VOL. 7, NO. 5, MAY 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

INFLUENCE OF PHOSPHORUS ON SELECTED DESMODIUM GROWTH AND NODULATION PARAMETERS

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ABSTRACT

Phosphorus is known to affect growth and formation of nodules although its role in the process is less understood. Several workers have, however, related phosphorus nutrition with rhizabial activities in leguminous plants. In other studies, it has been shown that nodules are stronger sinks for phosphorus-compared roots, shoots and even young mature leaves. We initiated this study to determine the effect of phosphorus on selected desmodium species growth and nodulation parameters in a pot experiment. A 2 by 8 factorial experiment was laid in a complete randomized design (CRD) at KARI-CYMMIT Research Station located in western Kenya. Pots were each filled with 7.5 kg 2 mm sieved dry soil. The treatments consisted of two desmodium species (*D. intortum* and *D. uncinatum*) and 8 levels of phosphorus applied at 0, 10.3, 20.6, 30.9, 41.2, 51.5, 61.8 and 72.1 mg P kg^{-1} soil. The experiment was replicated 3 times. Plants were top dressed with urea (at 85.5 mg N pot^{-1}) at 21 days after emergence (DAE). Shoots were harvested twice during the growth period (at 15 and 28 weeks after planting) while dry root weight and nodulation parameters were determined at 34 wap. Results showed that shoot biomass produced in the second harvest was 21% and 42% higher than production in the first harvest for *D. uncinatum* and *D. intortum*, respectively. Root biomasses was significantly ($p = 0.045$) increased at 41.2 mg P kg^{-1} soil compared to the control treatment in the *D. uncinatum* plant while *D. intortum* had significant increase in root weight at ≥ 30.9 mg P kg^{-1} soil. Addition of phosphorus in excess of these rates did not translate into gain in biomass except at 72.1 mg P kg^{-1} soil. Application of phosphorus at ≥ 20.6 mg P kg^{-1} soil significantly ($p = 0.039$) increased the number of nodules produced by *D. intortum* species from an average of 37 (control) to 86.8 $nodules^{-1}$ pot at 51.5 mg P kg^{-1} soil. The number of active nodules obtained in the control treatment was significantly ($p = 0.041$) low compared to that obtained at 30.9 mg P kg^{-1} soil in the *D. uncinatum* species. Whereas *D. uncinatum* produced few but heavy nodules, *D. intortum* had relatively large number and light nodules, phosphorus rates not withstanding. Relationships between nodule number and root biomass are also discussed.

Keywords: desmodium nodulation, phosphorus, effective nodules, root density, shoot biomass.

INTRODUCTION

Some of the main constraints limiting the capacity of smallholder farmers to combat declining soil fertility include limited range of alternative nutrient sources (Sanchez *et al*., 1997). Only mineral fertilizers and N_2-fixing legumes offer a realistic chance for raising agricultural production. Low levels of phosphorus in the soil are a hindrance to the growth and development of various leguminous species (Okalebo *et al*., 2009). Several studies have revealed that the most important nutrient for establishment of legumes in the tropical region is phosphorus (Haque *et al*., 1986, Gitari *et al*., 2003). Nodules are stronger sinks for phosphorus-compared roots, shoots and or even young mature leaves (Tsvetkova and Georgiev 2003). Indeed effect of P on rhizabial activities enhanced nodulation in *Trifolium subterraneum* L species (Robson *et al*., 1981).

MATERIALS AND METHODS

Experimental design and treatments

A 2 by 8 factorial pot experiment was laid in a complete randomized design (CRD) arrangement at KARI-CYMMIT Striga Research Station located in western Kenya (Kibos). Pots were each filled with 7.5 kg sieved (2 mm) dry soil. The treatments consisted of two desmodium species (*D. intortum* and *D. uncinatum*) and 8 levels of phosphorus (0, 10.3, 20.6, 30.9, 41.2, 51.5, 61.8 and 72.1 mg P kg^{-1} soil). Plants were all top dressed at 85.5 mg N pot^{-1} with urea at 21days after emergence (DAE). The experiment was replicated 3 times.

Establishment of desmodium plants

Desmodium seeds were drilled at the centre of each of the phosphorus treated soils. Emerged plants were thinned to three plants pot^{-1} at 7 DAE. To sustain growth, desmodium plants were irrigated with tap water at 700 ml pot^{-1} at alternate days. All pots were kept weed free by carrying out hand removal as and when weeds were noticed. Desmodium plants were maintained up to the 15^{th} weeks before shoots were harvested (1^{st} harvest). The re-growth plants were further maintained for another 13 weeks before shoots (2^{nd} harvest) and roots were harvested.

Data collection

Determination of shoot biomass

Shoots were separated from roots by cutting with a clean sharp knife. They were transferred into labeled brown paper bags for determination of fresh weight. The paper bags and contents were thereafter oven dried at 68^0 C to a constant weight and dry matter yields recorded.

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Determination of root biomass yield

Shoots were separated from roots by cutting the plant at the soil surfaces with a clean sharp knife. Roots in the pots were soaked with tap water for 30 minutes and further washed gently with excess water to remove the soaked/softened soils. Nodules were left intact on the root surfaces. The cleaned roots were allowed to drain off water for 10 minutes and fresh weights recorded. Fresh roots were transferred into labeled brown paper bags and contents oven dried at 68^0 C to a constant weight.

Determination of total nodule, effective nodule and nodule weight

Effective (pinkish) and non-effective nodules, associated with specific phosphorus treatments, were identified and separately harvested from the cleaned /washed roots. The nodules were counted using a tally counter. Fresh nodule weights for both effective and non-effective nodules were determined using electronic weighing balance (Model XL - 31000). The sum of effective and non-effective nodules for each treatment was considered as the total nodules for that treatment.

RESULTS

Effect of phosphorus on desmodium shoot biomass yield

Shoot dry matter production associated with various rates of phosphorus in the first and second harvests for *D. uncinatum* and *D. intortum* is presented in Table-1(a) and Table-1(b), respectively. It is evident from the results that mean shoot biomass produced in the second harvest was 21% and 42% higher than production in the first harvest in *D. uncinatum* and *D. intortum plants* respectively. *D. intortum* had significantly ($p = 0.05$) different shoot dry matter yields at phosphorus rates ≥ 20.6 mg kg^{-1} soil compared to control treatment in both first and second harvests (Table-1(b)). But such differences in dry shoot weight in *D. uncinatum* were observed only in the second harvest at P rates above 41.2 mg kg^{-1} soil (Table-1(a)). Both species of desmodium produced significantly more biomass in the second harvest compared to the first harvest.

Table-1(a). Effects of phosphorus on *D. uncinatum* shoot biomass.

Phosphorus mg kg^{-1}soil	15 WAP (g^{-1} pot)	28 WAP	Cumulative
0	22.15	32.63	54.78
10.3	31.07	39.66	70.13
20.6	36.57	40.10	76.67
30.9	34.47	41.53	76.02
41.2	36.28	45.63	81.9
51.5	37.87	40.96	78.14
61.8	38.94	43.83	82.17
72.1	35.79	45.40	81.09
Mean	34.14b	41.20a	37.7
LSD	NS	7.547	23.91
CV	5.3	6.8	7.7

Means followed with the same letter not significantly different

Table-1(b). Effects of phosphorus on *D. intortum* shoot biomass accumulation.

Phosphorus mg kg^{-1}soil	15 WAP (gm^{-1} pot)	28 WAP	Cumulative
0	25.10	29.50	54.6
10.3	45.70	51.00	96.7
20.6	54.53	69.18	123.71
30.9	53.56	76.97	130.4
41.2	51.63	70.04	121.67
51.5	58.88	81.48	140.2
61.8	50.08	77.10	127.18
72.1	45.13	88.68	133.81
Mean	48.07b	67.994a	58.01
LSD	21.374	30.992	44.602
CV	7.5	5.1	9.4

Means followed with the same letter not significantly different.
WAP = Weeks after planting

Effect of phosphorus on root biomass yield

Results presented in Table-2 below show that root dry matter yield for *D. uncinatum* was significantly ($p=0.045$) increased to 40 gm^{-1} plant at 41.2 mg P kg^{-1} compared to the control treatment which had a mean weight of 24.6 gm^{-1} plant while *D. intortum* root weight was significantly increased at a lower rate of 30.9 mg P kg $^{-1}$ soil. Addition of phosphorus in excess of these rates did not translate into any gain in weight except at 72.1 mg P kg^{-1} soil which resulted in total *D. intortum root* biomass being increased by about 84% while *D. uncinatum* had root weight increased by 20% (Table-2). Comparison of *D. uncinatum* and *D. intortum* dry root biomasses, across phosphorus rates, revealed that *D. intortum* roots was about 15% heavier than *D. uncinatum's* roots with more pronounced variations being observed at lower rates of fertilization.

Table-2. Effect of phosphorus on desmodium root biomass accumulation.

Phosphorus P mg kg^{-1}soil	D. *uncinatum*	D. *intortum*	Mean
	(gm^{-1}plant)		
0	24.56	19.13	21.85
10.3	28.86	32.53	32.2
20.6	30.43	41.5	35.96
30.9	33.3	43.93	38.61
41.2	39.9	43.13	41.51
51.5	34.63	37.36	36.0
61.8	29.56	35.16	30.86
72.1	29.66	34.56	32.11
Mean	31.366	35.916	33.641
LSD	NS	11.274	7.4975
CV	6.6	4.1	6.9
S.E	3.011	3.760	2.6231
P Level	0.0783	0.006	0.0003

Means followed with the same letter are not significantly different

Effect of phosphorus on nodulation

Effect of phosphorus application on nodulation varied with rate of phosphorus fertilization in both *D. intortum* and *D. uncinatum* species although number of nodules in *D. uncinatum* species did not respond significantly to phosphorus application (Table-3). The species produced a mean of 31.3 $nodules^{-1}$ pot at control treatment. But number of nodules increased to a peak of 71 $nodules^{-1}$ pot at 72.1 mg P kg^{-1} soil (Table-3). Application of phosphorus at ≥ 20.6 mg P kg^{-1} soil significantly ($p = 0.039$) increased the number of nodules produced by *D. intortm* species. The number of nodules was increased from an average of 37 (control) to 86.8 $nodules^{-1}$ pot at 51.5 mg P kg^{-1} soil (Table-3).

Fresh nodule weight was not significantly affected by phosphorus application in *D. intortum* (Table-3b) while *D. uncinatum* produced significantly lighter nodules at 41.2 and 51.5 mg P kg^{-1} soil (Table-3). While *D. uncinatum* produced few but relatively heavy nodules, nodules associated with *D. intortum* were large in number and relatively light in weight, phosphorus rates not withstanding.

VOL. 7, NO. 5, MAY 2012 ISSN 1990-6145
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Table-3. Effect of phosphorus on number of nodules and fresh nodule weight of two desmodium species.

Phosphorus	*D. uncinatum*	*D. ntortum*	*D. uncinatum*	*D. intortum*
	Total number of nodules		**Fresh nodule weight**	
P (mg kg^{-1}soil)	(nodules^{-1} pot)		(gm^{-1} nodule)	
0	31.33	37	0.7	0.5
10.3	45.67	33	0.78	0.47
20.6	46	52.3	0.71	0.43
30.9	62	51.8	0.69	0.41
41.2	61	68	0.55	0.52
51.5	55.33	86.8	0.55	0.33
61.8	50.33	70.6	0.67	0.38
72.1	70.67	73.6	0.62	0.5
Mean	53.541	60.041	0.662a	0.443b
LSD	NS	NS	0.142	0.159
CV	9.2	6.6	5.4	8.8

Means followed with the same letter not significantly different.

Comparison of effective and non-effective nodules

The two desmodium species produced ineffective and effective nodules (red nodules) which indicate that effective nodules were active in fixing nitrogen. Application of P at rates ≥ 41.2 mg P kg^{-1} soil did not affect number of nodules in *D. uncinatum* (Figure- 1). But the number of active nodules obtained in the control treatment was significantly ($p \leq 0.05$) low compared to the ones obtained at 20.6 and 30.9 mg P kg^{-1} soil in *D. uncinatum* . Application of P at 30.6 mg P kg^{-1} soil did not only increase the number of effective nodules (6 nodules per plant) but also induced the highest number (13 nodules per plant) of total nodules in *D. uncinatum*).

D. intortum had a significant (p $p \leq 0.05$) increase in total nodules at P rate ≥ 30.9 mg P kg^{-1} soil except at 41.2 mg P kg^{-1} soil (Figure- 2). A significant high number of effective nodules was produced by *D. intortum* at 30.9, 51.5 and 60.8 mg P kg^{-1} soil application rates fertilization compared to the control treatment. The number of effective nodules produced by either of the two species generally followed a trend similar to that of total nodules.

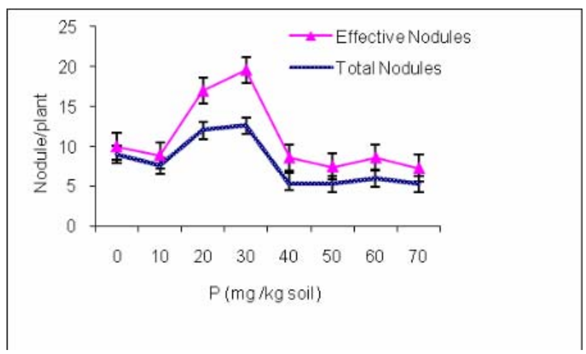

Figure- 1. Effect of phosphorus on *D. uncinatum* nodulation.

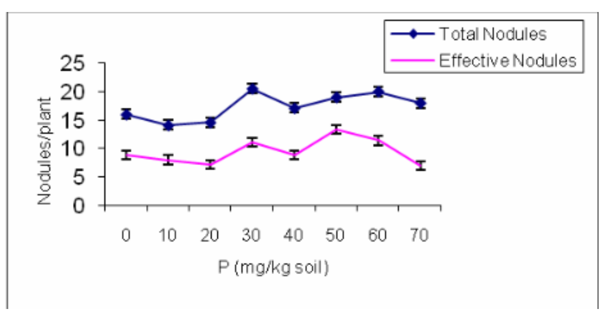

Figure- 2. Effect of phosphorus on *D. intortum* nodulation.

VOL. 7, NO. 5, MAY 2012 ISSN 1990-6145
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Relationships between nodule number and root biomass

Nodules and root biomass showed positive relationship at low rates of phosphorus application (Figures-3(a) and 3(b)). The number of nodules consistently increased with root weight and peaked at 41.2 mg P kg^{-1} soil. Further increase in phosphorus was less beneficial although at 72.1 mg P kg^{-1} soil, phosphorus application outstandingly increased the number of nodules in *D. uncinatum* by about 80%.

The highest number of nodules was obtained at 41.2 mg P kg^{-1} soil, which represents an increase of 112% compared to the control treatment (Figure- 3(b). The trend line showing relationship between nodule fresh weight and total number of nodules indicated a negative correlation with critical phosphorus rate (above which nodules became lighter) being obtained at 55 and 46 mg P kg^{-1} soil for *D. uncinatum's* and *D. intortum* species, respectively (Figure-3(c) and Figure-3(d)). Whereas response of root weight to phosphorus was positive, phosphorus fertilization did not influence nodule weight, desmodium species not withstanding (Figure- 4(a) and Figure 4(b)). It appears that nodule weight and root biomass followed a trend similar to that observed in the nodule density and shoot biomass relationship. The number of nodules tended to increase as root biomass increased and curved down wards as root weight reduced.

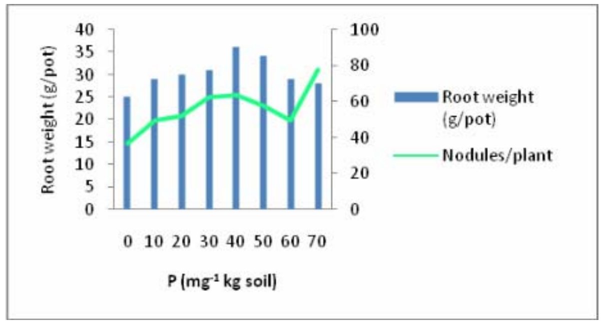

Figure-3a. Relationship between *D. uncinatum* root biomass and nodule number as affected by phosphorus.

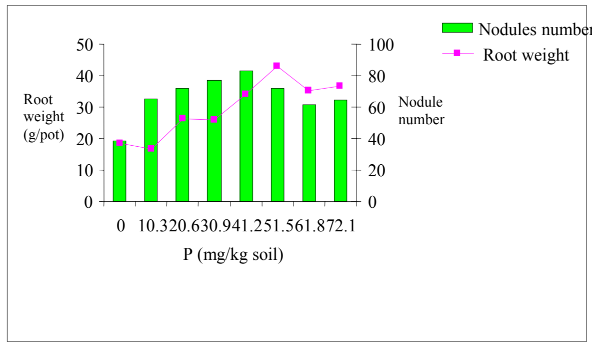

Figure-3b. Relationship between *D. intortum* root biomass and nodule number as affected by phosphorus.

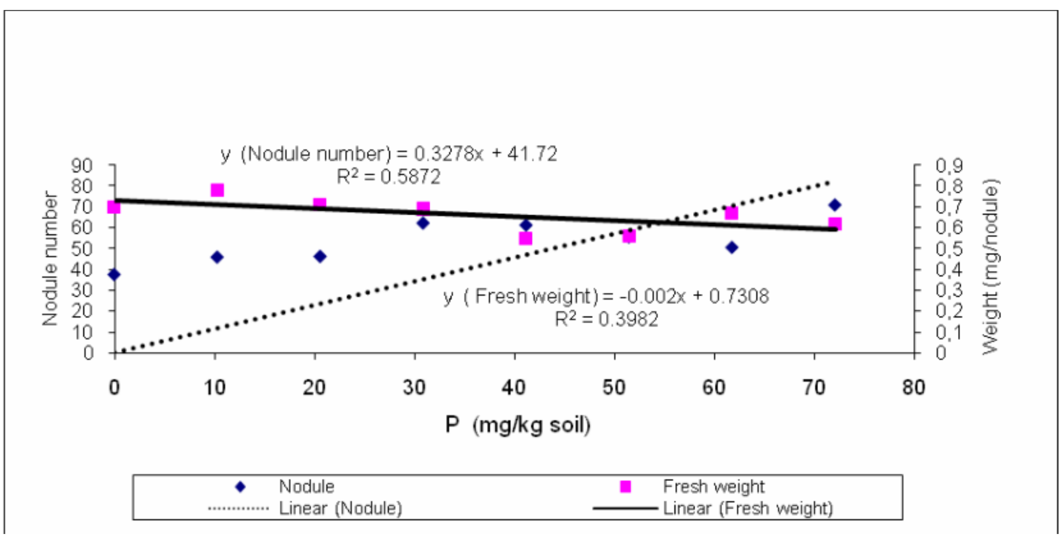

Figure-3c. Relationship between number of nodules and fresh nodule weight of *D. uncinatum* as affected by phosphorus.

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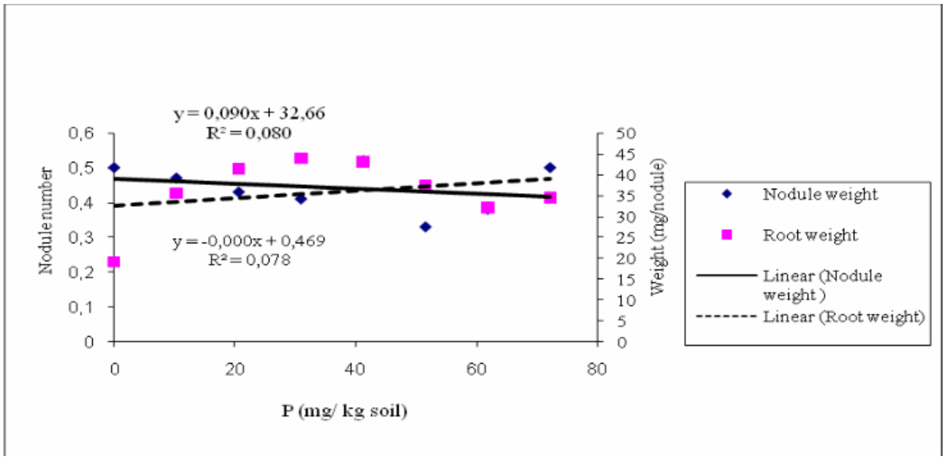

Figure-3d. Relationship between number of nodules and fresh nodule weight of *D. intortum* as affected by phosphorus.

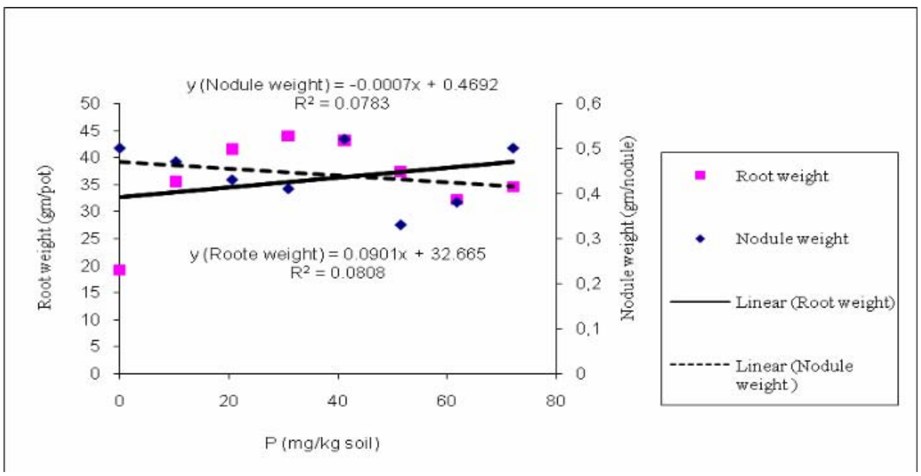

Figure-4a. Relationship between fresh nodule weight and *D. intorturm* root biomass as affected by phosphorus.

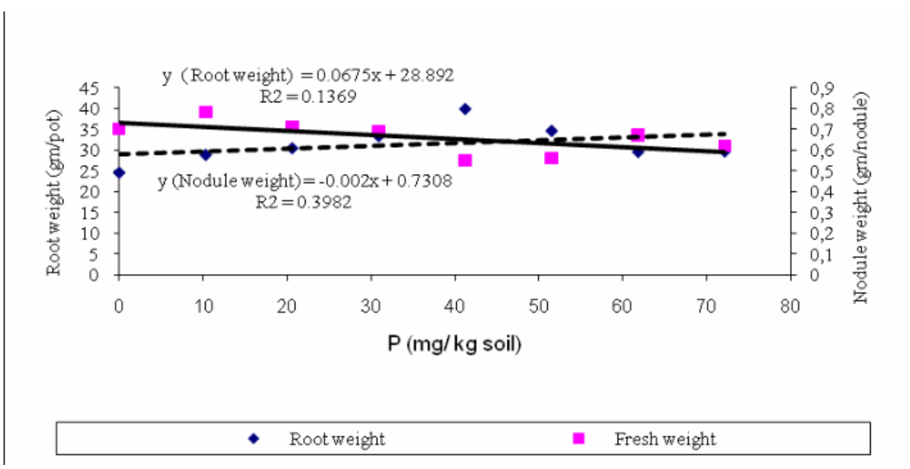

Figure-4b. Relationship between fresh nodule weight and *D. uncinatum* root biomass as affected by phosphorus.

DISCUSSIONS

Effect of phosphorus on biomass production

Effect of phosphorus on shoot biomass

Our results revealed consistent gains in shoot biomass enhanced by phosphorus especially at higher rates of application. We attributed the massive shoot biomass accumulation observed at high P rates to the enhanced root growth and increased nutrient uptake. We show in this study that both *D.intortum* and *D.uncinatum* had biomass accumulated gradually in apparent response to their growth and physiological development. As expected, mature plants produced higher herbage dry matter yields than the young ones. By the end of the first season, desmodium plants had matured and had roots fully established in the pot. It thus resulted in optimizing nutrient uptake and resulted in accelerating accumulation of shoot biomass. Our result is in agreement with previous findings from field studies by other workers (Haque *et al*., 1986, Otieno *et al*., 1989, Cadisch 2003 and Reda *et al*., 2005). In yet another study where effect of organic and inorganic fertilizers on D.*uncinatum* growth was investigated under field condition, Muyekho *et al*., (2003) reported that application of N beyond 40 kg N ha^{-1} resulted in a significant decline in total herbage dry matter yield while application of P at 23 and 46 kg P_2O_5 ha^{-1} induced (significantly) increase in herbage dry matter yield. They also observed no gain in shoot dry matter yield at P rates above 46 kg P_2O_5 ha^{-1}.

Effect of phosphorus on root biomass

Supplying phosphorus at 41.2 mg P kg^{-1} soil increased *D. uncinatum* and *D. intortum* root biomass by 62 and 131%, respectively. The increase in biomass was evidently more pronounced in the *D. intortum* treatment, which suggests that *D.intortum* was more responsive to phosphorus application than *D. uncinatum*. In a study that tested nine tropical and one temperate pasture legume species grown on soil with varying additions of phosphate, Ascencio (1996) found that phosphorus was positively correlation with biomass production of *Desmodium tortuosum* (sw.) dc in a phosphorus deficient soil while severe phosphorus stress reduced its root length by 50%.

Lack of response by root biomass to phosphorus application above 51.5 mg P kg^{-1} soil was probably caused by high P concentration (associated with the application rates) in the soil. This may have induced deficiency and or antagonized the uptake of others nutrients and resulted in compromising root growth. Alternatively, presence of mycorrhizae in the acutely P deficient soil used in the pot experiment might have had contribution in suppressing P uptake. A number of workers have reported suppression of phosphorus supply by some fungi in phosphorus deficient soils. Kaori *et al*., 2006, showed that both AM fungi and root holoparasitic plant *Orobanche minor* Sm., reduced supply of phosphorus but not of other elements (N, K, Mg, Ca) they examined using red clover plants (*Trifolium pratense* L.). Other workers who investigated mycorrhizae as potential resource for phosphorus acquisition in systems that incorporate legumes sps also reported similar results (Ciotola *et al*., 1995, Ciotola *et al*., 1996, Savard *et al*., 1997).

Desmodium nodulation

Many soils in the tropical region are low in both total and available phosphorus (Chien and Menon, 1995; Rao *et al*., 1999). This condition is worsened by continuous depletion of nutrients (Sanchez *et al.,* 1997, Smalling *et al*., 1997). It is appreciated that legumes require effective rhizobia strains and adequate soil phosphorus to fully exploit their nitrogen fixation potential. Our work revealed that supplying phosphorus induced production of not only large number but also small sized and lighter nodules. This observation was particularly holding in *D. intortum* treatments.

Although appreciable number of the total nodules produced by each desmodium species was red in colour, indicating potential of nitrogen fixation, the number tended to decrease with application of phosphorus above 41.5 mg and 51.8 mg P kg^{-1} soil in *D. uncinatum* and *D. intortum* respectively. The proportion of effective nodules was 59% and 64% of total nodules in *D. uncinatum* and *D. intortum* treatments. Interestingly, treatments that produced high number of effective nodules in *D. intortum* also produced lighter nodules while treatments that produced few nodules had relatively heavy nodules. This observation indicates that nodule production by *D. intortum* was inversely related to nodule fresh weight. It may be of interest to quantify and compare the biologically fixed nitrogen associated with the relatively few but big nodules in *D. uncinatum* and the small, but large in number, produced by D. *intortum*.

Relationship between nodule and nodule weight

The inverse relationship between number of nodules and nodule mass was apparent though more evident in *D.intortum* than in *D.uncinatum.* Supplying phosphorus at higher rates enhanced production of small and light nodules as well as induced emergence of primary and lateral roots on which most nodules were attached. This relation ship was holding as far as root dry mass benefited from phosphorus application particularly in the *D. intortum* treatment.

CONCLUSIONS

There were consistent gains in shoot biomass enhanced by phosphorus application. We attributed shoot biomass accumulation observed at optimum P rates to the enhanced root growth which increased nutrient uptake. We show in this study that both *D. intortum* and *D.uncinatum* had biomass accumulated gradually in apparent response to their growth and physiological development. Mature plants produced higher herbage dry matter yields compared to the young plants.

Phosphorus fertilization at rates an above 41.2 mg kg^{-1} soil evidently increased more biomass in *D. intortum* species compared to *D. uncinatum* sps. The observation suggests that *D. intortum* was apparently more responsive to phosphorus application than *D. uncinatum*. Supplying phosphorus induced production of not only large number but also small sized and light nodules in *D. intortum* while *D. uncinatum* had relatively large sized but few nodules. The number of nodules produced by *D. intortum* was inversely related to nodule fresh weight.

RECOMMENDATIONS

Further research is recommended to quantify and compare biologically fixed nitrogen by the relatively few but big nodules associated with *D. uncinatum* and that of the small sized nodules produced by D. *intortum*.

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