Nodule Distribution among Root Morphological Components of Field-grown Cowpeas

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Abstract. Field experiments were conducted in 1985 at Fort Pierce, Fla., and Bixby, Okla., to quantify and describe the distribution of nodules among root morphological components of cowpea [*Vigna unguiculata* (L.) Walp.]. Plants of 'Knuckle Purplehull', 'Mississippi Cream', and 'White Acre' were sampled by cultivar on separate dates at three growth stages: pre-anthesis, seed initiation, and harvest, when most pods were dry. Root masses were partitioned into adventitious, basal, lateral, and taproot components. Nodules were removed from roots, grouped according to root morphological component of origin, and weighed. No linear correlation was found between the weight of a particular root morphological component and the nodule weight associated with that component. Total root weight and total nodule weight also were not strongly correlated. Nodule weights usually were lower at harvest than at earlier stages of ontogeny, especially for nodules from taproots. Although $\approx$ 70% of the root mass was in the taproot and its associated laterals at both locations, the taproot per se was not the primary locus of nodulation. Instead, most nodules generally were located on the basal and lateral roots. When percentage distribution of total nodule weight was examined, neither growth stage nor cultivar was found to affect nodulation of basal or lateral roots.

Effective cowpea-*Rhizobium* symbiosis can result in the fixation of more than 150 kg N/ha and supply 80% to 90% of the host plant's N requirement (Eaglesham et al., 1977; Summerfield et al., 1977). The events in the symbiotic process have been reviewed (Bauer 1981; Vincent, 1980). Host plant interactions with *Rhizobium* affect which strains of *Rhizobium* infect the root, the extent of nodulation and the timing of its onset, and the potential for N₂ fixation (Graham, 1982). Host plant differences in nitrogenase activity, nodule number, nodule weight, and plant top weight in response to given rhizobial strains have been identified (Miller et al., 1982). Lawn and Bushby (1982) conducted grafting studies with four Asiatic *Vigna* species and found that root and shoot genotypes affected N₂ fixation, primarily through effects on nodule fresh weight.

Summerfield and Minchin (1976) suggested that *Rhizobiurn* infection of cowpea and subsequent nodule formation may be related to particular roots with inherent differences in elongation rate. Wolyn et al. (1989) found that values for acetylene reduction, specific nodule activity, leghemoglobin concentration, and nodule mass obtained from lateral roots were more highly correlated with total shoot N in common bean (*Phaseolus vulgaris* L.) than were values obtained from the whole root system. Kahn and Stoffella (1987) partitioned cowpea root systems into adventitious, basal, lateral, and taproot components. A description of nodule distribution among these root morphological compo-

nents could lead to improved N₂ fixation through development of cowpea plants with appropriately modified root systems. Increased N₂-fixing capacity and improved efficiency in the use of limited soil nutrients have been identified as important cowpea breeding objectives (Fery, 1985).

The objective of these studies was to describe the distribution of nodules among cowpea root morphological components and to determine whether this distribution was affected by cultivar or by growth stage.

Materials and Methods

Three cowpea cultivars ('Knuckle Purplehull', 'Mississippi Cream', and 'White Acre') from the same seed lots were grown at Fort Pierce, Fla., and Bixby, Okla., using a commercial cultural system of double rows. Double-row plots, with 0.46 m between the two rows within each plot and 1.64 m between the rows of adjacent plots, were used at both locations. The plots were on raised beds (1.09 m wide and 0.15 m high) in Florida and on the flat in Oklahoma. Plots were hand planted at 20 seeds per meter of row and thinned to 10 plants per meter of row shortly after emergence at both locations.

Cultural practices—Fort Pierce, Fla. The experiment was conducted at the Agricultural Research and Education Center during Spring 1985. The soil was an Oldsmar fine sand (sandy, siliceous, hyperthermic Alfic Arenic Haplaquods). Preplant fertilization consisted of 94N-62P-195K (kg-ha⁻¹) as a band application in the center of each bed. The rates reflected the leaching potential at Fort Pierce due to sandy soils and high seasonal rainfall. Seeds were planted on 28 Feb. Plots were 3.0 m long. No *Rhizobium* inoculants were used, since previous studies with cowpea at Fort Pierce had shown that native *Rhizobia* populations provided extensive nodulation. Seeds were dusted with the fungicide 3a,4,7,7a-tetrahydro-2-[trichloromethylthio]-1 H-isoindole-1,3(2 H)-dione (captan) before planting. Pentachloron-

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Abbreviations: H, harvest; PA, pre-anthesis; SI, seed initiation.

itrobenzene ($4.4 \text{ kg}\cdot\text{ha}^{-1}$) was sprayed on the furrows before the seeds were covered and on top of each bed after they were covered to control soil fungi. Weeds were manually controlled within the beds and with 1,1'-dimethyl-4,4'-bipyridinium salts (paraquat) ($280 \text{ g}\cdot\text{ha}^{-1}$) between the beds. Insects and foliar fungi were chemically controlled as required during crop growth. Subsurface irrigation was used throughout the experiment to maintain the water table at $\approx 0.38 \text{ m}$ below the soil surface.

Cultural practices—Bixby, Okla. The experiment was conducted at the Vegetable Research Station during Spring and Summer 1985. A Severn very fine sandy loam [coarse-silty, mixed (calcareous), thermic Typic Udifluvents] was prepared with a broadcast, preplant-incorporated application of 44N-19P-36K ($\text{kg}\cdot\text{ha}^{-1}$), plus preplant-incorporated *O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate (diazinon) at $2.80 \text{ kg}\cdot\text{ha}^{-1}$ for insect control and 2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl) benzenamine (trifluralin) at $560 \text{ g}\cdot\text{ha}^{-1}$ for weed control. Seeds were planted on 31 May. Plots were 3.5 m long. Previous studies with cowpea at Bixby had shown that native *Rhizobia* populations provided inconsistent modulation. Therefore, a peat-base granular cowpea-type *Rhizobium* implant material (Nitragin "EL", Nitragin Co., Milwaukee; minimum of 100 million viable cells/g) at $560 \text{ mg}\cdot\text{m}^{-1}$ of row was lightly spread over the seeds in the furrows before the seeds were covered. No fungicides were used at planting. Insects were chemically controlled as required during crop growth. Frequency of sprinkler irrigation was based on subjective soil observations. Plots received 20 to 25 mm of water each on 11 and 18 July and 2 and 27 Aug.

Root and nodule measurements. Plants were sampled at three growth stages: pre-anthesis (PA), just before initial bloom; seed initiation (SI); and harvest (H), when most pods were dry. Plants were sampled by cultivar on separate dates since cultivars differed in relative days to maturity. At each stage, four plants per plot were randomly chosen and excavated with a shovel at a 20-cm radius around each plant to a depth of $\approx 23 \text{ cm}$. Root breakage was minimized by wet soil conditions. Roots were soaked in water, gently rinsed to remove adhering soil, and separated from the shoots. The recovered root mass of each plant was partitioned into adventitious, basal, lateral, and taproot components (Kahn and Stoffella, 1987). Nodules were dissected from each root mass component and weighed. Roots and shoots were oven-dried at 55 to 65°C for 3 to 4 days and then weighed.

Statistical analysis. Plots were arranged in randomized complete blocks at both locations, with each cultivar replicated four times. Data were evaluated by an analysis of variance as a split plot in time, so that the main effect of growth stage (main plots) was tested using replications within growth stages as the error term. When main effects of growth stages or cultivars were significant, means were separated by Duncan's multiple range test at $P = 0.05$. Interaction LSD values were calculated when significant growth stages $\times$ cultivar interactions were evident.

Results

'Mississippi Cream' had smaller basal roots, lateral roots, and taproots than the other two cultivars at both locations (Table 1). 'White Acre' had relatively high adventitious root weights. Weights of root mass components increased between the PA and SI stages in Florida, then either remained unchanged or decreased between the SI and H stages. Weights of basal roots, lateral roots, and taproots also increased between the PA and

SI stages in Oklahoma, but further increases occurred between the SI and H stages.

Plants of 'Mississippi Cream' usually had lower shoot weights (data not presented) and total root weights than plants of 'Knuckle Purplehull' and 'White Acre' (Table 2). However, cultivar differences in total root weight were not always consistent over growth stages, as evidenced by the growth stage $\times$ cultivar interactions at both locations.

The percentage distribution of total root weight among the four root morphological components was little affected by cultivar, growth stage, or location (data not presented). Grand means were: adventitious roots, 3.5%; basal roots, 24%; lateral roots, 27.5%; and taproots, 45%.

Neither cultivar nor growth stage affected weights of nodules from adventitious roots (Table 3). 'Knuckle Purplehull' had higher weights of nodules from basal roots, lateral roots, and taproots than the other two cultivars in Florida. Fewer differences were evident among cultivars in Oklahoma. Weights of nodules from basal and lateral roots were unaffected by growth stage in Florida, while weights of nodules from taproots declined between the SI and H stages. Weights of nodules from lateral roots and from taproots declined between the PA and SI stages in Oklahoma, then did not change between the SI and H stages.

'Knuckle Purplehull' had a higher total nodule weight than the other two cultivars in Florida, but not in Oklahoma (Table 3). Total nodule weights did not differ between the PA and SI stages in Florida, but were greater at the SI stage than at the H stage. In contrast, total nodule weights were highest at the PA stage in Oklahoma and did not differ between the SI and H stages.

The percentage distribution of total nodule weight among the four root morphological components was calculated, based on the data in Table 3 (percentages not presented). Differences among cultivars were present only for nodules from adventitious roots and from taproots. 'White Acre' had a relatively low percentage (9%) of its nodule weight located on taproots at both locations. In Oklahoma, a greater percentage of the total nodule weight was located on taproots at the PA stage than at the SI and H stages, with a reciprocal trend evident for nodules from adventitious roots. A similar effect of growth stage on nodules from taproots was observed in Florida, but growth stage did not significantly affect nodule distribution among adventitious roots, basal roots, or lateral roots. On average, $\approx 50\%$ of the total nodule mass was associated with lateral roots in Florida, with another 31 % associated with basal roots. About 60% of the total nodule mass was associated with nodules from lateral roots plus nodules from basal roots in Oklahoma.

Discussion

Changes in root weights during ontogeny were similar to those previously reported (Kahn and Stoffella, 1987). Root growth may have ceased before harvest in Florida. Others have reported decreases in total root weight during late podfill in cowpeas (Minchin et al., 1980; Ryle et al., 1979).

Nodule counts were not recorded in our studies. Nodules sometimes were lobed, making differentiation difficult. Also, field studies have indicated that nodule weight is more relevant to N_2 fixation in cowpea than is absolute number of nodules (Miller et al., 1982, 1986). Strong linear relationships between total nodule fresh weight and quantity of N_2 fixed have been reported (Miller et al., 1982; Wadisirisuk and Weaver, 1985). Lawn and Bushby (1982) also suggested that nodule fresh weight was a better indicator of effects of different root and shoot

Table 1. Dry weights (grams/plant) of root mass components for three cowpea cultivars at three growth stages grown at two locations.^z

Main effects	Adventitious roots		Basal roots		Lateral roots		Taproots	
	Location							
	FL	OK	FL	OK	FL	OK	FL	OK
Grams/plant								
Cultivar								
Knuckle Purplehull	0.05 b	0.02 b	0.47 a	0.30 a	0.46 a	0.26 a	0.57 a	0.53 a
Mississippi Cream	0.02 b	0.01 b	0.18 b	0.10 c	0.24 b	0.15 b	0.34 b	0.23 b
White Acre	0.08 a	0.04 a	0.40 a	0.20 b	0.39 a	0.25 a	0.56 a	0.57 a
Growth stage								
PA	0.02 b	0.01 b	0.13 b	0.11 c	0.15 c	0.13 c	0.22 b	0.28 c
SI	0.06 a	0.03 ab	0.51 a	0.20 b	0.53 a	0.22 b	0.57 a	0.43 b
H	0.07 a	0.04 a	0.41 a	0.28 a	0.40 b	0.30 a	0.67 a	0.62 a
CV	60.5	70.0	37.9	47.0	40.2	27.7	38.4	43.9

^zLocations: FL = Fort Pierce, Fla; OK = Bixby, Okla. All growth stage $\times$ cultivar interactions were nonsignificant at $P = 0.05$. Mean separation in columns and among growth stages or among cultivars by Duncan's multiple range test, $P = 0.05$.

Table 2. Total dry weights of roots (grams/plant) at three growth stages for three cowpea cultivars grown at two locations.

Cultivar	Total dry wt of roots (g/plant)							
	Fort Pierce, Fla.				Bixby, Okla.			
	Growth stage				Growth stage			
	PA	SI	H	$\bar{X}$	PA	SI	H	$\bar{X}$
Knuckle Purplehull	0.77	2.03	1.83	1.55	0.64	1.10	1.57	1.10
Mississippi Cream	0.43	0.87	1.01	0.77	0.30	0.52	0.65	0.49
White Acre	0.36	2.09	1.84	1.43	0.66	1.01	1.50	1.06
$\bar{X}$	0.52	1.66	1.56	1.25	0.54	0.88	1.24	0.88
Interaction LSD ^z		0.56				0.24		
CV		29.9				18.0		

^zGrowth stage $\times$ cultivar interactions significant at $P = 0.05$.

Table 3. Fresh weights (grams/plant) of nodules from four root mass components for three cowpea cultivars at three growth stages grown in two locations.^z

Main effects	Nodules from adventitious roots		Nodules from basal roots		Nodules from lateral roots		Nodules from taproots		Total nodule wt	
	Location									
	FL	OK	FL	OK	FL	OK	FL	OK	FL	OK
Grams/plant										
Cultivar										
Knuckle Purplehull	0.05	0.02	1.08 a	0.11 a	1.35 a	0.09 a	0.32 a	0.07	2.80 a	0.30
Mississippi Cream	0.06	0.03	0.37 b	0.05 b	0.72 b	0.05 b	0.22 b	0.07	1.37 b	0.20
White Acre	0.09	0.08	0.25 b	0.06 b	0.46 b	0.06 ab	0.08 c	0.02	0.88 b	0.22
Growth stage										
PA	0.07	0.02	0.47	0.11	0.88	0.11 a	0.28 a	0.12 a	1.70 ab	0.37 a
SI	0.05	0.07	0.89	0.06	0.99	0.05 b	0.24 a	0.03 b	2.18 a	0.21 b
H	0.07	0.04	0.35	0.06	0.65	0.03 b	0.10 b	0.01 b	1.18 b	0.14 b
cv	109.8	140.7	56.5	63.1	47.8	58.6	46.6	96.4	35.5	48.6

^zLocations: FL = Fort Pierce, Fla; OK = Bixby, Okla. All growth stage $\times$ cultivar interactions were nonsignificant at $P = 0.05$. Mean separation in columns and among growth stages or among cultivars by Duncan's multiple range test, $P = 0.05$. Mean separation letters do not appear when main effects were nonsignificant (F test) at $P = 0.05$.

genotypes on N_2 fixation than was either specific or total nodule activity.

Cowpea nodule weights usually are highest from about anthesis to mid-pod fill (Eaglesham et al., 1977; Minchin et al., 1980; Summerfield et al., 1977), but apparently functional nodules have been observed even at final harvest (Dart et al., 1977). In our studies, nodule weights usually were lower at harvest than at earlier stages of ontogeny, especially in Oklahoma (Table 3). The decline in weight was especially noteworthy for nodules from taproots (Table 3). Others (Minchin and Summerfield, 1978; Minchin et al., 1980) had identified two distinct

nodule populations in growth-chamber-grown cowpeas. Nodules in "crown" positions on the main taproot and on earlier-formed secondary roots near the junction with the taproot predominated during vegetative growth. After anthesis, these nodules generally senesced and sloughed off, and "secondary" nodules (located on secondary, tertiary, and higher-order roots away from the primary root) became dominant. In one study, these secondary nodules accounted for more than 50% of total nodule dry weight at the time the first pods were maturing (Minchin et al., 1980). Our data indicate that while some nodules were present on the taproot following anthesis, the taproot was

proportionally less important as a locus of nodulation at the SI and H stages than the PA stage.

Dart and Wildon (1970) reported that many more nodules formed on "secondary roots" of cowpea than on the primary root (taproot). This pattern of nodulation may reflect the physical distribution of the root system in the soil. The taproot is a single structure growing in essentially one plane. In contrast, each plant has several basal and lateral roots (Kahn and Stoffella, 1987) that can extend in many directions, thereby providing more potential sites for infection by *Rhizobium*.

Adventitious roots accounted for only 4% of the total root weight in Florida and 3% in Oklahoma. However, nodules from adventitious roots accounted for $\approx 21\%$ of the total nodule weight in Oklahoma, compared to $\approx 6\%$ in Florida (based on data in Table 3). This difference may have reflected soil differences between the two locations. A more likely explanation, however, is the presence of rhizobial inoculant. Kamicker and Brill (1987) found that inoculant added to the seed furrow (as was done in Oklahoma) produced nodules mainly in the upper region of the soybean [*Glycine max* (L.) Merr.] root system, while inoculant tilled into the soil produced nodules primarily in the lower region of the root system. Whatever the explanation, adventitious roots were important as loci for nodulation in Oklahoma, particularly for 'White Acre'.

No consistent linear correlations were evident between the variables in Tables 1 through 3 (correlations not presented). Most values of r were below 0.50. Thus, no linear correlation was found between the weight of a particular root morphological component and the nodule weight associated with that component. Total root weight and total nodule weight also were not strongly correlated ($r = -0.35$ in Oklahoma, significant at $P = 0.05$; $r = 0.15$ in Florida, not significant at $P = 0.05$). Rosas and Bliss (1986) obtained a low correlation between root dry weight and nodule dry weight in field-grown common bean ($r = 0.38$, significant at $P = 0.01$). Genotypic effects on determinants of N_2 fixation are known to be complex. Lawn et al. (1974) suggested that the control of soybean nodule initiation occurs primarily in the root itself, but the control of nodule fresh weight occurs solely in the shoot and is related to the supply of assimilates.

These studies confirmed a previous report (Kahn and Stoffella, 1987) that the majority of the root mass in cowpea occurs in the taproot and its associated laterals. The taproot per se, however, was not the primary locus of nodulation, particularly after anthesis. Instead, most nodules generally were located on the basal and lateral roots. Also, when percentage distribution of total nodule weight was examined, neither growth stage nor cultivar was found to affect nodulation of basal and lateral roots. Breeders selecting for extensive root systems in cowpea in an effort to maximize sites for nodulation and to increase nutrient uptake efficiency (Adepetu and Akapa, 1977) should consider the importance of these root types in cowpea nodulation.

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