Rosannah Taylor,[1] *Ph.D.; John Lydon,*[1] *Ph.D.; and James D. Anderson,*[1] *Ph.D.*

Anatomy and Viability of *Cannabis sativa* Stem Cuttings With and Without Adventitious Roots

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ABSTRACT: The objective of this research was to determine the stage of root development of *Cannabis sativa* stem cuttings necessary to produce viable plants. Cuttings were made from one pistillate (female) plant and transplanted into pots when callus, newly emerging roots, or 1 to 2 mm long roots were present. Cuttings were grown for two weeks and survival and biomass accumulation were recorded. Cross sections of the stems and roots of cuttings and roots of seven-day-old seedlings were made for anatomical comparisons. Based on the cross sections, the general anatomical features of roots from seedlings and cuttings were similar. As in some other herbaceous species, adventitious roots of *C. sativa* stem cuttings develop from the vascular cambium. Two weeks after transplanting, all propagules from the three cutting types survived and grew. Total biomass accumulation and components of total biomass accumulation of the cuttings were not significantly different among cutting types. Thus, cuttings of *C. sativa* at the callus stage are capable of survival and growth comparable to rooted cuttings without any additional care beyond that normally given to plants established from seed.

KEYWORDS: criminalistics, marijuana, hemp, vegetative propagation, root development

Congressional law and U.S. Sentencing Guidelines dictate the level of punishment for possession of marijuana with the intent to manufacture: where 50 or more living marijuana (*Cannabis sativa* L.) plants are involved, each plant is treated as equivalent to 1 kg of marijuana regardless of age, weight, or size [*1,2*]. Minimum terms of imprisonment have been set at 5 years for possession of 100 to 999 plants and 10 years for possession of 1000 plants or more [*2*]. Seizures of indoor marijuana growing operations in the United States have increased from 951 in 1985 to 3849 in 1992, with an average of 90 plants per seizure in 1992 [*3,4*]. In many of the indoor growing operations, plants are produced vegetatively from stem cuttings of female stock plants, mainly to ensure the sex of the plants [*5*]. Female plants are preferred because they produce more potent marijuana due to the high Δ^9-tetrahydrocannabinol (Δ^9-THC) content of the flower bracts [*6–8*]. As a result, *C. sativa* stem cuttings at various stages are generally found in indoor growing operations [*5*]. Thus, the inclusion of *C. sativa* stem cuttings in the total count of plants seized often has a significant impact on the severity of the sentencing.

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[1]Research Associate, Plant Physiologist, and Research Leader, respectively, U.S. Department of Agriculture, Agricultural Research Service, Weed Science Laboratory, Beltsville, MD.

While the level of punishment for possession of marijuana is clearly stated in the sentencing guidelines [*2*], no definition of "plant" was included. As a result of a series of court cases, however, it has been established that cuttings from *C. sativa* must have roots in order to be considered plants [*9–11*]. Recently, it was determined that *C. sativa* stem cuttings are considered plants if there is "readily observable evidence of root formation" [*12*]. Nevertheless, no defined study exists on the establishment or anatomy of roots in *C. sativa* stem cuttings or as to how extensive the root system must be in order for cuttings to be viable. The work described here was conducted to address these points.

Materials and Methods[2]

Plant Propagation

All plant material was grown in a greenhouse at Beltsville, Maryland. When the natural daylength was less than 12 h, *Cannabis sativa* seeds of a high Δ^9-THC strain from Colombia, South America (CO-H631) were germinated in 10 cm pots containing horticultural vermiculite (The Schundler Co., Metuchen, NJ) and watered as needed. After seven days, seedlings (Fig. 1A) were transplanted one seedling per pot into 15 cm pots containing soilless potting medium (PROMIX™ BX, Premier Brands, Inc., Stamford, CT), watered as needed, and fertilized at 2.5 g l^{-1} with PETERS® 20-20-20 fertilizer (Grace-Sierra Horticultural Products Co., Milpitas, CA) every fourth watering after the first month of growth. When the plants flowered, one female plant was selected as a source of cuttings. To induce vegetative growth, the natural photoperiod was extended to 16 h with a 1000 W mercury vapor lamp that provided a photosynthetic photon flux density of 60 μmol m^{-2} s^{-1} at the canopy of the plant.

A clonal population was established from this plant as follows. The terminal meristems of the developing lateral branches were removed to encourage additional branching. The lateral branches were cut with a razor blade below the fourth or fifth internode, and the leaf at that node, as well as any developing meristems at the leaf axil, were removed. Cuttings were successively dipped into tap water and auxin-containing rooting powder (ROOTONE® F, Union Carbide, Agricultural Products Co., Inc., Raleigh, NC), shaken to dislodge excess rooting powder, and placed in a tray containing wet, horticultural vermiculite. To maintain a high relative humidity, the cuttings were misted and the tray covered with a clear plastic dome which allowed a head space of 12 cm above the cuttings. After four days, the vegetative cuttings were fertilized once with 0.25 g l^{-1} PETERS® 20-20-20. When roots developed in approximately 8 to 14 days, the cuttings were transplanted to 15 cm pots containing soilless medium, and watered and fertilized as described for the plants grown from seed.

Viability and Growth

For tests of viability, cuttings were made from the clonal population as described above. When callus, newly emerging roots, or 1- to 2-mm long roots were present, cuttings (Fig. 1B) were removed from the cutting chamber and senescent, chlorotic leaves removed. The cuttings were then blotted dry, weighed, and planted in 10 cm pots containing soilless medium, grown under the natural photoperiod, and watered as needed.

[2]Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

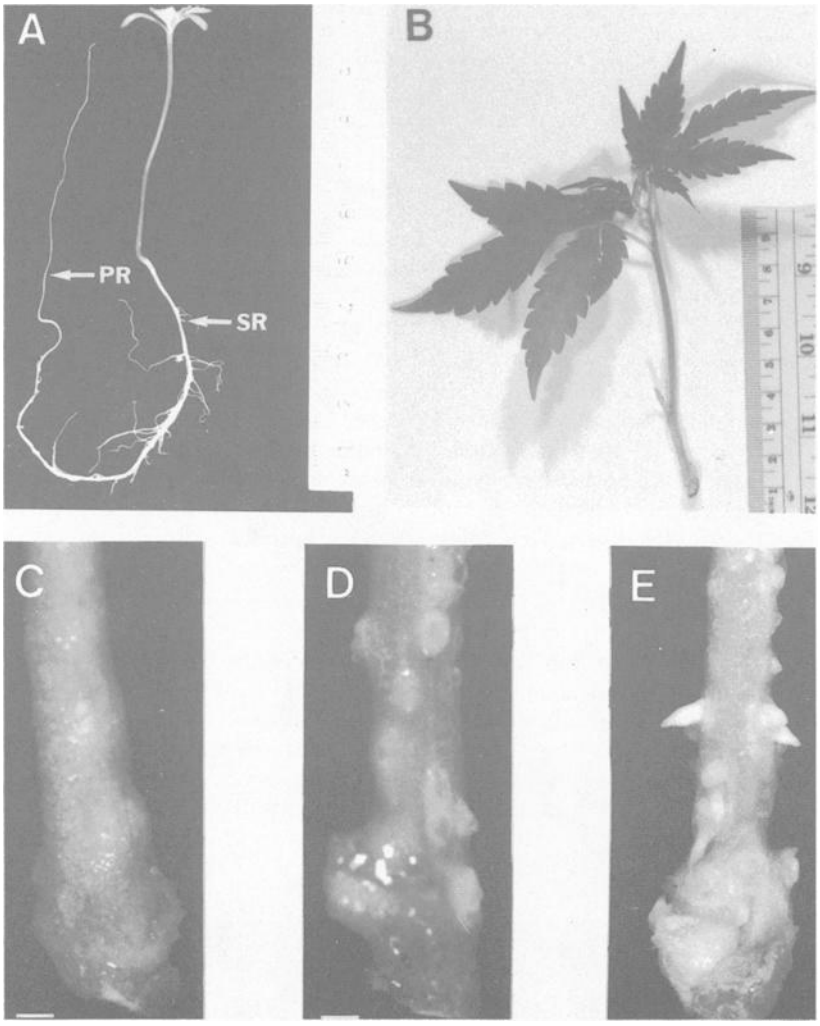

FIG. 1—Cannabis sativa: *seven-day-old seedling* (A); *stem cutting with newly emerging roots before transplanting* (B); *and enlargements of the base of stem cuttings showing callus* (C); *newly emerging roots* (D); *and 1 to 2 mm long roots* (E). *Abbreviations: PR = primary root and SR = secondary root. Horizontal bars equivalent to 1 mm.*

After two weeks, plants were washed free of potting media, blotted dry, weighed, the roots removed with a razor blade, and the remaining shoot reweighed. There were 30 observations per treatment and the data were analyzed by single-factor analysis of variance, with means tested at the 0.05 level.

Anatomy

Root and stem tissues were sectioned from the vegetative cuttings and seedlings with a razor blade and immediately placed in Bouin's fluid [*13*] at room temperature for 8 to 24 h. The solution was decanted, and the tissue successively transferred to a series of

TABLE 1—*Biomass accumulation of* Cannabis sativa *L. stem cuttings transplanted at the callus* (c), *newly emerging roots* (n), *and 1 to 2 mm roots* (r) *stages* (T_0) *and grown for two weeks* (T_1) *under greenhouse conditions.*[a] *Values represent the mean of 30 observations. Values within the same column and followed by the same letter are not significantly different* $P \geq 0.05$.

Cutting type	Biomass (fresh wt.)				
	Cutting T_0	Cutting T_1	Shoot T_1	Root T_1	ΔCutting
c	1.20 a	3.89 a	2.84 a	1.03 a	2.70 a
n	1.05 a	3.94 a	2.95 a	1.00 a	2.86 a
r	1.09 a	3.76 a	2.84 a	0.95 a	2.61 a

[a]Stem cuttings without callus or other visible root structures did not survive to T_1.

alcohol solutions at 30, 50, and 70% ethanol for 30 min each. The tissues were then infiltrated with and embedded in paraffin wax. Serial sections of 15 to 20 μm were made on a rotary microtome and collected on microscope slides. The paraffin was removed from the sections and the tissue stained with toluidine blue [*14*]. Sections were viewed and photographed with either a Leitz Sm-Lux compound microscope or a Zeiss Sv 8 compound stereo microscope equipped with 35 mm cameras. All chemicals were from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Results and Discussion

While stem cuttings taken from one plant are genetically identical, we found that the time required for *C. sativa* stem cuttings to develop roots varied. For example, cuttings required 5 to 9 days to form callus, 7 to 11 days to form newly emerging roots, and 8 to 14 days to produce 1 to 2 mm long roots (data not presented). Not surprisingly, preliminary studies demonstrated that stem cuttings at the pre-callus stage failed to survive when transplanted to pots and cultivated as plants started from seed. However, the

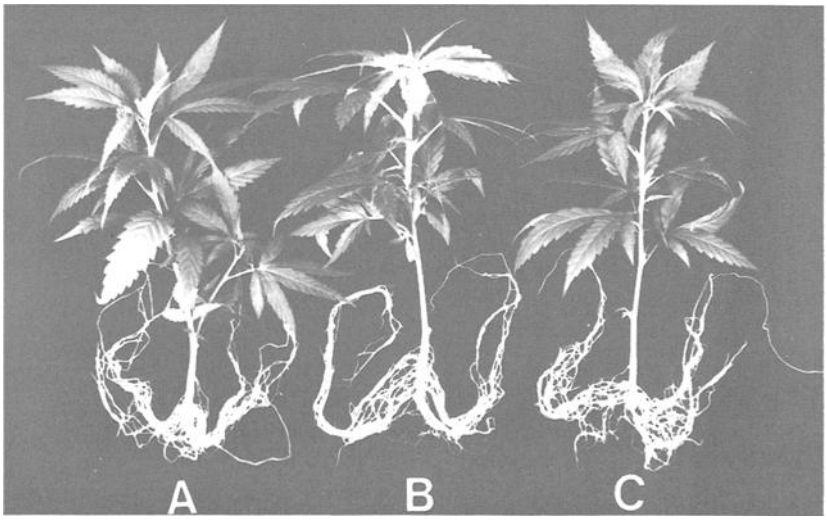

FIG. 2—*Greenhouse-grown* Cannabis sativa *plants two weeks after transplanting stem cuttings at the callus* (A), *newly emerging roots* (B), *and 1 to 2 mm roots* (C) *stages into soilless medium.*

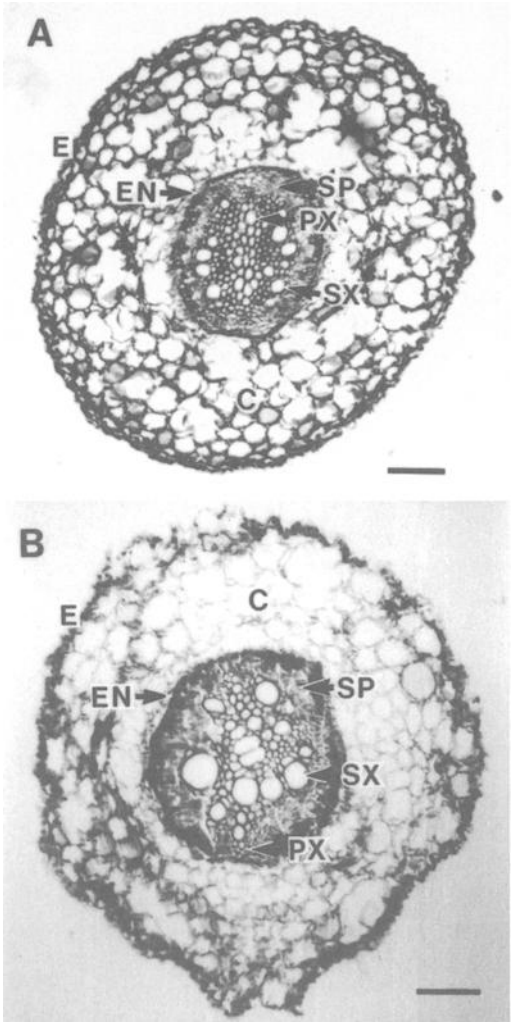

FIG. 3—*Transverse sections of root tissues from greenhouse-grown* Cannabis sativa: *primary root of a seven-day-old seedling above the zone of secondary roots* (A) *and an adventitious root from a plant two weeks after transplanting a stem cutting at the callus stage into soilless medium* (B). *Horizontal bars equivalent to 0.1 mm. Abbreviations: C = cortex, EN = endodermis, E = epidermis, SP = secondary phloem, PX = primary xylem, and SX = secondary xylem.*

viability of *C. sativa* stem cuttings changed dramatically once callus formation was observed. In this study, all stem cuttings transplanted at the callus, newly emerging roots, and 1 to 2 mm roots stages (Fig. 1C-E) survived and grew. Two weeks after transplanting, total biomass accumulation and components of total biomass accumulation (that is, shoot weight and root weight) of the cuttings were not significantly different among cutting types (Table 1) and cutting types were visually indistinguishable (Fig. 2). Thus, *C. sativa* stem cuttings at the callus stage are capable of survival and growth comparable to rooted cuttings without any additional care beyond that normally given to plants established from seed.

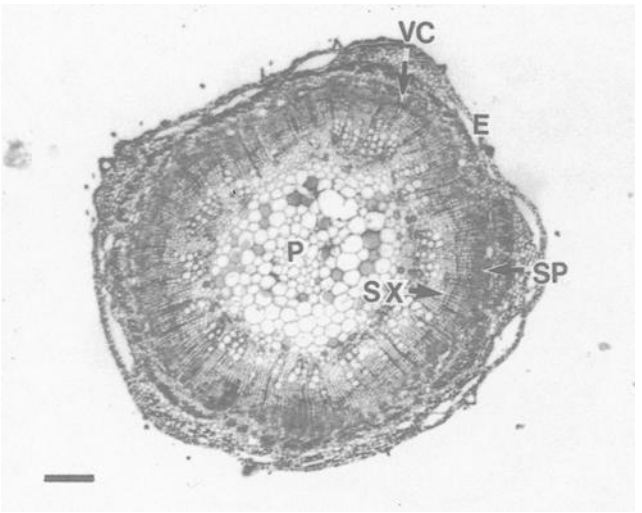

FIG. 4—*Transverse section of a greenhouse-grown* Cannabis sativa *stem. Section is 15–20 μm thick and stained with toluidine blue. The horizontal bar is equivalent to 0.5 mm. Abbreviations: E = epidermis, P = pith, SP = secondary phloem, VC = vascular cambium, PX = primary xylem, and SX = secondary xylem.*

Transverse sections of *C. sativa* root tissues from seedlings and cuttings (Fig. 3) demonstrate an anatomy common to dicotyledonous roots [*15*], with the stele, which contains the xylem and phloem, at the center of the tissue separated from the cortex by an endodermis. This anatomical arrangement differs from that of dicotyledonous stems, including *C. sativa*, [*16*] in which the xylem and phloem, separated by the vascular cambium, encircle a central path (Fig. 4).

Roots from tissues of a similar stage of development were anatomically similar. The oldest root tissue from the primary root of a seven-day-old seedling (Fig. 3A), that is, tissue above the zone of secondary roots, and a two-week-old transplanted cutting that was transplanted at the callus stage (Fig. 3B) contain primary and secondary xylem, as well as secondary phloem. However, younger root tissue from a seven-day-old seedling, that is, from the primary root below the zone of secondary roots (Fig. 5A) and a 2 mm long secondary root (Fig. 5B), and the 1 to 2 mm long adventitious root from a stem cutting before transplanting (Fig. 5C), do not contain secondary xylem. Although present in the younger root tissue, primary phloem cells cannot be visualized at this magnification (Fig. 5).

Adventitious roots of *C. sativa* stem cuttings do not develop from the exterior callus tissue, but internally, from the vascular cambium: growing through the phloem, epidermis, and callus tissues (Fig. 6A-C) as in chrysanthemums [*16*]. The results demonstrate that adventitious roots of *C. sativa* cuttings at the 1 to 2 mm roots stage and older have the same general anatomy as roots from seedlings. The ability of rootless cuttings to survive and grow when transplanted at the callus stage can be attributed to the presence of internal, rapidly developing, root primordia (Fig. 6A). The transition from the callus stage to the newly emerging roots stage can occur in as quickly as two days (data not presented).

In conclusion, the anatomical data demonstrates that *C. sativa* stem cuttings with a single 1 to 2 mm long root can be classified as plants. However, if the reference to "plant" in the Congressional law refers to a growing, vegetative, viable propagule, then cuttings at the callus stage should be considered for sentencing purposes.

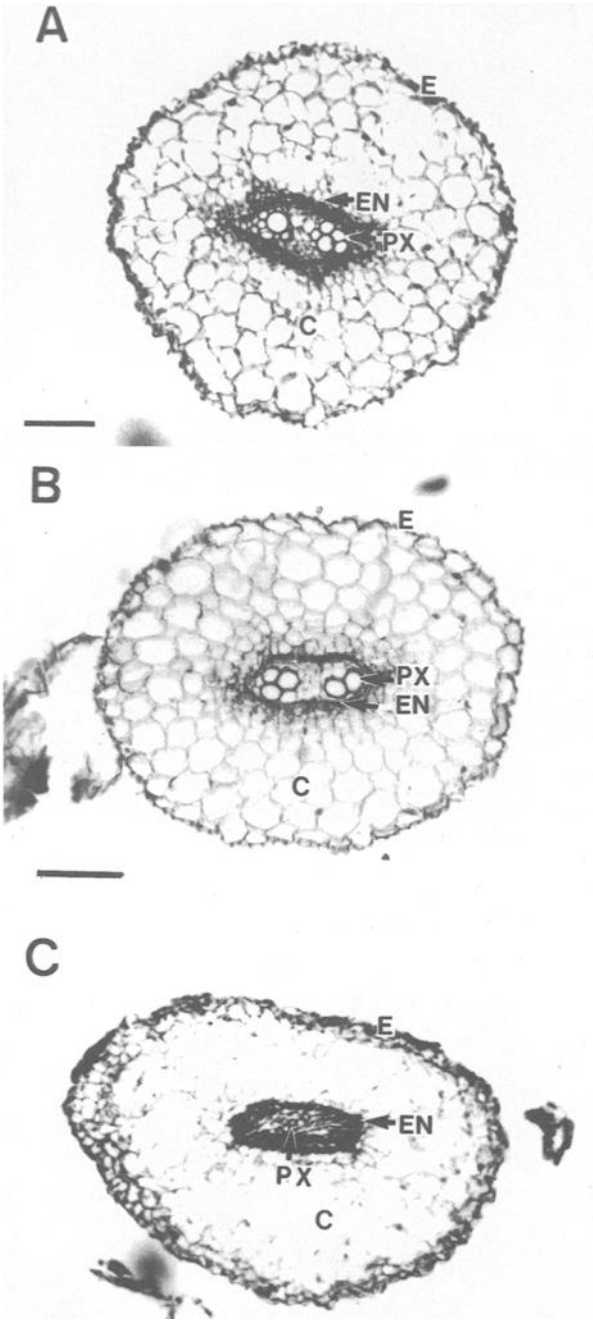

FIG. 5—*Transverse sections of root tissues from greenhouse-grown* Cannabis sativa: *primary root of a seven-day-old seedling below the zone of secondary roots* (A); *2 mm long secondary root of a seven-day-old seedling* (B); *and 1 to 2 mm long adventitious root from a stem cutting before transplanting cutting into soilless medium* (C). *Sections are 15–20 μm thick and stained with toluidine blue. Horizontal bars are equivalent to 0.1 mm. Abbreviations: C = cortex, EN = endodermis, E = epidermis, and PX = primary xylem.*

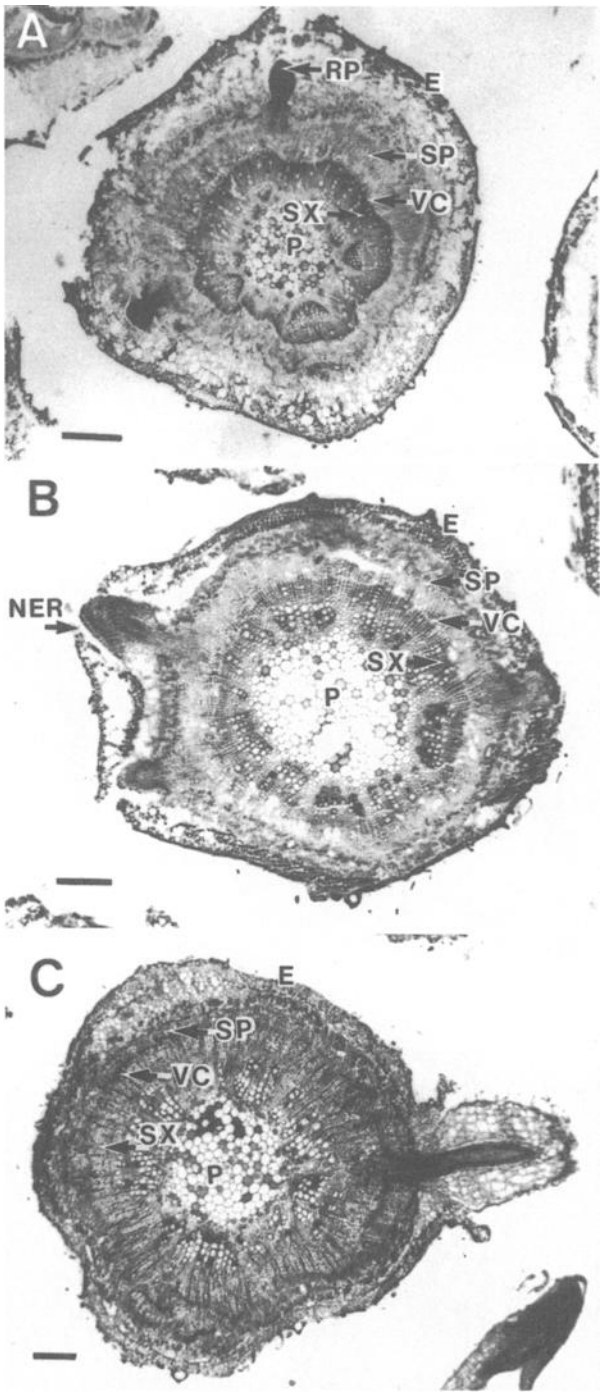

FIG. 6—*Transverse sections of greenhouse-grown* Cannabis sativa *stem cuttings at the callus* (A), *newly emerging roots* (B), *and 1 to 2 mm root* (C) *stages. Sections are 15–20* μm *thick and stained with toluidine blue. Horizontal bars are equivalent to 0.25 mm in plate A and 0.5 mm in plates B and C. Abbreviations: E = epidermis, NER = newly emerging root, P = pith, SP = secondary phloem, VC = vascular cambium, PX = primary xylem, and SX = secondary xylem.*

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Address requests for reprints or additional information to
John Lydon
USDA/ARS/WSL
Bldg. 001, Room 236
Beltsville, MD 20705