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Yield and nutritional responses to waterlogging of soybean cultivars

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Abstract Furrow irrigating soybean prior to a large, unexpected rainfall event can reduce nitrogen fixation and crop yield. The objective of this study was to evaluate the tolerance of soybean cultivars to waterlogged alluvial soils. Five cultivars were selected, which showed a range of tolerances to excessive soil water. Flood duration and flood timing experiments were conducted on clay and silt loam soils. Main plots were flooding duration and flood timing and subplots were soybean cultivars. Most cultivars were able to withstand flooding for 48–96 h without crop injury. Cultivars flooded during the V5 growth stage suffered the least amount of yield loss. The greatest yield losses from flooding occurred at the R5 growth stage. Soybean yields from cultivars flooded at R5 were reduced by 20-39% compared to non-flooded checks. Pioneer 94B73 (cv.) had no significant change in yield from flooding for 192 h at any of the three growth stages, compared to non-flooded controls.

Introduction

Soybean (*Glycine max* (L.) Merrill) fields in the Mississippi River delta region of the USA occasionally flood due to excess rain or irrigation followed by rainfall. Symptoms

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of flood in soybean may include leaf yellowing, reduced root growth, reduced nodulation, stunted growth, defoliation, reduced yields and plant death (Linkemer et al. 1998; Minchin and Pate 1975; Oosterhuis et al. 1990; Purcell et al. 1997; Stanley et al. 1980). Waterlogged soil may damage plants directly or indirectly through enhanced plant disease.

Flooding suppresses activity of the enzyme nitrogenase in soybean roots, due to the lack of oxygen in flooded conditions (Sprent 1969; Minchin and Pate 1975). A small amount of oxygen (0.0016 atm) is required for optimal nitrogen fixation (Keister and Rao 1977). Sallam and Scott (1987) reported that flooding at V1 completely inhibited soybean nodulation. Soybeans growing in flooded soil in the greenhouse showed an initial N deficiency and reduced growth rates (Nathanson et al. 1984). Nitrogen accumulation in the shoots of nodulated peas and other legumes is slowed by soil waterlogging due to reduced nodulation and nitrogenase activity (Minchin and Pate 1975). Sullivan et al. (2001) showed that the flood duration was positively correlated with soybean leaf tissue Ca, Mg, B, Fe, Cu and Al, but was negatively correlated with soybean leaf N concentration.

Waterlogged soil conditions can cause injury or death to soybean plants. In Louisiana, 3–5 cm of rain per day falling on poorly drained soil was sufficient to reduce soybean growth and seed yield (Linkemer et al. 1998). Most soybean cultivars grown in continuously saturated soil averaged 40% less seed yields than furrow-irrigated soybeans (Purcell et al. 1997). Leaf yellowing following flooding was observed and was associated with a lag in N accumulation in soybean plants. The only cultivar that was not reduced by waterlogged soil was Asgrow 6297. Oosterhuis et al. (1990) observed that within 48 h of flooding at V4 and R2, photosynthesis was reduced by 33

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and 32%, respectively. Although seed yields were reduced in both Essex and Forrest cultivars compared to nonflooded checks, Forrest yields were reduced less than Essex.

Heatherly and Pringle (1991) found that soybean genotypes differ in their response to soil saturation. Cultivars varied in yield increases due to flood irrigation during the dry years. Yields also decreased differently for different cultivars during periods of rain after flood irrigation. Shannon et al. (2005) determined that yields of all cultivars tested were reduced by flood at R1, but there were differences among cultivars. Yields were reduced to 39% for the most flood-tolerant cultivars and 77% for the most flood-sensitive cultivars. VanToai et al. (1994) documented that flooding tolerance can be defined as high yield under flooding stress, and that differences in flood tolerance can be found between US soybean cultivars.

Some studies have shown that flood tolerance could be attributed to a genetic trait. VanToai et al. (2001) identified quantitative trait loci (QTL) in isolines associated with soybean tolerance to soil waterlogging. A single QTL linked to marker Sat_064, from the cultivar Archer was associated with improved plant growth and grain yields in waterlogged environments. This marker is close to the *Rps4* gene for *Phytophthora (Phytophthora sojae)* resistance. They concluded, however, that flood tolerance was not due to disease resistance (QTL).

Although the impact of soybean genetics on flood tolerance is not fully understood, there are some physical characteristics in certain soybean cultivars that may attribute to flood tolerance. Rapid formation of adventitious roots and aerenchyma between the stem (immediately above the water line), roots and nodules in flooded soybean have been observed (Thomas et al. 2005; Bacanamwo and Purcell 1999). Weisz and Sinclair (1987) concluded from greenhouse research that soybean nodules can adjust to a wide range of rhizosphere oxygen concentrations.

Flood tolerance depends not only on the physical characteristics of a particular cultivar, but also on the duration of flooding and the growth stage at which the crops were flooded. In Louisiana, Griffin et al. (1988) found that soybeans were more tolerant to waterlogged soils during vegetative growth stages than during reproductive stages. They also found that floods during reproductive stages should be removed within 2 days to avoid significant yield reductions.

Identifying soybean cultivars tolerant to flooding is essential for optimal production under these conditions. The objective of this study is to evaluate tissue nutrient concentrations and yield of soybean cultivars under various flood durations and flood timings on alluvial soils.

Materials and methods

Preliminary screening

Beginning in 2002, a 3-year cultivar screening trial was conducted on a zero-grade Sharkey clay soil (very-fine, smectitic, thermic Chromic Epiaquert) at the University of Missouri-Delta Research Center Lee Farm, Hayward, Missouri (36°N, 89°W) to identify the flood tolerance of all soybean cultivars entered in the Missouri Soybean Variety Testing Program. Tests were divided into three soybean maturity groups and arranged in replicate hill plots with eight seeds per hill, spaced 61 cm apart in rows and 76 cm between rows. When the majority of cultivars began blooming in each maturity group, plots were flooded and water was allowed to stand for 14 days. The plot area was drained and cultivars were rated for injury after 2 weeks. Cultivars were visually rated on a 1-5 scale, with 1 for plants showing no apparent injury, and 5 for all plants dead (Shannon et al. 2005). Studies in 2002 consisted of two replications. In 2003 and 2004, three replications were used. Visual observations of cultivars tested in 2002 were used to determine which cultivars would be evaluated in flood duration and growth stage experiments.

Most farmers in the region produce group IV maturity soybean cultivars. Based on visual ratings made in 2002, group IV soybean cultivars, Manokin, Pioneer Brand 94B73 (P94B73), Merschman Denver RRSTS (Mersch-Denver), Delsoy 4710 and DeltaKing 4868 (DK4868), were selected for further evaluations of flood tolerance. These represented a range of waterlog tolerances. Delsoy 4710 was included as a control with minimal flood tolerance.

Flood duration and flood timing experiments

In 2003 and 2004, field experiments were conducted on a Sharkey clay soil in Hayward, Missouri and a Tiptonville silt loam (fine-silty, mixed, superactive, thermic Oxyaquic Argiudoll) soil in Portageville, MO (36°N, 90°W). Separate experiments were conducted to study flood duration and flood timing effects on injury to soybean cultivars. Rows were prepared in March with 76 cm spacing using a hipper. Before planting, rows were harrowed leaving 8 cm tall planting beds.

A network of levees, irrigation pipes and drainage gates was constructed in the border rows between main plots to control plot flooding during the growing season. Levees were placed around each main plot to allow a controlled flooding environment for the subplots. Lay-flat irrigation pipe was placed across each main plot for flooding treatments. Adjustable gates were inserted in the pipe to allow operators to shut off flooding in some plots, while still flooding others. Plots were flooded with 2–4 cm of water maintained in the furrow of each soybean row. Weed species were controlled with imazaquin, thifensulfuron methyl, fomesafen and clethodim.

Split plot designs were used for flood duration and flood timing experiments. Main plots were four rows wide and 23 m long. Each of the five soybean cultivars was randomly assigned to subplots (3.7 m long) in each main plot. All treatments were replicated four times. Plots were planted with a cone planter during the 1st week of May in both 2003 and 2004. Cultivars were planted at a seeding rate of 10 seeds per ft. Each soybean cultivar was monitored for vegetative and reproductive growth to determine flood timing. At maturity, subplots were mechanically harvested for yield with a plot combine.

In flood duration tests, main plot treatments were flooded at full bloom flowering stage (R2) for durations of 0, 2, 4, 6 or 8 days for each of the soybean cultivars. Irrigation water was applied on a daily basis in each plot based on flood duration treatments. The plots were then immediately drained at the end of the specified flooding by cutting a trench in the levees.

In flood timing experiments, main plot treatments were flooded at either five trifoliate vegetative stage (V5), full bloom flowering stage (R2) or pod fill reproductive stage (R5) (Fehr et al. 1971). Main plots were flooded for 8 days (192 h) at their respective stages. Subplot treatments included each of the five selected soybean cultivars. Irrigation water was applied on a daily basis to maintain flooding, and immediately drained after 8 days by cutting a trench in the levees. Rainfall was recorded using the weather station at the University of Missouri farms at Portageville and Hayward, Missouri.

Soybean plant sampling

Soybean cultivars were allowed 2 weeks to recover from waterlogging damages before sampling. After the recovery period, 20 trifoliate leaves were collected from each plot and digested for nitrogen, potassium and phosphorus analyses using a modified wet acid dilution procedure (Mills and Jones 1996). Soybean leaf samples were dried at 100°C, ground, digested with a Hach DigesdahlTM Digestion Apparatus, 115Vac, 50/60 Hz (Hach Company, Loveland, CO) using H₂SO₄ and H₂O₂. Leaf potassium content was tested with a Perkin-ElmerTM (Wellesley, MA) atomic absorption spectrophotometer (Thomas 1982). Phosphorus and nitrogen were tested colorimetrically (Laverty 1963; Keeney and Nelson 1982) with a GenesysTM 10 spectrophotometer (Thermo Spectronic, Rochester, NY). In 2003, visual observations were made to identify any Phytophthora infestations.

Statistical analysis

The statistical analysis of flood duration and flood timing data was performed using Mixed Model procedures of the Statistical Analysis System (SAS 1997). The Mixed Model procedure provides Type III F values, but does not provide mean square values for each element within the analysis or the error terms. Mean separation was evaluated though a series of pairwise contrasts among all treatments (Saxton 1998). Probability levels greater than 0.10 were categorized as non-significant.

Results

The five cultivars selected for flood duration and flood timing research, conducted in 2003 and 2004, were based on flood screening results from cultivars entered in the 2002 Missouri State variety trials. However, the flood screening was continued in 2003 and 2004 to gather information on new cultivars. Approximately 360 soybean cultivars were visually evaluated in flood-tolerance screening tests each year. During the 3 years, some soybean cultivars were added and others dropped each year by seed companies. Group IV cultivars that were entered consistently throughout the 3 years are shown in Table 1. A significant year by cultivar interaction (P = 0.0015) for visual injury rates was found. Cultivars Mersch-Denver and DK4868 showed varying degrees of tolerance in different years. Cultivar P94B73 remained at a steady level of flood tolerance in each of the 3 years (Table 1). When the years were analyzed separately, the cultivars showed significant differences based on visual ratings in 2002 and 2003. In 2004, the cultivars showed no significant differences based on visual observation. All cultivars showed signs of injury. This injury ranged from moderate tolerance (2) to severe intolerance (5). Manokin and Delsoy 4710 were only screened for flood tolerance in 2002 before being dropped from the variety testing program. In 2002, Manokin was rated as highly tolerant to flooding, while Delsoy 4710 was rated as very intolerant.

Effect of flood duration on soybean leaf N, P and K

Averaged across cultivars, soybean leaf P content in plots that had 8 days of flooding was significantly lower than leaves collected from non-flooded check plots on both soil types (Table 2). Averaged across cultivars, the 8 days of flooding treatment also significantly reduced leaf N and K concentration compared to the untreated check on clay, but not on silt loam soil. On the Sharkey clay soil, leaf N content decreased numerically as flood duration increased.

Table 1 Visual ratings of soil waterlogging injury to group IV soy-
bean cultivars entered in the Missouri Soybean Variety Testing Pro-
gram on Sharkey clay soils at Hayward, MO in 2002, 2003 and 2004

Cultivar ^a	Visual ratings of flood injury (1 to 5) ^b			
	2002 ^c	2003	2004	
Armor 44–R4	3.7 cdef	1.5 a	2.0 a	
Armor 47–G7	4.2 efghi	2.5 abc	2.0 a	
ASGROW AG4201	4.7 hij	3.0 bcde	2.7 a	
ASGROW AG4403	4.7 hij	3.7 cde	2.0 a	
ASGROW AG4603	4.7 hij	2.5 abc	3.3 a	
Delta Grow 4860RR	4.5 ghij	3.3 bcde	2.7 a	
Delta King DK4461RR	4.3 fghij	3.0 bcde	2.0 a	
Delta King DK4763RR	4.3 fghij	3.5 cde	2.0 a	
Delta King DK4868RR	4.0 defgh	3.0 bcde	1.7 a	
Delta Pine DP 4933 RR	3.8 defg	4.3 e	2.7 a	
Dyna Gro DG3443NRR	4.7 hij	2.0 ab	2.7 a	
Excel Brand 8499NRR	4.8 ij	4.0 de	2.7 a	
FFR 4922RR	4.5 ghij	4.0 de	3.0 a	
Golden Harvest H-4368RR	3.7 cdef	4.0 de	2.0 a	
Hornbeck HBK R4820	3.8 defg	2.5 abc	2.3 a	
Mersch. Austin RR	3.8 defg	3.0 bcde	2.7 a	
Mersch. Dallas RR	3.5 bcde	3.7 cde	2.7 a	
Mersch. Denver RRSTS	2.0 a	3.5 cde	3.0 a	
Mersch. Phoenix IIIRR	3.3 bcd	3.5 cde	2.7 a	
MFA Morsoy RT 4201N	4.8 ij	4.3 e	2.7 a	
MFA Morsoy RT 4480N	3.5 bcde	2.5 abc	2.3 a	
MFA Morsoy RT4731N	3.7 cdef	3.0 bcde	2.7 a	
Pioneer 94B73	1.8 a	2.3 abc	2.3 a	
Pioneer 94B74	2.8 b	2.3 abc	2.0 a	
Progeny 4401RR	3.7 cdef	2.3 abc	2.0 a	
Progeny 4910	3 bc	2.0 ab	2.3 a	
Southern Cross Aaron	3.5 bcde	2.0 ab	2.7 a	
Southern Cross Silas	4.2 efghi	2.7 abcd	2.3 a	
Southern Cross Titus	3.3 bcd	3.0 bcde	2.3 a	
UniSouth USG 7440nRR	5 ј	3.3 bcde	2.3 a	
UniSouth USG 7499nRR	5 ј	4.3 e	2.7 a	
Willcross RR2432N	4 defgh	4.0 de	2.7 a	

^a Only cultivars tested for all 3 years (2002–2004) are shown

^b Rated 1–5 with 1 = no injury, 5 = all plants dead

 $^{\rm c}$ Within columns, visual ratings followed by the same letter were not significantly different at the 0.05 level

This is in agreement with studies conducted in Arkansas, where saturated soil culture resulted in soybean leaf yellowing due to a lag in N accumulation (Purcell et al. 1997). These differences in the effect of temporary flood on leaf N among soil types may be due to the ability of these two soils to dry after flood. Sharkey clay has poor internal drainage with slow surface runoff (Pettry and Switzer 1996). Tiptonville silt loam soil is classified as moderately

Table 2 Soybean leaf N, P and K content 2 weeks after flooding for
0, 2, 4, 6 and 8 days on Sharkey clay and Tiptonville silt loam soils
averaged across cultivars (Delsoy 4710, Delta King 4868, Manokin,
Mersch-Denver and Pioneer 94B73) in 2003 and 2004

Flood duration (days)	Leaf content					
	N g kg ⁻¹	Pg kg ⁻¹	K g kg ⁻¹			
Sharkey clay						
0	4.68 a	0.20 a	1.59 a			
2	3.23 b	0.22 a	1.42 b			
4	2.78 b	0.19 ab	1.30 b			
6	3.08 b	0.20 a	1.35 b			
8	2.81 b	0.18 b	1.32 b			
Tiptonville silt loam						
0	2.77 a	0.30 a	1.43 a			
2	2.85 a	0.27 a	1.46 a			
4	2.92 a	0.27 a	1.38 a			
6	2.76 a	0.23 b	1.44 a			
8	2.56 a	0.23 b	1.38 a			

Within soil types, soybean leaf contents followed by the same letter were not significantly different at an α -level of 0.05

well drained by the US Natural Resource Conservation Service (Soil Survey Staff 1971).

No significant interaction was found between flood duration and cultivar for leaf N. The cultivar effect on leaf N was significant at the 0.12 level. The effect of flood duration on leaf N had a consistent negative linear relationship for most cultivars, but not for Manokin, P94B73 and DK 4868 (Fig. 1). These cultivars had no decrease in leaf N content after 6 days of flooding compared to plots with no flooding. Cultivar DK4868 continued to have increase in leaf N concentrations up to 4 days of floods. Overall, P94B73 plants produced the highest leaf N content under flooded conditions, averaging 18-40% higher than other cultivars tested. These differences in leaf N content among cultivars exposed to temporary flood conditions may be because: (1) more robust N-fixing rhizobium may be associated with some cultivars than others, and (2) some cultivars may be able to recover more quickly than others after flood. Another possibility is that that these cultivars began translocating leaf N to pods later in the season than the other Group IV cultivars. However, this discovery that some cultivars are better adapted to temporary flood than others is encouraging, since these conditions can develop in fields due to irrigation or excess rain.

Our purpose was to determine differences in tolerance to temporary flood among cultivars. More research is needed to determine the mechanism(s) by which some cultivars are able to tolerate these conditions.

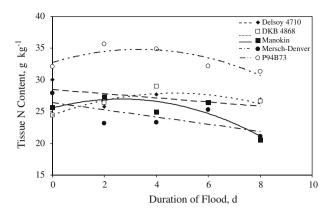


Fig. 1 Effect of flood duration at bloom stage on leaf N content of five group IV soybean cultivars averaged across loam and clay soils in 2003 and 2004 in Portageville and Hayward, Missouri. Best fit regression equations relating h of flood (*x*) to g kg⁻¹ leaf N (*y*) were Delsoy 4710 (y = 2.85 - 0.0014x), DK 4868 (2.44 + 0.0062 $x - 0.00003x^2$), Manokin (2.56 + 0.0045 $x - 0.00004x^2$), Mersch-Denver (y = 2.64 - 0.0024x), and P94B73 (3.27 + 0.0052 $x - 0.00003x^2$)

Effect of flood duration on soybean yields

The amount and timing of rainfall caused differences in soil waterlogging among years. A yield interaction among year, soil, cultivar and duration was found in the combined ANOVA from Proc Mix (P < 0.1). This interaction arose because of the weather conditions at Hayward, Missouri at the Sharkey clay site in 2004. The magnitude of soybean vield loss from flooding on the Sharkey clay soil in 2004 was much greater than in 2003. This is due to an excess of rainfall during the flooding and recovery stage of all treatments and main plots on the Sharkey clay soil. Although the total quantity of rainfall was less during May to August 2004 than in 2003, immediately after flood termination, 72 mm of rain fell on clay plots from 30 June to 6 July 2004. The plots remained under flooded conditions for more than 7 days after the planned flood termination due to the poor internal drainage of the Sharkey clay soil. Studies in Mississippi indicate that even relatively short flood durations can be detrimental if coupled with untimely rainfall (Heatherly and Pringle 1991).

Flood duration significantly affected soybean yields in both locations. Cultivar yield response to flooding on the silt loam site in 2003 and 2004 were similar to the clay site in 2003. Manokin, Mersch-Denver, and Delsoy 4710 cultivars had increased yield at 2 days of floods compared to check plots (Fig. 2). Heatherly and Pringle (1991) reported similar yield increases from short-term flooding. Cultivars P94B73 and DK 4868 had increased yield under flooding for up to 4 days' duration. Each of these cultivars then began to show declining yield at longer flood durations. Although standing water covered each plot during flood treatments, soil oxygen was not measured. It is possible

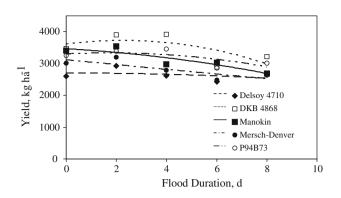


Fig. 2 Effect of flood duration on yield of five group IV soybean cultivars averaged across three environments (Tiptonville silt loam soil in 2003–2004 and Sharkey clay soil in 2003) at Portageville and Hayward, Missouri. Best fit regression equations relating h of flood (*x*) to kg ha⁻¹ soybean yield (*y*) were Delsoy 4710 (y = 2724.4 - 0.837x), DK 4868 (3612.4 + 4.2177 $x - 0.0387x^2$), Manokin (3517.8 - 4.0395x), Mersch-Denver (y = 3118.7 - 3.1047x), and P94B73 (3303.3 + 1.6348 $x - 0.0195x^2$)

that trapped oxygen under the soil surface could still have been in contact with plant roots, allowing further aerobic root respiration and yield increases. Yields from the clay site in 2004 were graphed separately due to the untimely excessive early July rainfall (Fig. 3). Yield responses for all cultivars on the clay soil in 2004 drastically declined at every flood duration. All cultivars were injured when extended soil waterlogging occurred due to excess rainfall after flooding treatments.

In agreement with a study conducted by VanToai et al. (1994), this study showed differences in flood tolerance among soybean cultivars. However, the criterion for ranking cultivar tolerance to environmental stress depends on the point of reference. A cultivar in waterlogged soil can be compared against itself without flood (check) or against

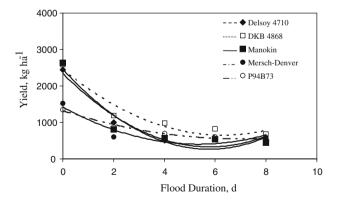


Fig. 3 Effect of flood duration on yield of five group IV soybean cultivars grown on a Sharkey clay soil in 2004 at Hayward, Missouri. Best fit regression equations relating h of flood (*x*) to kg ha⁻¹ soybean yield (*y*) were Delsoy 4710 ($y = 2343.6 + 28.982x - 0.1043x^2$), DK 4868 (2445.3 + 0.0785x - 23.74x²), Manokin (2460.9 - 31.748x + 0.1148x²), Mersch-Denver ($y = 1424.5 - 15.943x + 0.623x^2$), and P94B73 (1324.1 - 9.2527x + 0.0277x²)

other cultivars in the same flood treatment. Linear regression showed almost no yield loss for different flood durations for Delsoy 4710 in three moderate waterlogging environments (silt loam 2003, 2004, and clay 2003; Fig. 2). But, Delsoy 4710, which usually performs well in cultivar trials in the region, overall, yielded the lowest among the five cultivars. VanToai et al. (1994) documented that soybean flooding tolerance can be defined as high yield under flooding stress. The highest yielding cultivars in this study were DK4868 and P94B73 throughout the flood durations. Although these cultivars had decreased yield under 8 days of floods, their yields were overall higher than the other cultivars. This cannot be interpreted as complete tolerance, because DK4868 and P94B73 still lost yield under flooded conditions.

Influence of flood timing on flooding damage

Yields from the Sharkey clay location in 2004 for the flood timing experiment were separated from the other locations for evaluation, similar to the flood duration experiment. The Tiptonville silt loam tests in 2003 and 2004, along with the 2003 data from the Sharkey clay soil, were separated from the Sharkey clay tests in 2004 due to an interaction among year, soil, cultivar and growth stage found in the analysis of variance on yield (P < 0.0853). Flood timing experiment results from Sharkey clay in 2004 are not shown, because water stood in furrows longer at bloom than at V5 or R5 due to excessive rainfall and poor internal drainage.

Mean separation was conducted on leaf N, P and K across years to determine any differences within cultivars at various flood timings (Table 3). Nitrogen concentration

was significantly different for DK 4868 with flood at the R2 growth stage compared to the control. Leaf N content in other cultivars was numerically reduced by flooding at R2. No significant differences were found in leaf N for any cultivar at V5 and R5. Leaf P levels were significantly higher in control plots compared to flood treatments at V5 in cultivars DK 4868 and Mersch-Denver. Delsoy 4710 control plots had significantly higher leaf P than flooded plots at R2. Tissue K concentrations were significantly different at V5 for Delsoy 4710, DK 4868 and Manokin; however, those with flood treatments had higher leaf K than the controls. At R2, tissue K content was significantly higher in control plots than in flooded plots for cultivar Delsoy 4710. No significant differences were found for leaf K at R5 floods, compared to non-flood treatments. Cultivar P94B73 showed no statistical difference between flood and non-flood treatments in N, P or K contents.

A significant soybean yield interaction was found between the cultivar planted and flood timing. For each cultivar, the highest yielding flood timing treatment was usually either V5 or the control (Table 4). Leaves of most plants flooded at V5 turned either pale green or yellow in color after 1 week. However, unless a plant was killed by flooding at the vegetative stages, all cultivars were able to fully recover from damage. The greatest yield loss occurred when plants were flooded at the R5 growth stage. This confirms the work done by Griffin et al. (1988), which found that soybeans were more tolerant to waterlogged soils during the vegetative growth stages than during the reproductive stages. A study conducted by Linkemer et al. (1998) also found that early reproductive stages were the most susceptible to waterlogging damage. Yield reduction at R2 flood timing was generally moderate. Although,

	Flood (days)	Soybean leaf nutrient content								
		V 5 ^a			R2			R5		
		N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹	N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹	N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹
Delsoy 4710	0	3.28 a	0.33 a	1.78 b	1.69 a	0.26 a	1.30 a	2.00 a	0.26 a	1.17 a
	8	3.34 a	0.31 a	2.08 a	1.40 a	0.20 b	1.09 b	2.20 a	0.25 a	1.17 a
DK4868	0	3.68 a	0.33 a	1.73 b	1.81 a	0.24 a	1.22 a	1.78 a	0.25 a	1.18 a
	8	3.15 a	0.28 b	2.03 a	1.32 b	0.23 a	1.39 a	1.98 a	0.26 a	1.15 a
Manokin	0	3.11 a	0.29 a	1.64 b	1.53 a	0.27 a	1.21 a	2.20 a	0.24 a	1.07 a
	8	3.08 a	0.29 a	1.94 a	1.37 a	0.26 a	1.29 a	1.70 a	0.25 a	1.09 a
Mersch-Denver	0	2.79 a	0.32 a	1.80 a	1.48 a	0.20 a	1.29 a	1.33 a	0.22 b	1.41 a
	8	3.00 a	0.26 b	1.83 a	1.42 a	0.24 a	1.37 a	1.87 a	0.26 a	1.34 a
P94B73	0	2.57 a	0.31 a	1.48 a	1.74 a	0.25 a	1.35 a	1.23 a	0.22 a	1.17 a
	8	3.57 a	0.30 a	1.69 a	1.47 a	0.23 a	1.31 a	2.37 a	0.24 a	1.02 a

Table 3 Effect of flooding at V5, R2 and R5 soybean growth stages on nitrogen, phosphorus and potassium in soybean leaves

^a Values with different letters following control and flooded (192 h) in the same column and cultivar were significantly different at α -level of 0.05

Table 4 Yield effect of flooding five cultivars 8 days at three growth stages averaged across loam and clay soils in 2003 and loam soil in 2004 at Portageville and Hayward, Missouri

Cultivar	Growth stage	Yield ^a kg ha ⁻¹
Delsoy 4710	Check	2,557 b
	V5	3,387 a
	R2	2,192 b
	R5	2,037 b
DK 4868	Check	3,127 a
	V5	2,957 ab
	R2	3,437 a
	R5	2,385 b
Manokin	Check	3,258 a
	V5	2,711 ab
	R2	2,474 bc
	R5	1,984 c
Mersch-Denver	Check	2,390 bc
	V5	3,121 a
	R2	2,661 ab
	R5	1,837 c
P94B73	Check	3,035 a
	V5	3,009 a
	R2	2,663 a
	R5	2,428 a

^a Within cultivars, soybean yields followed by the same letter were not significantly different at an α -level of 0.05

cultivars Delsoy 4710 and Manokin flooded at R2 had significantly lower yield than their V5 flood and nonflooded counterparts, respectively. Cultivars DK 4868 and Manokin had significantly less yield during R5 floods, compared to the control plots. Delsoy 4710 and Mersch-Denver had significantly higher yield when flooded at V5 than without flooding. This may denote an instance of insufficient irrigation of non-flooded plots when compared to V5 flood plots. Compared to V5 flooding treatments for Delsoy 4710 and Mersch-Denver, R5 floods caused yields to significantly decrease. P94B73 produced the highest numerical yield of any cultivar flooded at R5. P94B73 also had no significant change in yield for any flooding treatments, including controls (Table 4). In this experiment, P94B73 was shown to be the most tolerant cultivar to flooding at each of the various growth stages.

Field observations

Field notes and digital images were recorded in the fields from the root systems of cultivars after flooding. These were compared to soybean roots in control plots and roots from a legume weed species (coffeeweed, *Sesbania herbacea*) that grow naturally under flooded conditions in rice



Fig. 4 Aerenchyma development on Manokin soybean roots after an 8-day flood duration

fields. We found that several Manokin cultivar plants contained spongy, white roots after 8 days of flooding (Fig. 4). The same thick, spongy root system was observed in healthy coffeeweed plants growing in water in a levee ditch. When flood-tolerant plants are grown in waterlogged soil, some plant species, such as coffeeweed, develop aerenchyma tissue to transfer oxygen from the plant canopy down to the roots (Kawase 1981). Aerenchyma tissue facilitates the transport of oxygen and other compounds between shoots and roots during periods of anaerobic soil conditions. This tissue is formed by either partial cell separation in ground parenchyma tissue or programmed cell death in the root cortex (Jackson and Armstrong 1999). If soybeans were able to develop this tissue in the later stages of flooding, plants would begin to receive air, increasing a limiting factor in a flooded situation. Observations made by Shimamura et al. (2003) have confirmed the formation of secondary aerenchyma tissue in soybeans under flooded conditions. Although development of aerenchyma tissue in soybean roots is not typical, these observations have shown another possible plant characteristic to look for when developing flood-tolerant cultivars of soybean.

Fields were monitored in 2003 to determine whether *Phytophthora* or *Pythium* incidence was enhanced under excess water. No signs of either were prominent due to increased flooding. However, it should be noted that one of the most soil waterlogging-tolerant cultivars, P94B73, contains the Rps1k gene for *phytophthora* resistance.

Conclusions

Typically, furrow irrigation in the delta region requires 48 h to allow water to flow down long rows in large soybean fields. Waterlogged soil conditions created by irrigation can be detrimental to N, P and K tissue concentrations and soybean yield at R2 and R5 growth stages, if followed by several days of rainfall, especially on clay soils. N concentrations in all cultivars were significantly reduced by extended flooding treatments on clay soils. No significant reduction in leaf N was found due to flooding on loam soils. Yields of cultivars DK 4868 and P94B73 were positively correlated with flooding up to 96 h during dry conditions. While other cultivars showed little or no damage at 48 h of soil waterlogging, yields of all cultivars were negatively correlated to some degree by 192 h of flood duration. In this study the cultivar that produced the highest yield under flooded conditions was DK 4868. The cultivar that lost the least yield compared to its nonflooded check was Delsoy 4710. However, this was the lowest yielding cultivar of the study. This may indicate that Delsoy 4710 could be used in future breeding programs for flood tolerance in higher yielding cultivars. Information from this research will be used to develop new cultivars, which will allow soybean farmers to irrigate with less concern about unexpected rainfall events.

References

- Bacanamwo M, Purcell L (1999) Soybean root morphological and anatomical traits associated with acclimation to flooding. Crop Sci 39:143–149
- Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971) Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci 11:929–931
- Griffin JL, Habetz RJ, Regan RP (1988) Flood irrigation of soybeans in Southwest Louisiana. La Agric Experiment Stn Bull 795
- Heatherly L, Pringle H (1991) Soybean cultivars' response to flood irrigation of clay soil. Agron J 83:231–236
- Jackson MB, Armstrong W (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biol 1:274–287
- Kawase M (1981) Anatomical and morphological adaptation of plants to waterlogging. Hortic Sci 16:30–34
- Keeney DR, Nelson DW (1982) Nitrogen-inorganic forms: indophenol blue method. In: Page A, Miller R, Keeney D (eds) Methods of soil analysis: chemical and microbiological properties, part 2. Agronomy Monograph No. 9. American Society of Agronomy, Madison, pp 674–676
- Keister DL, Rao VR (1977) Nitrogen fixation in *Rhizobium japonicum x planta*. In: Newton W, Postgate JR, Rodriguez-Barrucco C (eds) Recent developments in nitrogen fixation. Academic, New York, pp 419–430
- Laverty JC (1963) A modified procedure for the determination of phosphorus soil extracts. Soil Sci Soc Am Proc 27:360–361
- Linkemer G, Board JE, Musgrave ME (1998) Waterlogging effects on growth and yield components in late-planted soybean. Crop Sci 38:1576–1584

- Mills H, Jones J (1996) Plant analysis handbook II. MicroMacro Publishing, Athens
- Minchin FR, Pate JS (1975) Effects of water, aeration and salt regime on nitrogen fixation in a nodulated legume: definition of an optimum root environment. J Exp Bot 26:60–69
- Nathanson K, Lawn RJ, DeJabrun PLM, Byth DE (1984) Growth, nodulation and nitrogen accumulation by soybean in saturated soil culture. Field Crops Res 8:73–92
- Oosterhuis DM, Scott HD, Hampton RE, Wullschleger SD (1990) Physiological responses of two soybean [*Glycine max* (L.) Merr] cultivars to short-term flooding. Environ Exp Bot 30:85–92
- Pettry DE, Switzer RE (1996) Sharkey soils in Mississippi. Pp1. Miss Agric For Experiment Stn Bull 1057
- Purcell LC, Vories ED, Counce PA, King CA (1997) Soybean growth and yield response to saturated soil culture in a temperate environment. Field Crops Res 49:205–213
- Sallam A, Scott H (1987) Effects of prolonged flooding on soybeans during early vegetative growth. Soil Sci 144:61–66
- SAS Institute (1997) SAS/STAT: procedures. Release 6.12. SAS Institute, Cary
- Saxton AM (1998) A macro for converting mean separation output to letter groupings in PROC MIXED. Paper 230 In: Proceedings of 23rd Annual SAS Users Group Int Conf ISBN 1-58025-149-8. SAS Institute, Cary, pp 1243–1246
- Shannon JG, Stevens WE, Wiebold WJ, McGraw RL, Sleper DA (2005) Breeding soybeans for improved tolerance to flooding. Proceedings of 35th Soybean Seed Research Conference American Seed Trade Association, Chicago
- Shimamura S, Mochizuki T, Nada Y, Fukuyama M (2003) Formation and function of secondary aerenchyma in hypocotyl, roots and nodules of soybean (*Glycine max*) under flooded conditions. Plant Soil 251:351–359
- Soil Survey Staff (1971) Soil Survey of Pemiscot County, Missouri. USDA-SCS. U.S. Government Printing Office, Washington
- Sprent JI (1969) Prolonged reduction of acetylene by detached soybean nodules. Planta 88:372–375
- Stanley CD, Kaspar TC, Taylor HM (1980) Soybean top and root response to temporary water tables imposed at three different stages of growth. Agron J 72:341–346
- Sullivan M, VanToai T, Fausey N, Beuerlein J, Parkinson R, Soboyejo A (2001) Evaluating on-farm flooding impacts on soybean. Crop Sci 41:93–100
- Thomas G (1982) Exchangeable cations. In: Page A, Miller R, Keeney D (eds) Methods of soil analysis: chemical and microbiological properties, part 2. Agronomy Monograph No 9. American Society of Agronomy, Madison, pp 160–161
- Thomas AL, Guerreiro SM, Sodek L (2005) Aerenchyma formation and recovery from hypoxia of the flooded root system of nodulated soybean. Ann Bot 96:1191–1198
- VanToai T, Beuerlein JE, Schmitthenner AF, St Martin SK (1994) Genetic variability for flooding tolerance in soybeans. Crop Sci 34:1112–1115
- VanToai T, St Martin S, Chase K, Boru G, Schnipke V, Schmitthenner A, Lark K (2001) Identification of a QTL associated with tolerance of soybean to soil waterlogging. Crop Sci 41:1247– 1252
- Weisz PR, Sinclair TR (1987) Regulation of soybean nitrogen fixation in response to rhizosphere oxygen. II. Quantification of nodule gas permeability. Plant Physiol 84:906–910