ABSTRACT

SUN, MINGHUI Seed quality issues associated with high-oleate peanut (*Arachis hypogaea* L.) (Under the direction of Dr. JANET SPEARS)

The high-oleate trait of peanut is of great interest to the peanut processing industry because it produces greater oxidative stability of the oil without adversely affecting flavor. Most US peanut breeding programs have incorporation of the high-oleate trait into existing cultivars and future releases as an objective. While much of the peanut industry is concerned about peanut dietary oil quality, seed technologists are concern that altering peanut seed fatty acid or total lipid composition could influence germination rate, seed and seeding vigor, and seedling survival, especially if the seed are planted in stressful soil conditions. An experiment was designed to evaluate temperature effect on seed oil quality of high-oleate and normal peanut cultivars in controlled greenhouse environment. Two cultivars, NC-V 11 and Gregory, along with their paired backcross-derived high-oleate lines were planted in greenhouses maintained at 22/18^{o}C, 26/22^{o}C and 30/26^{o}C day/night temperature. A split-plot experimental design with two replications was used. Peanut kernels were analyzed for fatty acid composition of the whole seed and axis lipids. The whole seed oleic to linoleic acid (O/L) ratio of normal peanuts grown in 30/26^{o}C, 26/22^{o}C, and 22/18^{o}C, measured 1.9, 1.5, and 1.3, respectively. The O/L for their high-oleate pairs decreased from 24.7 when grown in 30/26^{o}C to 15.9 in 26/22^{o}C and to 13.7 in 22/18^{o}C. Temperature did not affect the fatty acid composition of axis total lipid or

phospholipid fractions. The high-oleate trait was however, expressed in the axis lipids. The average O/L of axis from normal peanut was 1.1 while that of high-oleate lines was 4.6. Likewise, axis phospholipids for normal and high-oleate lines were 1.0 and 5.9. Decreased production environment temperature in this study decreased the O/L ratio of seed oil of high-oleic peanut lines, and the high-oleate trait expressed in peanut seed storage lipids is also expressed in axis membrane lipids to a lesser degree. The second experiment was designed to determine if the production methods applicable to traditional peanuts will hold for high-oleate cultivars. Six Virginia-type peanut cultivars and their paired backcross-derived high-oleate lines were grown at the Peanut Belt Research Station near Lewiston, NC in 2003 and 2004. A split-plot experimental design was used with 2 × 2 factorial combinations of planting and harvest date as whole plot treatments, and 2 × 6 factorial combinations oleic acid and cultivars as subplot treatments. Seed quality evaluation included standard germination (SG), cool germination (CG), and electrical conductivity (EC). Oleic acid level had no influence on SG but did significantly alter CG and EC of high-oleate lines. Averaged across background genotypes, high-oleate lines had lower seed vigor than their paired lines with normal oleic content. The high-oleate lines of three of the six pairs had significantly lower CG and higher EC. Planting and harvest date affected all the seed quality traits measured. SG of both normal and high-oleate lines was reduced in 2004 when harvest was delayed, but was not affected in 2003. In 2003, CG of the high-oleate lines was significantly lower than that of normal lines in three of the four production environments; EC was significantly

higher in the high-oleate lines in all planting date and harvest date combinations. In 2004, there was no statistical difference between the CG of normal and high-oleate lines, but EC was significantly higher in the high-oleate lines for three of the four environments.

SEED QUALITY ISSUES ASSOCIATED WITH HIGH-OLEATE PEANUT (*ARACHIS HYPOGAEA* L.)

by

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BIOGRAPHY

Minghui Sun was born and raised in Laixi County, Shandong Province, China. He obtained a B.S. degree in Agronomy from Laiyang Agriculture University in July, 1988. Minghui worked at the Department of Agriculture of Laixi, Shandong as a agronomist until he came to the United States in April 2000. He first worked as a research assistant in the Department of Crop Science at North Carolina State University. In January 2003, he was accepted by graduate school of North Carolina State University. He obtained a Master of Statistics from Department of Statistics in May 2005, and is expecting to receive his Master of Science in the Department of Crop Science in December 2005.

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INTRODUCTION

Peanut Production

Arachis hypogaea L, commonly referred to as peanut or groundnut, is a self-pollinating, annual, herbaceous legume. The center of origin for *Arachis* spp. is thought to be in the Mato Grosso region of Brazil or northeastern Paraguay (Gregory et al., 1980). *A. hypogaea*, the most widely cultivated peanut species, probably originated in the region of southern Bolivia or northern Argentina (Hammons, 1982) and was subsequently taken to Europe, Africa, and Asia. Today, peanut is grown world wide, in the tropics and subtropics on approximately 26,416,700 Ha with total annual production around 37,058,000 Mt. Though the U.S. produces only 5 percent of the world's peanuts, the U.S. ranks first in average yield per Ha and second in world percentage of export peanut (Revoredo and Fletcher, 2002).

Four market types of peanut are commonly grown in the U.S. The most widely grown (70 percent of the U.S. production) is the small-seeded runner type, which is produced mainly in Georgia, Alabama, Texas, Florida, and South Carolina. The large seeded Virginia types produced in North Carolina and Virginia represent about 20 percent of the U.S. peanut production. The Spanish and Valencia market types are grown primarily in the southwestern areas of the U.S. and represent ten and one percent of our production, respectively (Knauft and Gorbet, 1989).

Peanut Seed Chemical Composition

Peanut is grown primarily for human consumption either as whole seeds or processed to make peanut butter, oil, and other products. Peanut seeds are a rich source of edible oils and contain 42 to 52 percent oil, 25 to 32 percent protein on a dry seed basis and provide minerals such as phosphorus, calcium, magnesium, potassium, and vitamins (Savage and Keenan, 1994). In the U.S., peanut is primarily used for peanut butter and snacks while it is used mainly as an oil source in most other peanut production areas of the world.

Seed oxidative stability is closely associated with oil composition; therefore, fatty acid composition in peanut seed is an important quality attribute, regardless of whether the seed is used as food or oil. The two predominant fatty acids in the peanut seed oils are oleic acid (18:1) and linoleic acid (18:2) which together comprise about 80 percent of peanut fatty acid composition (Ahmed and Young, 1982). Oleic acid, the 18-carbon monounsaturated oil precursor to linoleic acid, is less reactive with oxygen and therefore significantly more stable than linoleic acid. Peanut kernels with high oleic acid content were determined to have improved stability against lipid oxidation that lead to adverse flavors (Mugendi et al., 1998; O'Keefe et al., 1993). In addition, monounsaturated oils are desirable for both improved shelf-life and potential health benefits. For example, high-oleate oils have been reported to be associated with lowered blood serum cholesterol in human (O'Bryne et al., 1997). The relationship between the two fatty acids is direct and negative. Increased seed oleic acid content is accompanied by a highly correlated decrease in seed linoleic acid content. Therefore, the oleic to linoleic acid ratio (O/L)

accurately reflects the relative contents of these two fatty acids in peanut (Knauft et al., 1993). Peanut seed with high O/L (20 to 30) have nearly twice the oil stability and shelf-life than those with normal O/L (1.5 to 2.5) (Braddock et al., 1995; Branch et al., 1990; James and Young, 1983).

It has been reported that Virginia-type peanuts naturally produce oil with slightly lower linoleic percentage and therefore have greater oil stability than Spanish and Valencia-types (Norden et al., 1982). In response to the request of the peanut industry, most U.S. peanut breeding programs have attempted to incorporate the high-oleate trait into existing cultivars to enhance oleic acid content. A high-oleate trait in peanut was first identified in F 435, a University of Florida breeding line that contained 80 percent oleic acid and 2 percent linoleic acid (Norden et al., 1987). The first high-oleate peanut cultivar was SunOleic 95R released in 1995 (Gorbet and Knauft, 1997). Developing Virginia-type cultivars with elevated levels of oleic acid and depressed levels of linoleic acid is one of the goals for peanut breeding program at North Carolina State University. High-oleate peanut lines have been developed through backcrossing the trait from the high-oleate line, F 435, into large-seeded Virginia cultivars. High-oleate lines were selected after two to four backcrosses to the commercial cultivars. These efforts have resulted in several advanced high-oleate peanut lines with 80 to 85 percent oleic acid content in their seed oil (Isleib, personal communication, 2005).

Seed Quality and Seed Quality Test

A uniform stand of healthy, vigorous seedlings is essential if growers are to achieve the yield and quality needed for profitable peanut production. Thus, seed quality is critical for growers. According to Spears et al. (2002), peanut seed quality can be separated into five related components: germination, vigor, genetic purity, crop purity and health. Of these components, germination and vigor have the greatest impact on seedling emergence and survival.

The Association of Official Seed Analysts (2002) defines germination as an indication of a seed's ability to produce a normal plant under favorable conditions. To a peanut grower, germination percentage is the value printed on the seed tag and represents the maximum germination rate of the seed lot if seeds are planted in fields with optimal temperature and soil moisture. However, field conditions are rarely optimal and the germination rate may at times over estimate field emergence and seedling survival (AOSA, 2002). It is not unusual for peanut seed lots with very similar germination percentages and planted in similar field conditions to have very different seedling emergence and survival rates. Those differences in field performance are often attributed to the physiological quality component known as seed vigor (Spears et al., 2002).

Seed vigor is defined "those properties of seeds that determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (AOSA, 2002). When planted in fields with stressed environmental conditions, especially cool, wet conditions, a high-vigor seed lot can withstand the stress

during germination and early seeding development longer than a low vigor seed lot (Spears et al., 2002). Thus, emergence is generally higher and seedling growth is more rapid. Vigor tests are used extensively in the seed industry to provide a sensitive, consistent, fast, simple and economic method that can be used to predict the seed performance in the field environment (McDonald, 1980). The AOSA Seed Vigor Testing Handbook (2002) has included a consideration of the many possible uses of seed vigor tests. These include company decisions on where to plant and market seeds, as well as seed storage potential. For example, high vigor seed lots may be planted earlier or in the most northern region of cultivar adaptation where soils are often cooler. They also retain their quality potential in storage for a longer period of time, and vigor tests can be used to evaluate seed lot carry over potential (Copeland and McDonald, 2001).

The cold test, cool germination test, and electrical conductivity test (EC) are among the most widely used vigor tests in the commercial seed industry (Copeland and McDonald, 2001). The cold test was developed to assess seedling ability to tolerate low temperature stress, which often occurs in early spring planting. Seeds are placed in soil or paper towels lined with soil and exposed to cold temperature for a specified period, often in the presence of soil pathogens. Seeds are then placed under favorable growth conditions and allowed to germinate. The results are useful for predicting the quality of the seed since the test evaluates the seed and seedling behavior under sub-optimal conditions. The cold test is widely used in the corn and soybean seed industry. Martin et al. (1988) reported that the cold test was a superior predictor of field emergence of corn.

The cool germination test, the most widely used vigor test for cotton, is conducted at low temperatures (18^{o}C) but unlike the cold test, the cool germination test does not expose the seed to pathogens. This test procedure was developed to provide less demanding, but sufficiently severe test conditions to separate seeds on the basis of vigor (ISTA, 1995).

Low vigor seeds have been shown to possess decreased membrane integrity due to injury or deterioration under storage (Copeland and McDonald, 2001). EC test is a biochemical test that measures the amount of electrolytes that leak through the seed coat during imbibition. Higher conductivity, caused from increased cell leakage, would indicates a low vigor seed lot. The EC test is a suggested vigor test for large-seeded legumes (Matthews and Powell, 1987) and protocols have been developed to evaluate seed vigor for peas (*Pisum sativum* L.) (Bradnock and Mattews, 1970), soybean (Yaklick, and Abdul-Baki, 1975), common beans (*Phaseolis vulgaris* L.) (Kolasinska et al., 2000), and field bean (*Vicia faba* L.) (Hegarty, 1977).

Although the cold test, cool germination test and EC test are used routinely in the corn, cotton, and soybean seed industries, respectively, to identify seed lots with potential field emergence problems, no protocol has been developed for evaluating peanut seed vigor.

Peanut Seed Production

Spears et al. (2002), indicates that peanut seed production has many critical components. Optimizing seed quality requires that seed growers pay close attention to

pre-planting decisions, cultural practice during growing season, digging and harvest procedures, and post harvest handling. However, peanut seed growers know that weather conditions during the peanut growing season can have a dramatic influence on subsequent peanut seed quality.

Seed germination and vigor are greatly influenced by environmental factors that occur during seed growth, development, and maturation (Copeland and McDonald, 2001; TeKrony et al., 1984). High temperature during development has been shown to reduce soybean seed germination and vigor (Egli et al., 2005; Keigley and Mullen, 1986; Spears et al., 1997). In their study, Spears et al. (1997) reported that soybean seed that developed under 27/22^{o}C day/night temperature had significantly higher germination and accelerated aging germination and significantly lower EC than those grown at 38/33^{o}C. Peanut is sensitive to temperature with the optimum temperature range for vegetative and reproductive growth and development between 25 and 30^{o}C (Cox, 1979). Temperature also has a significant influence on peanut plant and pod growth rates (Leong and Ong, 1983). In an earlier study, high temperature reduced the numbers of pegs and pods (Ketring, 1984), and the reduction in pod and seed dry weight was due to reductions in total dry matter and harvest index (Craufurd et al., 2002). According to Nigam et al. (1994), cultivars differ in their sensitivity to heat stress during vegetative and the reproductive phases.

In addition to influencing seed planting quality, production environment can also affect seed composition. Dwivedi et al. (1993) reported significant genotype, environment, and genotype by environment interaction effects on oil content, individual

fatty acid content and derived oil quality parameter in peanut. The change of seed chemical composition is one of the factors that may influence the seed germination and vigor (Copeland and McDonald, 2001).

While much of the peanut industry is concerned about peanut dietary oil quality, seed technologists know that lipids are important energy source for germination. Consequently, altering peanut seed fatty acid or total lipid composition could influence germination rate, seed and seedling vigor, and seedling survival, especially if the seed is planted in stressful soil conditions. Alterations of seed lipid fatty acid composition brought about by traditional or molecular techniques could also change membrane lipid composition and therefore affect membrane function and permeability. Membrane integrity is linked to seed quality, seedling vigor, and germination tolerance to environmental stress. However, there is little information available in the literature on the effect of genetically altering peanut seed lipid composition on seed membrane function, germination, or vigor.

With the increasing number of high-oleate peanut cultivars, there is a need to analyze high-oleate peanut seed quality produced in various environments. In addition, it is not known if seed production methods (planting date and harvest date, for example) applicable to traditional peanuts will hold for high-oleate cultivars.

Large-seeded peanuts are planted in diverse climates across the United States. Therefore, a better understanding of how temperature during seed growth will influence seed and oil quality is needed.

Objectives of this study

1) Investigate temperature effect on seed oil quality of high-oleate and normal peanut cultivars in controlled greenhouse environment.

2) Provide information for optimizing seed production practices of high-oleate peanut cultivar.

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Temperature Effect on Oil Quality of Normal and High-Oleate Large Seeded Virginia-Type Peanut (*Arachis hypogaea* L.) During Seed Development

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ABSTRACT

Because of its greater oxidative oil stability, the high-oleate trait is of great interest to the peanut (*Arachis hypogaea* L.) processing industry. It is not known how production environment will alter oil quality of high-oleate peanuts. Therefore, an experiment was designed to evaluate oil quality of both normal and high-oleate peanuts produced in three temperature regimes. Two cultivars, NC-V 11 and Gregory, along with their paired backcross-derived high-oleate lines were planted in greenhouses maintained at 22/18^{o}C, 26/22^{o}C and 30/26^{o}C day/night temperature. A split-plot experimental design with two replications (two years) was used. Peanut kernels were analyzed for fatty acid composition of the whole seed and axis lipids. The whole seed oleic to linoleic acid (O/L) ratio of normal peanuts grown in 30/26^{o}C, 26/22^{o}C, and 22/18^{o}C, measured 1.9, 1.5, and 1.3, respectively. The O/L for their high-oleate pairs decreased from 24.7 when grown in 30/26^{o}C to 15.9 in 26/22^{o}C and to 13.7 in 22/18^{o}C. Temperature did not affect the fatty acid composition of axis total lipid or phospholipid fractions. However, the high-oleate trait was expressed in the axis lipids. The average O/L of axes from normal peanut was 1.1 while that of high-oleate lines was 4.6. Axis phospholipids for normal and high-oleate lines were 1.0 and 5.9. Decreasing production environment temperature in this study decreased the O/L ratio of seed oil of high-oleic peanut lines, and the high-oleate trait expressed in peanut seed storage lipids is also expressed in axis membrane lipids to a lesser degree.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is grown world-wide in the tropical and sub-tropical regions for oil and human food. Though the U.S. produces only five percent of the world's peanut supply, it is an important cash crop for growers in the southern and southwestern states.

Peanut is grown primarily for human consumption either as whole seeds or processed to make peanut butter, oil, and other products. Peanut seeds are a rich source of edible oils and contain 42 to 52 percent oil, 25 to 32 percent protein on a dry seed basis and provide minerals such as phosphorus, calcium, magnesium, potassium, and vitamins (Savage and Keenan, 1994). In the US, peanut is primarily used for peanut butter and snacks while it is used mainly as an oil source in most other peanut production areas of the world.

Seed oxidative stability is closely associated with oil composition; therefore, fatty acid composition in peanut seed is an important quality attribute, regardless of whether it is used as food or oil. The two predominant fatty acids in the peanut seed oils are oleic acid (18:1) and linoleic acid (18:2) which together comprise about 80 percent of peanut fatty acid composition (Ahmed and Young, 1982). Oleic acid, the 18-carbon monounsaturated oil precursor to linoleic acid, is less reactive with oxygen and therefore significantly more stable than linoleic acid. Peanut kernels with high oleic acid content were determined to have improved stability against lipid oxidation that lead to adverse flavors (Mugendi et al., 1998; O'Keefe et al., 1993). In addition, monounsaturated oils

are desirable for both improved shelf-life and potential health benefits. The relationship between the two fatty acids is direct and negative. Increased seed oleic acid content is accompanied by a highly correlated decrease in seed linoleic acid content. Therefore, the oleic to linoleic acid ratio (O/L) accurately reflects the relative contents of these two fatty acids in peanut (Knauft et al., 1993). Peanut seed with high O/L (ranging from 20 to 30) have nearly twice the oil stability and shelf-life than that with normal O/L (ranging from 1.5 to 2.5) (Braddock et al., 1995; Branch et al., 1990; James and Young, 1983). It has been reported that Virginia-type peanuts naturally produce oil with slightly lower linoleic percentage and therefore have greater oil stability than Spanish and Valencia-types (Norden et al., 1982).

Environment during peanut production can greatly influence crop productivity and quality. For example, Cox (1979) found that peanut vegetative and reproductive growth is sensitive to temperature, with the optimum temperature range between 25 and 30^{o}C. Temperature was also shown to significantly influence peanut plant and pod growth rates (Leong and Ong, 1983) and cultivars were found to differ in their sensitivity to heat stress during vegetative and the reproductive phases (Nigam et al., 1994). Spears and Sullivan (1995) also showed that peanut seed quality, especially seed vigor, can be affected by production environment. In their study, conducted over three years, peanut seed germination and vigor were higher in years when soils were warm with normal moisture and lower when conditions were wet and cool.

In addition to influencing seed planting quality, production environment can also affect seed oil composition (Brown et al., 1974). Dwivedi et al., (1993) reported

significant genotype, environment, and genotype by environment interaction effects on peanut oil content, individual fatty acid content, and derived oil quality. The change of seed chemical composition is one of the factors that may influence seed germination and vigor (Copeland and McDonald, 2001). Seed lipids are an important energy source for germination; consequently, altering peanut seed fatty acid or total lipid composition could influence germination rate, seed and seedling vigor, and seedling survival, especially if the seed are planted in stressful soil conditions.

A high-oleate trait in peanut was first identified in F 435, a University of Florida breeding line with 80 percent oleic acid and 2 percent linoleic acid (Norden et al., 1987). The North Carolina State University peanut breeding program has successfully incorporates the high-oleate trait into several commercial large seeded peanut cultivars. But, alterations of seed lipid fatty acid composition brought about by traditional or molecular techniques could also change membrane lipid composition and therefore affect membrane function and permeability. Membrane integrity is associated with seed quality, seedling energy, and germination tolerance to environmental stress. The scientific literature, however, offers little information on the effect altering peanut seed lipids might have on seed membrane lipid composition.

The objective of this study was to investigate the effect of temperature on seed oil quality of high-oleate and normal Virginia-type peanut cultivars grown in three controlled greenhouse environments.

MATERIALS AND METHODS

Four peanut cultivars, NC-V 11, high-oleate NC-V 11 (NC-V 11 HO), Gregory, and high-oleate Gregory (Gregory HO) were used in this experiment. NC-V 11 and Gregory are two popular large-seeded Virginia type cultivars grown in North Carolina and Virginia and have oleic acid content of approximately 50 to 55 percent. NC-V 11 HO and Gregory HO were developed through backcrosses transferring the high-oleate trait from the University of Florida line F 435 (Norden et al., 1987). Selection was made after two to three successive backcrosses resulting in NC-V 11 HO and Gregory HO having oleic acid levels of 80 to 85 percent (Table 1) (Isleib, personal communication 2005).

Experimental design

Studies were established in 2003 and 2004 in greenhouses located at the Southeastern Plant Environment Laboratory at North Carolina State University main campus. A split plot experimental design was used, with greenhouse as the whole plot (three levels) and cultivar as subplot (four levels). All greenhouses were maintained at 80% RH and natural photoperiod plus 3 h night interruption (11PM to 2 AM). The three temperature levels in this study were 22/18°C, 26/22°C, and 30/26°C day/night with 12 hours of high and low temperatures. Single plants were grown in 45 cm diameter pots filled with three parts sand (steam sterilized) and one part peat. Plants were watered twice daily with a complete nutrient solution (Downs and Thomas, 1991). In each greenhouse, randomized complete block designs were used with seven blocks in 2003 and five blocks

in 2004. The four cultivars were randomly arranged within each block. The days to peanut harvest (maturities) varied with growth temperature (Table 2). Hull scrape and peanut maturity profile method were used to determine maturity. Following harvest, pods were dried with unheated forced air, shelled by hand, and the seeds tored at 10^{o}C until analyzed for oil quality.

Fatty acid profile of peanut seed analysis

Ten sound mature kernels and 15 embryos of each of the four lines produced under three environments in 2003 and 2004 were analyzed for fatty acid profile using the technique of Zeile et al. (1993). Samples were extracted for 12 hr in 1 ml of solvent (chloroform: hexane: methanol, 8:5:2 vol:vol:vol) in stoppered test tubes. Fatty acid methyl esters of the lipid extracts were prepared using sodium methoxide. The samples were analyzed by gas chromatography using an HP 5890 Series II GC (Agilent Technologies, Inc., 2850 Centerville Rd., Wilmington, DE 19808) equipped with an AT-Silar 30m × 0.53mm column (same source). Operating conditions were 1µl injection volume, a 20:1 split ratio, and He carrier gas flow of 6 ml min^{-1}. Temperatures were 250°C, 200°C, and 275°C for the injector, oven and flame ionization detector (FID), respectively. Chromatograms were analyzed using HP ChemStation software.

Ten axes were crushed to a powder and extracted with 5ml of 2:1 chloroform: methanol and filtered. Water was added until the solvent formed two phases. The lower choroform phase was removed and the chloroform evaporated at 50 C under a stream of nitrogen and redissolved in 0.5ml of 2:1 chloroform: methanol. A 100µl aliquot was

removed for total lipid fatty acid composition. Fatty acid methyl esters were prepared by transesterification using sodium methoxide as the derivitization reagent and analyzed by GC. Phospholipids were isolated by Silica TLC using petroleum ether:diethyl ether: acetic acid (80:20:1) as the mobile phase. The TPL remained at the origin and the silica was scraped in to screw-capped vials and fatty acid metyl esters were prepared by acid methanolysis using 1ml of 5% HCl in methanol. Samples were heated to 80C for 90min, cooled to room temperature, 2ml of 1.5%NaCl were added and the FAMEs were partioned twice into 1ml hexane. Hexane upper layers were pooled, evaporated to dryness under nitrogen, resuspended in 100µl hexane and analyzed by GC.

Gas chromatographic analysis of FAMEs was performed with an Agilent 6890N GC equipped with FID, using a 0.53mm x 30m DB-23 column in a 200 C oven, 250 C injector, 10:1 split ratio, 300 C FID, and He carrier flow of 6.7ml/min.

Statistical analysis

For statistical analysis of collected data, we used pooled analysis of variance over two years and three temperatures based on a mixed linear model with temperature and cultivar as fixed effects of variance and year, replication (year) as random effects. Analyses were conducted using the general linear models procedure (PROC GLM) with random statement of SAS (SAS Institute, Inc., Cary, NC). Treatment means were separated by t-test using significance level of $\alpha=0.05$.

RESULTS AND DISCUSSION

Temperature effect on whole seed fatty acid composition

There was no significant temperature or temperature by cultivar interaction effect on whole seed levels of palmitic acid (16:0) (Table 3). When averaged across the three temperatures, palmitic acid levels of NC-V 11, NC-V 11 HO, Gregory, and Gregory HO, were 9.7, 5.5, 8.5, and 5.5%, respectively. Cultivar effect for palmitic acid was significant. The high-oleate lines had significantly lower 16:0 content than their paired normal lines in all environments (Table 4).

There was a significant temperature effect on stearic acid (18:0) content ($P<0.01$), but no cultivar or temperature by cultivar interaction (Table 3). Stearic acid content increased slightly but significantly with increasing growth temperature (Table 4). Average stearic acid levels across the four cultivars for three temperature regimes, 22/18˚C, 26/22˚C, and 30/26˚C were 2.1, 2.3, and 2.6%, respectively.

Temperature by cultivar interactions were significant for whole seed oleic acid ($P<0.05$) and linoleic acid levels ($P<0.05$) (Table 3). Therefore, we analyzed the temperature effect sliced by cultivar (Table 5). The oleic acid level of all four cultivars increased when the temperature increased from 22/18˚C to 30/26˚C. For NC-V 11 and Gregory, these increases were significant ($P<0.0138$ and $P<0.0006$ respectively). For their corresponding high-oleate lines however, the increases were not significant (Table 4 and 5). Linoleic acid levels of the four cultivars were reduced when the temperature increased from 22/18 to 30/26˚C. The reduction was significant for NC-V 11 ($P<0.006$),

Gregory (P<0.0002), and Gregory HO (P<0.0297), but not for NC-V 11 HO (Table 4 and 5). The low growth temperature resulting in decreased oleic content and increased linoleic levels were also observed in soybean (*Glycine max* L.) (Wilson, 2004), rapeseed (*Brassica mapus* L.) oil (Marie et al., 1999), and sunflower (*Helianthus annus* L.) (Harris et al., 1978). The increase in linoleic content for seeds grown in low temperature may be due to increased activity of desaturase enzymes involved in the conversion of oleic to linoleic acid (Harris et al., 1978). Earlier studies indicated that the increase in desaturase activity at lower growth temperature could be associated with the increased availability of O_2 in the tissue as a result of its greater solubility at low temperature (Harries and James, 1969).

Temperature effects on oleic/linoleic ratio varied depending on cultivar (Table 4). O/L of all cultivars increased with the increased growth temperature, however, only the increases for the high-oleate lines, NC-V 11 HO (P<0.0217) and Gregory HO, (P<0.0049) were significant. The average O/L ratio of these two normal cultivars grown in 22/18˚C, 26/22 ^{o}C, and 30/26^{o}C, measured 1.3, 1.5, and 1.9, respectively. The O/L for their high-oleate pairs increased from 13.8 when grown in 22/18^{o}C to 16.2 when grown in 26/22^{o}C and 25.4 when grown in 30/26^{o}C (Tables 3-5).

Temperature effect on total axis lipid fatty acid composition

There were no significant temperature or temperature by cultivar interaction effects on the axis lipid fatty acid composition or on O/L ratio, except for the temperature effect on palmitic acid. When averaged across the three temperature regimes for NC-V

11, NC-V 11 HO, Gregory, and Gregory HO, palmitic levels were 18.8, 12.7, 17.4, and 12.0%, respectively. The average levels of palmitic acid across the four cultivars were 13.9, 15.3, 16.6% in three temperature regimes, 22/18, 26/22, and 30/26^{o}C. Thus, seed produced in the low growth temperature had significantly lower amounts of palmitic acid in their axis (Tables 3 and 6).

The high-oleate trait expressed in the oils of the whole seed (primarily storage oils) was also found in the total lipid fraction of embryonic axis. The average oleic acid content for whole seed of NC-V 11 and Gregory was 47.5 and 51.5% (Table 4) and for their axis, these values were 31.0 and 32.1% (Table 6). Similarly, whole seed values of oleic for NC-V 11 HO and Gregory HO were 80.3 and 80.2%, while axis values were 62.1 and 62.5%. Likewise, the lower linoleic acid levels in the high-oleate whole seed lipids were also expressed in the axis total lipid fraction. The respective changes in axis oleic and linoleic acid content are reflected in the O/L ratio. While the whole seed O/L ratio of the high-oleate cultivars was much greater than that of the axis, our data shows that axis O/L ratio is much higher for the high-oleate lines than for their paired normal lines.

Temperature effect on axis phospholipid fatty acid composition

The effects of temperature on axis phospholipid fatty acids were similar to the effects on axis total lipid composition. There were no significant interactions between temperature and cultivar on any axis phospholipid fatty acid components, nor on O/L ratio. Except for stearic acid, temperature had no significant effect on axis phospholipids fatty composition or O/L ratio (Table 3 and 7). Like the axis total lipid fraction, NC-V

11 HO and Gregory HO axis phospholipids had much higher levels of oleic acid (63.6 and 63.2%) compared to their normal paired cultivars (30.5 and 32.4%). The comparable reduction in linoleic acid content was also seen and reflected in the O/L ratio.

Phospholipids are primarily located in membranes, and membrane function and fluidity are greatly influenced by fatty acid composition. Membranes with high levels of unsaturated fatty acids (18:2) are more fluid and more functional (Somerville, 1992). Our study shows that the high-oleate trait is expressed, not only in the storage lipids of the peanut seed cotyledons and axis, but also the membranes of the axis. The embryonic axis is the seed component where seedling growth begins. Therefore, it is possible that changing axis membrane fatty acid composition and function will impact seedling growth rate and the ability of the seedling to tolerate less than optimum growth temperatures. Low seed supply in this study prevented accurate evaluation of seed quality. NC-V 11 and NC-V 11 HO seed from the 2004 study were analyzed for electrical conductivity of seed soak water (data not shown). We found that the high-oleate line had higher conductivity (lower seed vigor) than the normal cultivar at all production temperature regimes and that conductivity increased five fold for seeds grown in the 22/18^{o}C environment compared to those grown in 30/26^{o}C. Sun et al. (2005) found that four out of six high-oleate peanut lines were significantly lower in vigor than their paired normal cultivar in field experiments conducted in 2003 and 2004.

Conclusion

This study showed that low growth temperature significantly affected whole seed fatty acid composition, but had little or no effect on axis total or phospholipid fatty acids.

Seed grown in low temperatures had decreased whole seed oleic and increased linoleic content, resulting in a decrease in the O/L ratio for seeds grown in cooler temperatures. In our study we also found significant temperature by cultivar interactions for O/L. The whole seed O/L ratio of high-oleate cultivars decreased significantly with decreasing growth temperature, but the corresponding decrease in the normal cultivars was not significant.

The high oleate trait seen in the storage lipids of the whole seed was also expressed in the embryonic axis. We saw a significant increase in the O/L ratio of NC-V 11 HO and Gregory HO axis total lipids and axis phospholipid when compared to their normal paired cultivars.

Further studies are needed to determine if the change in axis phospholipids O/L ratio seen in the high-oleate lines is responsible for any change in membrane function and peanut seed vigor.

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Table 1. Pedigrees of high-oleate lines developed by back-crossing the Florida high-oleate F 435 line with large-seeded Virginia-type cultivars.

Line	Background Genotype	Parentage[a]	Pedigree[b]
NC-V 11 HO	NC-V 11	NC-V 11*4/ F435	BC3F1-02-01: F05
Gregory HO	Gregory	Gregory*3 // NC 9*2 / F 435	BC2F1-01-03: F04

[a] Purdy et al.'s method for illustrating parentage is used.

[b] Pedigrees indicate the BC_nF_1 and BC_nF_2 plants selected and the current generation of the test material

Table 2. Average days to maturity of the four cultivars grown in three temperature environments.

Temperature	2003	2004
	Days	Days
22/18˚C	175	174
26/22˚C	161	160
30/26˚C	129	130

Table 3. Mean squares from analysis of variance of fatty acid and O/L for four peanut cultivars grown in three temperature environments.

Source	df	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Oleic/Linoleic (O/L)
Whole Seed Fatty Acids						
Temperature	2	20.29	3.23**	213.22**	243.48**	423.38
Cultivar	3	152.82**	0.38	10873.11**	8519.56**	3117.76**
Temp*Cultivar	6	0.29	0.25	27.68*	26.51*	119.08
Error		0.14	0.05	4.28	3.27	10.97
CV		5.07	9.99	3.18	9.68	33.16
Axis Fatty Acids						
Temperature	2	15.40*	0	1.27	2.4	0.09
Cultivar	3	68.53**	0.11	1891.34**	1371.85**	29.49**
Temp*Cultivar	6	0.17	0.01	1.07	0.59	0.08
Error		0.50	0.02	3.23	1.55	0.25
CV		4.63	9.80	3.83	4.65	18.38
Axis Phospholipid Fatty Acids						
Temperature	2	55.06	0.05*	36.48	3.59	0.01
Cultivar	3	281.86**	1.08**	2045.43**	754.45**	47.41**
Temp*Cultivar	6	1.91	0.02	2.16	0.49	0.05
Error		1.00	0.02	2.22	2.0	0.10
CV		3.54	7.41	3.15	6.92	9.18

*, ** significant at 0.05 and 0.01 levels, respectively

Table 4. Adjusted means of whole seed fatty acids of four peanut cultivars grown in three temperature environments.

Source Temperature Day/night	Palmitic (16:0)		Stearic (18:0)		Oleic (18:1)		Linoleic (18:2)		Oleic/linoleic (O/L)	
	NO	HO	NO	HO	NO	HO	NO	HO	NO	HO
NC-V 11					%					
22/18˚C	9.0	5.0	2.0	2.1	45.5	79.5	36.4	5.8	1.3	14.3
26/22˚C	9.4	5.3	2.2	2.1	46.8	80.7	35.1	5.3	1.3	16.0
30/26˚C	10.6	6.3	2.4	2.6	50.1	80.9	31.0	4.3	1.6	23.5
Mean	9.7	5.5	2.2	2.3	47.5	80.3	34.2	5.1	1.4	17.9
Gregory										
22/18˚C	8.0	5.1	2.0	2.1	48.0	78.6	34.0	6.6	1.4	13.4
26/22˚C	8.5	5.2	2.4	2.3	50.3	80.3	32.0	5.2	1.6	16.4
30/26˚C	9.1	6.2	2.5	2.9	56.2	81.7	25.9	3.5	2.2	27.2
Mean	8.5	5.5	2.3	2.4	51.5	80.2	30.6	5.1	1.7	19.0

NO = Normal cultivar
HO = High-oleate cultivar

Table 5. Probability associated with their F-statistic for temperature by cultivar interaction effect for oleic acid, linoleic acid, and O/L in whole seed of each cultivar

Cultivar	df	Pr > F		
		Oleic Acid	Linoleic Acid	O/L
NC-V 11	2	0.0138	0.0026	0.9910
Gregory	2	0.0006	0.0002	0.9463
NC-V 11 HO	2	0.4225	0.2902	0.0217
Gregory HO	2	0.0692	0.0297	0.0049

Table 6. Adjusted means of axis fatty acids of four peanut cultivars grown in three temperature environments.

Source	Palmitic (16:0)		Stearic (18:0)		Oleic (18:1)		Linoleic (18:2)		Oleic/linoleic (O/L)	
Temperature Day/night	NO	HO	NO	HO	NO	HO	NO	HO	NO	HO
	%									
NC-V 11										
22/18˚C	17.3	11.6	1.5	1.4	31.7	62.3	40.7	14.0	0.8	4.5
26/22˚C	18.8	12.8	1.5	1.3	31.3	62.5	39.9	13.8	0.8	4.5
30/26˚C	20.4	13.7	1.5	1.3	30.2	61.6	39.8	14.0	0.8	4.5
Mean	18.8	12.7	1.5	1.3	31.0	62.1	40.1	13.9	0.8	4.5
Gregory										
22/18˚C	15.8	10.7	1.5	1.3	33.2	61.7	39.9	14.6	0.8	4.3
26/22˚C	17.3	12.1	1.6	1.2	31.6	63.2	40.0	13.0	0.8	4.9
30/26˚C	19.1	13.3	1.5	1.3	31.5	62.6	38.7	12.5	0.8	5.0
Mean	17.4	12.0	1.5	1.3	32.1	62.5	39.5	13.4	0.8	4.7

NO = Normal cultivar
HO = High-oleate cultivar

Table 7. Adjusted means of axis phospholipid fatty acids of four peanut cultivars grown in three temperature environments.

Temperature	Palmitic (16:0)		Stearic (18:0)		Oleic (18:1)		Linoleic (18:2)		Oleic/linoleic (O/L)	
Day/night	NO	HO	NO	HO	NO	HO	NO	HO	NO	HO
	%									
NC-V 11										
22/18˚C	31.1	21.1	2.2	1.4	33.6	64.2	31.1	11.3	1.1	5.7
26/22˚C	34.4	20.9	2.1	1.4	30.3	64.6	31.3	11.2	1.0	5.8
30/26˚C	37.5	24.4	2.1	1.3	27.7	62.0	30.4	10.1	0.9	6.2
Mean	34.3	22.1	2.1	1.4	30.5	63.6	30.9	10.9	1.0	5.9
Gregory										
22/18˚C	31.9	21.3	2.1	1.5	34.2	64.3	29.8	10.9	1.1	5.9
26/22˚C	32.5	20.9	2.0	1.2	33.2	64.9	30.5	10.8	1.1	6.0
30/26˚C	37.7	25.3	2.3	1.5	29.9	60.5	27.9	10.4	1.1	5.9
Mean	33.0	22.5	2.1	1.4	32.4	63.2	29.4	10.7	1.1	5.9

NO = Normal cultivar
HO = High-oleate cultivar

Effect of Production Environment on Seed Quality of Normal and High-Oleate Large Seeded Virginia-Type Peanut (*Arachis hypogaea* L.)

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ABSTRACT

Six Virginia-type peanut (*Arachis hypogaea* L.) cultivars and their paired backcross-derived high-oleate lines were grown at the Peanut Belt Research Station near Lewiston, NC in 2003 and 2004. A split-plot experimental design was used with 2 × 2 factorial combinations of planting and harvest date as whole plot treatments, and 2 × 6 factorial combinations oleic acid and cultivars as subplot treatments. Seed quality evaluation included standard germination (SG), cool germination (CG), and electrical conductivity (EC). Oleic acid level had no influence on SG but did significantly alter CG and EC of the high-oleate lines. Averaged across background genotypes, high-oleate lines had lower seed vigor than their paired lines with normal oleic content. The high-oleate lines of three of the six pairs had significantly lower CG and higher EC. Planting and harvest date affected all the seed quality traits measured. SG of both normal and high-oleate lines was reduced in 2004 when harvest was delayed, but was not affected in 2003. In 2003, CG of the high-oleate lines was significantly lower than that of normal lines in three of the four production environments, EC was significantly higher in the high-oleate lines in all planting date and harvest date combinations. In 2004, there was no statistical difference between the CG of normal and high-oleate lines, but EC was significantly higher in the high-oleate lines for three of the four environments.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a rich source of edible oils, protein, and minerals such as phosphorus, calcium, magnesium, potassium, and vitamins (Savage and Keenan, 1994). In the U.S., peanut is an important cash crop and is largely used for peanut butter and snacks while it is used primarily as an oil source in most other peanut production areas of the world.

Seed oxidative stability is closely associated with its oil composition, therefore, fatty acid composition in peanut seed is an important quality attribute regardless of whether it is used as food or oil. The two predominant fatty acids in peanut seed oil are oleic acid (18:1) and linoleic acid (18:2) which together comprise about 80% of the total fatty acid composition (Ahmed and Young, 1982). Typically, peanut oleic acid to linoleic acid ratio, commonly referred to as O/L, is about 1.5 to 2.0(approximately 50% oleic acid to 30% linoleic acid). Oleic acid, the 18-carbon monounsaturated fatty acid and precursor to linoleic acid, is less reactive with oxygen and as a result, significantly more stable than linoleic acid. Therefore, peanut seeds with high oleic acid content and high O/L have improved stability against lipid oxidation that lead to adverse flavors (Mugendi et al., 1998; O'Keefe et al., 1993) and nearly twice the oil stability and shelf-life as those with normal O/L ratio (Braddock et al., 1995; Branch et al., 1990; James and Young, 1983). Virginia-type peanut cultivars naturally produce oil with slightly lower linoleic percentage and therefore have greater oil stability than other market types (Norden et al., 1982).

Norden and coworkers in the late 1980s, identified a high-oleate peanut trait in a runner breeding line (Norden et al., 1987). The line, F 435, was found to have 80% oleic acid and 2% linoleic acid. Developing Virginia-type cultivars with higher levels of oleic acid and depressed levels of linoleic acid is one of the goals of the peanut breeding program at North Carolina State University. High-oleate peanut lines were developed through backcrosses using the high-oleate line F 435 as the source of the trait and large-seeded Virginia cultivars such as NC-V 11 and Gregory as the recurrent parents. High-oleate lines were selected after two to four backcrosses to the commercial cultivar. The program has resulted in several advanced high-oleate peanut lines with 80 to 85% oleic acid content in their seed oil (Isleib, personal communication, 2005).

A uniform stand of healthy, vigorous seedlings is essential if growers are to achieve the yield and quality needed for profitable peanut production. Thus, seed quality is critical for growers. According to Spears et al. (2002), peanut seed quality can be separated into five related components: germination, vigor, genetic purity, crop purity and health. Of all the components, germination and vigor have the greatest impact on seedling emergence and survival. Germination tests are standardized throughout the U.S.. Vigor tests, however, are common only for major commodities and high value crops.

The cold test, cool germination test (CG), and electrical conductivity test (EC) are among the most widely used vigor tests in the commercial seed industry. The cold test was developed to assess the seedling's ability to tolerate low temperature stress, which often occurs in early spring planting. Seeds are placed in soil or paper towels lined with

soil and exposed to cold temperature, often in the presence of soil pathogens for a specified period of time then placed under favorable growth conditions and allowed to germinate. The results are useful for predicting the quality of the seed since the test evaluates the seed and seedling behavior under sub-optimal conditions. The cold test is widely used in the corn and soybean seed industry.

The cool germination test, the most widely used vigor test for cotton (*Gossypium hirsutum* L.), is conducted at low temperatures (18ºC) but unlike the cold test, the cool germination test does not expose the seed to pathogens. This test procedure was developed to provide less demanding but sufficiently severe test conditions to separate seeds on the base of vigor (ISTA, 1995).

Low-vigor seeds have been shown to possess decreased membrane integrity due to deterioration or injury (Copeland and McDonald. 2001). The electrical conductivity (EC) is a biochemical test that measures the amount of electrolytes that leak through the seed coat during imbibition. Higher conductivity, caused by increased cell leakage, would indicate a low-vigor seed lot. EC is a suggested vigor test for large-seeded legumes (Matthews and Powell, 1987) and protocols have been developed to evaluate seed vigor for peas (*Pisum sativum* L.) (Bradnock and Matthews, 1970), soybean (*Glycine max* L.) (Yaklich and Abdul-Baki, 1975), common beans (*Phaseolus vulgaris* L.) (Kolasinska et al., 2000) and broad bean (*Vicia faba* L.).

Although cold test, cool germination test and electrical conductivity test are used routinely in the corn (*Zea mays* L.), cotton, and soybean seed industries, respectively, to

identify seed lots with potential field emergence problems, no tests have been developed for the routine evaluation of peanut seed vigor.

Seed quality and vigor are greatly influenced by environmental factors that occur during seed growth, development and maturation (Copeland and McDonald, 2001; TeKrony et al., 1984). High temperature during development has been shown to reduce soybean seed germination and vigor (Egli et al., 2005; Keigley and Mullen. 1986; Spears et al., 1997). In their study, Spears et al. (1997) reported that soybean seeds grown in 27/22ºC day/night temperature had higher germination and accelerated aging germination and lower EC than those grown at 38/33ºC high temperature stress environment. Temperature also has a significant influence on peanut plant and pod growth rates (Leong and Ong, 1983). High temperature reduces the numbers of pegs and pods (Ketring, 1984) and the reductions in pod and seed dry weight were reportedly due to reductions in total dry matter and harvest index (Craufurd et al., 2002).

In addition to influencing seed planting quality, production environment can also affect peanut seed chemical composition. Dwivedi et al. (1993) reported significant genotype, environment and genotype-by-environment interaction effects on oil content, individual fatty acid content and derived oil quality in peanut. Change in seed chemical composition has been identified as one of the factors that can influence the seed germination and vigor (Copeland and McDonald, 2001).

While much of the peanut industry's concern has been about peanut dietary oil quality, seed technologists know that lipids are an important source of energy for

germination. Consequently, altering peanut seed fatty acid or total lipid composition could influence germination rate, seed and seedling vigor, and seedling survival, especially if the seeds are planted in stressful soil conditions. Alterations of seed lipid fatty acid composition brought by traditional or molecular techniques could also change membrane lipid composition and therefore affect membrane function and permeability. Membrane integrity is linked to seed quality, seedling energy, and tolerance to environmental stress during germination and emergence. However, there is little information available in the literature on the effect of altering peanut seed lipid on seed membrane function, germination, or vigor.

With the increasing number of high-oleate peanut cultivars, there is a need to analyze quality of high-oleate peanut seed produced in various environments. The objective of this study was to 1) determine the influence of high oleic acid on peanut seed germination and vigor and 2) evaluate planting date and harvest date affect on normal and high-oleate peanut seed germination and vigor.

MATERIALS AND METHODS

High-oleic peanut lines NC 7 HO, NC 9 HO, NC 10C HO, NC-V 11 HO, NC 12C HO, and Gregory HO were developed by backcrossing the high-oleate trait from F 435 into six large-seeded Virginia-type cultivars: NC 7, NC 9, NC 10C, NC V-11, NC 12C, and Gregory. These six large-seeded Virginia-type cultivars are grown in the North Carolina / Virginia peanut production area and each has 50 to 55% oleic acid content in

the seed oil. High-oleic lines were selected after two, three, or four backcrosses to the cultivar and their seed oils contain 80 to 85% oleic acid.

Experimental design

Experiments were conducted at the Peanut Belt Research Station near Lewiston, N C in 2003 and 2004. The treatment design included two planting dates, early May and early June, and two harvest dates early October and late October. A split-plot experimental design was used with 2 × 2 factorial combinations of planting and harvest date serving as whole plot treatments. The factorial combination for two peanut oleic acid levels and six cultivars were subplot. Each subplot consisted of two rows, each on a raised bed spaced on 91 cm centers and 10 m in length. Experimental test plots were established according to the treatment design of the experiment and standard production practices appropriate for the region were followed.

Harvest procedure

At harvest, plants were dug with a commercial digger/inverter and pods were removed by machine several days after digging, placed in mesh bags, and dried over non-heated forced air to approximately 8% seed moisture content (fwb). Sound mature seeds were collected from each plot, placed in plastic bags, and stored at 12ºC until evaluation. Seed quality evaluation included standard germination (SG), cool germination (CG), and electrical conductivity (EC).

Determination of seed standard germination

Prior to SG evaluation, seeds were treated with Vitavax PC [45% captan, 15% PCNB, 10% carboxin (5, 6-dihydro-2-methyl -N- phenyl-1, 4-oxathiin-3-carboxamide)]. SG tests were conducted by placing four 25-seed subsamples in rolled towels at alternating 30°/20°C (day/night) with 16 h at 20°C. The standard germination test procedure followed Association of Official Seed Analysts Rules for Testing Seeds (AOSA, 2002). Seedlings were evaluated at 8 days after planting and germination was assessed as the percentage of seeds producing a normal seedling with a radical at least 3.5 cm long.

Determination of seed cool germination rate

Seeds were randomly sampled from the same seed lot used for SG and pretreated with Vitavax PC. CG tests were conducted on four 25-seed subsamples placed in rolled towels at 18°C (± 0.5°C). The test was conducted in the dark. Seedlings were evaluated at 9 days after planting and only those seedlings with radicals 2.5 cm or longer were considered to have germinated.

Determination of seed electrical conductivity

Seed vigor was also evaluated by EC of seed soak water. Seeds were adjusted to about 7.5% moisture content in a chamber at 20°C and near 95% RH prior to measuring seed leakage. Fifty seeds were weighed and placed 250 ml of 25°C distilled water. Containers were covered and held at 25°C (±0.5°C) for 24 (±0.5) h. Following incubation, the containers were gently swirled and the conductivity of the seed soak water was measured using a Cole Palmer Conductivity meter model 1481-60 (Cole-Palmer

Instrument Company, Niles, IL). Conductivity is reported as μmhos cm^{-1} g^{-1} of seed. Two randomized subsamples from each field plot as two replications and a completely randomized design (CRD) were used in this experiment.

Statistical analysis

Prior to statistical analysis, SG and CG percentages were transformed using the angular (arcsin of square root) transformation and EC values were transformed using natural log. Analysis of variance was conducted using the general linear models procedure (PROC GLM) of SAS (SAS Institute, Inc., Cary, NC). Years and replications were considered random effects while planting and harvest dates, oleic acid level, and cultivar were considered fixed effects. Treatment means were separated by t-test using a significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

High-oleate trait influence of seed quality

There were no significant oleic acid levels by cultivar interactions for any trait measured (Table 2). Likewise, peanut seed oleic acid level had no effect on SG, but did have a significant effect on CG and EC.

All 12 lines, regardless of production environment or oleic acid level, had standard germination percentages above 88 % (adjusted mean) and there were no significant differences for SG between the paired normal and high-oleate lines (Table 3). Thus, it

appears that SG is not affected by the elevated oleic acid content found in the high-oleate lines.

When averaged across production environments, peanut seed CG was lower than SG for each cultivar used in this study (Table 3). The differences between SG and CG were larger for high-oleate lines than for those corresponding normal lines. The high-oleate lines had adjusted means for ranging from 66.4 to 76.0% while the normal lines had values ranging from 76.2 to 82.3%. Three high-oleate lines, NC 10C HO, NC V 11 HO, and NC 12C HO had significantly lower CG (66.9, 66.4, and 67.4%) than their paired NC 10C, NC V 11, and NC 12C parents (82.3, 77.8, and 76.2%).

Oleic acid level also had significant effect on peanut seed EC (Table 3). Three high-oleate lines NC V11 HO, NC 12C HO and Gregory HO had significantly higher EC (2.98, 2.93, and 2.61 μmhos cm^{-1} g^{-1}) than their three paired normal lines (2.23, 2.32, and 2.10 μmhos cm^{-1} g^{-1}).

There was no correlation between SG and CG or between SG and EC. However, CG and EC was significantly and negatively correlated ($r = -0.73$, $P<0.01$) (Fig.1). Although they measure different aspects of seed vigor, the two vigor tests appear to be in agreement when evaluating the seed lot vigor potential for the seed used in our study. It is interesting that the two outlying points are NC 10C and NC 10C HO. Why this cultivar pair does not fit into the pattern of the other five cultivar pairs is not known. Further tests might reveal any genetic influence on subsequent seed quality.

The standard germination test is designed to provide the maximum germination percentage for the seed lot tested. If seeds are planted in fields with near ideal conditions, SG will provide a good estimate of seedling establishment. However, soil environments are rarely ideal and the SG test may not accurately reflect potential seedling emergence and survival. Seed vigor tests were developed to help seedsmen better evaluate seed lot field emergence potential. In our study, seed vigor of four of the six paired high-oleate, normal lines (NC 10C, NC-V11, NC 12C and Gregory) was significantly reduced when the high-oleate trait was incorporated into the cultivar.

Production environment influence on seed quality

Planting and harvest dates had significant effects on SG, CG and EC. There were however, no genotype by oleic interactions (Table 2). Because of the wide temperature and rainfall pattern variations seen in 2003 and 2004 (Figure 2), the data is presented as eight environments (two years by two planting dates by two harvest dates) for mean comparisons.

In 2003, there was no difference in peanut SG for planting date or for harvest date (Table 4). Germination was above 90% in all production environments. In 2004, however, germination of both normal and high-oleate peanut seed harvested on October 26 was significantly lower than those harvested 20 days earlier. In addition, peanut seed planted in June and harvested on October 26 had lower SG than those planted in May and harvested on October 26. The 2004 peanuts harvested on October 6 were dug 4 days prior to combining. However, rain delayed combining on the late October harvest. Peanuts

remained in the windrows for 10 days before combining. It appears that this delay and exposure to wet conditions dramatically reduced peanut quality for all samples.

In the 2003 production environments, CG for normal cultivars was significantly higher than that of the high-oleate lines, with the exception of CG for peanuts planted on May 8 and harvested on October 7 (Table 4). Delaying harvest of peanut planted in May resulted in a large increase on CG for the normal cultivars (67.7 to 81.4%) but only a slight increase in CG for the high-oleate lines (62.5 to 63.9%). When peanuts were planted in June, delaying harvest date resulted in a large increase in CG for the high-oleate lines (67.0 to 79.7%) but only a slight increase for the normal cultivars (85.3 to 87.8%). The highest CG value for the high-oleate lines (79.7%) occurred when peanuts were planted in June and harvested on October 21.

EC was significantly lower in normal cultivars compared to the paired high-oleate line in 2003 for all planting and harvest dates (Table 4). EC values of May and June planted normal and high-oleate peanuts decreased when the harvest date was delayed. One would predict that the peanut seed with the most time to develop (planted in May and harvested October 21) would have the highest vigor potential. Indeed, the lowest EC, 1.42 and 1.62 μmhos cm^{-1} g^{-1} for normal and high-oleate lines, respectively, was found in these peanuts. However, highest CG was found in peanuts, both normal and high-oleate, planted in June and harvested on October 21. EC and CG tests measure different mechanisms associated with seed vigor. It is possible that the components of seed vigor reach maximum potential at different times in peanut seed development or that they vary in their response to production environment.

In 2004, there were no significant differences between the CG of normal and high-oleate lines for any planting date/harvest date combination. EC of the normal cultivars was significantly lower than that of the high-oleate lines with the exception of the May planting, October 26 harvest. For both planting dates, CG was significantly reduced when harvest date was delayed and the lowest CG (54.8 and 52.4 % for normal and high-oleate, respectively) and highest EC (3.40 and 3.87 μmhos cm^{-1} g^{-1}) occurred when peanuts were planted in June and harvested on October 26. Highest CG values (92.0 % for both normal and high-oleic) and lowest EC (2.23 and 2.45 μmhos cm^{-1} g^{-1}) occurred when peanuts were planted in May and harvested on October 6.

Seed lot quality of most species is influenced by production environment. The two planting and harvesting dates for 2003 and 2004 in this experiment represented diverse production environments (Fig 2). Cold and wet weather occurred between the first and second harvest dates in 2004, which resulted lower SG, CG and higher EC for seeds harvested on October 26 compared to those harvested on October 6, regardless of planting date. In contrast, 2003 weather conditions between the first and second harvests were cool with only trace amounts of rainfall. SG of peanuts harvested on October 21 was not significantly different than that of seed harvested on October 7, regardless of planting date. Seed vigor of 2004 peanuts for both planting dates, as measured by CG and EC actually, increased when harvest date was delayed. However, CG and EC tests for the two years revealed that across genotype, high-oleate lines had lower vigor than normal peanut lines in each planting date and harvest date environment.

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Table 1. Pedigrees of high-oleate lines developed by back-crossing the Florida high-oleic genes into large-seeded Virginia-type cultivars.

Line	Background Genotype	Parentage [a]	Pedigree [b]
NC 7 HO	NC 7	NC 7*5 / F-435	BC4F1-03-11: F04
NC 9 HO	NC 9	NC 9*5 / F-435	BC4F1-01-03: F04
NC 10C HO	NC 10C	NC 10C*4 / F-435	BC3F1-01-22: F05
NC-V 11 HO	NC-V 11	NC-V 11*4/ F-435	BC3F1-02-01: F05
NC 12C HO	NC 12C	NC 12C*3 // NC 9*2 / F-435	BC2F1-01-01: F04
Gregory HO	Gregory	Gregory*3 // NC 9*2 / F-435	BC2F1-01-03: F04

[a] Purdy *et al.*'s method for illustrating parentage is used.

[b] Pedigrees indicate the BC_nF_1 and BC_nF_2 plants selected and the current generation of the test material.

Table 2. Mean square from pooled analysis of variance of standard germination (SG), cool germination (CG), and electrical conductivity (EC).

Source	df	SG	CG	EC
		%		μ mhos cm^{-1} g^{-1}
Year	1	0.2495**	0.0002	77.1779**
Planting date in year	2	0.0963**	0.6335**	15.6289**
Harvest date in year	2	0.5304**	1.8133**	18.2305**
Plant × harvest in year	2	0.0416**	0.0171	0.4384
Oleic acid level	2	0.0008	0.4889**	10.3654*
Genotype	5	0.0111	0.0260	1.0588
Oleic × genotype	5	0.0059	0.0566	1.8595
Error		0.0027	0.0134	0.0818
CV(%)		5.7347	15.6118	11.54

* and ** Denote mean squares that are significant at $P<0.05$ and $P<0.01$, respectively

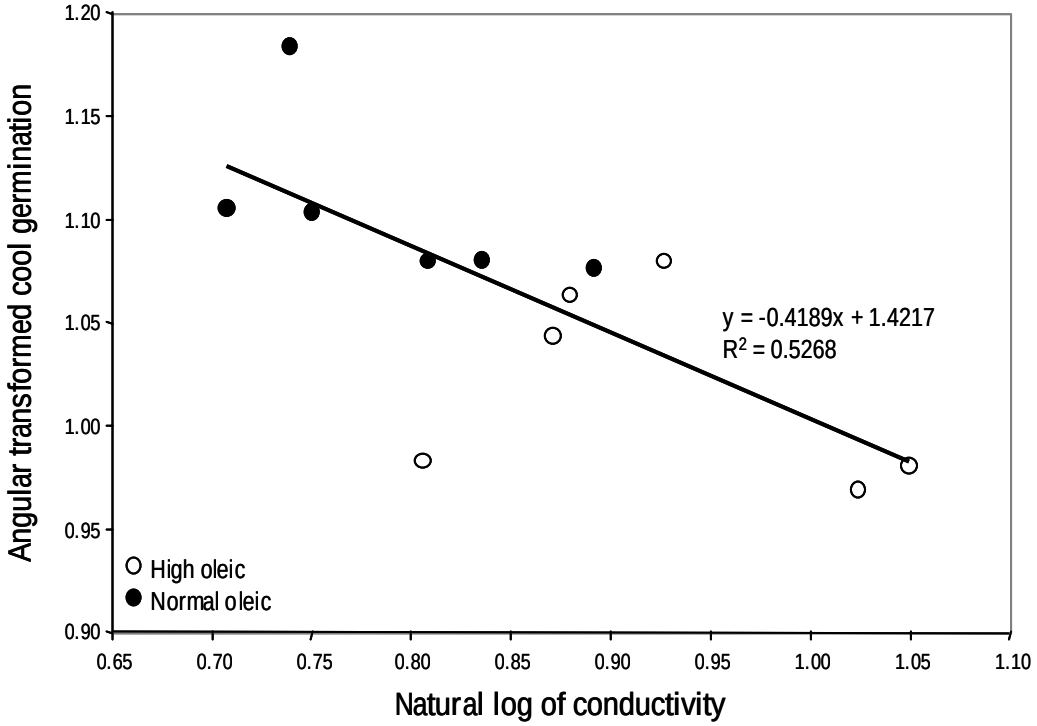

Fig. 1. Regression of log of conductivity and angular transformation ($\arcsin\sqrt{X}$) of cool germination for normal and high-oleic peanut.

Table 3. Adjusted means of standard germination (SG), cool germination (CG), and electrical conductivity (EC) of six normal and high-oleic peanut lines.

Oleate level	Genotype	SG	CG	EC
		%	%	$\mu mhos\ cm^{-1}\ g^{-1}$
Normal	NC 7	88.2	76.0	2.42
High	NC 7 HO	89.0	72.9	2.47
Normal	NC 9	89.4	76.0	2.58
High	NC 9 HO	93.3	74.5	2.47
Normal	NC 10C	91.6	82.3**	2.22
High	NC 10C HO	91.6	66.9	2.40
Normal	NC-V 11	90.7	77.8*	2.23*
High	NC-V11 HO	89.0	66.4	2.98
Normal	NC-12C	91.1	76.2*	2.32*
High	NC-12C HO	89.4	67.4	2.93
Normal	Gregory	92.2	78.3	2.10*
High	Gregory HO	92.8	76.0	2.61

* and ** Denote significant differences between the normal- and high-oleate variants within a background genotype by t-test at the 0.05 and 0.01 α-levels, respectively. Statistical difference is based on angular transformation of SG and CG and natural log transformation of EC.

Table 4. Difference of seed quality between normal and high-oleate seed produced in varied planting and harvest dates, estimated by adjusted means of standard germination (SG), cool germination (CG) and electrical conductivity (EC).

Planting	Harvest	Oleate level	SG	CG	EC
			—— % ——		*µmhos* cm^{-1} g^{-1}
2003 season					
May 8	October 7	Normal	93.0	67.7	1.92**
May 8	October 7	High	92.6	62.0	2.27
May 8	October 21	Normal	91.4	81.4**	1.42**
May 8	October 21	High	92.3	63.9	1.62
June 3	October 7	Normal	94.3	85.3**	2.40**
June 3	October 7	High	94.9	67.0	2.78
June 3	October 21	Normal	94.3	87.8*	1.71**
June 3	October 21	High	94.9	79.7	2.08
2004 season					
May 12	October 6	Normal	97.5	92.1	2.23**
May 12	October 6	High	97.9	92.0	2.45
May 12	October 26	Normal	84.5	71.3	2.75
May 12	October 26	High	85.8	65.0	2.9
June 7	October 6	Normal	94.9	82.7	2.65**
June 7	October 6	High	96.1	83.0	3.16
June 7	October 26	Normal	74.4	54.8	3.40**
June 7	October 26	High	72.4	52.4	3.87

* and ** Denote significant differences between the normal- and high-oleate variants averaged across background genotypes by t-test at the 0.05 and 0.01 α-levels, respectively. Statistical difference is based on angular transformation of SG and CG and natural log transformation of EC.

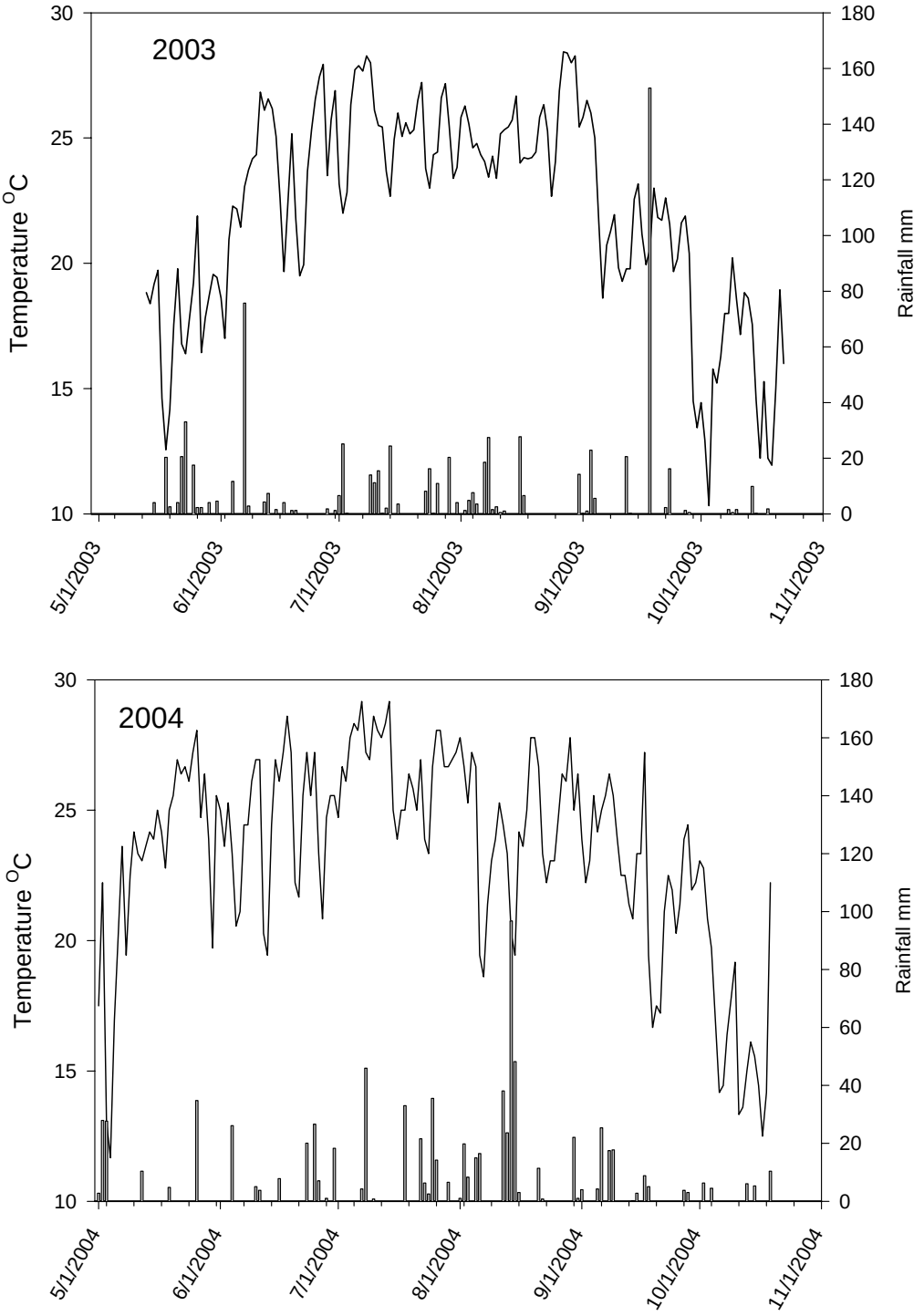

Fig.2. Weather data for Peanut Belt Research Station, Lewiston, NC in 2003 and 2004.