Growth Response of Peanut to Field Inoculation with Endomycorrhizal Fungi, *Bradyrhizobium*, and Supplemental Phosphorus in Texas[1]

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ABSTRACT

Field studies were conducted at four locations in Texas over a two year period to assess the response of five peanut cultivars to inoculation with four species of vesicular-arbuscular endomycorrhizal fungi (VAMF) with or without *Bradyrhizobium* sp., and *Bradyrhizobium* alone. Supplemental phosphorus treatments were also included. Replicated treatments were superimposed upon indigenous microflora in five nonfumigated field plots. Soil phosphorus (up to 50 ppm) did not necessarily stimulate peanut growth nor negate growth stimulation by mycorrhizal fungi. VAMF species differed in their effectiveness for increasing peanut growth characteristics such as root, shoot, and pod weights but did not affect peanut yield. Cultivars also responded differently to inoculation. Shoot and root weights of inoculated plants increased more rapidly than the controls early in the growing season. Increased dry pod weights were obtained at two locations; however, yields of peanut from all treatments at harvest were statistically similar. The value of field inoculation of peanut with vesicular-arbuscular endomycorrhizal fungi used in this research is discussed.

Key Words: Peanut, *Arachis hypogaea*, mycorrhizae, vesicular-arbuscular mycorrhizal fungi, field inoculation.

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The beneficial effects of vesicular-arbuscular endomycorrhizal fungi (VAMF) associated with plant roots in a number of crops have been well documented (2-7, 9, 11-14, 19-32, 35, 36). Although their presence in the roots of peanut plants has been known for many years (1, 4, 8-10, 15-18, 23, 27, 33, 34), little information is available about their effects on peanut plant growth. The positive growth response of peanut has been demonstrated in the greenhouse and some experiments have shown interactions among VAMF, fungal pathogens, and nematodes (8, 15-18, 33, 34). No information is available on the effects of these fungi on peanut growth when inoculated into nonfumigated soils in farmer's fields. Information relevant to large-scale field application of these fungi on any crop is meager because availability and/or quality of sufficient inocula for field use can be unpredictable, time-consuming, and expensive. The fact that these fungi cannot be cultured on artificial media in the laboratory, but must be grown and maintained on living plant roots in the greenhouse, has been a major problem. This has also delayed progress on commercial development of these fungi for on-farm use. Powell (25) stated that most VAMF researchers in the U.S. have failed to move away from experimental work in sterilized soil and listed seven factors that have received too little consideration in previous field trials: use of very small plots, lack of replication, inappropriate use of pre-inoculated seedlings, short growth period (harvested too early), excessive rates of randomly placed inoculum, incompatibility with agricultural technology and economics, and lack of correlation of mycorrhizal responses to growth responses obtainable from phosphorus fertilizer alone. These shortcomings were especially considered in the studies reported here involving the growth response of peanut after field inoculation with VAMF in nonfumigated farmer's fields and experimental plots in Texas.

Materials and Methods

Field trials were conducted at four locations, representing distinct geographical areas in Texas. Five field plots in the four areas constituted five independent 1 yr experiments in which comparisons were made among variables within each field plot. Comparisons were not made between geographical areas. Experimental variables imposed at each site included different peanut cultivars, mycorrhizal fungi with or without *Bradyrhizobium* inoculation, *Bradyrhizobium* alone, and/or phosphorus soil supplements (Table 1). The four locations were: (a) south Texas (at Poth, a high commercial peanut production area south of San Antonio), (b) southeast Texas (at the Texas A&M University Agricultural Research Station, Yoakum), (c) east Texas (farmer's field at Grapeland), and (d) the northern Panhandle (at Etter, north of Amarillo, near an emerging production area).

Soil samples from each field were analyzed for pH and nutrients (Table 2) at the Texas A&M University Soil Testing Laboratory prior to planting. Mycorrhizal fungi included *G. etunicatum* Becker & Gerdmann (GE), *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe (GM), *G. deserticola* Bloss and Menge (GD), and *G. intraradices* Schenck & Smith (GI). All isolates were maintained and increased on sudan grass (*Sorghum vulgare* var. *sudanese*

Table 1. Field plot locations and treatments.

Treatment	Location in Texas: East (Grapeland) yr 1	North (Etter) yr 1	North (Etter) yr 2	South (Poth) yr 2	Southeast (Yoakum) yr 2
1. *Glomus etunicatum*	X				
2. *G. mosseae*				X	X
3. *G. deserticola*				X	X
4. *G. intraradices*				X	X
5. *Bradyrhizobium*	X	X	X	X	X
6. *B. etunicatum* + B[a]	X				
7. *G. mosseae* + B			X	X	X
8. *G. deserticola* + B			X	X	X
9. *G. intraradices* + B		X	X	X	X
10. Mix of 7, 8, 9			X		
11. No inoculation	X			X	X
12. Phosphorus - 20 ppm				X	X
13. Phosphorus - 50 ppm				X	X

[a] *Bradyrhizobium.*

Hitchcock) in the greenhouse. The species used as inocula were influenced by availability of isolates that performed well in the greenhouse for inoculum production. Most probable numbers (24) were determined for the VAMF and all inocula were added to the soil at equivalent rates. *Bradyrhizobium* sp. (B) inoculum was supplied by the Nitragin Company, Milwaukee, WI and applied at recommended rates. Production practices varied with the site but were consistent with those followed by producers in the area.

South Texas

The soil type at Poth was an Alfisol, Paleustalf, Miguel fine sandy loam, pH 8.6. Available soil phosphorus was low (4 ppm). See Table 2 for other elemental values.

The field plot design was a split plot randomized block with four replicates per treatment. Rows were 5.2 m x 91.5 cm. Cultivars Florunner and Starr seed were planted and inoculated with a V-belt planter. Mycorrhizal treatments included inoculation with GM, GD, GI, GMB, GDB, GIB, and B. Two additional treatments included two phosphorus supplements to bring the total phosphorus (applied as triple super phosphate) in the soil to both 30 ppm and 50 ppm. The VAMF inocula were increased from inocula supplied by Native Plants Incorporated, Salt Lake City, UT.

Southeast Texas

The test plot was established at the Texas A&M University Plant Disease Research Station, Yoakum, TX. The soil type was an Alfisol, Straber fine loamy sand, pH 7.3. Available soil phosphorus was considered moderate (10 ppm). See Table 2 for other elemental values. The plot design was a split plot randomized block and treatments were replicated four times. Rows were 5.2 m x 91.5 cm. Treflan and Dual 8E were applied preplant at rates of 1.2 L/ha and 1.8 L/ha, respectively. Cultivars Tamnut-74 and Florunner seed were planted and inoculated with a V-belt planter. VAMF included GM, GD, GI alone and each in combination with B. In two additional treatments phosphorus was added to the soil as triple superphosphate to increase the phosphorus level to both 30 ppm and 50 ppm. Seven applications of Bravo 500 were applied at the rate of 5.2 L/ha for management of early and late leaf spot. Plants were harvested at 5 1/2 Mo.

East Texas

The soil type at Grapeland was an Alfisol, Paleudalf, Nacagadoches soil, pH 6.8. The available soil phosphorus was considered high (21 ppm). See Table 2 for other elemental values. The test plot was established in a farmer's field and an isolate of GE obtained from a home garden in Paw Paw, West Virginia was used as the inoculum. It was cultured on sudan grass in a soil:sand (2:1) pot culture in the greenhouse for 3.5 mo before collection. The plot design was a split plot randomized block with four replications/ treatment. Rows were 6.1 m x 91.5 cm. Seed of two cultivars, Florunner and Tamnut-74, were planted. GE inocula with and without B were first distributed by hand into the row, covered by hand with 3 cm soil, seeded and covered by hand with a 5 cm layer of soil.

North Texas

The test plots were established north of Amarillo at Etter, TX for 2 yr. The previous crop in both fields was irrigated wheat.

The year 1 experimental design was a split plot randomized block with three replications/treatment. The soil at the year 1 site was an Alfisol, Haplustalfs, Dalhart fine sandy loam, pH 8.1. The available soil phosphorus was considered moderate (ll ppm). See Table 2 for other elemental values. Treflan was broadcast as a preplant treatment at 1.8 L/ha, the plot was fertilized with 45.5 kg 10-34-0 liquid, and pre-irrigated 3 weeks prior to planting in May. Seed of Pronto and McRan cultivars treated with recommended rates of B were hand-planted in 6.1 m rows after hand-application of GI inoculum. An iron foliar spray and 1.5 L/ha Bravo were applied during the growing season for pepperspot control.

The year 2 experiment was a randomized block design with four replications/treatment.

Soil in the second year plot was the same soil type as that of the previous year, with a pH of 7.8. Available phosphorus was higher (38 ppm) than in the year 1 plot. Other elemental values are given in Table 2. Preplant treatments of 18.2 kg N/ha and 1.8 L/ha Treflan were applied. Pronto seed were hand-planted in 5.8 m rows after in-row hand-inoculation with four VAMF (GM, GD, GI, and an equivalent mixture of three species). Plots were irrigated five times (a total of 50.8 cm water) during the growing season. Plants were harvested at 45, 90, and 130 DAP.

Table 2. Soil analyses for five test sites inoculated with vesicular-endomycorrhizal fungi.

Element	Locations in Texas				
	East (yr 1)	North (yr 1)	North (yr 2)	South (yr 2)	Southeast (yr 2)
	ppm				
Nitrogen	14	20	2	3	5
Phosphorus	21	11	38	4	10
Potassium	71	400	450	144	76
Calcium	984	5784	2274	548	5
Magnesium	56	555	555	92	50
Iron	6	-	13	8	16
Manganese	5	-	22	3	1
Sodium	37	80	40	245	70
pH	6.9	8.1	7.8	8.6	7.3

Results and Discussion

South Texas

Shoot Weights. Shoot fresh weights of Florunner inoculated with two of the VAMF (GD and GI) and all VAMF treatments in combination with B were statistically higher than the controls (Fig. 1A). Plants treated with *Bradyrhizobium* were very similar to the controls. Florunner responded slightly positive to both applications of phosphorus. Starr responded best to inoculation with all GM, the lower phosphorus application, and also to the three VAMF and B mixes (Fig. 1B). *Bradyrhizobium* plants did not weigh more than control plants. P30 plants were better than P50 plants.

Shoot dry weights of all treated Florunner plants were greater at harvest than the controls (Fig. 1C). Both the GD and GDB treatments were better than either of the phosphorus treatments. Plants inoculated with GM were little better than controls. Compared with 30 ppm P, GD and GI (each alone and in combination with B) produced more shoot dry weight (Fig. 1C). Starr shoot dry weights of GMB and GIB were significantly better than the control, followed by P30 and GDB (Fig. 1D).

Root Weights. All treatments except GD significantly stimulated root production and weights of Florunner (Fig. 1E). All treatments of Starr stimulated root production (Fig. 1F).

Pod Weights. All VAMF treatments applied to Florunner resulted in production of greater pod weights than those from uninoculated plants. Florunner also responded to the higher rate of P (Fig. 1G). Starr cultivar showed no increase in pod dry weights regardless of treatment (Fig. 1H).

Southeast Texas

Heavy rainfall at Yoakum necessitated replanting. The possible intermixing of applied inoculum in the soil was of considerable concern; however, the decision was made to monitor all parameters originally planned. As shown in the following data sets, the responses of peanuts to the inoculations were, in general, similar to responses at the other three locations and therefore are still considered valid.

Shoot Weights. Shoot fresh weights of Florunner plants in soil inoculated with all VAMF and VAMF + B were greater than those of controls (Fig. 2A). Shoot fresh weights of Florunner plants inoculated with B alone were no better than controls. GI alone and GDB and GIB significantly increased fresh shoot weights of Tamnut-74 (Fig. 2B). Dry shoot weights of Florunner were increased only by GDB whereas Tamnut-74 responded to both GDB and GIB (Figs. 2C and 2D). Added phosphorus had no effect on dry shoot weights of either cultivar.

Root Weights. Root fresh weights of Florunner grown in soils inoculated with GI, GDB and GIB were greater than controls (Fig. 2E), as were the P30 plants. Neither B nor added phosphorus influenced root weights.

Root fresh weights of Tamnut-74 were greater only in plants in the GDB and GIB treatments (Fig. 2F).

Yield. Trends towards increased yields of Florunner were observed; however, values were not statistically different according to Duncan's multiple range test at the 5% level. Trends towards increased yields/ha were also

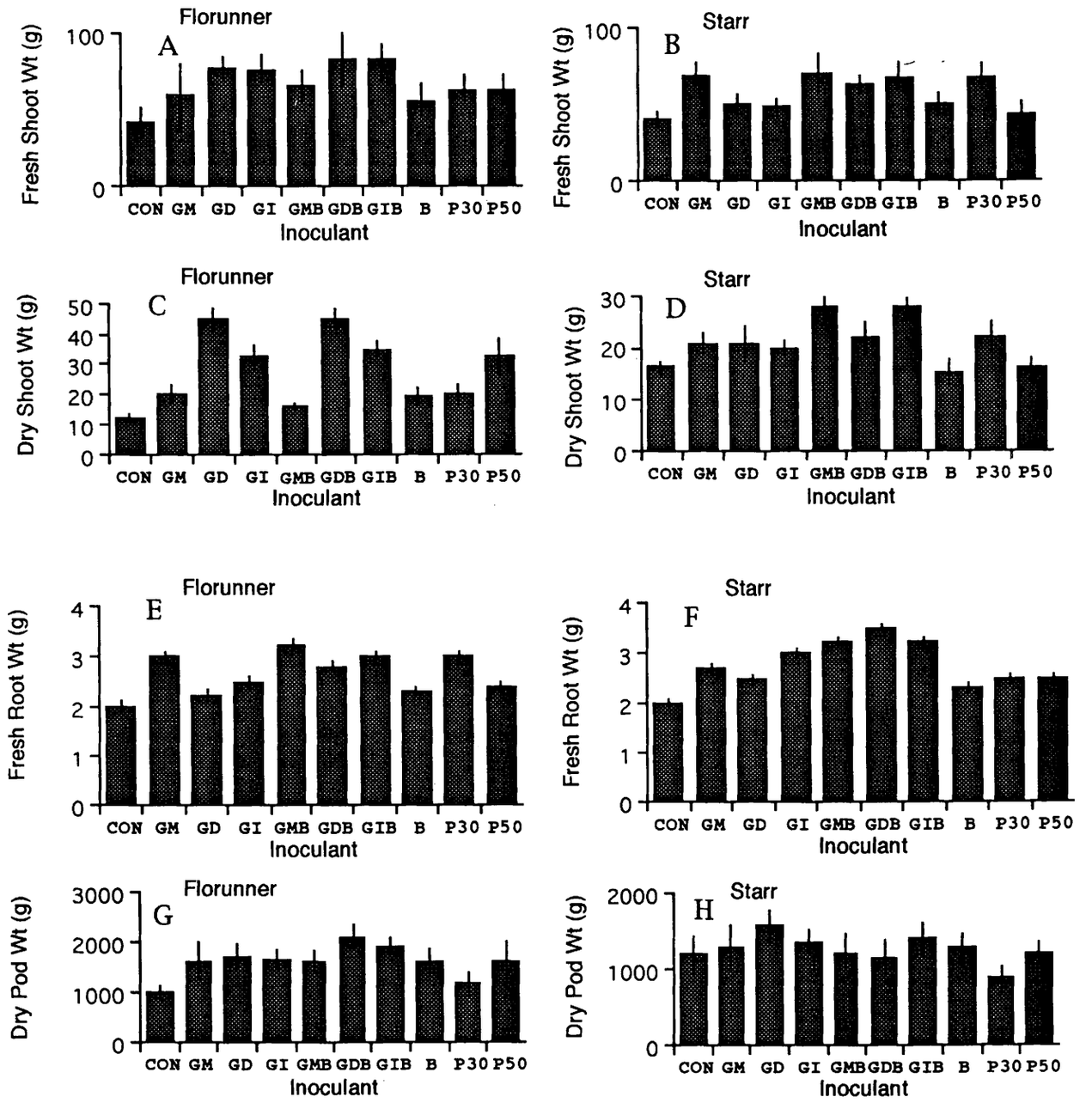

Fig. 1. Growth response of Florunner and Starr peanuts to inoculation in the field with mycorrhizal fungi, *Bradyrhizobium*, and added phosphorus. Nonfumigated soil in South Texas (Poth). CON = control, uninoculated; GM = *Glomus mosseae*; GD = *G.deserticola*; GI = *G.intraradices*; B = *Bradyrhizobium*. P30 = 30 ppm phosphorus; P50 = 50 ppm phosphorus. Vertical lines are standard errors.

observed in Tamnut-74, particularly with two VAMF fungi (GD and GIB); however, again, they were not statistically different.

East Texas

Shoot Weights. Fresh shoot weights of all inoculated Florunner plants were significantly greater than controls at Grapeland at 80 and 120 DAP (Fig. 3A). Fresh shoot weights of Tamnut-74 plants at 80 and 120 days were significantly greater only when inoculated with GEB (Fig. 3B). At 80 DAP in Tamnut-74 only GEB was better than the controls; however, at 120 DAP, as with Florunner, all VAMF treatments were better than the control. Some of the inoculated Florunner plants (at 80 and 120 DAP) showed over 100% increase in shoot growth (Fig. 3A).

Dry shoot weights of Florunner were greater than controls with GEB only at 80 and 120 DAP, whereas by 120 days all treatments of Tamnut-74 were better than controls.

Root Weights. Weights of Florunner GEB and B fresh root systems at 80 DAP were significantly greater than controls; however by 120 days all treatments eclipsed the

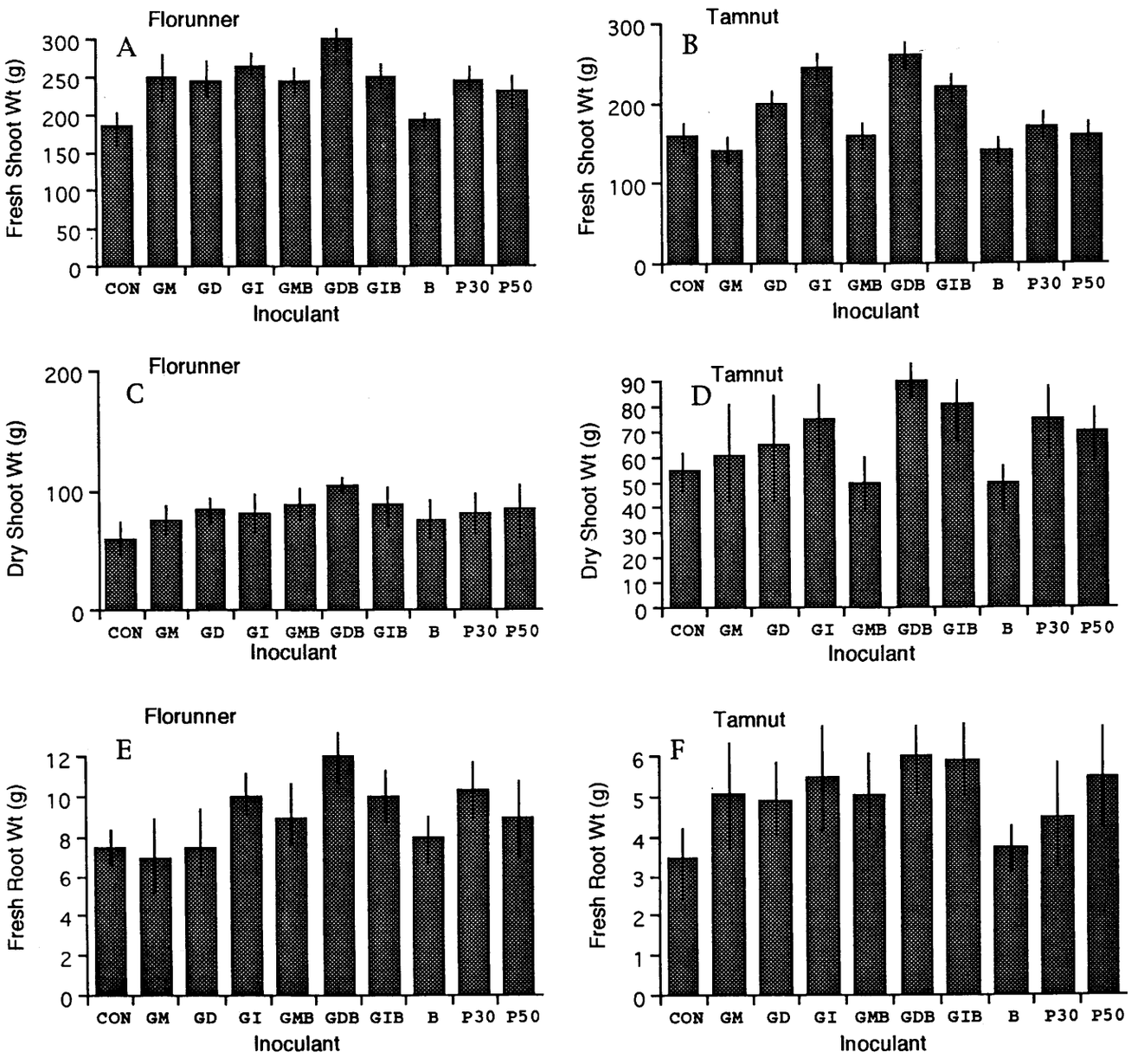

Fig. 2. Growth response of Florunner and Tamnut-74 peanuts to inoculation in the field with mycorrhizal fungi, *Bradyrhizobium*, and added phosphorus. Nonfumigated soil in Southeast Texas (Yoakum). CON = control, uninoculated; GM = *Glomus mosseae*; GD = *G. deserticola*; GI = *G. intraradices*; B = *Bradyrhizobium*. P30 = 30 ppm phosphorus; P50 = 50 ppm phosphorus. Vertical lines are standard errors.

control (Fig. 3E). At 30 and 80 days Tamnut-74 GE and GEB were significantly higher than the control (Fig. 3F). At 120 days, controls caught up to GEB and only GE was significantly different from controls. In general, Florunner responded more positively to inoculation with VAMF than Tamnut-74.

North Texas

In year 1 experiment, cultivars responded differently to inoculation.

Shoot Weights. Fresh shoot weights were not obtained at Etter in this experiment. Dry weights of inoculated McRan were significantly higher than those of control plants (Figs. 4A and B). Shoot dry weights of both the inoculated McRan and Pronto cultivars were significantly greater (108% and 50%, respectively) at harvest than those of the uninoculated controls (Fig. 4B) - that is, the addition of GI to the indigenous VAMF population resulted in a stimulation of top growth.

Root Weights. The root systems of inoculated McRan were stimulated before the first sampling date and this trend was evident at the second sampling date (85 DAP). At harvest, the root systems of inoculated plants were still larger than the controls (Fig. 4C). Root systems of inoculated Pronto plants had a delayed and less pronounced stimulation

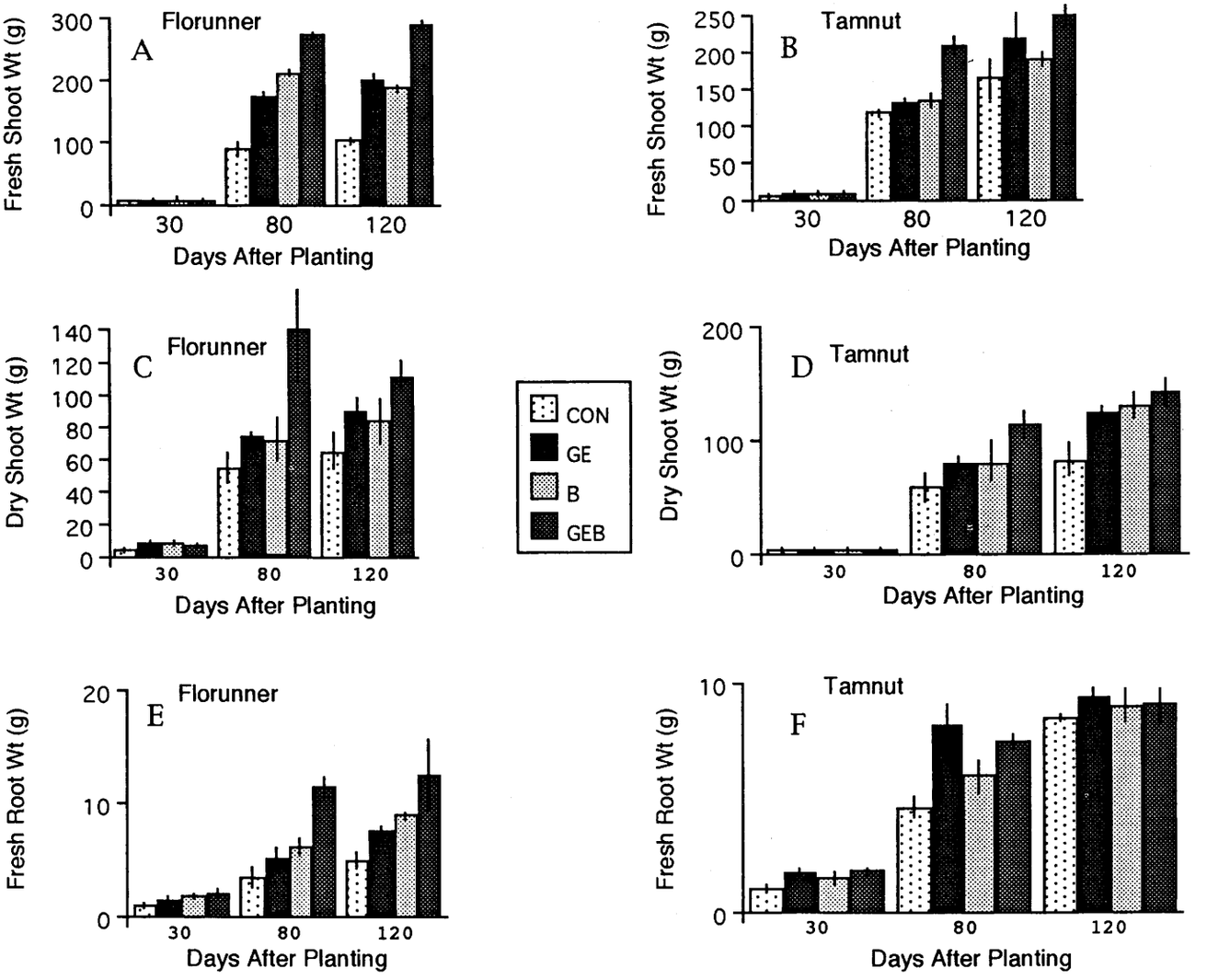

Fig. 3. Growth response of Florunner and Tamnut-74 peanuts to inoculation in the field with *Glomus etunicatum* with and without *Bradyrhizobium* and with *Bradyrhizobium* alone. Nonfumigated soil in East Texas (Grapeland). CON = control, uninoculated; GE = *Glomus etunicatum*; B = *Bradyrhizobium*; P30 = 30 ppm phosphorus; P50 = 50 ppm phosphorus. Vertical lines are standard errors.

when compared with those of McRan (Fig. 4C). Early root stimulation, as exhibited by McRan, is considered a desirable response.

In the year 2 experiments (Fig. 5), all treatments increased mean plant fresh weights at 45 DAP and the mixture of VAMF species was the best treatment applied.

Shoot Weights. Fresh shoot weights of all inoculated plants were significantly greater than those of the CONB (Figs. 5A and 5C). Shoot dry weights also were greater in plants from the GDB and MIXB plants than those of the control at 45 days. At harvest, shoot dry weights of all VAMF inoculated plants were higher than those of B (Fig. 5B).

Pod Weights. Pod weights from inoculated plants were all significantly greater than those of the B controls (Fig. 5D); however, yields of peanut from all treatments were statistically similar (Fig. 5E) and grade factors were also similar.

The overall results from these 2-yr field studies support the hypothesis that the addition of mycorrhizal inocula to the indigenous species in field soil may result in a positive plant growth response in peanut. Pod dry weights of inoculated plants at two locations were greater than those of control plants. Early stimulation of shoot and/or root systems was pronounced with some treatments. Weber *et al.* (36), working with chickpea, also noted this response in fumigated fields. They showed that, at maturity, chickpea seed yields from all treatments were similar and concluded that susceptibility of legume reproductive growth to water stress in the pod-fill period tended to give less seed yield despite greater shoot biomass and that early infection with VAMF increased water demands during seed production. Fitter (7) discussed the fact that some plants start to benefit from inoculation in the seedling stage and early vegetative growth stage because P inflow rates into roots may limit growth of plants without mycorrhization. Dinkelaker (6) stated that, in large-seeded species such as chickpea, P reserves may sustain growth during the first few weeks after

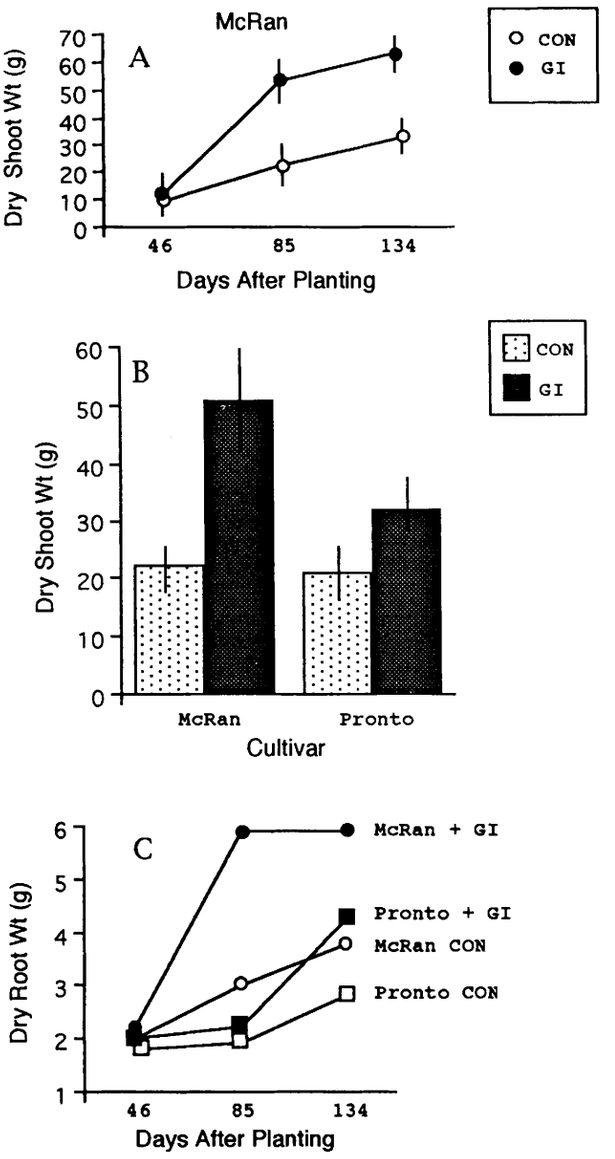

Fig. 4. Growth response of McRan and Pronto peanuts to inoculation in the field with *Glomus intraradices*. Nonfumigated soil in North Texas (Etter). Vertical lines are standard errors.

germination. Later, during flowering, the chickpea plants retain a high growth rate but become dependent on P from the soil. Thus, both adequate water and phosphorus at pod-fill become increasingly important with increased shoot and root biomass due to mycorrhization. This may also be true in the case of peanuts. Yield responses were not obtained with the combinations of species of fungi and cultivars used in this study, the same situation as reported in experiments with other crops. Powell (25) and Safir (28) provide extensive reviews of the responses of many other crop plants.

It is evident that vegetative growth responses do not always lead to yield increases under conditions of the tests. Growth response in peanut was sometimes greater when mixed inocula were employed. This is in keeping with the results of greenhouse experiments conducted by Koomen *et al.* (12). They concluded that multiple mycorrhizal inocula may be superior to single species inocula and then extrapolated from the greenhouse studies to speculation about results in the field. In our studies, the positive effect of inoculation in the field was pronounced regardless of soil pH (pH 6.8-8.6). High soil phosphorus did not necessarily negate the growth stimulation produced by the mycorrhizal inoculum (see 25 for discussion). Inoculation with *Bradyrhizobium* did not guarantee increased plant growth, nor did the addition of phosphorus.

These experiments demonstrate that inoculation with mycorrhizal fungi can increase peanut growth (including root, shoot, and pod biomass) under field conditions in nonfumigated soil. Although yield increases have been obtained in fumigated and nonfumigated soil in the greenhouse using these fungi and cultivars (unpublished information), extension to field studies introduces many more variables. Research is needed to manipulate the crop management system to obtain significant increases in yield and develop cost-effective methods for production and application of inocula.

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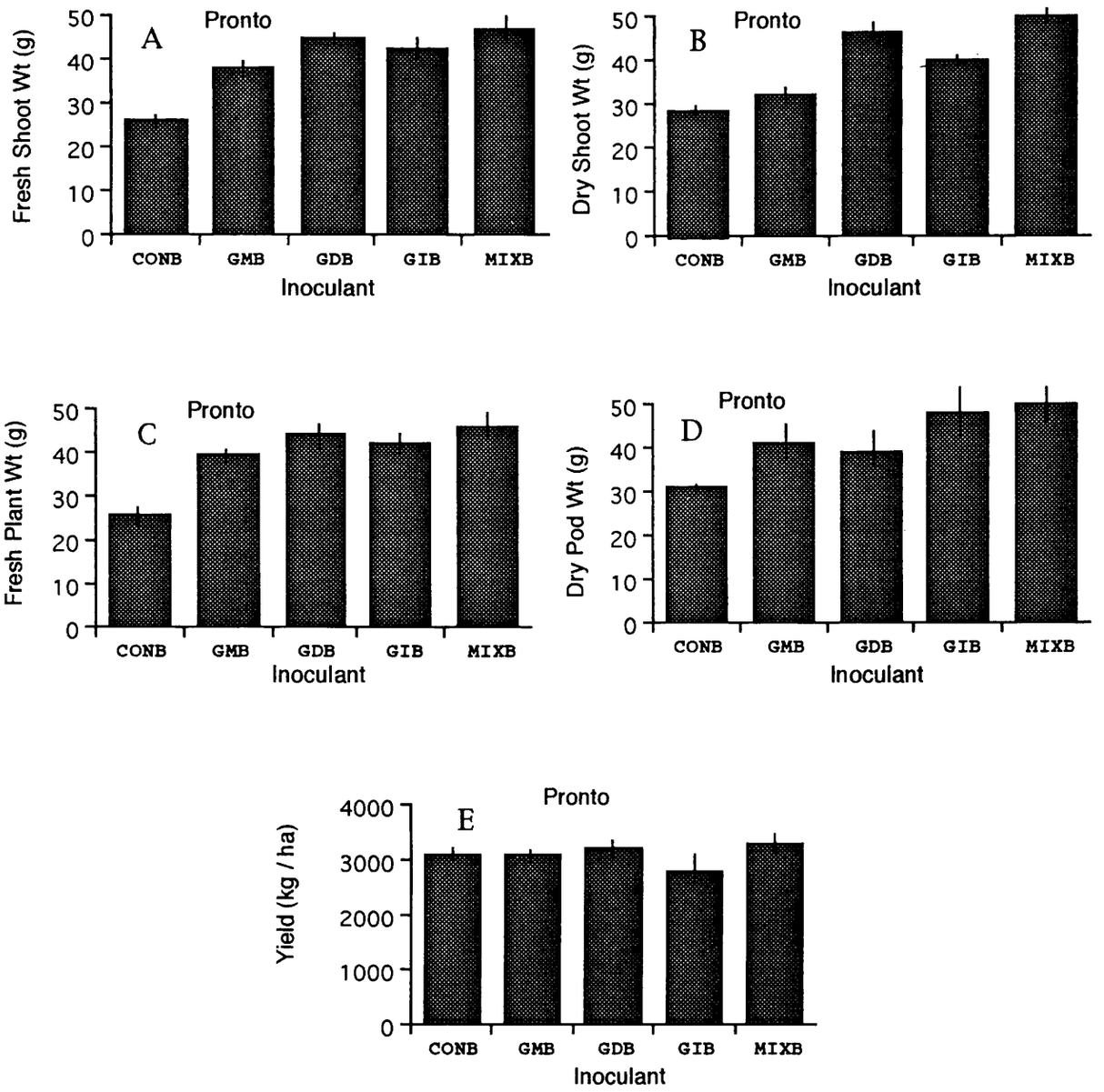

Fig. 5. Growth response of Pronto peanuts to inoculation in the field with mycorrhizal fungi and *Bradyrhizobium*. Nonfumigated soil in North Texas (Etter). GM = *Glomus mosseae*, GD = *G. deserticola*, GI = *G. intraradices*, MIX = mixture of all three mycorrhizal fungi. Vertical lines are standard errors.

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