MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 33-2016—YEAR 2
FINAL REPORT

Title of Project: Post-infection Development of Reniform Nematode on Resistant Soybean Lines JTN-5203, PI 404166, and 02011-1-1-5-1-1

Principal Investigator: Dr. Salliana R. Stetina (Sally.Stetina@ars.usda.gov)

EXECUTIVE SUMMARY

The objectives of this research project were to (1) characterize the resistance in soybean lines JTN-5203, PI 404166, and 02011-126-1-1-5-1-1 with respect to effects on post-infection development and fecundity of reniform nematode, and (2) evaluate and advance superior lines from crosses to these three lines to develop improved breeding lines with resistance to reniform nematode.

Three soybean lines, previously identified by project scientists as having resistance to Mississippi isolates of reniform nematode, were used as parents in crosses to transfer that resistance into soybean lines adapted for Mississippi.

Results from fecundity assessments showed that both the mean and maximum number of eggs per female was at least twice as high on the susceptible cultivar Braxton as on any of the resistant lines at 25 DAI (**Table 3**). This trend was the same 30 DAI (**Table 3**). All three of the resistant soybean genotypes had significantly fewer eggs per egg mass than was observed on the susceptible cultivar Braxton. The resistance sources apparently limited reniform nematode fecundity.

More nematodes were in the reniform and gravid cohorts on resistant genotypes JTN-5203 and PI 404166 than on 02011-126-1-1-5-1-1, suggesting that one factor contributing to the resistance in 02011-126-1-1-5-1-1 could be delayed development of the nematode.

All three resistant genotypes had significantly reduced numbers of nematodes infecting the roots compared to the susceptible cultivar Braxton.

Delayed development was noted as early as 10 DAI, with 50% of the population on the susceptible cultivar Braxton classified as gravid while only 20% to 25% of the nematodes in populations developing on the resistant lines had begun laying eggs.

Resistant breeding line 02011-126-1-1-5-1-1 maintained a high percentage of nematodes in the swelling phase of development late into the infection cycle, suggesting that the delay in nematode development could be making a greater contribution to the resistance observed in this line compared to JTN-5203 and PI 404166.

Thus, it appears that the mechanisms contributing to resistance in these lines include fewer infections, slower development, and reduced reproduction. The greatly delayed development in 02011-126-1-1-5-1-1 suggests this line has different or additional mechanisms contributing to reniform nematode resistance compared to JTN-5203 and PI 404166.

Superior genotypes (**Table 5**) were selected based on a combination of nematode resistance and agronomic data. For progeny currently at the $F_{5:6}$ generation, rows will be bulk-harvested in 2017 to begin yield testing in 2018.

The results showing that the resistance mechanism(s) in 02011-126-1-1-5-1-1 may function differently from the other resistance sources examined suggest that there may be future opportunities to either combine different types of resistance or rotate between them to achieve greater suppression of the reniform nematode population in soybean and prolong the utility of each type of resistance.

BACKGROUND AND OBJECTIVES

Many soybean growers, particularly in the Delta region, are faced with the challenge of producing a profitable crop in fields infested with reniform nematode (*Rotylenchulus reniformis*). Three soybean lines, previously identified by project scientists as having resistance to Mississippi isolates of reniform nematode, were used as parents in crosses to transfer that resistance into soybean lines adapted for Mississippi.

The objectives of this research project are to (1) characterize the resistance in soybean lines JTN-5203, PI 404166, and 02011-126-1-1-5-1-1 with respect to effects on post-infection development and fecundity of reniform nematode, and (2) evaluate and advance superior lines from crosses to these three lines to develop improved breeding lines with resistance to reniform nematode.

REPORT OF PROGRESS/ACTIVITY

Characterize the effects of three sources of resistance on reniform nematode development

Development and fecundity of reniform nematode were evaluated on resistant soybean lines JTN-5203, PI 404166, and 02011-126-1-1-5-1-1, and the susceptible control Braxton in three growth chamber experiments (day length = 16 hours, temperature = 28 °C). In each experiment, seeds were planted into a container (Ray Leach SL-10 Cone-tainer) containing 120 cm^3 of a steam-pasteurized soil mixture composed of one part sandy loam soil and two parts sand. Upon stand establishment, 500 reniform nematodes (mixed vermiform life stages) were added to the soil in each container by suspending them in 1 ml of water, pipetting the suspension into a 5-cm-deep hole made near the plant stem, and filling the hole with additional pasteurized soil mix. Mississippi reniform nematode isolate RR04, maintained in greenhouse culture on Rutgers tomato, was used for all trials.

A completely randomized design was used for each experiment, with 10 replications at each sampling date. Experiments were conducted twice, and data from both trials were combined for final analysis after confirming that there were no significant interactions between trials and soybean lines. In each experiment, data from each sampling date were analyzed independently. Nematode counts were transformed [$\log_{10}(x + 1)$] prior to analysis of variance (ANOVA) to normalize data. Where ANOVA indicated significant differences among soybean lines, differences of least squares means ($P \leq 0.05$) were used to compare means. Analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC). Patterns of nematode development also were examined based on developmental cohorts. For each developmental stage, the percentage of the nematode population was calculated and graphed to visualize changes in the predominant developmental stage on each genotype over time.

To assess reniform nematode development early in the infection cycle, fifty plants of each soybean line were established. Beginning 2 days after inoculation, root infection was measured on 10 individual plants of each line at 2-day intervals through day 10. Plant roots were separated from plant shoots at the soil line, and the roots were stained with red food coloring using published protocols to help locate the nematodes. Root-associated nematodes in each of four developmental stages were counted at ×200 magnification. Nematodes were classified as either vermiform (attached but not yet beginning to swell), swelling (enlargement of body but not yet assuming the kidney-shape characteristic of this species), reniform (kidney-shaped female with no egg mass), or gravid (kidney-shaped female with associated egg mass) (**Figure 1**). After counting, fresh weights were recorded after roots were drained briefly on paper towels to remove excess water. Counts were expressed as females per g of fresh root tissue.

Reniform nematode development early in the infection cycle is summarized in **Table 1**. Vermiform nematodes infected the susceptible cultivar Braxton 2 days after inoculation (DAI), but no nematodes were observed on the resistant lines until 4 DAI. At 4 DAI, swelling nematodes were seen on all four genotypes, and the reniform stage of development had been reached on JTN-5203 and PI 404166. By 8 DAI, gravid females were observed on all four genotypes, though Braxton had significantly higher numbers of gravid females than the resistant lines. When all developmental stages were considered together, the susceptible and resistant genotypes could be distinguished from each other as early as 8 DAI.

To assess reniform nematode development late in the infection cycle, fifty plants of each soybean line were evaluated using the same test establishment, inoculation, and root infection measurements as described for assessment of the early stages of reniform nematode development. However, assessment of nematode development on roots began 15 DAI, with root infection measured on 10 individual plants of each genotype at 5-day intervals through day 30.

Table 2 shows the nematode developmental stages later in the infection cycle. By 15 DAI, all nematodes had progressed beyond the vermiform stage of development. When all developmental stages were considered together, the susceptible and resistant genotypes could be distinguished from each other at all intervals from 15 to 30 DAI. There were no significant differences among the resistant genotypes based on total nematode counts or the number of nematodes in any specific life stage during this interval.

Because the infection levels on susceptible and resistant soybean lines differed, patterns of population development also were examined on a percentage basis. **Figures 2 and 3** show the proportion of the nematode population in each developmental stage during the early and late phases of the infection cycle, respectively. Early in the infection cycle (**Figure 2**), the nematode population composition on all four genotypes was similar at 4 and 6 DAI. At 8 DAI, fewer nematodes had reached the reniform and gravid stages of development on 02011-126-1-1-5-1-1 than on the other genotypes, and this pattern held true at 10 DAI. By 10 DAI, almost 50% of the nematode population on the susceptible cultivar Braxton was producing eggs, and approximately 95% had reached maturity. In comparison, only 20% to 25% of the nematode population had reached the gravid stage on the resistant genotypes.

Differences in the developmental cohorts also were evident later in the infection cycle (**Figure 3**). The susceptible cultivar Braxton consistently supported the highest proportions of gravid nematodes (ranging from approximately 70% to 90% of the population). In contrast, 02011-126-1-1-5-1-1 maintained a high percentage of nematodes in the swelling phase of development at 15, 20, and 25 DAI. More nematodes

were in the reniform and gravid cohorts on resistant genotypes JTN-5203 and PI 404166 than on 02011-126-1-1-5-1-1, suggesting that one factor contributing to the resistance in 02011-126-1-1-5-1-1 could be delayed development.

Characterize the effects of three sources of resistance on reniform nematode fecundity

Reniform nematode fecundity was assessed using the plants from Experiment 2. At 25 and 30 DAI, egg production by individual females was measured. At each sampling interval, up to 50 randomly selected gravid females were hand-picked from the roots of each line. Because all three resistant lines limited infection by the nematode, it was not possible to examine 50 individuals from all genotypes. A single gravid female was crushed under a cover slip on a glass slide to disperse the gelatinous matrix surrounding the eggs, and the number of eggs per female was recorded.

Results from the fecundity assessments showed that both the mean and maximum number of eggs per female was at least twice as high on the susceptible cultivar Braxton as on any of the resistant lines AT 25 DAI (**Table 3**). This trend was the same 30 DAI (**Table 3**). All three of the resistant soybean genotypes had significantly fewer eggs per egg mass than was observed on the susceptible cultivar Braxton. The resistance sources apparently limited reniform nematode fecundity.

Key findings from the reniform nematode development and fecundity experiments are as follows:

- There is a slight (1 or 2 day) delay in root infection on the three resistant genotypes compared to the susceptible cultivar Braxton.
- All three resistant genotypes had significantly reduced numbers of nematodes infecting the roots compared to the susceptible cultivar Braxton.
- Delayed development was noted as early as 10 DAI, with 50% of the population on the susceptible cultivar Braxton classified as gravid while only 20% to 25% of the nematodes in populations developing on the resistant lines had begun laying eggs.
- Resistant breeding line 02011-126-1-1-5-1-1 maintained a high percentage of nematodes in the swelling phase of development late into the infection cycle, suggesting that the delay in nematode development could be making a greater contribution to the resistance observed in this line compared to JTN-5203 and PI 404166.

Thus, it appears that the mechanisms contributing to resistance in these lines include fewer infections, slower development, and reduced reproduction. The greatly delayed development in 02011-126-1-1-5-1-1 suggests this line has different or additional mechanisms contributing to reniform nematode resistance compared to JTN-5203 and PI 404166.

Develop improved soybean breeding lines with resistance to reniform nematode

Single-row plots representing more than 100 families from crosses to JTN-5203, PI 404166, or 02011-126-1-1-5-1-1 were successfully established in the soybean breeding nursery at Stoneville in 2016. Adapted parents were chosen based on traits such as high germination (DS25-1), resistance to reniform (DS-880) or soybean cyst nematode (DB04-10836, DS-880), and exotic yield genes (LG01-5087-5). Progeny were evaluated in the field for agronomic properties including height, pod load, shattering resistance, yellow seed coat, and lodging resistance.

Concurrently, tests were established in the growth chamber to evaluate the reaction of at least 5 plants from each family to the reniform nematode. Infection by reniform nematode was measured on progeny from each cross in three growth chamber experiments. Each experiment included the resistant parent, the resistant control Hartwig, and the susceptible control Braxton. In each experiment, seeds were planted into a container containing 120 cm^3 of a steam-pasteurized soil mixture composed of one part sandy loam soil and two parts sand. Upon stand establishment, 1,000 reniform nematodes were added to the soil in each container by suspending them in 1 ml of water, pipetting the suspension into a 5-cm-deep hole made near the plant stem, and filling the hole with additional pasteurized soil mix. Plant roots were harvested 28 DAI, stained with red food coloring using published protocols, and nematodes were counted. Families that were at least as resistant as the resistant parent were identified (**Table 4**) based on the mean number of nematodes infecting the roots.

Superior genotypes (**Table 5**) were selected based on a combination of nematode resistance and agronomic data. For progeny currently at the $F_{5:6}$ generation, rows will be bulk-harvested in 2017 to begin yield testing in 2018.

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

According to the National Agricultural Statistics Service, approximately 2,020,000 acres of soybean were harvested in Mississippi in 2016, primarily in the Delta region where reniform nematode is ubiquitous. Mississippi's soybean crop was valued at $964,752,000. Yield losses to reniform nematode estimated at 5% translated to a potential annual loss of $48,237,600 to Mississippi growers.

Losses to reniform nematode could be drastically reduced if resistant soybean varieties adapted for Mississippi were available; our breeding program is focused on meeting that challenge. Further, the results showing that the resistance mechanism(s) in 02011-126-1-1-5-1-1 may function differently from the other resistance sources examined, suggesting that there may be future opportunities to either combine different types of resistance or rotate between them to achieve greater suppression of the reniform nematode population and prolong the utility of each type of resistance.

END PRODUCTS–COMPLETED OR FORTHCOMING

The ultimate end products from this work will be soybean germplasm with resistance to reniform nematode derived from thee different soybean lines, though that end product is still several years in the future. Conference presentations and journal publications describing the germplasm releases will also be prepared. A manuscript describing the effects of these sources of resistance on development and fecundity of the reniform nematode will be prepared and submitted to a journal no later than December 31, 2017.

Table 1. Number of reniform nematodes in four developmental stages (vermiform, swelling, reniform, gravid) and total number of nematodes per g of root on susceptible (Braxton) and resistant (JTN-5203, 02011-1-1-5-1-1, and PI 404166) soybean in growth chamber tests at 2-day intervals during the first 10 days after inoculation (DAI).

Developmental stage	Soybean genotype	2 DAI		4 DAI		6 DAI		8 DAI		10 DAI	
Vermiform	Braxton	0.1		0.4		0.6		0.2		0.0	-[a]
	JTN-5203	0.0		0.3		0.6		0.0		0.0	-
	PI 404166	0.0		0.7		1.1		0.0		0.0	-
	02011-126[b]	0.0		0.2		0.6		0.1		0.0	-
Swelling	Braxton	0.0	-[a]	1.3	b	4.8		2.0		0.7	b
	JTN-5203	0.0	-	1.4	b	7.6		1.8		3.0	a
	PI 404166	0.0	-	5.4	a	4.8		2.1		0.7	b
	02011-126[b]	0.0	-	1.2	b	4.3		3.2		2.5	a
Reniform	Braxton	0.0	-[a]	0.0		0.6		5.7	a	4.1	
	JTN-5203	0.0	-	0.1		0.8		1.0	b	3.6	
	PI 404166	0.0	-	0.1		0.5		1.6	b	1.4	
	02011-126[b]	0.0	-	0.0		0.4		0.9	b	1.7	
Gravid	Braxton	0.0	-[a]	0.0	-[a]	0.0	-[a]	2.5	a	4.1	a
	JTN-5203	0.0	-	0.0	-	0.0	-	0.2	b	1.6	ab
	PI 404166	0.0	-	0.0	-	0.0	-	0.4	b	0.6	b
	02011-126[b]	0.0	-	0.0	-	0.0	-	0.1	b	1.0	b
All stages combined	Braxton	0.1		1.6	b	7.1		14.3	a	10.0	a
	JTN-5203	0.0		1.9	b	9.9		4.3	b	9.9	a
	PI 404166	0.0		6.5	a	7.0		5.3	b	2.4	b
	02011-126[b]	0.0		1.5	b	6.7		5.4	b	5.2	ab

[a] All values were zero so no means separation was possible
[b] 02011-129 is breeding line 02011-126-1-1-5-1-1
Within each developmental stage and interval, means (20 plants; pooled data from two tests) followed by the same letter are not significantly different (differences of least squares means, $P \leq 0.05$).

Table 2. Number of reniform nematodes in three developmental stages (swelling, reniform, gravid) and total number of nematodes per g of root on susceptible (Braxton) and resistant (JTN-5203, 02011-1-1-5-1-1, and PI 404166) soybean in growth chamber tests at 5-day intervals from 15 to 30 days after inoculation (DAI).

Developmental stage	Soybean genotype	15 DAI	20 DAI	25 DAI	30 DAI
Swelling	Braxton	1.0	0.4	0.1 b	0.3 a
	JTN-5203	1.0	0.2	0.2 b	0.0 b
	PI 404166	0.4	0.1	0.0 b	0.0 b
	02011-126[a]	2.5	0.3	0.6 a	0.1 b
Reniform	Braxton	2.4	1.5 a	0.5	1.5 a
	JTN-5203	1.0	0.3 b	0.2	0.1 b
	PI 404166	0.7	0.1 b	0.1	0.2 b
	02011-126[a]	1.5	0.2 b	0.2	0.1 b
Gravid	Braxton	9.8 a	6.1 a	5.8 a	3.7 a
	JTN-5203	2.0 b	0.4 b	0.2 b	0.2 b
	PI 404166	0.9 b	0.1 b	0.1 b	0.1 b
	02011-126[a]	0.5 b	0.3 b	0.2 b	0.5 b
All stages combined	Braxton	13.7 a	8.2 a	6.4 a	5.6 a
	JTN-5203	3.9 b	0.7 b	0.5 b	0.3 b
	PI 404166	1.9 b	0.2 b	0.2 b	0.2 b
	02011-126[a]	4.3 b	0.7 b	0.9 b	0.6 b

[a] 02011-126 is breeding line 02011-126-1-1-5-1-1

Within each developmental stage and interval, means (20 plants; pooled data from two tests) followed by the same letter are not significantly different (differences of least squares means, $P \leq 0.05$).

Table 3. Reniform nematode egg production on susceptible (Braxton) and resistant (JTN-5203, 02011-1-1-5-1-1, and PI 404166) soybean in two growth chamber tests.

		25 days after inoculation					30 days after inoculation				
			Eggs per egg mass					Eggs per egg mass			
Test	Soybean line	Number of egg masses counted	Mean		Max[a]	Min[b]	Number of egg masses counted	Mean		Max[a]	Min[b]
1	Braxton	50	28.4	a	99	0	50	35.7	a	217	3
	JTN-5203	45	9.2	b	50	0	37	4.1	b	51	0
	PI 404166	32	6.2	b	26	0	4	7.1	b	20	0
	02011-126[c]	13	9.2	b	40	0	10	2.3	b	17	0
2	Braxton	50	38.5	a	136	3	50	56.2	a	181	3
	JTN-5203	6	2.1	c	21	0	8	1.3	b	6	0
	PI 404166	1	3.0	bc	--	--	1	10.0	ab	--	--
	02011-126[c]	8	9.4	b	20	2	16	4.8	b	16	0

[a] maximum number of eggs per egg mass
[b] minimum number of eggs per egg mass
[c] 02011-126 is breeding line 02011-126-1-1-5-1-1
Within each test and interval, means followed by the same letter are not significantly different (differences of least squares means, $P \leq 0.5$).

Table 4. Selection of families with reniform nematode resistance from eleven different crosses based on plant infection by the nematode in growth chamber tests conducted in 2016.

Pedigree[a]	2016 generation	Number of families evaluated	Number of resistant families selected
DS24-2//**JTN-5203**/DS-880[b]	$F_{3:4}$	2	2
LG04-1459-8/**JTN-5203**	$F_{4:5}$	11	1
R99-1613F/**JTN-5203**	$F_{4:5}$	24	11
DS25-1/**02011-126-1-1-5-1-1**	$F_{3:4}$	8	2
DS30-1/**02011-126-1-1-5-1-1**	$F_{3:4}$	2	2
DB04-10836/**02011-126-1-1-5-1-1**	$F_{3:4}$	15	9
DS-880[b]/**02011-126-1-1-5-1-1**	$F_{3:4}$	5	2
04025-1-1-4-1-1/**02011-126-1-1-5-1-1**	$F_{3:4}$	10	0
DS-880[b]/**PI 404166**	$F_{3:4}$	10	4
LG01-5087-5/**PI 404166**	$F_{3:4}$	15	3
DB04-18036/**PI 404166**	$F_{3:4}$	20	11

[a] resistant parent in **bold** type
[b] DS-880 is moderately resistant to reniform nematode; these crosses are designed to combine the two sources of resistance

Table 5. Summary of breeding material in the reniform nematode resistance program at the end of the 2016 cropping season, USDA ARS, Crop Genetics Research Unit, Stoneville, MS.

Pedigree[a]	2016 generation	Number of resistant lines
R99-1613/**JTN-5203**	$F_{5:6}$	54
DS25-1/**02011-1-1-5-1-1**	$F_{4:5}$	9
DB04-18036/**02011-1-1-5-1-1**	$F_{4:5}$	15
DS-880[b]/**PI 404166**	$F_{4:5}$	12
LG01-5087-5/**PI 404166**	$F_{4:5}$	8
DB04-18036/**PI 404166**	$F_{4:5}$	45
LG01-5087-5/**PI 404166**	$F_{3:4}$	48

[a] resistant parent in **bold** type
[b] DS-880 is moderately resistant to reniform nematode; these crosses are designed to combine the two sources of resistance

CAPTIONS FOR FIGURES

Figure 1. Progressive stages of development of female reniform nematodes from immature to mature are: vermiform (attached but not yet beginning to swell), swelling (enlargement of body but not yet assuming the kidney-shape characteristic of this species), reniform (kidney-shaped female with no egg mass), or gravid (kidney-shaped female with associated egg mass).

Figure 2. Proportion of the reniform nematode population in four developmental cohorts ranging from vermiform (immature) to gravid (mature) at two-day intervals from 4 to 10 days after inoculation (DAI) on susceptible soybean cultivar Braxton and the resistant soybean genotypes 02011-126-1-1-5-1-1 (02011), JTN-5203, and PI 404166.

Figure 3. Proportion of the reniform nematode population in three developmental cohorts ranging from swelling (immature) to gravid (mature) at five-day intervals from 15 to 30 days after inoculation (DAI) on susceptible soybean cultivar Braxton and the resistant soybean genotypes 02011-126-1-1-5-1-1 (02011), JTN-5203, and PI 404166; none of the nematodes observed were in the vermiform stage of development on any genotype during this later stage of the infection cycle.

Figure 1

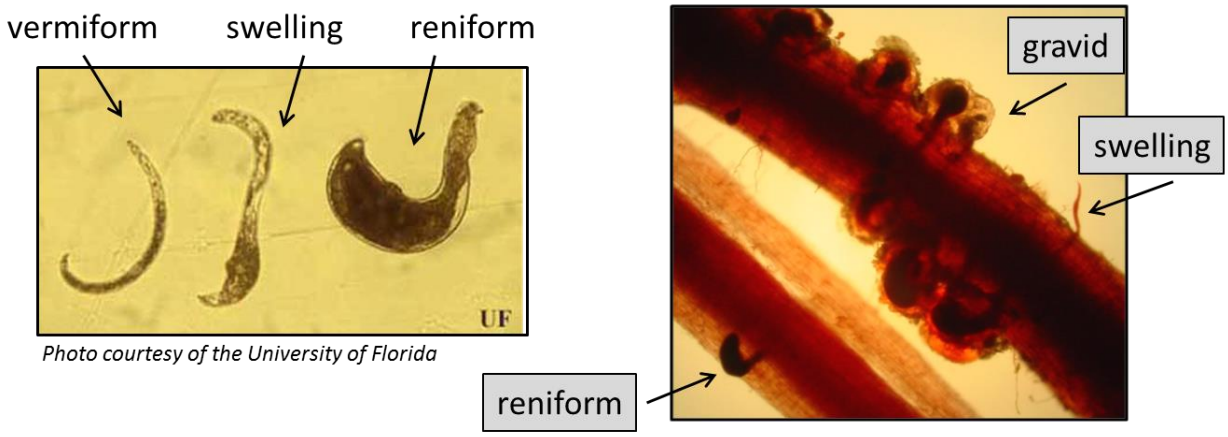


Photo courtesy of the University of Florida

Figure 2

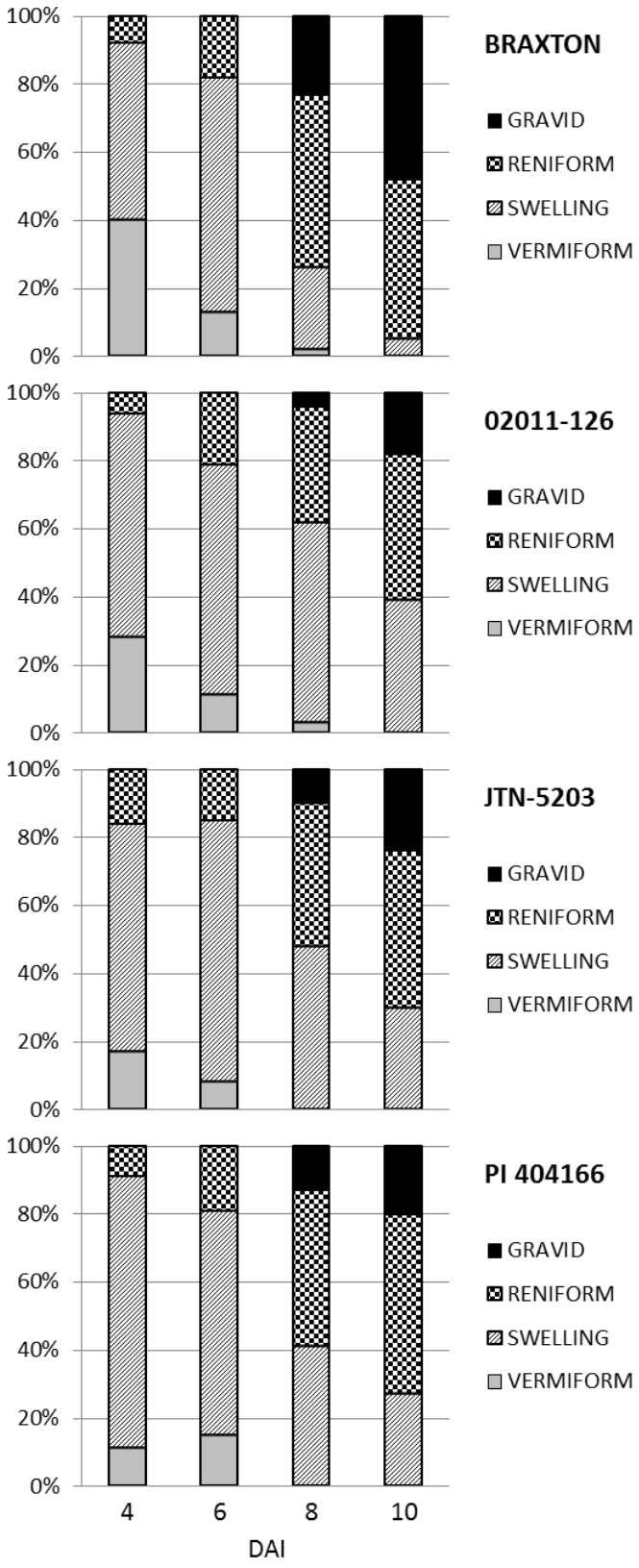
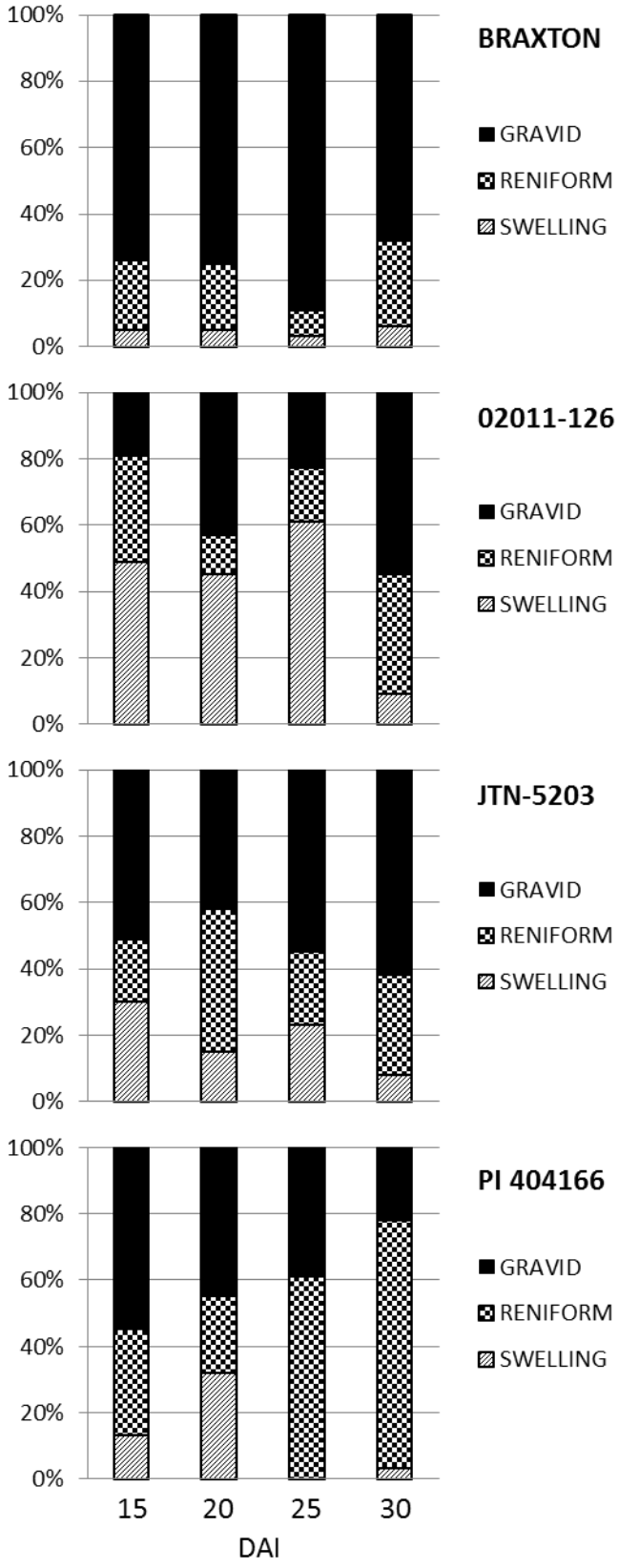

BRAXTON
02011-126
JTN-5203
PI 404166
GRAVID
RENIFORM
SWELLING
VERMIFORM
100%
80%
60%
40%
20%
0%
4
6
8
10
DAI

Figure 3


BRAXTON
02011-126
JTN-5203
PI 404166
GRAVID
RENIFORM
SWELLING
100%
80%
60%
40%
20%
0%
15
20
25
30
DAI