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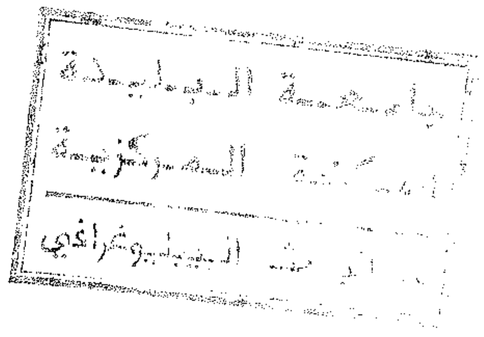

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FUNCTION OF ROOT BORDER CELLS IN PLANT HEALTH: Pioneers[1] in the Rhizosphere

M. C. Hawes, L. A. Brigham, F. Wen, H. H. Woo, and Y. Zhu
Departments of Plant Pathology and Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721; e-mail: mhawes@u.arizona.edu

KEY WORDS: root caps, root exudates, microbial gene expression, rhizosphere ecology

ABSTRACT

Plants dedicate a large amount of energy to the regulated production of living cells programmed to separate from roots into the external environment. This unusual process may be worth the cost because it enables the plant to dictate which species will share its ecological niche. For example, border cells can rapidly attract and stimulate growth in some microorganisms and repel and inhibit the growth of others. Such specificity may provide a way to control the dynamics of adjacent microbial populations in the soil to foster beneficial associations and inhibit pathogenic invasion. Plant genes controlling the delivery of border cells and the expression of their unique properties provide tools to genetically engineer plants with altered border cell quality and quantity. Such variants are being used to test the hypothesis that the function of border cells is to protect plant health by controlling the ecology of the root system.

"We think of plants, like we think of ourselves, as ending at the epidermis. What you're saying is that border cells are a literal example of a situation where that isn't true."
(Ian Sussex, personal communication)

[1]Pi-o-neer [fr. OF *peonier* foot soldier] 1: a member of a military unit usually of construction engineers. 2 a: person or group that originates or helps open up new line of thought or activity or a new method or technical development. b: one of the first to settle in a territory. 3: a plant or animal capable of establishing itself in a bare or barren area and initiating an ecological cycle (63).

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INTRODUCTION

Plant roots face a perpetual dilemma: In order to move into new territory to obtain nutrients and to anchor the growing plant, new tissue must be generated by the root meristem. The architecture of the entire plant, in fact, depends in part on the ability of root tips to sense signals from its environment and respond by directed growth of the root (2). Such newly synthesized plant tissue, unfortunately, is notoriously susceptible to attack by the array of soilborne organisms that are awakened in response to signals released by the invading root. One mechanism by which plants may cope with this problem is by the production of a "front line" of detached living cells (Figures 1 and 2). Border cells export a diverse array of biological chemicals that influence the behavior of fungi and bacteria (Table 1). Border cells released in advance of the vulnerable root tip may protect plant health by inhibiting tip infection by pathogens that would halt further growth, or by stimulating the development of beneficial associations. Under controlled conditions, border cells and their associated products can contribute up to 98% of the carbon-rich material that is released by plants as "root exudates," so their potential impact on plant-microbe interactions is large (25). This review summarizes what is known about the properties of border cells and the signals that regulate their production and release into the "rhizosphere," defined as "the region surrounding a root that is affected by it" (30). Evidence supporting the hypothesis that border cells constitute a uniquely differentiated root "tissue" whose function is to engineer the ecology of the rhizosphere is discussed in the context of experimental approaches that are being used to test predictions of this model.

ROOT BORDER CELLS

Border cells, originally called "sloughed root cap" cells, are living cells programmed to separate from the periphery of roots into the external environment (17, 22, 50). Because the structural linkages with the root and with each other are broken, border cells disperse into suspension upon immersion of the root tip into water (Figure 1). The cells do not become detached *en masse* in the absence of free water, unless the tip is wiped repeatedly with a damp tissue. The cells are enmeshed in a mucilage that can hold 1000 times its weight in water but does so only when actually immersed in water (26). Otherwise, the mucilage remains rather dry and border cells tightly adhere to the root periphery (Figure 3*A*). Immersion in water results in a release of cells that looks like an expulsion—within seconds, the mucilage swells dramatically and border cells disperse quickly into suspension (Figure 1). We proposed the new

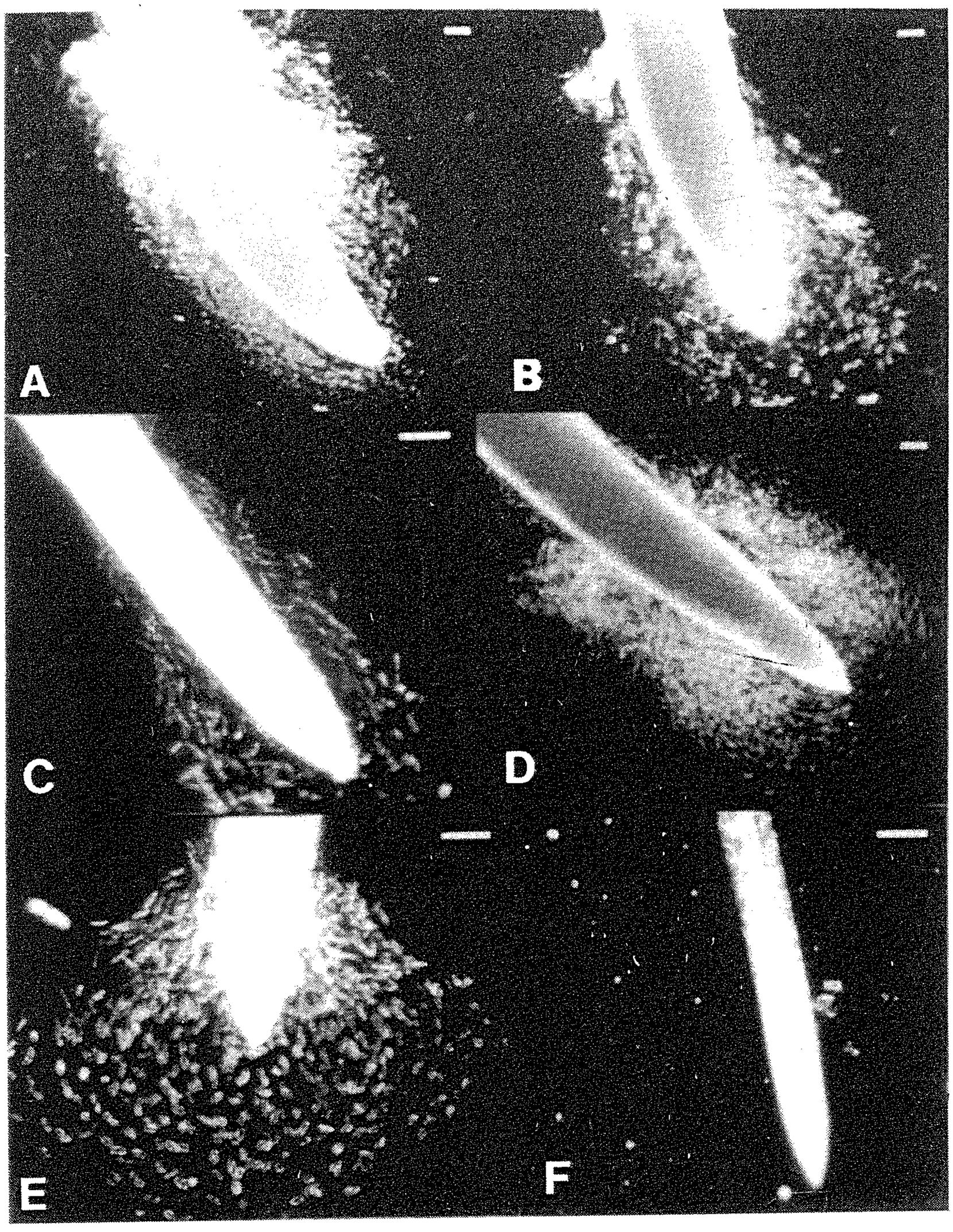

Figure 1 Root tips of (*A*) maize; (*B*) cucumber; (*C*) tomato; (*D*) cotton; (*E*) alfalfa; and (*F*) *Arabidopsis thaliana* grown and treated to reveal border cells, if present. Seeds were germinated on 1% water agar overlaid with filter paper, then immersed for 30 seconds in water without agitation, so that all border cells present are visible. *A. thaliana* root tips do not release border cells under the conditions used (10). Bar = 1 mm.

Table 1 Documented effects of border cells*

Effects on bacteria
Stimulation of sporulation
Stimulation of growth
Chemoattraction
Repulsion
Signals controlling gene expression
Effects on fungi
Chemoattraction
Repulsion
Stimulation of growth
Decoy for pathogenic infection
Substrate for mycorrhizal mantle development
Exported products
Signals for control of mitosis
Extracellular enzymes
Mucilage
Antibiotics
Phytoalexins
Unknown proteins

*Reviewed in this paper and References 31–37.

term, "border cells" in part because, by definition, they are not a part the root cap, so it is a misnomer to call them root cap cells (34, 38). In addition, the implied connotation of "sloughed"—synonyms are moribund, gangrenous, and putrid—interfered with conveying the reality that these are metabolically active cells (Figure 2*A*) that can survive as long as they are provided with appropriate nutrients and are protected from predators or other stresses (27, 39, 43, 45, 60). Viability of border cell populations generally is higher than 90% (Figure 2*A*, Figure 3*B*), and in culture the cells can undergo cell division (Figure 2*B*) and differentiate into organized tissue (39, 42). Nevertheless, border cells have been proposed to carry out an active pathway of programmed cell death (62). Border cell death can result from damage to the embryo, and in some species viability is lower than 90% in the absence of obvious stress (10, 39). It is possible that programmed cell death occurs in such plants in response to unknown stimuli. Cellular suicide could facilitate the rapid release of specific products from border cells into the rhizosphere.

REGULATION OF BORDER CELL PRODUCTION

Early surveys revealed that under standard laboratory conditions, the number of border cells produced varied from 0 to 10,000 per root among different species,

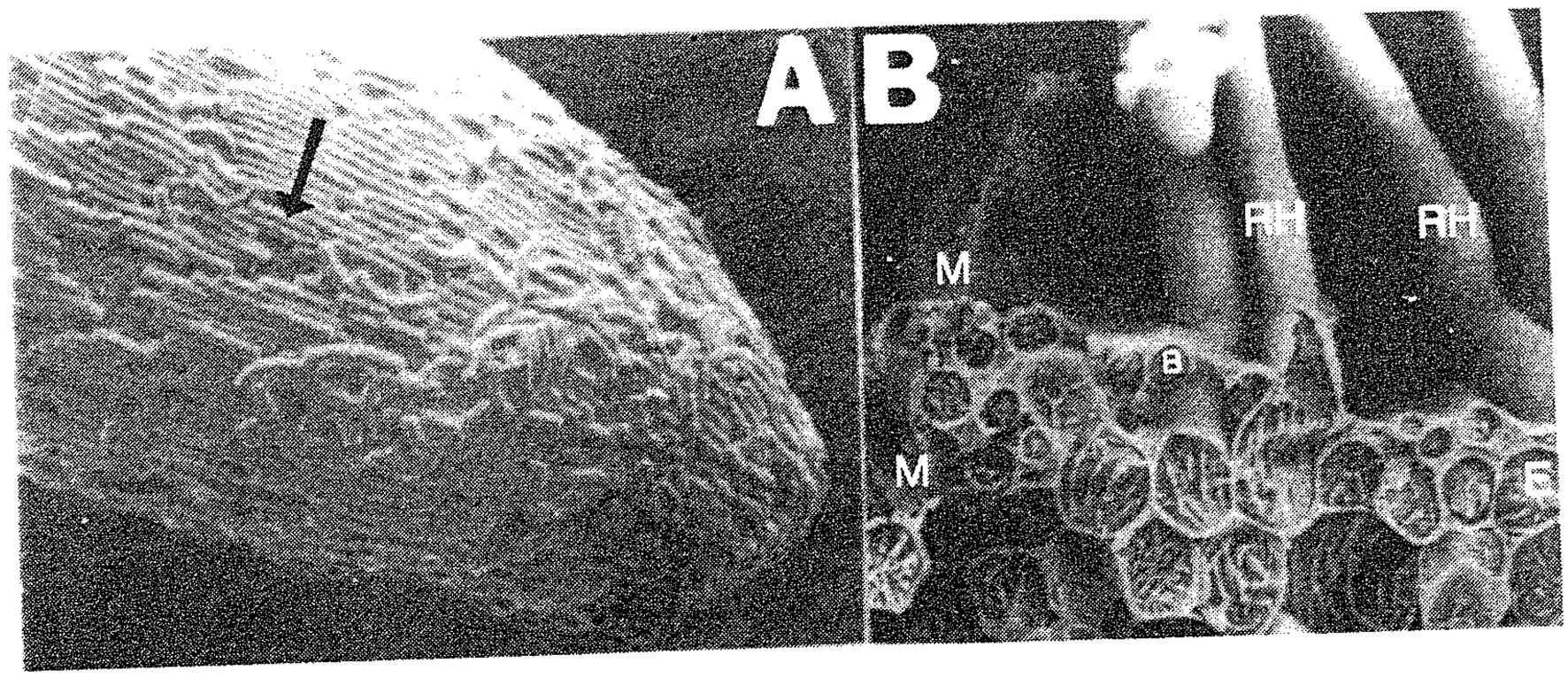

Figure 3 (*A*) Appearance of border cells of soybean in the absence of free water; cells are tightly appressed to the root surface (*arrows*). (*B*) Cross section of a soybean root grown through a clay particle matrix; border cells (*B*) embedded within mucilage (*M*) can be seen resting on epidermal cells (*E*), among root hairs (RH). (Photos by S Perkins, HE Calvert, WD Bauer; details in Reference 34.)

but the species-specific number of border cells produced daily was conserved at the family level: If one species released several thousand cells, other species in that family generally did too (10, 39). During early development, when seeds of pea and other species are germinated on water agar overlaid with filter paper, border cells can be collected from the tap root by the time the root is ca 5 mm long, but not before. Then cell number increases to a species-specific maximum, at which point cell production is turned off unless the existing cells are removed, such as by immersing the root tip in water and agitating gently (12, 38, 56). This treatment induces renewed border cell production. Within 1 h after removal of existing border cells, new border cells can be collected from the cap periphery and cell separation continues until a full set has again accumulated on the root periphery within 24 h. A similar developmental sequence occurs on lateral roots and on cultured hairy roots (47).

Border cells are produced by the root cap meristem, a layer of meristematic cells within the root tip that is physically distinct from the apical meristem that generates elongating root tissue (6, 19). After cell proliferation in the meristem, cells differentiate progressively through a series of developmental stages until the cells at the cap periphery separate as border cells. Contrary to a long-standing assumption that both meristems operate continuously (51, 55), the root cap meristem is regulated independently of the apical meristem (12, 34, 35, 38, 56). Thus, border cell production can be turned on and off independently of root growth. In response to the removal of border cells, mitosis in the root cap meristem (but not the apical meristem) is activated within 15 min and is preceded by

a switch in gene expression throughout the cap (12, 65). Many of the induced genes undoubtedly play roles in root cap development. One example is *rcpme1*, a gene encoding a pectinmethylesterase (PME) whose expression is localized in peripheral root cap cells and is required for border cell separation: When its expression is inhibited by antisense mRNA in transgenic roots, border cells do not separate from each other or from the root cap (36, 56, 64). Instead, cells accumulate in a pronounced ball and do not disperse into water like normal border cells (Figure 4).

SIGNALS CONTROLLING BORDER CELL SEPARATION

A chemical that acts to inhibit mitosis in the root cap meristem accumulates extracellularly during the process of border cell production and is released from washed border cells (8). When this repressor, which we call "Factor B," is added back to induced root caps, the normal induction of renewed border cell production is inhibited. This signal may explain in part why border cell separation is a self-limiting process: The more border cells accumulate, the more Factor B is released, until it reaches a critical level that blocks cell division and differentiation. Recently, however, it has become clear that environmental signals can override the normal regulation of border cell separation by endogenous signals such as Factor B. In wheat, for example, border cell production can be altered by up to two orders of magnitude, apparently in response to changes in growth medium, colonization by bacteria, and/or genotype (MC Hawes, E Milus, E Pierson & LS Pierson III, unpublished data). Pea roots respond to the type of increase in CO_2 levels that develops in shallow soils (14, 16), by producing an entire new set of border cells on top of the existing set of 3400 (Figure 5*A*) (67). Interestingly, not all plants respond as does pea to increased CO_2: Alfalfa border cell production is impervious to the same levels of CO_2 that stimulate border cell synthesis in pea (Figure 5*B*). Pea border cell production also is influenced by extracellular pH (J Chen & MC Hawes, unpublished data). A substantial change in extracellular pH within the root cap actually occurs normally during the process of border cell separation and may play a role in self-regulation of the process (64).

An emerging understanding of the molecular framework of border cell production has revealed that the process is highly responsive to environmental and endogenous signals. Understanding how the interplay of exogenous and endogenous signals acts to regulate border cell production in vitro, let alone in soil, will be a long-term process. The work to date already has made clear that the rhizosphere of even a single plant may vary drastically as a result of changes in border cell production in response to normal stimuli facing roots.

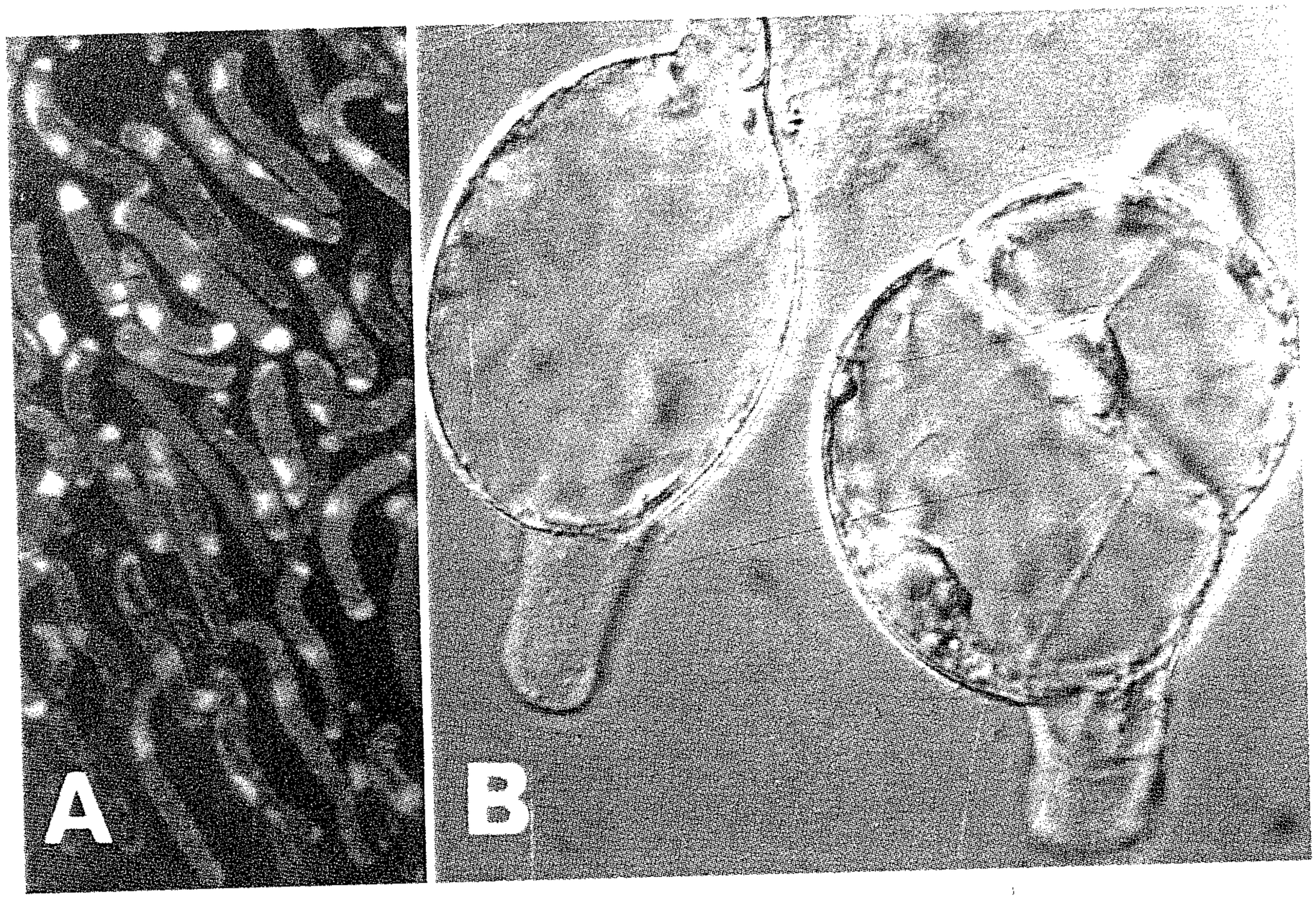

Figure 2 Viability of border cells of pea measured by staining with fluorescein diacetate (*A*) and by ability to undergo cell division in response to plant hormones (*B*) (From References 39, 43).

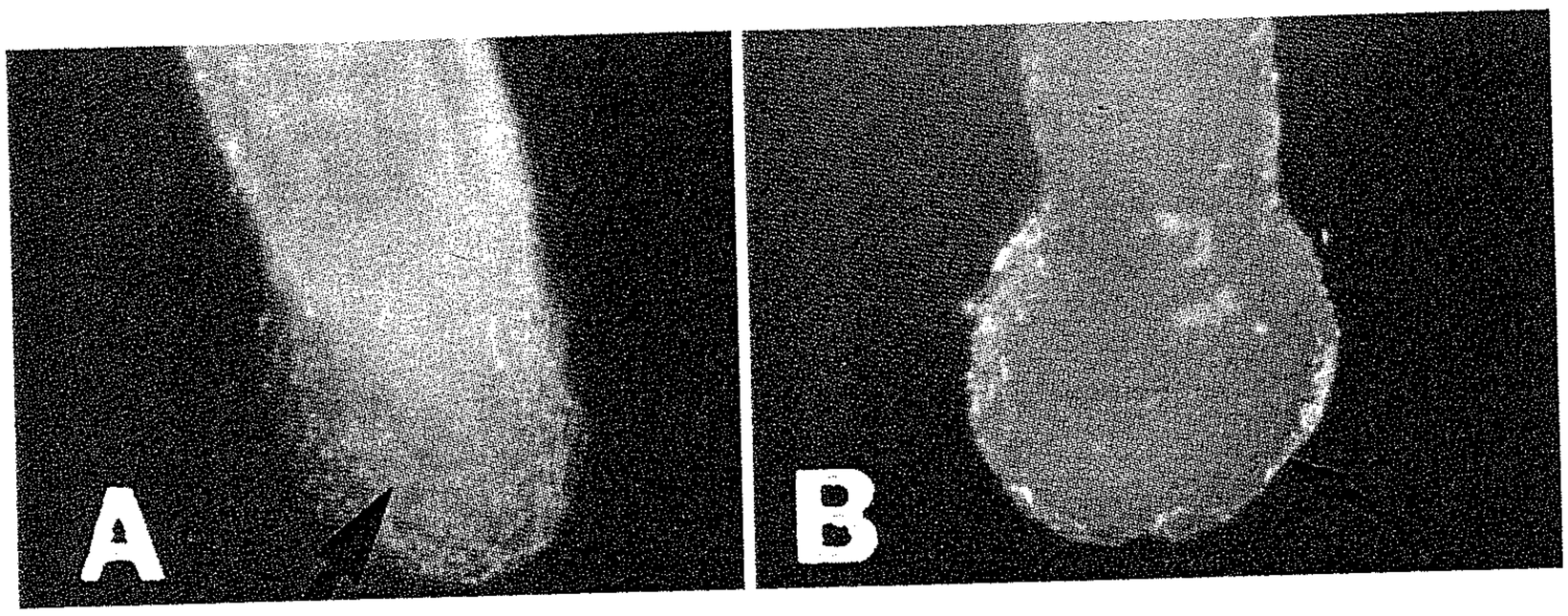

Figure 4 Requirement for an inducible, root cap localized pectinmethylesterase gene (*rcpme1*) in border cell separation and release. Whereas normal hairy root border cells disperse readily when the root tip is placed into water (*A*), border cells of roots expressing *rcpme1* antisense mRNA accumulate in a pronounced ball at the tip, and do not disperse into water (*B*) (64).

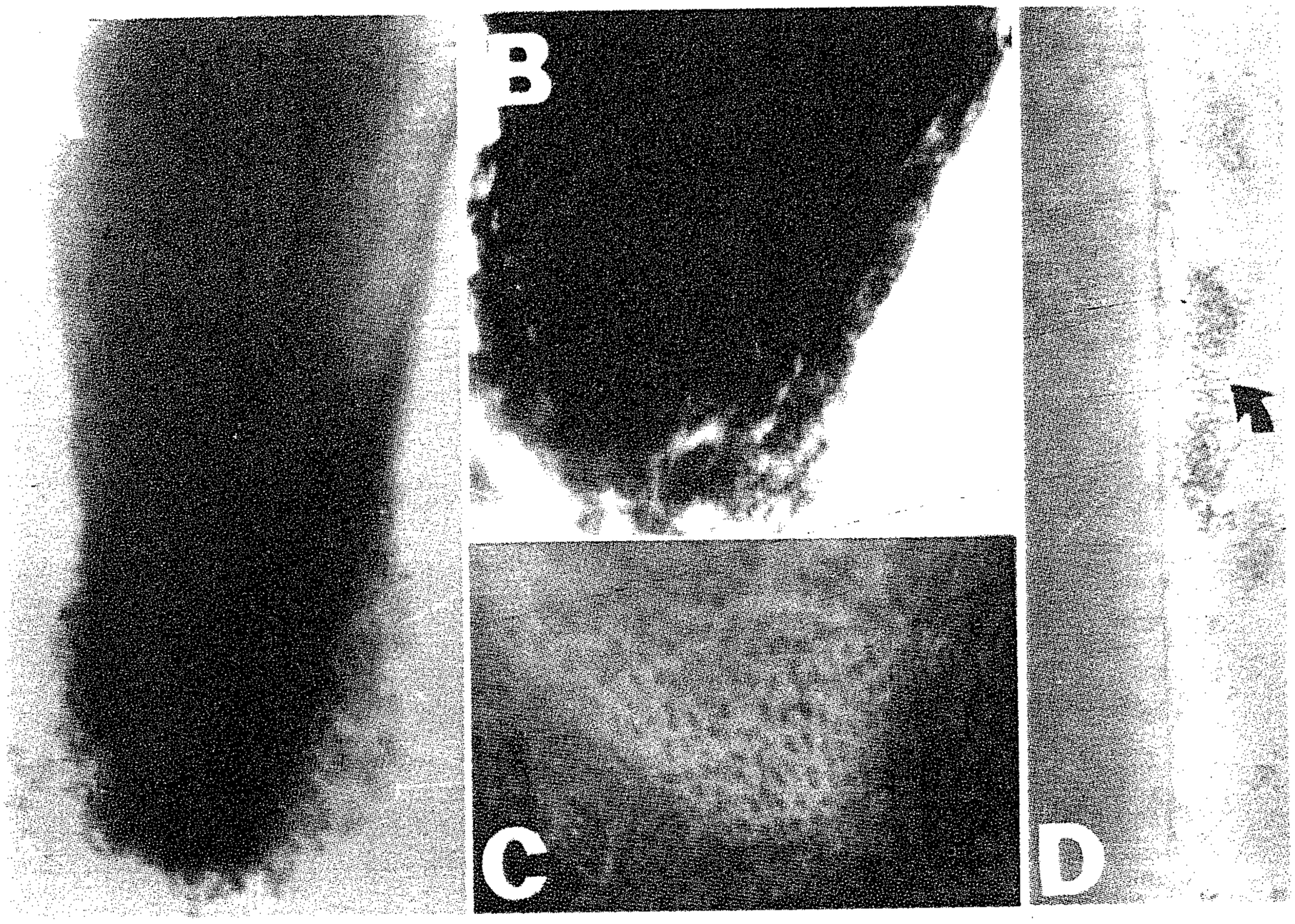

Figure 6 Gene expression in border cells. (*A*) Constitutive border cell-specific production of anthocyanins in sorghum roots; red border cells can be seen against a background of white root tissue (MC Hawes, unpublished). (*B*) Stress-inducible expression of bean *pal1*-glucuronidase in pea hairy roots (details in Reference 47). Expression in border cells results in their appearance as a contiguous blue-black fringe against the blue-black root. (*C*) Border cell-specific expression of galactosidase (J Chen & MC Hawes, unpublished). Under certain conditions, enzyme activity is only expressed in border cells, which are seen as blue against the white root cap. (*D*) Galactosidase continues to be expressed in border cells after they have been left behind in more mature regions of the root (*arrow*).

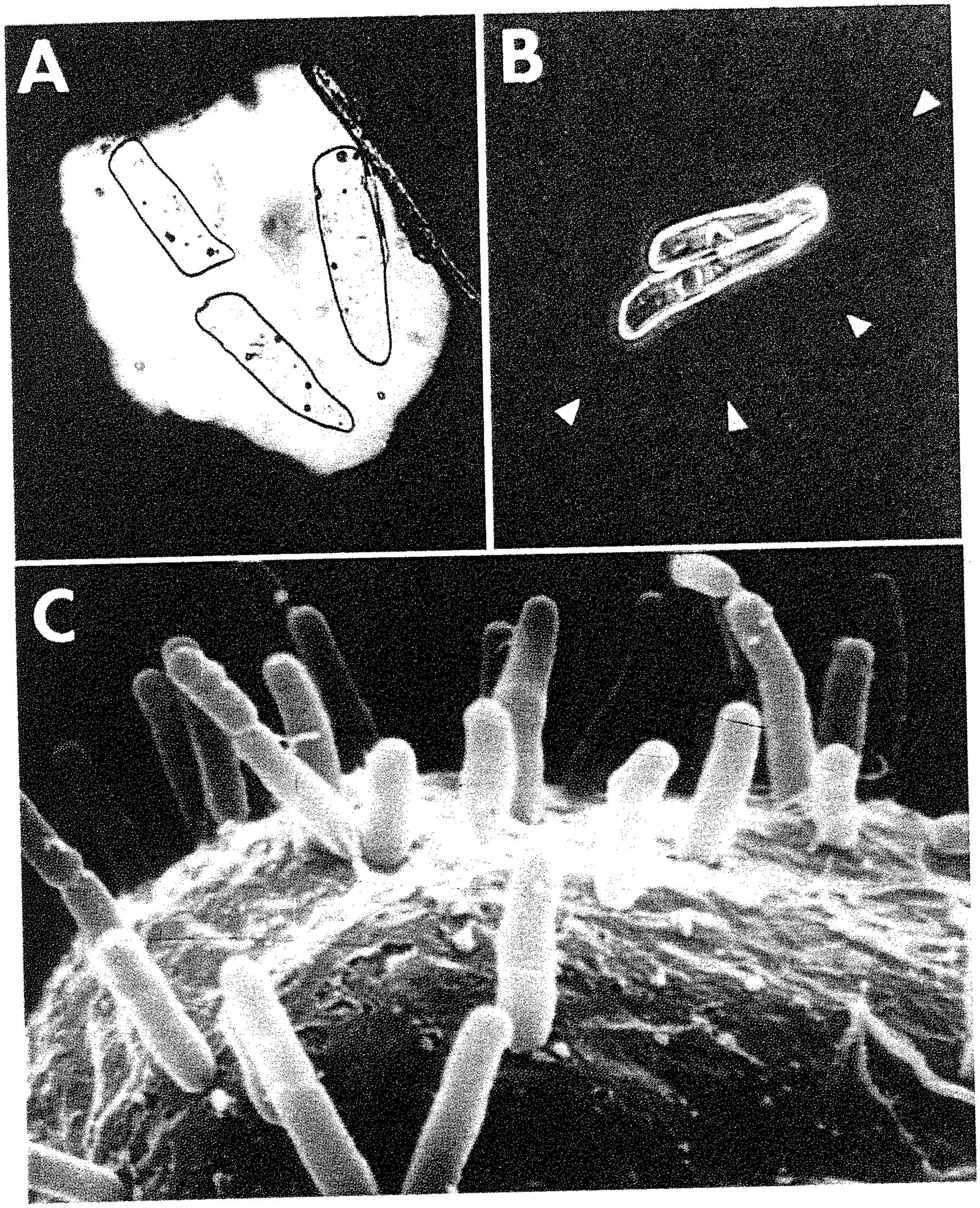

Figure 9 Specificity in bacterial binding and repulsion by border cells and surrounding mucilage. Some border cells, such as those from oats, have a large, variable polysaccharide boundary that can be visualized when stained with india ink (*A*). This layer repulses *Agrobacterium tumefaciens*, which is present in large numbers outside the boundary (*B*, *white triangles*) but not within it. Bacteria that enter the layer exit quickly (34, 40). In pea, the same bacteria bypass the mucilage layer and bind tightly to the surface of the border cell wall (*C*) (SEM photograph by Jerry White, details in Reference 34).

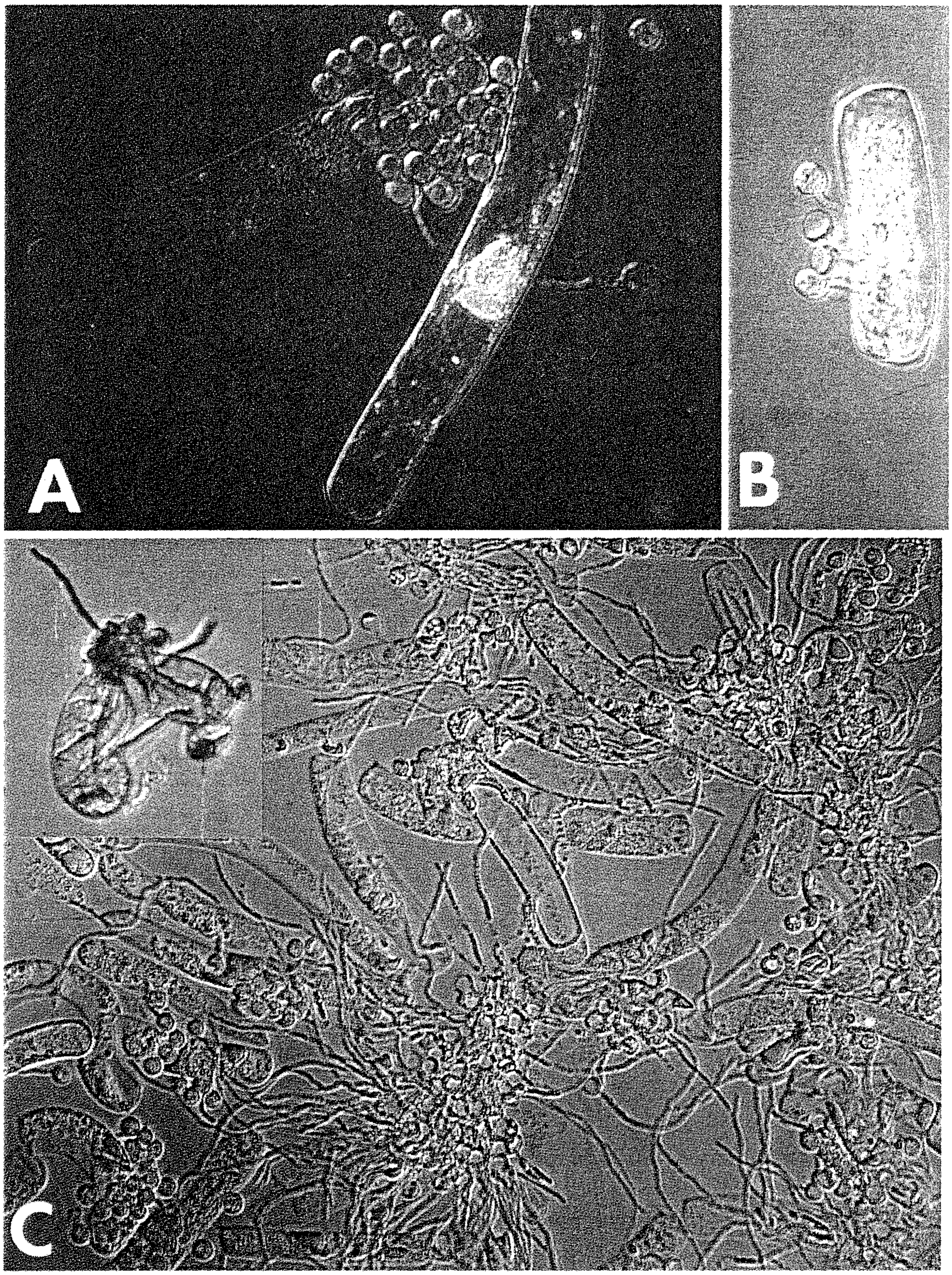

Figure 10 Rapid host-specific accumulation, infection, and multiplication of soilborne fungi in response to border cells. When a suspension of cotton border cells is mixed with a suspension of *Pythium dissotocum* zoospores, accumulation on individual cells is obvious within 1 min (*A*). Zoospores germinate and penetrate within 2 to 5 min (*B*) and within 15 min hyphae ramify throughout the cytoplasm (*C*, *inset*). After a 2-h incubation, most border cells are dead and many are digested to the point of being barely recognizable as cells (*C*) (24).

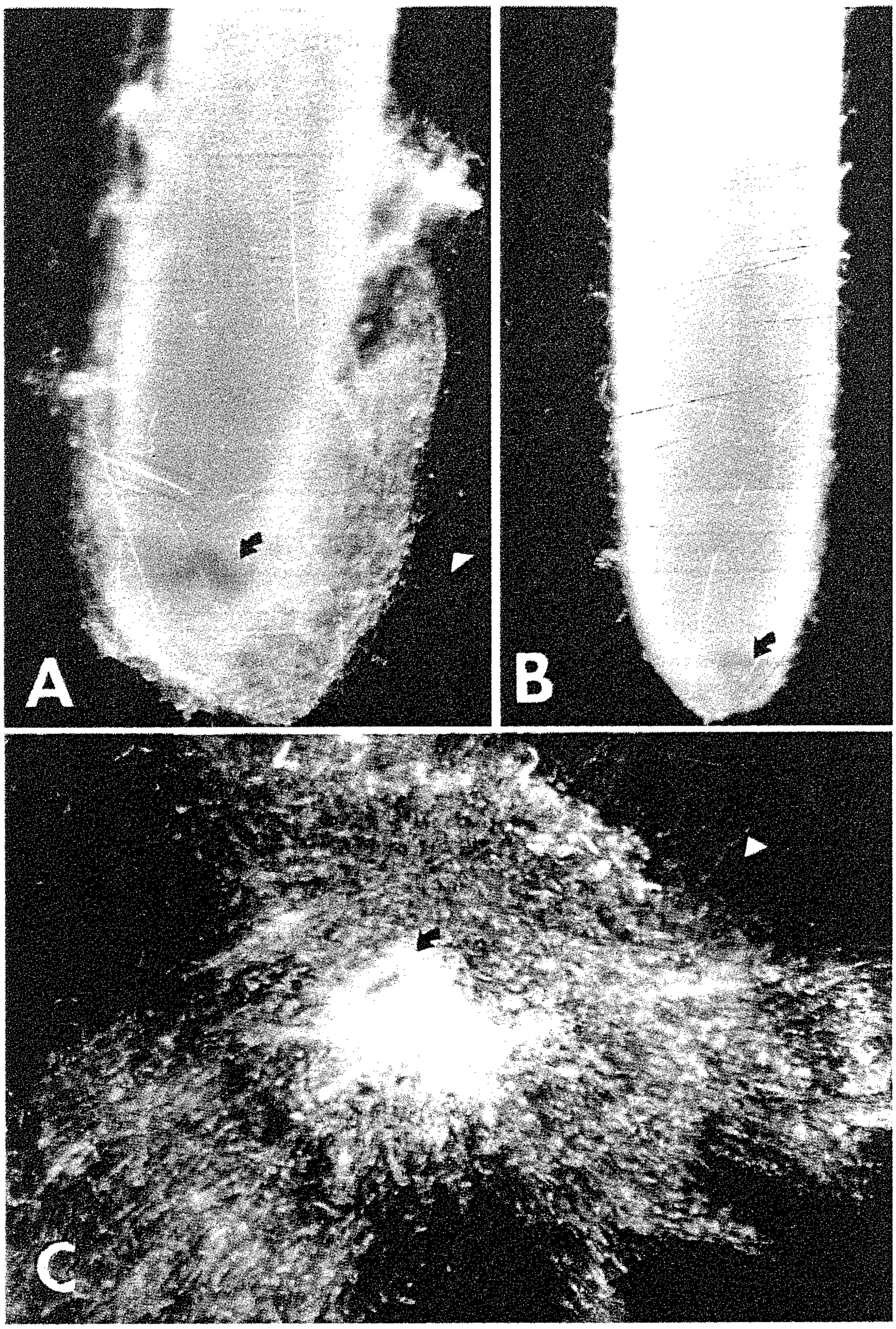

Figure 11 Removal of root tip infection by removal of border cells. (*A*) Three days after inoculating whole roots with spores of *Nectria haematococca*, the apex was covered by a mantle with hyphae extending outward (*white triangle*), and a small brown lesion (*arrow*) was present within the root cap. (*B*) When placed in water, the mantle spontaneously dropped away, taking half of the lesion (*arrow*) and leaving the surface of the root free of visible fungal hyphae. (*C*) The separated mantle consisted of digested border cells held together in a mass by fungal hyphae (*white triangle*). A darkened segment matching the shape of the lost lesion was visible at the tip of the mantle (*arrow*) (28).

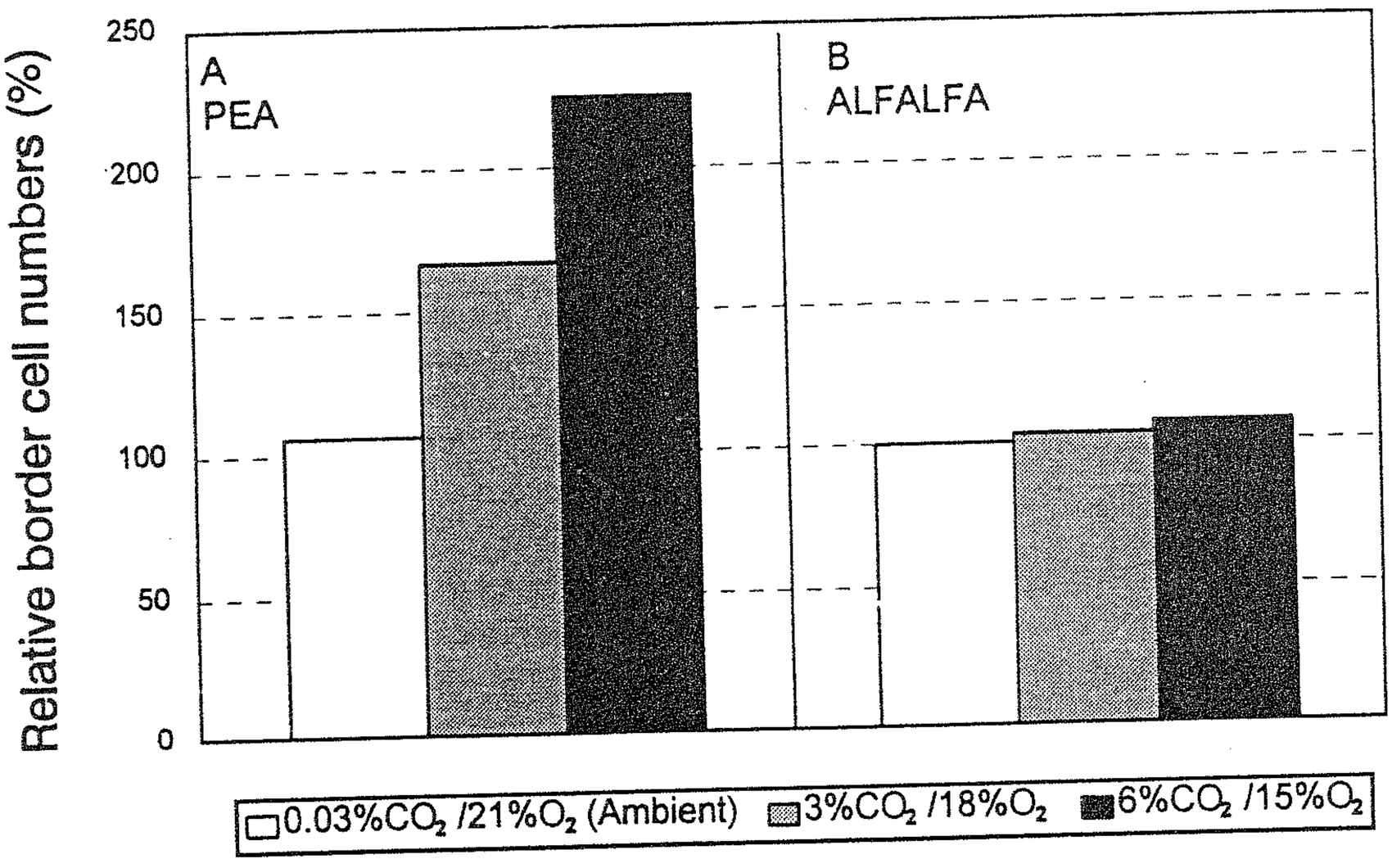

Figure 5 Species-dependent regulation of border cell separation by controlled atmospheres. Seedlings (root length ca 20–25 mm) germinated under ambient conditions were transferred into indicated treatments and border cell numbers were counted three days later. Mean border cell numbers present on roots prior to treatment (3760 for pea and 1780 for alfalfa) are used as 100% (67).

GENE EXPRESSION IN BORDER CELLS

It is natural to assume that anything that is not attached to the plant is not a viable part of the root system and this assumption was made about border cells for many years (18, 29, 51). In the face of prevailing wisdom that border cells were dead or dying, some early experiments focused in part on demonstrating that border cells could do anything that other cell types can do. These experiments revealed that, in addition to a capacity to respond to plant hormones by initiating cell division in culture, just like other cells, border cells also expressed specific genes that left them susceptible or resistant to host-specific toxins, just like other cells (32–34, 43, 59).

Only later did it become clear that in some cases, border cells exhibit traits that are not like other cells, even their immediate progenitors in the root cap (32–37). One example is the production of the red pigmented antibiotics shikonin (9) and anthocyanin (MC Hawes, unpublished data) in roots of *Lithospermum erythrorhizon* and sorghum, respectively, which occurs specifically in border cells under certain conditions (Figure 6*A*). We tested the hypothesis that such border cell–specific phenotypes occur because the cells are specialized for distinct functions that are reflected in distinct patterns of gene expression (11). A

dramatic switch in gene expression occurs upon differentiation of root cap cells into border cells: Many of the mRNAs and proteins made in the root cap are degraded, and a new set is synthesized in border cells. The predominant change is toward smaller acidic proteins that are exported almost immediately into the extracellular environment. Sequences of some border cell–specific genes are unique, and their functions are unknown (8), but several proteins whose expression is known to be influenced by biotic and abiotic signals are expressed in border cells. These include a heat shock protein (HSP70), isoflavone reductase (IFR) (11), phenylalanine ammonia lyase (PAL) (47), a galactosidase (BGAL) (J Chen & MC Hawes, unpublished data), and a rhizobium-induced peroxidase (RIP1) (15). PAL is not expressed constitutively in border cells but is induced in response to stress as it is in other tissues (Figure 6*B*). BGAL is expressed specifically in border cells under certain conditions (Figure 6*C*) and continues to be expressed in cells that have been shed from the cap for a day or more (Figure 6*D*). RIP1 expression also is detectable in border cells for some time after they have been shed from the cap (Figure 7).

It is easy to imagine ways that the products of such genes could influence the biological properties of the rhizosphere. For example, border cells are a source of flavonoid-based *nod* gene-inducing signals that are products of the pathway that includes PAL and IFR (68). Release of such signals from pea border cells increases in response to incubation with *R. leguminosarum* bv *viciae* (Figure 8*A*) and is much higher at cool temperatures that are physiologically appropriate

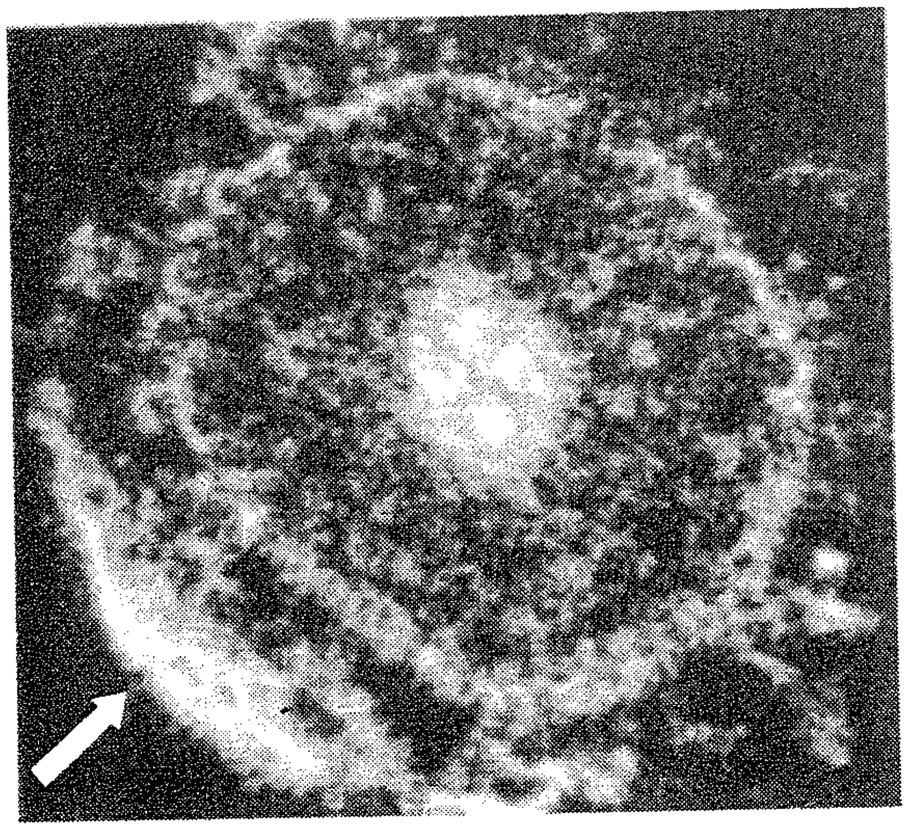

Figure 7 Expression of a rhizobium-induced peroxidase gene (*rip1*) in border cells of *Medicago trunatula*, approximately 1 cm behind the root apex, 12 h after inoculation with the compatible *Rhizobium meliloti* symbiont. Micrograph depicts in situ hybridization (seen as white against the dark field) (details in Reference 15) in the epidermis, vascular tissue, and in detached border cells (*arrow*) outside the periphery of the root (unpublished photos courtesy of D Cook & K Vanden Bosch).

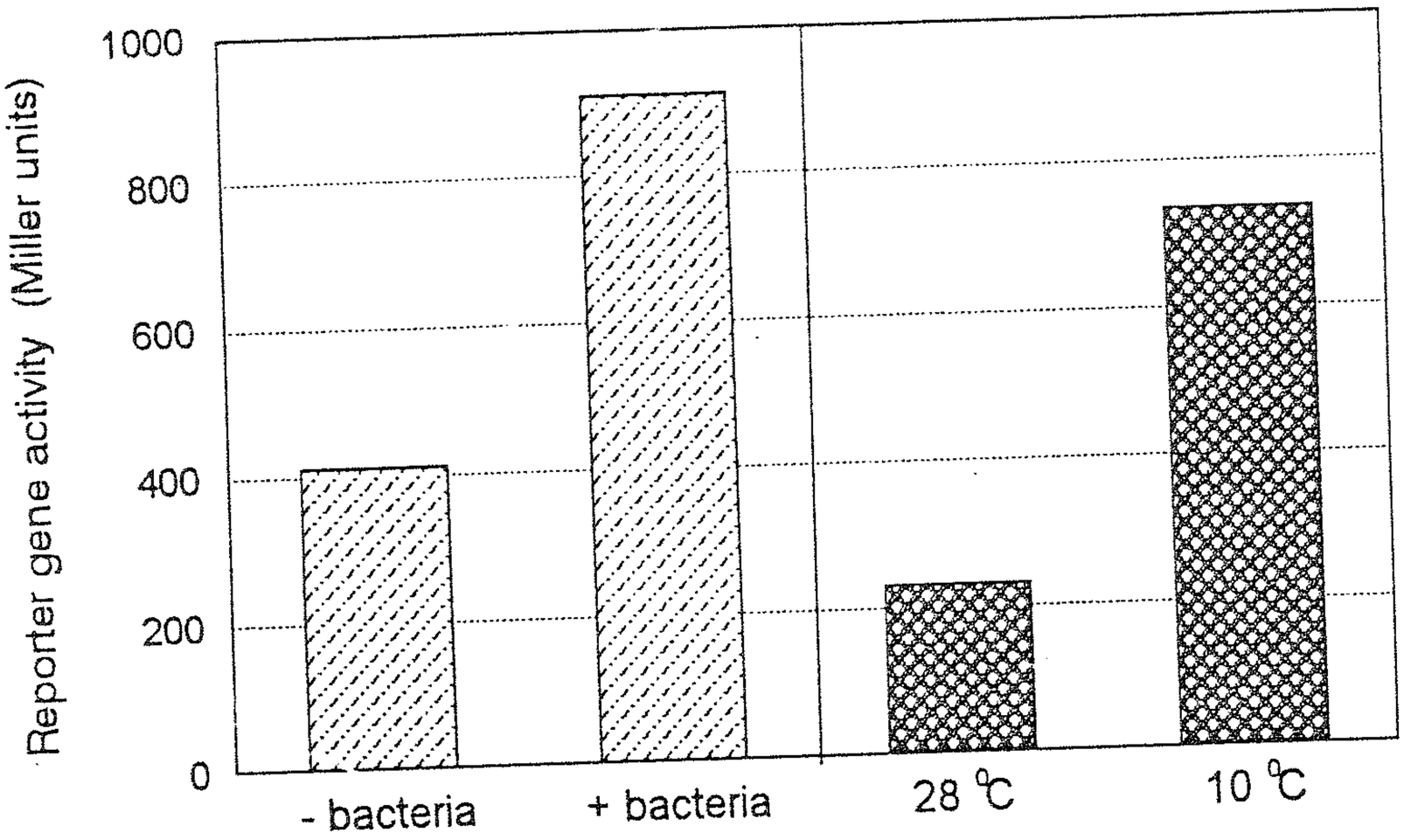

Figure 8 Release of nodulation gene–inducing chemicals from pea border cells. A low constitutive level of extracellular *nod* gene–inducing activity released during a 12-h period increases more than twofold in response to cocultivation with *Rhizobium leguminosarum bv viciae* (*A*). Extracellular *nod* gene–inducing activity released from pea border cells over a 24-h period is more than threefold higher at 10°C than at 28°C (*B*) (68).

for pea roots than at temperatures conducive to microbial growth (Figure 8*B*). As a root tip moves through the soil, the products of border cells are likely to be the first plant signals detected by soilborne bacteria. Rhizobia infect in the region behind the root tip where the first root hairs emerge, but not in older tissue, resulting in a narrow window of time and place where the nitrogen-fixing symbiosis is initiated (7). An ability to begin the signal exchange in advance, so that *nod* genes are being expressed actively at the time of contact with susceptible tissue, could greatly improve the chances that nodulation will actually occur. Border cells also may be a source of specific flavonoid-based chemicals needed to stimulate vesicular arbuscular mycorrhizal (VAM) fungi, which also infect in the region behind the root tip (58). The number of border cells produced by a given species is highly correlated with its ability to develop VAM associations (3, 48). Such observations may help to explain the striking specificity that border cells exhibit in their responses to fungi and bacteria.

HOST-SPECIFIC RECOGNITION, CHEMOTAXIS, BINDING AND GROWTH OF PATHOGENS AND SYMBIONTS IN RESPONSE TO BORDER CELLS

The host- and tissue-specific responses of border cells to microorganisms have been reviewed in detail (10, 31–37, 41). In summary, as with other plant cells, the ability of any given bacterium or fungus to associate with and/or

utilize the nutrients in border cells is strictly dependent on the genotypes of the plant and the microorganism. This applies not only to the plant cells per se, but also to associated exudates including a large polysaccharide layer that surrounds border cells (Figure 9*A*). This layer forms a boundary that inhibits accumulation of some bacteria (Figure 9*B*) but has no effect at all on others (23, 34, 40). In some cases, bacteria can penetrate the mucilaginous layer to bind tightly to the outer surface of the cell wall (Figure 9*C*). Such variation almost certainly could influence how readily bacteria can utilize the nutrients border cells have to offer.

Specific responses of border cells to pathogenic fungi are even more dramatic (34). For instance, single maize border cells, in the absence of any exogenous nutrients, can synthesize defense structures that repel penetration by *Colletotrichum graminicola* hyphae (54). In other cases, border cells can specifically attract fungal propagules (24): Zoospores of *Pythium dissotocum* accumulate on border cells of cotton almost instantaneously (Figure 10*A*). Within minutes, the spores germinate, penetrate (Figure 10*B*), and proliferate using only border cells as a carbon source (Figure 10*C*). Zoospores of *P. catenulatum* exhibit exactly the same behavior in response to cucumber border cells but are completely unresponsive to cotton border cells. These zoospores sit in suspension with the border cells and do not attach, germinate, or grow, even after the border cells die and release their cell contents (24). Since these contents include simple sugars and other nutrients that *P. catenulatum* can utilize easily, the inability of the fungus to grow even on dead border cells suggests that the cell contents may contain an inhibitor that actively prevents growth of this organism. The potential power of such selective stimulation of movement, attachment, and growth of microorganisms in controlling the dynamics of complex rhizosphere communities is obvious.

FUNCTION(S) OF BORDER CELLS: WHY SEPARATED CELLS?

At this time the biological basis for the shedding of border cells is unknown. Though it is easy to conjure scenarios in which signals from border cells might influence plant health by controlling microbial behavior, there is no obvious selection pressure to do it via detached border cells. Chemicals such as antibiotics that are present in border cells also are secreted from the root itself (9, 16), and there is no a priori reason to assume that detached border cells can secrete it more effectively. For many years the process was presumed to provide a lubricating function, based on logic rather than evidence. As Rogers et al (50) put it, "Early recognition of this sloughing away of certain root tissues as a normal process during root growth led to little more than mere speculation as to what

the role of such a process might be in the nutrition of the plant. The process was assigned, quite summarily it seems, the function of lubricating or protecting the advancing root tip as it forced its way into the soil." If correct, predictions of the model would be that the mucilage encasing border cells is slimy under most conditions, and that the number of border cells is correlated with the ability of roots to penetrate solid media. Neither prediction is true (26, 34).

A hint of a unique function that would explain the need for separated cells comes from work describing the events leading to root colonization by ecto-mycorrhizal fungi (13, 21, 44). Prior to invasion of the root, such fungi establish colonies external to the root surface by producing a sheath or "mantle" over the root apex. Direct time-sequence analysis reveals that border cells provide the structural and nutritional base for the development of this mantle. Though the authors, unaware that border cells are alive, called this early stage a "necrotrophic" phase, they clearly demonstrated that a period of living on border cells external to the root tip precedes the development of a stable biotrophic relationship. Border cells that are physically detached from the root tissue per se may allow the fungus to grow in close association and engage in a stabilizing signal exchange while circumventing the rapid early defenses that plants launch in response to cellular attack.

An even more straightforward hypothesis is that border cells act as decoys that lure potentially dangerous soilborne organisms away from the vulnerable root tip (32–34). Foster et al (20) noted that root tips of soil-grown plants tend to remain sterile even when the rest of the root has been colonized, and proposed that this may occur because organisms that colonize or invade the root tip are shed from the tip as border cells separate. Only cells that separate from the root physically could accomplish this. It is important to reiterate that only in the presence of free water are border cells actually dispersed away from the root and from each other; this also is the only time that microorganisms in the soil can initiate relationships with roots because free water is needed for movement and germination. Many organisms including nematodes, bacterial and fungal pathogens, and symbionts initiate infection in the region of elongation, just behind the root tip (16). This selectivity in infection has been attributed to an abundance of root exudates released in this region, primarily based on one frequently cited histological study (49). An alternative explanation is that this is just the first place where microorganisms can invade young tissue that stays put.

Surprising results from our laboratory are consistent with the hypothesis that border cells can act as a decoy that keeps fungal infection physically separate from the root cap (28). Whole roots of pea were inoculated uniformly with a suspension of virulent *Nectria haematococca* spores and then incubated in cellophane growth pouches. Within three days, visible necrotic lesions

developed behind the root tip, but to the naked eye the root tip appeared uninfected and root growth was indistinguishable from that of uninoculated controls. Microscopic examination, however, revealed that the tip actually was covered with fungal hyphae (Figure 11*A*). The hyphal growth was confined to the apex, and was remarkably similar in appearance to mantles that form in response to ectomycorrhizal infection (13, 21, 44). A microscopic brown lesion within the root cap also was evident (Figure 11*A, arrow*). When the root was placed into water, the mantle spontaneously fell off as a unit. Afterwards, only newly emergent border cells, not fungal hyphae, were evident at the surface of the root (Figure 11*B*). In contrast, prolific hyphal strands were obvious throughout the detached mantle, which remained as an intact entity rather than dispersing as a population of separated border cells (Figure 11*C*). Part of the root cap lesion also came away with the border cell mantle; one whole side of the brown spot disappeared altogether, and the remaining part (Figure 11*B, arrow*) was lighter brown. The lost half of the lesion was visible at the surface of the detached mantle (Figure 11*C, arrow*). It appeared that, just as predicted (20), the fungus that infected border cells or soon-to-be border cells of the root cap, was jettisoned along with the separating cells.

FUTURE PERSPECTIVES: GENETIC ENGINEERING OF BORDER CELLS

Understanding Border Cells: Rhizosphere Dynamics

The best method to test the hypothesis that border cells regulate root-microbe interactions is to compare the ecology of isogenic plants with and without border cells. Developing such variants has been the focus of our laboratory for several years. Two categories of genes are being used to manipulate the production and properties of border cells: Those that play a role in border cell separation and those that are expressed in border cells (35). Experiments designed to understand the molecular basis for the synthesis and separation of border cells, and for the expression of their unique properties, have yielded several classes of genes that provide tools to test predictions of our model (8, 10, 11, 12, 36, 64, 65, 66). Thus, if *nod* gene-inducing chemicals from border cells are needed to pre-induce rhizobia before they reach susceptible tissue, then roots that do not release border cells would be predicted to be ineffective in nodulation. If border cells protect root tips by shedding pathogenic fungi, then roots whose border cells do not separate would be predicted to be highly vulnerable to tip infection. Such predictions can be tested directly now that roots with altered border cell separation are becoming available (Figure 3).

Sorting out exactly how border cells influence soil ecology will be complex, to put it mildly. A single population of border cells, isolated under a single set of controlled conditions, can release an array of chemicals that have multiple effects on pure microbial cultures (Table 1). Under natural conditions border cells encounter extremely complex and multifaceted populations of bacteria, fungi, and other organisms (16, 30). Taking into consideration the ways that biotic and abiotic signals may act to turn border cell production on and off within distinct microenvironments, let alone influence border cell gene expression, the possible combinations of effects and counter-effects quickly overwhelm the mind's ability to formulate a comprehensive model. However, not fully understanding a process never has inhibited the human capacity to make effective use of it.

Using Border Cells: Rhizosphere Loading for Nutrition, Disease Resistance, and Bioremediation

The process of border cell release provides a natural mechanism to equip the leading edge of the rhizosphere—the root tip region where growth, nutrient and water uptake, and initiation of microbial relationships occur—with chemicals that foster plant and/or human health. This region is a primary target for agricultural additives, which currently must be broadcast throughout soils where most inevitably go to waste. Border cells, in contrast, can be used to deliver chemicals directly to the rhizosphere, without polluting vast acreages. The promoters of genes expressed in border cells can be linked to structural genes encoding products such as antibiotics, pesticides, and nutrient-solubilizing enzymes. The use of border cell–specific promoters that are expressed only after the cells separate from the root minimizes problems inherent in overexpressing such products within plant tissue. Potential applications are wide-ranging (34–37). These include altering physical properties of the environment such as pH to facilitate nutrient uptake, detoxication of contaminated soils, and establishment or inhibition of specific microbial relationships. As more genes encoding specific metabolites known to play a role in plant-microbe recognition are characterized, more opportunities to utilize border cells to create plant cultivars designed for specific environments will become available. For example, border cells can be used to deliver antibiotics like phenazines that foster colonization by beneficial microorganisms (LS Pierson III, personal communication).

CONCLUDING REMARKS

In the rhizosphere as elsewhere in the world, the key problem facing microorganisms is the need to obtain water and nutrients, and the key problem facing plants is that they are water and nutrients. The availability of the resources that plants offer is known to depend on specific plant and microbial genes that

condition the development of any kind of intimate association. It also is understood that a large amount of nutritious material is released from plants into the rhizosphere as root exudates. However, the fact that in many species most of this material is packaged in living cells with the same capacity to resist or foster microbial colonization as other plant cells still is not widely appreciated. This is despite the fact that it has been nearly 80 years since Knudson (45) set the record straight by saying, "One can find in various texts the statement that the root cap cells of plants die and are sloughed off, and it is probably the general opinion that the root cap cells are either dead when they are sloughed off or that they die soon thereafter.... That the root cap cells, when sloughed off... may persist for many days seems to be substantiated by observations with a number of different plants. In view of the increasing attention being devoted to the subject of root excretions, it seems desirable to make record of these incidental observations." Eight decades later, one can still find in plant biology textbooks the statement that these cells are dead, though in most cases they are not mentioned at all (46, 52, 57). In view of current worldwide efforts to alter plant productivity by genetic engineering, it is critical that plant scientists realize that there is this unusual part of plant root systems whose potential impact on the health of plants cannot be overestimated. Plant pathologists especially will continue to be hampered in efforts to utilize root-colonizing organisms agriculturally if they remain unaware of the existence, viability, and regulated release of border cells into the environment. Efforts to improve plant health by adding exogenous populations of beneficial microorganisms (biological control) are notoriously unreliable (30). It is surprising that such approaches ever work, in the absence of careful consideration of the plant's ability to influence microbial growth selectively by the release of root border cells and their associated products.

The capacity of plants to release thousands of healthy somatic cells into the external environment is unique among higher organisms, as far as we know. That they do not fit readily into a known category undoubtedly has contributed to a persistent lack of recognition of border cells as a viable component of root systems, even in current reviews (1, 2, 4, 5, 53, 55, 61). In a time when we are facing the awful consequences of not spending much energy taking care of our own external environment, the concept that it could be biologically worthwhile to an organism to waste so much energy on the world beyond itself is not as shocking as it once was. If our model is correct, it may be possible to improve plant health with a relatively small impact on the environment by using border cells to facilitate the plant's natural ability to regulate its own ecology.

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