

SPECIAL INVITED PAPER—RHIZOSPHERE INTERACTIONS

**INTRASPECIES VARIATION IN COTTON BORDER CELL
PRODUCTION: RHIZOSPHERE MICROBIOME IMPLICATIONS¹**

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- *Premise of the study:* Border cells, which separate from the root cap, can comprise >90% of carbon-based exudates released into the rhizosphere, but may not provide a general source of nutrients for soil microorganisms. Instead, this population of specialized cells appears to function in defense of the root tip by an extracellular trapping process similar to that of mammalian white blood cells. Border cell production is tightly regulated, and direct tests of their impact on crop production have been hindered by lack of intraspecific variation.
- *Methods:* Border cell number, viability, and clumping were compared among 22 cotton cultivars. Slime layer “extracellular trap” production by border cells in response to copper chloride, an elicitor of plant defenses, was compared in two cultivars with divergent border cell production. Trapping of bacteria by border cells in these lines also was measured.
- *Key results:* Emerging roots of some cultivars produced more than 20000 border cells per root, a 100% increase over previously reported values for this species. No differences in border cell morphology, viability, or clumping were found. Copper chloride-induced extracellular trap formation by border cells from a cultivar that produced 27921 ± 2111 cells per root was similar to that of cells from a cultivar with 10002 ± 614 cells, but bacterial trapping was reduced.
- *Conclusions:* Intraspecific variation in border cell production provides a tool to measure their impact on plant development in the laboratory, greenhouse, and field. Further research is needed to determine the basis for this variation, and its impact on rhizosphere community structure.

Key words: root border cells; rhizosphere; root cap; extracellular trap

“The potential environmental consequences of exudation of Cry proteins and differences in border cell numbers of cotton requires further investigation.” (Knox et al. 2007, p. 89).

As roots of cereals and legumes penetrate the soil environment, root border cell populations separate from the root cap periphery by the action of endogenous plant cell wall degrading enzymes (Wen et al., 1999). Border cells and their associated extracellular matrix can produce >90% of the total mass of carbon-based material released as “exudates” from seedling roots and may facilitate variable patterns of microbial colonization of the rhizosphere (Lynch and Whipps, 1990; Odell et al., 2008; Somasundaram et al., 2008b; Van Bruggen et al., 2002). Long-standing models have assumed that these “sloughed” cells provide a nonspecific source of nutrients for any microorganisms within close proximity to the root surface (Hawes et al., 1998).

Instead, border cells exhibit distinct gene and protein expression patterns that confer host–microbe-specific properties that can be stimulatory or inhibitory to bacteria, nematodes, and fungi (Goldberg et al., 1989; Hawes and Pueppke, 1987; Zhu et al., 1997; Brigham et al., 1998; Knee et al., 2001; Zhao et al., 2000b). The cells can survive for extended periods after separation into hydroponic culture or into the soil environment (Knudson, 1919; Gautheret, 1933; Vermeer and McCully, 1982). Laboratory studies have implicated the cell populations in defense of the root tip against parasitic nematodes, fungal and oomycete pathogens, and metals including arsenic, boron, lead, copper, nickel, and zinc (Hawes et al., 1998; Llugany et al., 2003; Wuyts et al., 2006; Kopittke et al., 2011, 2012). Conversely, the cells appear to facilitate colonization by beneficial bacteria, mycorrhizal fungi, and protozoa (Nagahashi and Douds, 2004; Gunawardena et al., 2005; Somasundaram et al., 2008a; Jaroszuk-Scisel et al., 2009; Hawes et al., 2012).

Recent studies have revealed that border cells function by a mechanism that appears to be similar to that of mammalian immune responses, in which extracellular DNA (exDNA) together with antimicrobial proteins trap invading pathogens and inhibit infection (Brinkmann et al., 2004, 2010; Hawes et al., 2011). As with neutrophil extracellular traps (NETs), border cells export exDNA and proteins that assemble into a matrix, which is eliminated within minutes of adding DNase or protease (Wen et al., 2007, 2009). Treatment of pea seedlings with DNase or protease

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during inoculation with soilborne fungal pathogens including *Fusarium solani* f. sp. *lisi* and *Phoma medicaginis* eliminates the normal root tip resistance to infection and results in an increase in disease from mild local lesions in <5% of inoculated roots to severe root rot in 100% (Wen et al., 2007, 2009).

Variation in border cell production by cultivars within the same crop species is of interest for its potential use as a tool to test the impact of border cells on root–rhizosphere interactions in field conditions. Knox and coworkers (2007) were the first to report significant intraspecific variation in border cell production, in a survey of 14 cultivars of *Gossypium hirsutum*. Conventional elite parent lines and their transgenic offspring expressing the insecticidal Cry proteins from *Bacillus thuringiensis* (Bt) were compared. Border cells from transgenic lines were found to produce and release Cry proteins into the extracellular matrix. The potential impact of this trait on root system properties was tested by comparing border cell numbers in transgenic lines with those of the parental lines. In most cases, daily production of several thousand cells per root was found, with no significant differences between donor and parent lines (Knox et al., 2007). In one cultivar, however, the elite parent Sicot 189 used in the development of several transgenic cultivars including Sicot 189 Roundup Ready (189RR), individual root caps yielded >10000 border cells.

Our primary goal for the present study was to determine whether variation in border cell production could be detected among cotton cultivars currently in production in the United States and whether such differences are correlated with altered properties including root growth and morphology and border cell viability, morphology, and function. Two cultivars with divergent border cell production were used to compare border cell responses to the plant defense elicitor copper chloride (Cruickshank, 1963) and to bacteria. Extracellular trapping of bacteria exhibits host–microbe specificity, but its significance in root–rhizosphere interactions is not clear (Hawes and Pueppke, 1987). Two bacteria, identified as *Erwinia* sp. and *Bacillus* sp., were found to predominate among colonies isolated from surface-sterilized roots of greenhouse- and field-grown cotton (Misaghi and Donndelinger, 1990). In the present study, we found that *Erwinia carotovora* (*Pectobacterium carotovora*) and *Bacillus subtilis*, are trapped within minutes upon exposure to cotton border cells. We used this phenotype as a marker to measure cotton border cell trapping responses in two cultivars with divergent border cell numbers.

MATERIALS AND METHODS

Plant materials—Seeds of *Gossypium hirsutum* L. (upland cotton) and *G. barbadense* L. (Pima cotton), Malvaceae; were surface sterilized for 5 min in 95% ethanol followed by 10 min in 0.5% sodium hypochlorite, then rinsed in six changes of sterile distilled water. Seeds were germinated on 1% water agar overlaid with sterile paper and incubated at 25°C until roots were 20–25 mm long (24–36 h). Transgenic varieties, made available for this study by Ayman Mostafa and Randy Norton, University of Arizona Cooperative Extension, were produced by Dow AgroSciences (PHY499, PHY375, PHY565), Dyna-Gro (DG 2450), Bayer CropScience (FM 2484, ST 5458, ST 4498), and Monsanto (DP 1032, DP 1044, DP 0949). In this paper, varieties that are commercially available and do not have transgenic expression of Bt toxin are referred to as “conventional” cultivars (van Deynze et al., 2011). Root growth and development were measured as described (Curlango-Rivera et al., 2010, 2013).

Enumeration of border cells—Border cells were isolated into suspension and counted as described previously (Hawes and Pueppke, 1987; Hamamoto et al., 2006). Seedling root tips (ca. 5–10 mm from the apex) were immersed into 500 μ L of sterile deionized water on a petri plate for 2 min and observed

with a dissecting microscope as the cells dispersed from the tip. The water was gently agitated with a pipet to disperse all the cells into suspension, which was transferred to a microfuge tube. The plate was washed with 500 μ L of water to collect residual cells. Seedlings with discolored or damaged root tips or root tips that had been exposed to free water (i.e., water droplets that disperse border cells) were not included. Suspensions were agitated, and triplicate counts from at least five replicate samples in at least three independent experiments were enumerated by direct counts. Differences between cultivars were analyzed using between-groups one way analysis of variance (ANOVA) (Scheffe, 1999). Border cell viability was assessed based on observation of cytoplasmic streaming in individual cells; at least 100 cells were evaluated for each sample. The same protocols were used to screen 1365 pea (*Pisum sativum* L., Fabaceae) cultivars for variation in border cell production (the same cultivars were screened for resistance to crown gall as described by Robbs et al. (1991).

Measurement of cotton border cell response to copper chloride—Seedling root tips (as above) were observed as they were immersed in 100 μ L of water, or copper chloride (1 mmol/L and 3 mmol/L). After agitation to disperse border cells into suspension, triplicate samples were examined at 5, 15, 30, and 60 min and at 24 h to measure cell viability. At least three replicate samples with at least 100 border cells for each of four independent experiments were evaluated. Dimensions of the slime layer in at least 100 cells in each of at least three replicate samples were measured by using india ink to visualize the size and shape, as described (Miyasaka and Hawes, 2001).

Extracellular trapping of bacteria by cotton border cells—Trapping assays were carried out as described (Curlango-Rivera et al., 2011; Hawes and Pueppke, 1987). Log phase bacterial cultures grown overnight in nutrient medium were diluted in water to 10^6 bacteria per milliliter, then added to border cell suspensions. Trapping of bacteria was measured by direct microscopic observation. A trapping response was counted as positive when the border cell surface was covered in immobilized bacteria. To confirm the role of exDNA in the trapping process, DNase I (1 unit per 10 μ L of plant–bacteria mixture) was added to the sample and reversal of trapping (as in Fig. 1D) was confirmed by direct microscopic observation. *Erwinia carotovora* (*Pectobacterium carotovorum*) and *Bacillus subtilis* strain 1085B (Cisneros et al., 2011) were used as test organisms.

RESULTS

Border cell number in cotton cultivars—In the first line tested, PHY499, an unusual abundance of border cell mucilage was evident by direct observation upon immersion of the root tip into water (Fig. 1). Within seconds, a mass of cells >2 cm in diameter extended several centimeters up the root and beyond the root apex and could be seen without microscopic magnification. Mean border cell number per root tip was 27921 ± 2111 (Table 1), a level not previously reported for any plant under the conditions tested (Hawes and Pueppke, 1986; Hamamoto et al., 2006). Nine additional transgenic lines from four different producers were tested. PHY499 produced significantly higher numbers of border cells than the other lines ($F_{1,198} = 76$, $P < 0.05$). With the exception of PHY 375 and DP 1032 ($F_{1,147} = 79$, $P < 0.05$), mean cell numbers of the other transgenic lines did not differ significantly (Table 1).

To ensure that our protocols for counting border cells were congruent with those used by Knox et al. (2007), cell numbers from conventional cultivars obtained from the CSIRO, Australian Cotton Research Institute in Narrabri, Australia in 1999 were measured for the present study. The mean number for Sicot 189 (10783 ± 457) was virtually identical to the 11000 ± 500 found for this cultivar by Knox et al. (2007). Sicala V-2 mean border cell number (9792 ± 1038) was not significantly different from Sicot 189 ($F_{1,48} = 0.765$, $P = 0.39$). Mean border cell number in Delta Topaz (18650 ± 2077) was significantly higher (Table 1) ($F_{1,149} = 362$, $P < 0.001$).

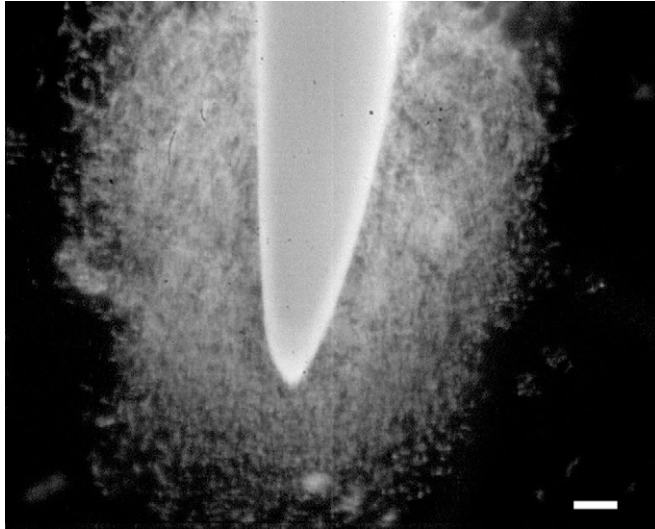


Fig. 1. Mass of border cell–root cap mucilage covering a cotton root tip, 30 s after immersion into water. Size marker = 3 mm.

Factors associated with variation in border cell number—One factor that could underlie the variation in number of border cells is standard seed treatment of commercial cultivars with fungicides (Olsen et al., 2011). For example, even a transient exposure of pea root tips to the antifungal phytoalexin pisatin triggers an overnight increase in production from 3988 ± 420 to 7767 ± 195 border cells per root (Curlango-Rivera et al., 2010). A second factor is the possible expression of Bt toxin, which was found in border cells of all the transgenic lines tested by Knox et al. (2007). If either one or both factors play a role, then roots developing from seeds without the Bt transgene or fungicide treatment would be predicted to yield cell populations in the range documented previously (Knox et al., 2007). To examine this prediction, six cotton varieties, including two *G. barbadense* lines, were obtained from MRC Seeds, a company that guarantees their seeds to be free of fungicide treatment and of genetic modifications. In each case, cell numbers were within the range seen for the transgenic varieties (Table 1). The results did not support the premise that Bt expression or fungicide treatment in general underlies the variation in border cell production reported here.

The use of different cultivars is an obvious factor that could underlie the differences seen in border cell production. Seeds of *G. hirsutum* cv. Acala 44 obtained from Margaret Essenberg, Oklahoma State University, were tested in a 1986 survey and found to yield 2900–3100 cells (Hawes and Pueppke, 1986), in the range found by Knox et al. (2007) for most of their varieties. For the present study, Acala 44 seeds harvested in 1984, 2008, and 2011 were made available by the Crop Germplasm Station, College Station, Texas. Yields of border cells were, respectively, 4270 ± 1790 , 10270 ± 1877 and $23\,134 \pm 3564$ cells per root (Table 1) ($F_{1,72} = 414$, $P < 0.001$).

Influence of border cell number on border cell morphology and properties—Altered cell cycle dynamics and increased carbon and energy involved in producing increased border cell numbers could be predicted to affect root development as well as border cell morphology, viability, and properties (Brigham et al., 1998; Curlango-Rivera et al., 2010). No differences in

TABLE 1. Yield and viability of root border cells from cotton.

Cultivar	Mean no. border cells/root (SE)	Viability (%)	Seed source ^a
<i>Transgenic G. hirsutum</i>			
PHY 499	27 921 ^a (2111)	95–100	1
PHY 375	23 521 ^b (2077)	95–100	1
PHY 565	21 417 ^b (3350)	95–98	1
DG 2450	21 585 ^b (3022)	95–100	2
FM 2484	21 909 ^b (2645)	96–100	3
ST 5458	21 430 ^b (2189)	96–100	3
ST 4498	23 400 ^b (3754)	95–100	3
DP 1032	20 406 ^b (2985)	95–100	4
DP 1044	19 216 ^b (2417)	97–100	4
DP 0949	19 146 ^b (2828)	95–100	4
<i>Gossypium hirsutum</i>			
Acala 44 (1984)*	4270 ^c (1790)	100	5
Acala 44 (2008)*	10 270 ^d (1877)	97–100	5
Acala 44 (2011)*	23 134 ^b (3564)	91–100	5
MRC-5156	15 345 ^e (1921)	98–100	6
Chandan	18 348 ^a (1846)	98–100	6
Kundan	17 849 ^{bc} (1287)	96–99	6
Buranda	16 978 ^{bc} (2014)	95–100	6
Sicot 189	10 002 ^d (614)	100	7
Sicala V-2	9792 ^d (1038)	100	7
Delta Topaz	18 650 ^{bc} (2077)	96–100	7
<i>Gossypium barbadense</i>			
Pima Long	20 563 ^b (2178)	100	6
Egyptian Fine'	20 287 ^b (1578)	99–100	6

Notes: Sources of seed: Data for border cell numbers are means, with standard errors in parentheses. Within a column, means followed by the same letter do not differ ($P > 0.05$); those with distinct letters different significantly ($P < 0.05$). Values for viability represent range for percentage of cells with cytoplasmic streaming. (1) Dow AgroSciences; (2) Dyna-Gro; (3) Bayer CropScience; (4) Monsanto; (5) USDA, ARS, Crop Germplasm Research, College Station, TX 77845; (6) MRC Seeds Company, Houston, TX 770795; (7) D. Nehl, CSIRO, Australian Cotton Research Institute, Narrabri, Australia 2390. Asterisk (*) denotes harvest date of seed lot. Seeds from nos. 1–4 were made available by A. Mostafa and Randy Norton, University of Arizona Cooperative Extension, Phoenix, AZ 85040.

rate of germination, growth, and development of roots were detected among the tested lines (data not shown). Border cell morphology also appeared to be unaffected among lines with divergent cell production. Significant variation in clumping can occur among border cells of some species and in some environmental conditions (e.g., Endo et al., 2011). Among the cotton lines tested, >95% of the border cells within populations separated as single cells, with a few small clumps of 3–5 cells each. No differences in cell clumping were found. Cell viability was 95–100% for all tested cultivars (Table 1).

Border cells produce a slime layer “trap”, that has been implicated in protecting the root tip from damage by trapping metals including aluminum, lead, zinc, cadmium, nickel, copper, and arsenic (Miyasaka and Hawes, 2001; Kopitke et al., 2011, 2012). Copper chloride, a standard elicitor of biochemical pathways involved in defense (Cruickshank, 1963; Carlson and Dolphin, 1981), was used here to compare the ability of a conventional and a transgenic cultivar with divergent cell production (10783 ± 457 vs. 27921 ± 2111). to respond to metal exposure by production of a slime layer. When placed into water, border cells from Sicot 189 (Fig. 2A) and PHY499 (Fig. 2B) dispersed within seconds as a population of single cells. Border cell viability in both cultivars at time 0 and after 24 h was 100% (328/328 and 417/417, respectively).

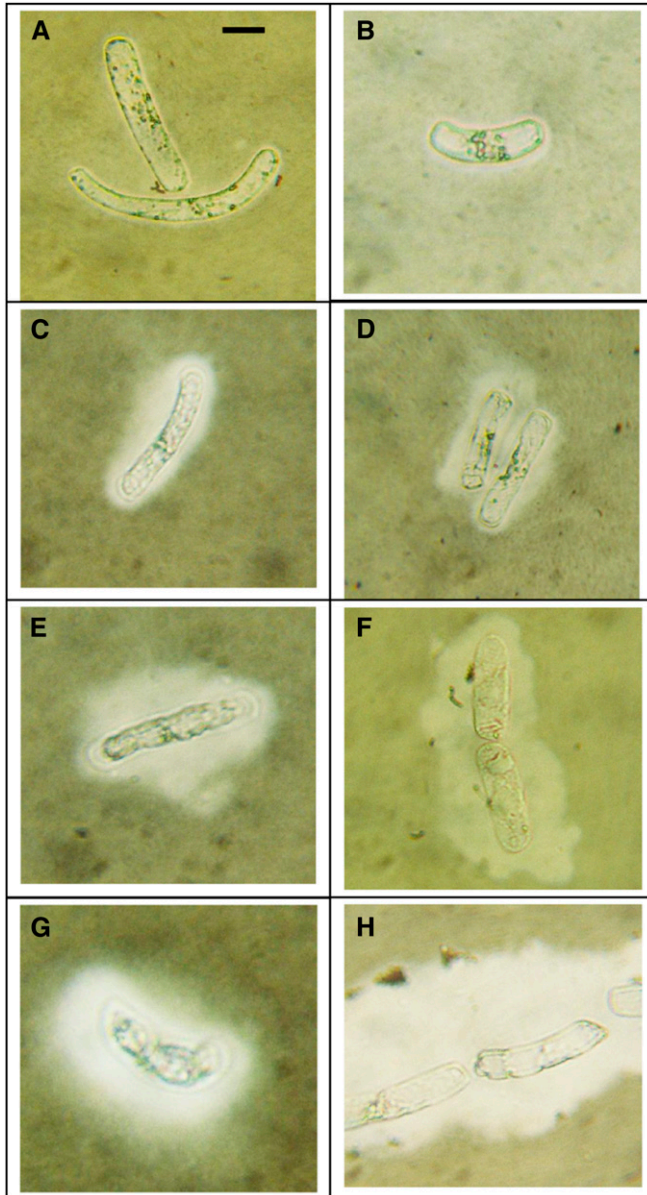


Fig. 2. India ink staining to monitor slime layer (“extracellular trap”) formation on individual border cells from root tips of conventional cultivar Sicot 189 (left) and transgenic cultivar PHY499 (right) immersed for (A, B) 20 min in water; (C, D) 1 mmol/L copper chloride; or (E, F) 3 mmol/L copper chloride. (G, H) Increased dimensions of slime layers remained stable after 24 h in 3 mM copper chloride. Size marker = 20 μ m.

When placed into 1 mmol/L copper chloride, border cell aggregation occurred instantaneously, such that detachment of the cells from the root tip was delayed in both cultivars, and even when dispersed by vigorous agitation, the cells primarily separated in clumps of 8–20 cells. Single cells of both cultivars responded within minutes by production of a uniform slime layer readily visible in response to india ink (Fig. 2C, D). The induced slime layers were approximately the same diameter as the cells ($20 \pm 1 \mu$ m). The dimensions of the layer remained stable over 24 h of observation even though the border cells were no longer viable (Table 2).

TABLE 2. Diameter of extracellular slime layer induced by copper chloride or water in border cells of two cotton cultivars at 15 min or 24 h after treatment.

Time after treatment	Sicot 189	PHY499
Water control		
15 min	negative ^{a*}	negative
24 h	negative	negative
1 mmol/L CuCl ₂		
15 min	$\geq 20 \mu$ m on 100%	$\geq 20 \mu$ m on 100%
24 h	$\geq 20 \mu$ m on 100%	$\geq 20 \mu$ m on 100%
3 mmol/L CuCl ₂		
15 min	$\geq 40 \mu$ m on 100%	$\geq 40 \mu$ m on 100%
24 h	$\geq 40 \mu$ m on 100%	$\geq 40 \mu$ m on 100%

^a >98% of cells had no slime layer when tested with india ink; ca. 2% had a minimal (<2 μ m in diameter) layer (as in Fig. 2A). Values for positive responses are the diameter of slime layer surrounding each border cell.

In response to 3 mmol/L copper chloride, border cells did not disperse readily into suspension but instead detached primarily (>90%) as sheets containing dozens of aggregated cells enmeshed within slime layers hundreds of microns in diameter and visible to the naked eye (Fig. 3). Single cells of Sicot 189 (Fig. 2E) and PHY499 (Fig. 2F) produced a uniform large slime layer (>40 μ m in diameter) within 15 min, and the layer remained stable 24 h later in both cultivars (Fig. 2G, H). No differences in border cell responses from the two cultivars were identified.

Border cell trapping of bacteria by conventional cultivar Sicot 189 vs. transgenic cultivar PHY499—Within 30 min of adding *Erwinia* populations to border cells of Sicot 189, a 100% trapping response had occurred (Fig. 4A–C). Every cell in the population was enmeshed in a continuous layer of bacteria and did not disperse even with vigorous agitation (Table 3). The trapping remained in place after 24 h and was reversed by DNase I; within 5 min of adding the enzyme, all cells were free of bacteria (as in Fig. 4D).

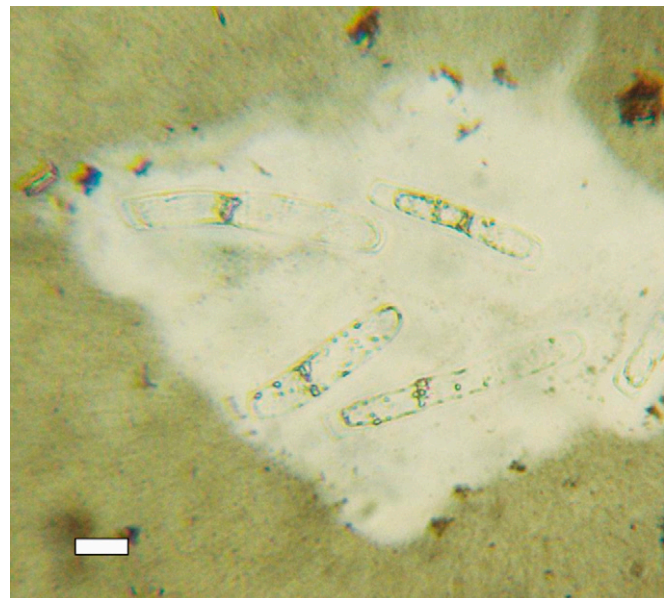


Fig. 3. India ink staining reveals a border cell aggregate with large slime layer (>100 μ m in diameter) induced in response to immersion for 2 min in 3 mmol/L copper chloride. Size marker = 20 μ m.

TABLE 3. Number and percentage of total border cells of conventional cv. Sicot 189 and transgenic PHY 499 with positive trapping response to *Erwinia carotovora* or *Bacillus subtilis* 30 min and 24 h after bacteria were added.

	Expt. no.	Sicot 189		PHY 499	
		No. positive/ Total no.	Positive (%)	No. positive/ Total no.	Positive (%)
<i>Erwinia</i>					
30 min	1	178/178	100	170/360	47
	2	224/224	100	128/205	62
	3	153/153	100	116/232	50
24 h	1	148/148	100	138/138	100
	2	155/155	100	160/160	100
	3	127/127	100	86/86	100
<i>Bacillus</i>					
30 min	1	244/310	77	139/307	45
	2	87/120	73	46/129	36
	3	92/116	79	40/102	39
24 h	1	365/631	57	139/480	29
	2	128/260	49	29/126	23
	3	206/387	53	62/250	25

Note: A trapping response was counted as positive when an individual border cell was covered in a layer of trapped bacteria, as seen in Fig. 4C, 4E (Curlango-Rivera et al., 2011).

There was a delay in maximum trapping of *Erwinia* in PHY499 border cells, with 47–62% having a positive response within 30 min. After 24 h, the responses were identical in both cotton cultivars (Table 3).

In response to *Bacillus*, trapping by PHY499 border cells was reduced by around 50%, compared with those of Sicot 189, at 30 min and at 24 hours (Table 3). As with *Erwinia*, all trapping was reversed within 5 min by the addition of DNase I.

DISCUSSION

Intraspecific variation in border cell number: Causes and effects?—The root cap of higher plants is the primary source of carbon-based material as young roots penetrate soil and establish an incipient rhizosphere (Lynch and Whipps, 1990; Odell et al., 2008). The impact of this carbon channeling on aboveground processes is unknown, and direct information about how the cells impact root development under natural soil conditions has remained elusive in part due to lack of plant variants with divergent production of border cells (Hawes et al., 2012). The availability of within-species variation in cotton border cell number provides an important tool to test the impact of their early delivery in the soil environment on plant growth and development.

The basis for this variation is unknown and could include environment, heredity, or both. Border cells are produced by a dedicated meristem within the root cap, whose cell cycle activity is regulated independently of the adjacent apical meristem (Guinel and McCully, 1987; Stephenson and Hawes, 1994; Brigham et al., 1998; Hawes et al., 1998; Ponce et al., 2005; Hamamoto et al., 2006). Cell number produced by a given root tip within a 24-h period under controlled conditions was found in early surveys to be conserved within families and to range from several hundred for tobacco, tomato, and other solanaceous species to several thousand for cereals, legumes, and cucurbits (Hawes and Pueppke, 1986). An effort to identify intraspecific variation within legumes by screening 1365 pea lines (cultivars described by Robbs et al., 1991) for changes in

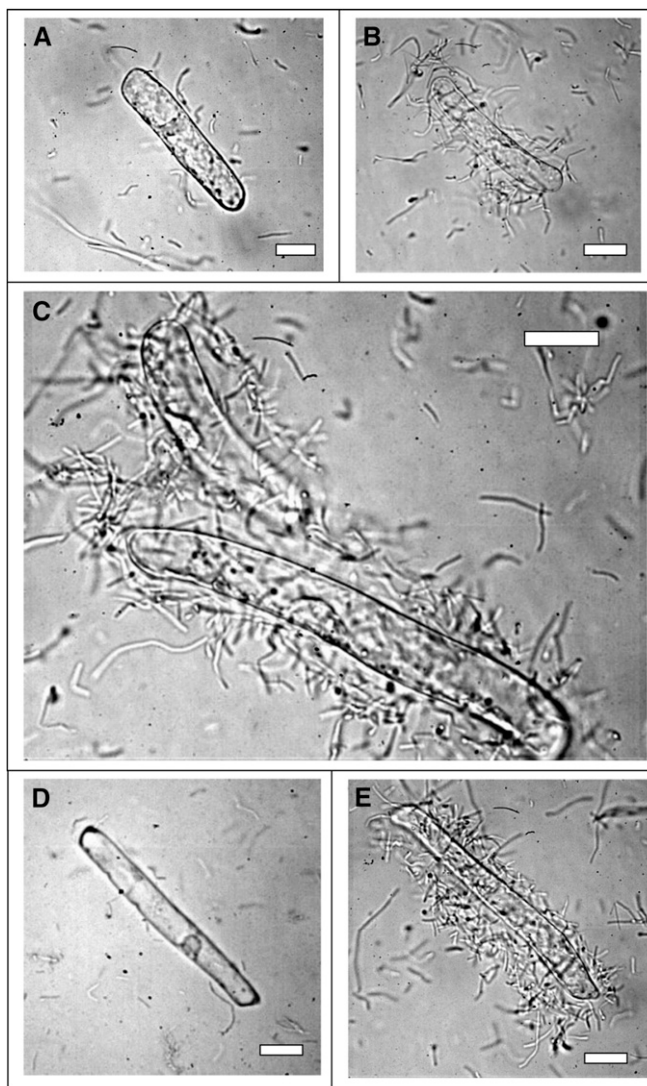


Fig. 4. Assay for trapping of bacteria by border cells and reversal of trapping by DNase treatment. Border cells were harvested as described and mixed with *Bacillus subtilis*. Responses were monitored over time using digital video imaging. Attachment of bacteria to a single border cell at (A) 12 min, (B) 22 min, and (C) 56 min is shown. (D) Reversal of attachment is evident within 10 min after adding DNase I. (E) After a recovery period of 45 min, attachment is restored. Size marker = 20 μ m.

border cell number yielded negative results: no significant differences were found (M. Hawes, unpublished data). Efforts to create variants by altering expression of genes controlling border cell production or properties in each case yielded pleiotropic or lethal phenotypes (Curlango-Rivera et al., 2010). Inhibiting expression of a gene that controls the cell cycle in alfalfa yielded plants with reduced border cell production and was correlated with increased susceptibility to a fungal pathogen and reduced nodulation by *Sinorhizobium meliloti* (Woo et al., 2004). However, root growth also was inhibited so effects of reduced border cell production were unclear.

Activation or suppression of cell cycle in the root cap meristem leading to changes in border cell number can occur in response to transient changes in environmental signals including soluble metabolites, increased carbon dioxide, microbial

colonization of roots, root age, availability of free water, and soil type (Guinel and McCully, 1986; Zhao et al., 2000a; Ijima et al., 2003; Odell et al., 2008; Somasundaram et al., 2008b; Curlango-Rivera et al., 2010; Endo et al., 2011). The differences seen here could result from unknown differences in environmental conditions of cultivation or harvest, seed treatments or storage, and/or natural or directed selection for phenotypic traits that also result in increased border cell numbers (Van Deynze et al., 2011).

Border cells and resistance of cotton to root disease—Border cells can be isolated within minutes as a population of detached living cells whose phenotypes reflect specific genotypes of the host plant (Hawes and Pueppke, 1986). As such, they comprise a convenient system for analysis of dynamic cellular responses to pathogens and other environmental stimuli under controlled laboratory conditions (Goldberg et al., 1989; Guinel and McCully, 1986, 1987; Gochnauer et al., 1990). Cotton cultivars with altered border cell number can be used in future studies to determine whether such phenomena function in variable root–soil interface properties and in crop performance in the field (Unruh and Silvertooth, 1996a, b; Norton and Silvertooth, 1998). Of particular interest are observations of differential resistance to fungal pathogens including *Verticillium* and *Fusarium* (Bolek et al., 2005). Knox et al., (2007) reported a limited correlation between border cell number and *Fusarium* wilt resistance in Sicot 189. If border cells play a significant role in protection of roots from fungal infection, then cotton lines with increased border cell production would be predicted to have a higher disease resistance index.

Bt toxin delivery into the soil environment by border cells—In the present study, border cell viability, morphology, and response to copper chloride were similar in a conventional cultivar, Sicot 189, producing 10002 ± 614 cells per root and a transgenic line, PHY499, producing 27921 ± 2111 cells per root. However, reduced trapping of bacteria by individual PHY499 border cells was apparent during 24 h of observation. In previous studies, adding antibody to a single protein among the >100 proteins secreted by border cells resulted in a significant increase in *Fusarium* infection of pea root tips (Wen et al., 2007). These data suggested that the border cell mucilage comprises an organized multidimensional structure such that altering a single component can significantly alter its function in defense of the emerging root (Knee et al., 2001). All of the transgenic Bt cotton cultivars evaluated by Knox et al. (2007) were found to express Cry proteins in border cells and its associated mucilage at varying levels among different cultivars. The impact of this introduced protein in the rhizosphere is unknown, but if border cells of the transgenic cultivars used here are found to express Bt toxin, it would not be surprising if Bt toxin delivered into the mucilage affected surface properties important in function(s) such as microbial trapping. Several recent studies have surveyed Bt cultivars of rice, corn, and cotton and found no significant impact on the chemistry and microbiology of the rhizospheres (Li et al., 2011; Verbruggen et al., 2012; Wei et al., 2012). By contrast, Chen et al., (2012) reported that repeated cultivation of transgenic cotton significantly altered rhizosphere microbiology and enzymology. A need for case-by-case analyses of transgene expression and its effects on rhizosphere biology has been emphasized (Bruinsma et al., 2003; Kabouw et al., 2012). The possibility that such divergent outcomes are influenced by altered patterns of border cell delivery of Cry proteins will be of interest in future studies.

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