INVASIVE EARTHWORM SPECIES EFFECTS ON THE DENSITY AND MICROSCALE HABITAT OF *IXODES SCAPULARIS* NYMPHS IN DUTCHESS COUNTY, NY

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Abstract. The black-legged tick (*Ixodes scapularis*) is the primary vector of *Borrelia bugdorferi*, the bacteria that causes Lyme Disease. The nymphal stage of the tick's life cycle plays a large role in the transmission of Lyme Disease. Suitable nymph microhabitat includes lower vegetation layers, upper layers of the soil column, and leaf litter, which maintains a desirable humidity and temperature. Invasive earthworms affect the forest floors by decreasing leaf litter and small plants. This study proposes that invasive earthworms change tick habitats in deciduous forests, which in return, affect tick densities. Nymphal tick density and invasive earthworm density were sampled at 16 sites in Dutchess County, NY. Plant density and leaf litter depth were also measured at each site. Plant density and leaf litter depth both had a correlation with nymphal tick density; however, in this study invasive earthworm density did not significantly change the plant density or leaf litter depth of deciduous forest floors. Thus, nymphal tick density was found to have no clear relationship with invasive earthworm density.

INTRODUCTION

In recent years, Lyme Disease (LD) incidence has increased in Dutchess County, NY. From 1992 to 2000, Dutchess County reported more cases of Lyme disease than any other county in the U.S., consistently ranking among the top ten counties in incidence rates (Chow et al. 2003). The bacteria spirochete *Borrelia burgdorferi* causes LD and in New York State is transmitted through the black-legged tick, *Ixodes scapularis*. Black-legged ticks complete three life-stages (larvae, nymph and adult) throughout its two year life. Activity of nymphs in the northeast U.S. usually begins in May and peaks in June and declines slowly through the months of July and August (Stafford et al. 1998). Human cases of LD correspond with the nymphal activity peak, and is considered the primary vector of LD. Nymphal ticks require suitable habitats in order to quest to find a host. Tick environment typically includes the soil-vegetation part of a forest floor. The microscale habitat includes lower vegetation layers, upper layers of the soil column, and leaf litter. From this environment, ticks rely on relative humidity and temperature levels during their questing activity (Schulze and Jordan, 2005).

In the northeastern U.S., there are no native species of earthworms. Forests in Dutchess County, NY, have lacked these native earthworms since the last glacial period. Human activity and movement of European settlers have allowed for the invasion of exotic earthworms from Asia and Europe (Bohlen et al. 2004). There are three different ecological earthworm groups: anecic, endogeic, and epigeic. Anecic species live in deep burrows in soil and feed on leaf litter as well as soil. Endogic species reside within the soil, usually avoiding the soil surface, and consume highly organic soil. Epigeic species are found on the soil surface within litter and feed on leaf litter (Hendrix and Bohlen, 2002). These three earthworm groups have varied impacts on the soil and forest floor.

Exotic earthworm activity has been shown to increase leaf litter disappearance. The leaf litter layer seems to provide both a suitable microclimate and protection from adverse environment conditions for nymphal ticks. The apparent importance of this microhabitat in the survival of *I. scapularis* suggests that the disruption or denial of the habitat may serve as a suitable control strategy (Schulze et al. 1995). Exotic earthworms affect leaf litter and change the forest floors, which include varying small plant cover, which explains their influence on ticks, but limited information is available on how different species of earthworms affect this litter disappearance (Suarez et al. 2006). Also, exotic earthworm effects on forest floors have consequences for plant communities and little is known about these consequences of plant communities in natural habitats (Eisenhauer et al. 2007).

We examine how invasive earthworms affect and change tick habitats in deciduous forest floors, which in return, affect tick densities. Ticks use their habitat of small plants, leaf litter, and the upper layer of the soil column to survive in order to quest for a host, which we expect to be altered by the presence of worms. This project proposes three hypotheses as shown in Figure 1. H_1: The density of invasive earthworms (DIE) affects the leaf litter depth and plant density in Dutchess County, NY. H_2: The leaf litter depth and plant density affects the density of nymphs (DON) in Dutchess County, NY. H_3: The density of invasive earthworms (DIE) affects the density of nymphs in Dutchess County, NY.

METHODS AND MATERIALS

Experimental Sites and Selection

Experimental sites were chosen in Dutchess County, southeastern NY (41°50’ N. 73°45’ W). Figure 2 shows a total of 16 sites were selected from 40 sites of a 2006 Institute of Ecosystem Studies nymphal tick survey. Eight sites with the highest nymphal tick densities and eight sites with the lowest nymphal tick densities were selected.

Tick Collection

In June and July 2007, nymphal black-legged ticks were sampled at 16 field sites within Dutchess County, NY. Questing ticks were collected using a drag sampling method that involved pulling 1 m by 1 m white corduroy cloth over low vegetation while walking 15 m transects. Transects were selected randomly. Ticks were counted, identified, and removed from drag cloths and investigator’s clothing with forceps and contained in microcuvettes for later identification. Density of nymphs (DON) was calculated as number of tick nymphs per square meter dragged.

Earthworm Collection

Earthworms were collected in three randomly selected plots within each of the 16 sites using the 'Hot' mustard extraction method. The method involved placing 30 grams of mustard powder—allyl isothiocyanate—in 3.8 L of water and mixed thoroughly (WEEB 2005). The mixture was poured throughout the 1 ft x 1 ft plot and allowed to soak into the soil. The earthworms collected from the mixture and hand sorting were rinsed in water and placed into iso-propyl alcohol for lab identification with the aid of a 10-20x dissecting scope. Density of earthworms was calculated as number of earthworms per meter squared. Physical features of earthworms were observed and taxonomic keys were used for species identification (Hale 2007).

Density of Leaf Litter and Small Plants

Leaf litter depth (cm) was measured from the center of each plot. Also, small plants in the herbaceous layer were counted within each plot. Small plant density was calculated as plants per meter squared.

DATA AND RESULTS

Earthworm data

One hundred eighty two adult and juvenile earthworms were collected. Five species of invasive earthworms (Figure 3) were identified: *Lumbricus terrestris*, *Dendrobaena octaedra*, *Amynthus* sp., *Eisenia fetida*, and *Bimastos parvus*. The majority (57%) of the sampled earthworms was *Dendrobaena octaedra*, while Amynthas sp. made up 25% followed by *Eisenia fetida* (13%) then *Lumbricus terrestris* (4%). Density of earthworms ranged from 0 to 3.96 (Table 1).

Tick data

Ticks were found at 14 of the 16 sites (Table 2). There was a large range in the DON from 0.44 to 0.00; DON varied in 10 of the 16 sites.

H_1: Leaf litter depth / plant density and DIE data

With a P value of 0.66 and a R^2 coefficient of 0.014, leaf litter depth did not have a significant relationship with DIE (Figure 4). Similarly, a P value of 0.19 and a R^2 coefficient of 0.1192 (Figure 5) represented that plant density did not have a significant relationship with DIE. The first hypothesis of DIE affects the leaf litter depth and plant density was rejected upon the results of Figures 4 and 5.

H_2: Leaf litter depth / plant density and DON data

With a P value of 0.045 and a R^2 coefficient of 0.2563, Figure 6 represents a weak positive correlation between leaf litter depth and DON. Figure 7, with a P value of 0.096 and a R^2 coefficient of 0.1854, results with plant density having no significant relationship with DON. The results of Figure 7 support the rejection of the second hypothesis of plant density affecting DON. With a P value of 0.99 and a R^2 coefficient of 7 e -06, Figure 8 represents no relationship between DIE and DON.

DISCUSSION

Invasive earthworm distribution can be affected by a number of environmental variables. Earthworm distribution has been studied heavily in agricultural; however, little is known about earthworm distribution in natural ecosystems like the sixteen experimental sites in Dutchess County, NY. Previous studies have shown that earthworm presence or abundance depends on colonization sources along with habitat quality (Suarez et al. 2006). Hence, invasive earthworms may vary from the sixteen different sites based on road, stream, and agricultural locations that promote invasion from creating ditch environment that accumulates moisture and leaf litter (Gundale et al. 2005). Suarez also mentions that soil moisture, topography, soil pH and texture, vegetation diversity, and forest type all play a role in invasive earthworm suitable habitat. Climatic conditions, including severe drought, may affect the time period before the sampling period and influence observed earthworm distributions (2006). These factors change from location to location and are difficult to understand how earthworm distribution occurs in natural ecosystems. A definite density of earthworms in each site was not known; thus, it was difficult to assess if earthworms did affect leaf litter and plant density. Since earthworms did not affect leaf litter and plant density, the DIE and DON relationship does not exist.

Environmental variables unrelated to earthworms may have affected the number of nymphal ticks sampled at each site. Although previous nymphal tick surveys have been completed from these sixteen experimental sites, subsequent sampling periods may provide nymphal density distribution changes. Nymphal distribution changes may occur by differences in host variability and movement; nymphal tick vertebrate hosts are capable of moving ticks across habitat boundaries (Ostfeld et al. 1995). Brownstein and associates add that composition and abundance of these hosts are influenced by landscape structure, the spatial arrangement of land cover types (2005). The experimental sites varied in forest fragmentation size from small, remote forest stands surrounded by urbanized or agricultural landscapes to constant, uninterrupted forest. Studies have shown that white-footed mice (*Peromyscus leucopus*) population densities are higher in small, isolated woodlots embedded in agricultural or urbanized landscapes than they are in continuous forested fragments (Ostfeld and Keesing 2000). Variation within the experimental sites occurred among the forest floor, where some sites included thick shrubbery and plants along the ground surface and other sites contained leaf litter-dominated forested fragments. The effect of the different types of ground cover influences moisture and temperature levels suitable for nymphal tick survival. In addition, climate also affects moisture and temperature, which may affect vegetation and habitat (McCabe and Bunnell 2004). The discussed factors may have changed nymphal tick numbers sampled from previous sampling periods in a number of different ways.

As represented in Figure 1, the proposed mechanism action among DIE, leaf litter depth/plant density, and DON was rejected. Hypothesis I-III are all rejected according to sampled data. Figure 4 and Figure 5 provide that DIE does not affect leaf litter depth and plant density. Acting as a missing link in the mechanism chain reaction, the rejection of H_1 causes the failure of the mechanism. However, DON was affected by leaf litter depth (Figure 6), but DON was not affected by plant density (Figure 7); so, H_2 was also rejected. To confirm DIE does not affect DON, Figure 8 statistical tests and data represents no significant relationship. The density of invasive earthworms does not affect the density of nymphal ticks within the sixteen selected sites of Dutchess County, NY.

ACKNOWLEDGEMENTS

This material is based upon work supported by the National Science Foundation under Grant No. DBI 0552871.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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APPENDIX

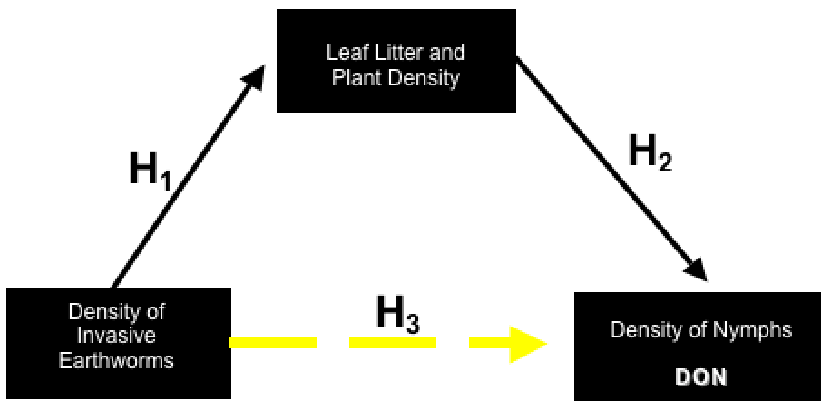

FIGURE 1. Proposed mechanism of action between DIE, leaf litter / plant density, and DON.

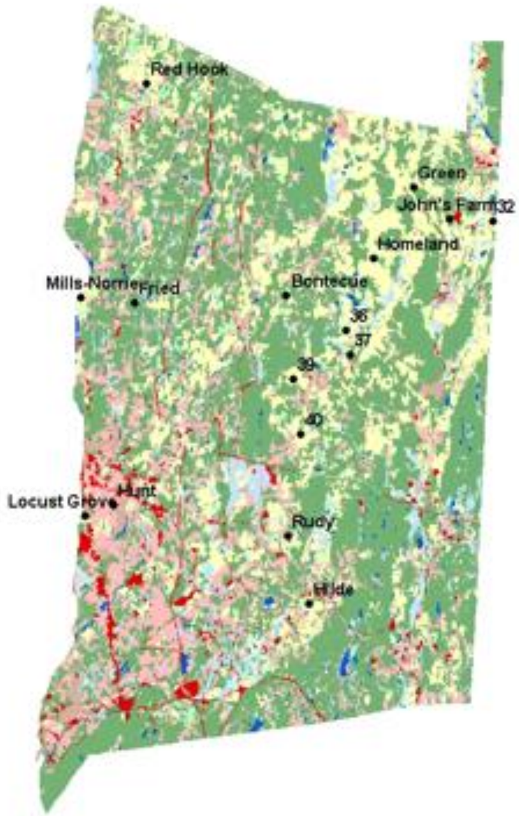

FIGURE 2. 16 selected experimental plots of Dutchess County, NY.

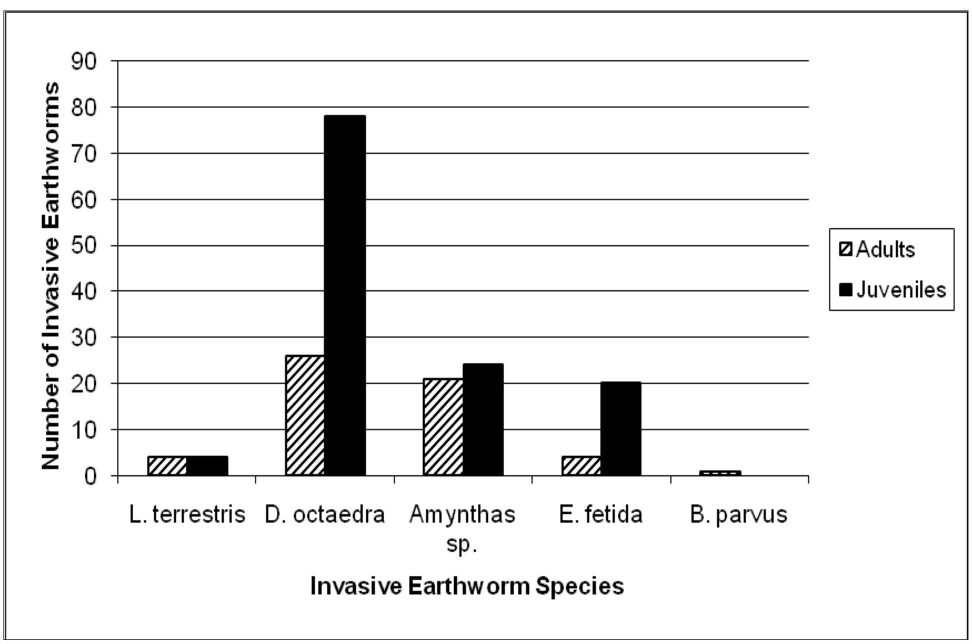

FIGURE 3. Total number of invasive earthworm adults and juveniles surveyed in June-July 2007 of Dutchess County, NY.

TABLE 1. Total number of collected invasive earthworms and density of invasive earthworms per selected site of Dutchess County, NY in June and July, 2007.

Site	**Total number of earthworms**	**DIE (earthworms/m^2)**
Bontecue	11	1.12
40	39	3.96
39	15	1.52
36	3	0.3
37	1	0.1
Rudy	5	0.51
Hilde	5	0.51
Green	22	2.24
John’s Farm	19	1.93
32	7	0.71
Homeland	0	0
Locust Grove	13	1.32
Mills-Norrie	4	0.41
Hunt	3	0.3
Fried	16	1.63
Red Hook	19	1.93

TABLE 2. Total number of collected *I. scapularis* nymphs and density of nymphs per selected site of Dutchess County, NY in June and July, 2007.

Site	**Total number of nymphs**	**Drag Length (m)**	**DON (nymphs/m^2)**
Bontecue	44	120	0.37
40	1	180	0.01
39	19	180	0.11
36	53	120	0.44
37	7	150	0.05
Rudy	0	75	0
Hilde	7	165	0.04
Green	42	180	0.23
John’s Farm	40	180	0.22
32	0	165	0
Homeland	4	180	0.02
Locust Grove	3	120	0.025
Mills-Norrie	11	120	0.09
Hunt	10	180	0.06
Fried	40	180	0.22
Red Hook	2	180	0.01

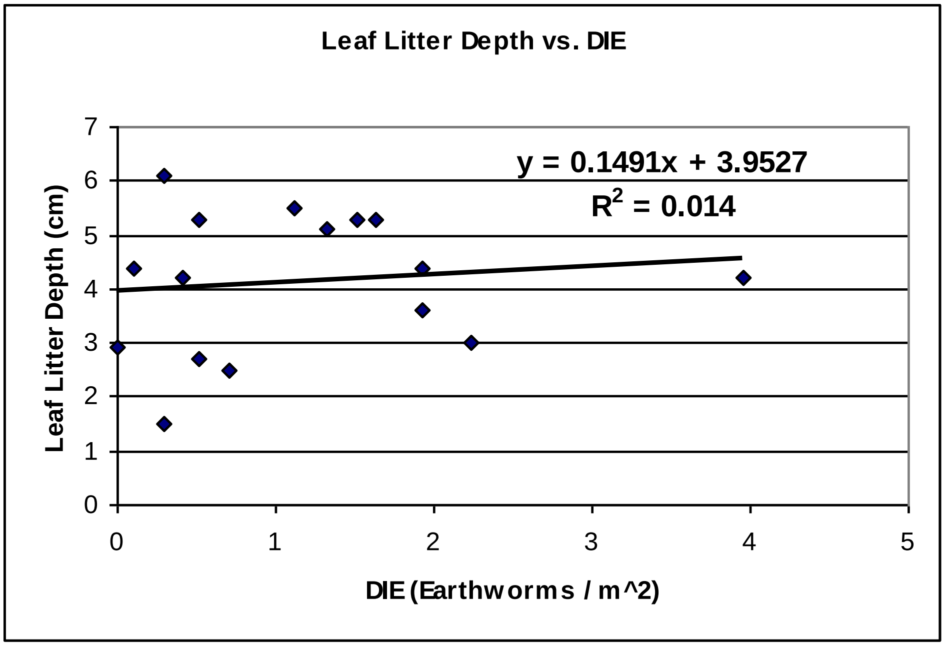

FIGURE 4. Density of invasive earthworms effects on leaf litter depth. P value = 0.66.

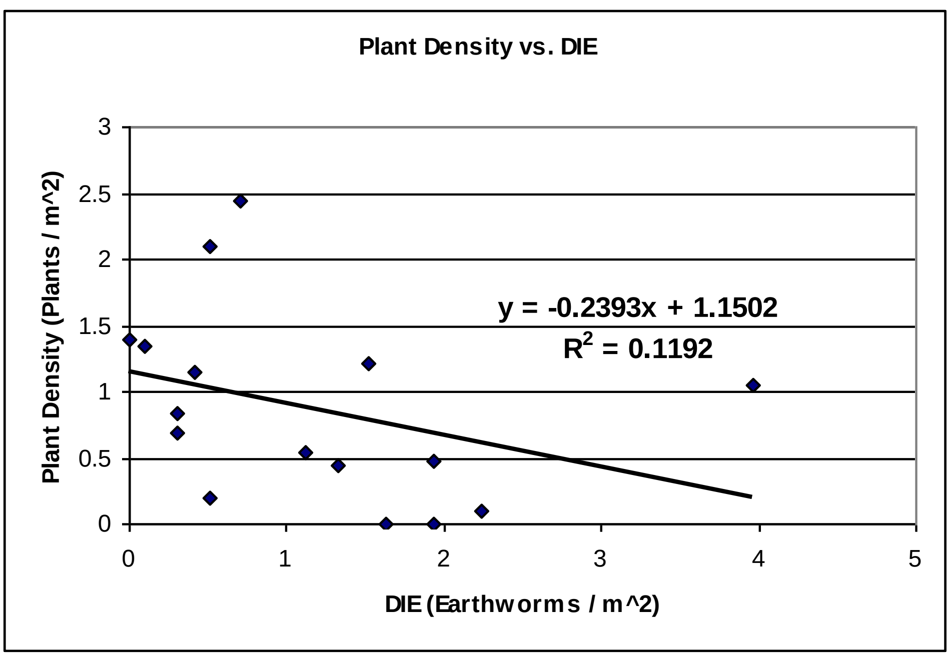

FIGURE 5. Density of invasive earthworms effects on plant density. P value = 0.19.

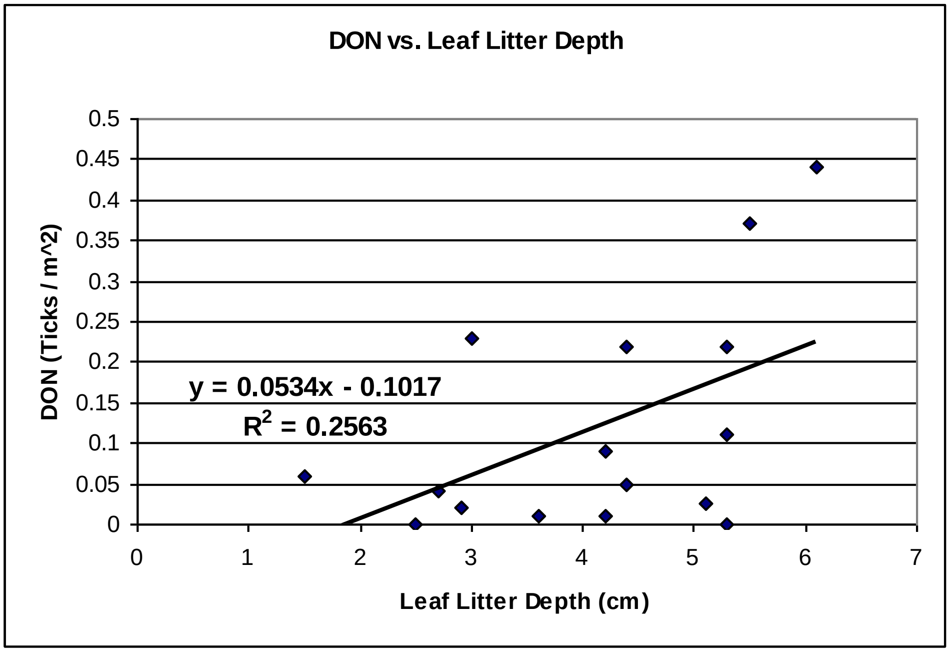

FIGURE 6. Leaf litter depth effect on density of nymphs. P value = 0.045.

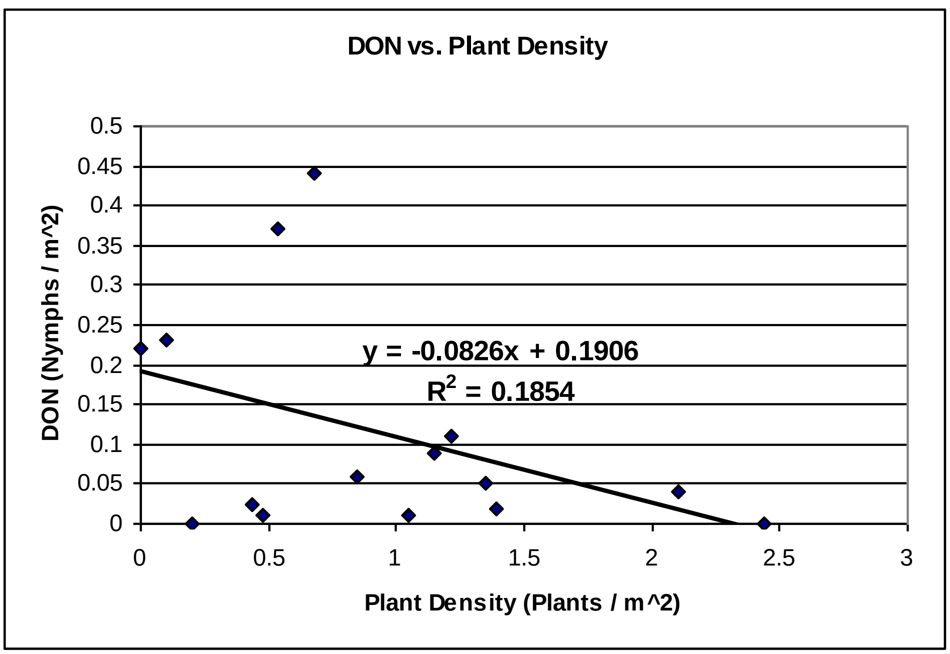

FIGURE 7. Plant density effects on density of nymphs. P value = 0.096.

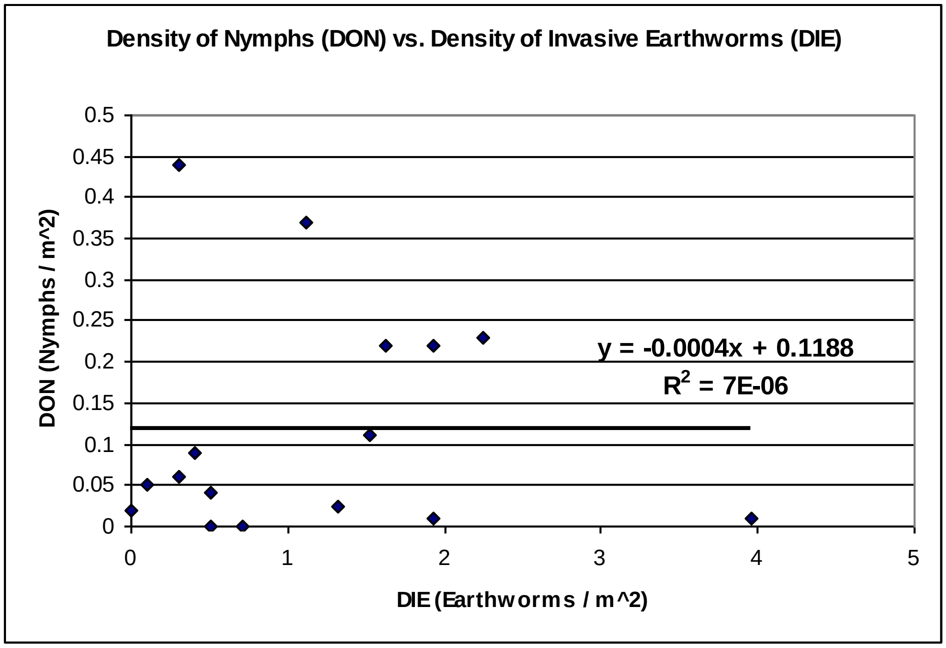

FIGURE 8. Density of invasive earthworms effects on density of nymphs. P value = 0.99.