temperatures of Fe deficient soils were increased from 16 to 24°C, plant dry weight increased and Mn concentration decreased. However, in the presence of chelated
Fe, plant Mn concentration increased with root temperature. There was a significant reduction in Mn concentration, but apparently no differences in magnitude across temperatures. The results of this study and previously reported experiments suggest that optimal root temperatures for nutrient uptake will differ based on the type of nutrient being absorbed by the root system (Nielsen and Cunningham, 1964; Del Valle and Harmon, 1968; Choi et al., 1995)

**Nutrient solution**

The main effects of providing soybeans with nutrient solution during the grain fill stage of development was significant on all response variables except grain Cu concentration and seedling vigor of progeny seeds. Of the significant responses to nutrient solution, only root dry weight and grain Fe concentration were decreased as a result of increased fertility. The data indicate that plant nutrient status during the grain fill stage is critical for reproductive development.

Based on observations from a study conducted by Konno (1969), deficiencies of individual macronutrients beginning at the pod fill stage in soybeans can affect plant dry weight, nutrient composition and grain yield. Their data indicated that grain yields, number of seeds and pods per plant were significantly reduced by nitrogen deficiency. Grain yields were reduced to 61, 79, and 74%, relative to yields of control plants, when N, P and Ca were limited, respectively. Stem dry weights were reduced to 85 and 95% of stem dry weights from control plants when P and Ca were deficient, respectively. When N, P, Ca, and Mg were deficient root dry weights were also reduced relative to roots from control plants.
The reduction in soybean grain yield and macronutrient concentration of seeds, in this study, indicate that leaves and stems were not capable of providing the full source of nutrients needed, via translocation to seeds, to sustain reproductive development in a nutrient stressed environment. Based on experimental work by Hammond et al. (1951) and Hanway and Weber (1971), approximately 50% or more of grain N, P, and K are translocated from vegetative plant parts and the remaining supply of nutrients must come from the soil and nitrogen fixation (Hanway and Weber, 1971). Hanway and Weber (1971) indicated that N, P, and K uptake for grain fill would occur sometime during late R5. Hammond et al. (1951) indicated that Ca and Mg uptake by vegetative plant parts continued through leaf senescence and essentially no losses of these elements were observed in senesced leaves suggesting that Ca and Mg uptake from the soil would be the principle method for accumulation of these nutrients in the seed. Therefore, because almost half of the macronutrients must come from the soil during the grain fill stage, it is critical that these elements be at sufficient levels in order for the plant to reach its genetic potential.

Providing plants with fertilizer during the grain fill stage of development in this experiment resulted in a significant reduction in root dry weights as compared with roots from nutrient stressed plants. The higher weights of non-fertilized roots in the potted plants are likely due to nutrient stressed plants having rapid rates of leaf senescence and increased numbers of aborted pods and seeds, thereby reducing the sink strength of aerial plant parts, thus making it possible for increased dry
weight retention in the roots (Hanway, 1976). Additionally, as leaves senesce and fruiting structures abort, the nutrient demand would be lowered thereby reducing the amount of energy needed in the roots for nutrient uptake (Veen, 1980).

Grain Fe concentration was reduced when plants received nutrient solution during the grain fill stage. This may have been the result of an antagonistic effect of phosphorous on Fe. Previous studies have shown that there may be an antagonistic relationship between P and Fe, where high levels of P may bring about an Fe deficiency (Wallace, 1990; Wallace et al., 1978; Wallace et al., 1981).

Seeds produced from maternal plants receiving nutrients during the grain fill stage had significantly greater rates of germination as compared with seeds from nutrient stressed maternal plants. Smicklas et al. (1989) tested relationships between germination and the concentration of nutrients in soybean seeds, and found that Ca was the only nutrient related to germination. Smicklas et al. (1989) observed that as Ca concentration of seeds dropped from 1648 to 1305 µg g\(^{-1}\), percent germination decreased from 96 to 85%, respectively. In this experiment Ca concentration of seeds was 1763 and 1089 µg g\(^{-1}\) from fertilized and non-fertilized plants respectively, with the lower germination rates coming from seeds with reduced Ca concentration. Observations by Keiser and Mullen (1993) support the findings of Smiklas et al. (1989) in that soybean germination rates increased with seed Ca concentration. Therefore, it is possible that the reduction in germination rates of seed harvested from nutrient stressed plants may primarily be due to significant declines in seed Ca concentration.
GENERAL CONCLUSIONS

The timing of root temperature treatments in the present study greatly reduced any effects root temperatures may have on soybean reproductive development. The fact that root temperature did not influence root and stem dry weight, reproductive structures, grain nutrient concentration and germination and vigor of progeny seed may be due to stems and roots reaching maximum dry weight near the time of treatment onset, which would have made the fruit the dominant sink. Additionally, root growth being confined to 7.5-liter buckets may have been the most limiting for root development, and would have masked or nullified root temperature effects on nutrient uptake.

Based on the results of this experiment, when soybeans are exposed to root temperatures of 18 to 36°C between R5 and late R6, seed production, nutrient concentration and subsequent germination may not be adversely affected. This may indicate that once canopy closure occurs in irrigated agriculture, soil cooling as a result of irrigation (Burke and Upchurch, 1995) would not have an effect on soybean grain yields and quality. The lack of a root temperature effect on potted plants and the significant improved performance of fertilized plants suggest if soil moisture and nutrient levels are adequate enough to sustain the plant through maturity, the plant growth effects from early season barriers to root expansion may be minimized.
It is also evident from this experiment that soil nutrient status during the seed filling stage of soybeans is critical for the plants to achieve their genetic potential. Remobilization of nutrients from vegetative tissues in itself is not enough to produce maximum grain yields and seed quality.
Figure 4. Estimated means of stem dry weight at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 5. Estimated means of root dry weight at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 6. Estimated means of pod count per plant at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 7. Estimated means of seed count per pod three at root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 8. Estimated means of total seeds per plant at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 9. Estimated means of large seed count per plant at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 10. Estimated means of large seed yield per plant at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 11. Estimated means of large seed count per gram at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 12. Estimated means of small seed count per plant at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 13. Estimated means of small yield per plant at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 14. Estimated means of small seed count per gram at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 15. Estimated means of grain nitrogen concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 16. Estimated means of grain phosphorus concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 17. Estimated means of grain potassium concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 18. Estimated means of grain calcium concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 19. Estimated means of grain magnesium concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 20. Estimated means of grain iron concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 21. Estimated means of grain copper concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 22. Estimated means of grain zinc concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 23. Estimated means of grain sodium concentration at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
Figure 24. Estimated means of germination rate at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.

Figure 25. Estimated means of seedling dry weight at three root temperatures and two levels of fertility. Error bars represent ± 1 standard error of the mean.
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