

Development of Plant-Parasitic Nematode Populations on Forage Crops under Field Conditions

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Abstract

The continuous increase in cost and scarcity of mineral fertilizers resulting from the use of high-cost energy sources has renewed interests in organic recycling and biological nitrogen-fixation to improve soil fertility and productivity. Forage legumes can fix nitrogen from atmosphere which has the merit to offer nitrogen for both crop production and soil fertility. However, plant-parasitic nematodes (PPNs) interactions with leguminous crops can be agronomically damaging under various conditions. The present study examined population development of each of the PPN genera *Meloidogyne* spp., *Paratrichodorus* spp., *Criconemella* spp., *Pratylenchus* spp., *Tylenchorhynchus*, and *Tylenchus* spp. on fourteen legume cultivars (i.e. 'Florida' red clover, 'Nolin' red clover, 'Kenland' red clover, 'Kenstar' red clover, 'Tibbee' crimson clover, 'Dixie' crimson clover, 'Mt. Barker' Subterranean clover, 'Amclo' arrowleaf clover, 'FL - 77' alfalfa, 'Abon' persian clover, 'Wood ford' big flower vetch, 'Sequest' ball clover, 'Kondinin' rose clover, and 'Chief' crimson clover). The nematode initial and final populations varied considerably among the legume cultivars. Therefore, we adjusted for the rate of nematode multiplication by using analysis of covariance. However, we could not use one of the statistical multiple comparison methods because each treatment had different mean of squares for the experimental error. Consequently, a matrix was designed to test the significance of nematode populations on the cultivars using the t-test. Using a computer program, the exact (numerical) level of statistical significance for nematode densities among the cultivars could be established. Relatively lower populations of the nematode genera were found on 'FL-77' alfalfa plants.

Key words: analysis of covariance, forage legumes, nematode population development.

Introduction

Many legumes rank high among other forage crops as they offer the best quality feed for livestock since they are palatable, help maintain proper functioning

of the ruminant digestive processes and stimulate high production of both meat and milk. Also, they are used as green manure by ploughing them into the soil. Forage legumes contain symbiotic bacteria called rhizobia within nodules in their root systems, producing nitrogen compounds that help the plant to grow and compete with other plants. When the plant dies, the fixed nitrogen is released, making it available to other plants; this helps to fertilize the soil. Therefore, in many traditional and organic farming practices, fields are rotated through various types of crops, which usually include one consisting mainly or entirely of clover, often referred to as green manure. Eventually, in view of the continuing increase in cost and scarcity of mineral fertilizers resulting from the use of high-cost fossil and renewed energy, there is renewed interest in organic recycling and biological nitrogen-fixation to improve soil fertility and productivity. Clearly, forage legumes can fix nitrogen from atmosphere which has the merit to offer nitrogen for both crop production and soil fertility.

The efficiency of nitrogen fixation by these symbiotic bacteria is dependent on many factors, including the legume, air, soil conditions and soil microorganisms. Among biotic factors, the absence of the required rhizobia species constitutes the major constraint in the nitrogen fixation process. Plant-parasitic nematodes (PPNs) are also considered among other limiting factors since these parasites have been reported to interfere with nodule formation, development, and functions (**Taha *et al.*, 1974**). Clearly, many forage legumes are very susceptible to damage by plant parasitic nematodes, especially the root-knot nematodes (*Meloidogyne* spp.) which are endoparasitic forms capable of reproducing not only on forage crops but also on a wide variety of host plants (**Abd-Elgawad and Askary, 2015**). From an agronomic perspective, the interactions of PPNs with leguminous crops can be devastating (*e.g.*, **Allison, 1956; Baxter and Gibson, 1958; Baltensperger *et al.*, 1985a and b; Barker, 1998; Davis and Mitchum, 2005**).

The objective of this study was to determine the population development of plant-parasitic nematodes on very common cultivars of forage crops grown in Florida, USA. Taking into consideration the aggregated distribution usually shaping the nematode patterns, we adjusted also for the error resulting from this uneven distribution *via* the use of analysis of covariance.

Materials and Methods

The experiment was established at the main Agronomy Farm, Gainesville, Florida, USA. Each plot measured 2.1 × 6.1 m (7 × 20 ft) with each forage legume cultivar and the fallow control replicated four times. The soil type is Arredondo fine sand. The plots had been planted to pigeon pea, *Cajanus cajan*, for eight months and then left fallow for four months before the experiment was established. Soil samples were taken twice, the first time when the plants were still in the seedling stage (January, 1984) and the second time at the first harvest of the foliage (April,

1984). Nematodes were extracted from 100 cm³ soil from each sample using a centrifugal-flotation technique (Caviness and Jensen, 1955), placed in vials, and stored in a refrigerator at 4 °C for no more than three days until identified to genera and counted. Data were analyzed by analysis of covariance. Initial populations of nematodes were plotted as covariates with their final populations as dependent variables for the analysis of covariance. As treatments (the cultivars) were found to have different mean of squares for the experimental error, a matrix was designed to test the statistical significance of nematode populations on these cultivars using the t-test. Using a computer program (ACOVs), the exact probability level of significance for nematode densities among the cultivars could be established.

Results

Differences in the initial population levels of plant-parasitic nematodes (P_i) among tested legume cultivars resulted in differences in their final populations (P_f) for these cultivars. However, since there was a regression relationship ($P = 0.0001$) between the P_i and the P_f ; the P_f was adjusted using analysis of covariance to make the P_f the best estimate of what it would have been if the P_i had been the same for each genus of nematodes (Table 1). Consequently, the adjusted means of nematode populations for each genus were significantly different ($P \leq 0.05$) on various planted cultivars, i.e., the cultivars had an effect on nematode populations as indicated by the adjusted populations of nematodes in Table (1). This statistical analysis was applied separately for *Meloidogyne* spp., using square root transformation, and for *Paratrichodorus* spp. Populations of each other nematode genera, *Criconemella* spp., *Tylenchorhynchus* spp., *Tylenchus* spp. and *Pratylenchus* spp., were too low to be treated statistically. Therefore, these genera were combined and analyzed statistically as one group (Table 1). A matrix of 14 rows representing the tested cultivars was designed to test for significance of nematode population levels between plant cultivars (Tables 2-4). Relatively lower population densities were found on 'FL-77' alfalfa. Most of the tested legume cultivars had non-significant difference between them for nematode reproduction at $P \leq 0.05$, e.g. 'Florida' red clover and 'Nolin' red clover ($P = 0.66$) for *Meloidogyne* spp. (Table 2); 'Dixie' crimson clover and 'Wood ford' big flower vetch ($P = 0.99$) for *Paratrichodorus* spp. (Tables 3), and 'Tibbee' crimson clover and 'Chief' crimson clover ($P = 0.86$) for the total populations of *Criconemella* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp. and *Tylenchus* spp. (Table 4). However, differences in the readiness of some legume cultivars for nematode reproduction were so great that the significant levels were less than 0.01; e.g. 'Kenstar' red clover and 'Tibbee' crimson clover ($P = 0.007$) for *Meloidogyne* spp. (Table 2); 'Abon' persian clover and 'Chief' crimson clover ($P = 0.003$) for *Paratrichodorus* spp. (Table 3); and 'Kenland' red clover and 'Kenstar' red clover ($P = 0.0006$) for the total populations of *Criconemella* spp., *Tylenchorhynchus* spp. and *Tylenchus* spp. (Table 4).

Table (1): Genera and means of adjusted⁺ final populations of nematodes on forage legume cultivars.

Legume (cultivar)	<i>Meloidogyne</i> *	<i>Paratrichodorus</i>	Other genera**
'Florida' Red Clover	9.5	53.0	40
'Nolin' Red Clover	8.6	110.3	80
'Kenland' Red Clover	8.1	71.0	0
'Kenstar' Red Clover	5.2	109.6	70
'Tibbee' Crimson Clover	11.4	46.0	31
'Dixie' Crimson Clover	11.3	58.2	73
'Mt. Barker' Subterranean Clover	5.5	60.0	88
'Amclo' Arrowleaf Clover	8.0	45.0	54
'FL - 77' Alfalfa	3.6	48.7	14
'Abon' Persian Clover	7.4	33.5	26
'Wood ford' Big flower vetch	10.2	57.9	48
'Seqrest' Ball Clover	6.6	91.1	47.
'Kondinin' Rose Clover	6.3	95.9	58
'Chief' Crimson Clover	6.1	117.1	27

+ Nematode genera per 100 cm³ soil. The adjustment was based on analysis of covariance, using the initial nematode populations as covariates and their final populations as dependent variables since there was regression relationship between them (P = 0.0001).

* Square root transformation was used for the original mean numbers of *Meloidogyne*.

** The populations included the total nematodes of *Criconemella*, *Tylenchorhynchus*, *Tylenchus* and *Pratylenchus*.

Table (2): Matrix table of statistical probability levels to compare populations of *Meloidogyne* spp. on fourteen* cultivars of forage legumes.

Cultivar number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.6546	0.5471	0.0516	0.3782	0.4051	0.0686	0.0674	0.0098	0.3256	0.7653	0.1873	0.1454	0.1169
2	0.6546	0	0.8426	0.1246	0.1862	0.2047	0.1642	0.4042	0.0271	0.5896	0.4572	0.2792	0.2076	0.2565
3	0.5471	0.8424	0	0.2555	0.1420	0.1826	0.2845	0.0758	0.0619	0.7659	0.3571	0.3434	0.4561	0.3307
4	0.0518	0.1290	0.2335	0	0.0065	0.0069	0.8957	0.2992	0.4642	0.3195	0.0282	0.5117	0.6071	0.8928
5	0.3782	0.1862 0.2047	0.1429 0.1826	0.0065	0	0.9542	0.0092	0.4202	0.0008	0.0673	0.5545	0.0321	0.0228	0.0167
6	0.4051	0.1642	0.2845	0.0059	0.9542	0	0.0097	0.4310	0.0099	0.0739	0.6005	0.0349	0.0252	0.0193
7	0.0686	0.8042	0.9759	0.8967	0.0092	0.0097	0	0.2489	0.3894	0.3365	0.0384	0.5999	0.7014	0.7916
8	0.4874	0.0271	0.8619	0.2002	0.1202	0.1310	0.2489	0	0.0477	0.7700	0.3258	0.5266	0.4377	0.3720
9	0.0090	0.6896	0.7659	0.4642	0.0008	0.0009	0.3894	0.0477	0	0.0881	0.9042	0.1688	0.2154	0.2614
10	0.3256	0.4572	0.3571	0.3195	0.0873	0.0739	0.3865	0.7700	0.0891	0	0.2055	0.7311	0.6271	0.5460
11	0.7653	0.3702	0.5434	0.0282	0.5545	0.6005	0.0384	0.3258	0.0042	0.2055	0	0.1118	0.0839	0.0647
12	0.1873	0.8076	0.4581	0.5117	0.0321	0.0349	0.5990	0.5256	0.1688	0.7311	0.1118	0	0.8866	0.7939
13	0.1454	0.2535	0.3897	0.6071	0.0229	0.0252	0.7014	0.4377	0.2154	0.6271	0.0838	0.8866	0	0.9058
14	0.1169			0.6928	0.0167	0.0193	0.7916	0.8720	0.2614	0.5460	0.0647	0.7039	0.9053	0

* The fourteen cultivars as numbered in the matrix are as follows; 1 = 'Florida' red clover, 2 = 'Nolin' red clover, 3 = 'Kenland' red clover, 4 = 'Kenstar' red clover, 5 = 'Tibbee' crimson clover, 6 = 'Dixie' crimson clover, 7 = 'Mt. Barker' Subterranean clover, 8 = 'Amclo' arrowleaf clover, 9 = 'FL - 77' alfalfa, 10 = 'Abon' persian clover, 11 = 'Wood ford' big flower vetch, 12 = 'Seqrest' ball clover, 13 = 'Kondinin' rose clover, and 14 = 'Chief' crimson clover.

Table (3): Matrix table of statistical probability levels to compare populations of *Paratrichodorus* spp. on fourteen* forage legume cultivars.

Cultivar number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.0343	0.5078	0.0374	0.7986	0.8537	0.7942	0.7782	0.8758	0.4992	0.8607	0.1781	0.1294	0.0224
2	0.0343	0	0.1489	0.9700	0.9223	0.0681	0.0653	0.0250	0.0274	0.0098	0.0641	0.4877	0.5941	0.8916
3	0.5078	0.1489	0	0.1499	0.3464	0.6328	0.6781	0.3342	0.4009	0.1684	0.6833	0.4608	0.3461	0.0852
4	0.0374	0.9790	0.1499	0	0.0214	0.0653	0.0661	0.0230	0.0287	0.0086	0.0619	0.4041	0.6065	0.7786
5	0.7986	0.0223 0.0681	0.3464	0.0214	0	0.6441	0.5961	0.9687	0.9168	0.6379	0.6515	0.0830	0.0636	0.0096
6	0.9527	0.0653	0.6328 0.6781	0.0652	0.6441	0	0.9462	0.6149	0.7208	0.3601	0.9908	0.2167	0.1606	0.0312
7	0.7942	0.0250	0.3342	0.0661	0.5961	0.9462	0	0.5764	0.6692	0.3293	0.9365	0.2474	0.1783	0.0351
8	0.7782	0.0274	0.4009	0.0230	0.9687	0.6149	0.5764	0	0.8868	0.6626	0.6225	0.0861	0.0614	0.0095
9	0.8758	0.0098	0.1684	0.0267	0.9168	0.7209	0.6692	0.8868	0	0.5680	0.7288	0.1186	0.9788	0.0125
10	0.4992	0.0641	0.6233	0.0086	0.6379	0.3501	0.3293	0.6626	0.5689	0	0.3579	0.9341	0.0238	0.0031
11	0.8607	0.4877	0.4500	0.0619	0.6515	0.9908	0.9369	0.6236	0.7235	0.3570	0	0.2123	0.1559	0.0298
12	0.1781	0.5941	0.3461	0.4941	0.0980	0.2167	0.2474	0.0861	0.1186	0.0341	0.2123	0	0.8546	0.2275
13	0.1204	0.8916	0.0852	0.6065	0.0636	0.1606	0.1783	0.0614	0.0788	0.9238	0.1869	0.8546	0	0.4222
14	0.2224			0.7786	0.0096	0.0312	0.0351	0.0095	0.0125	0.0031	0.9228	0.3275	0.4222	0

* The fourteen cultivars as numbered in the matrix are as follows; 1 = 'Florida' red clover, 2 = 'Nolin' red clover, 3 = 'Kenland' red clover, 4 = 'Kenstar' red clover, 5 = 'Tibbee' crimson clover, 6 = 'Dixie' crimson clover, 7 = 'Mt. Barker' Subterranean clover, 8 = 'Amclo' arrowleaf clover, 9 = 'FL - 77' alfalfa, 10 = 'Abon' persian clover, 11 = 'Wood ford' big flower vetch, 12 = 'Seqrest' ball clover, 13 = 'Kondinin' rose clover, and 14 = 'Chief' crimson clover.

Development of Plant-Parasitic Nematode Populations on Forage Crops under Field Conditions

Table (4): Matrix table of statistical probability levels to compare nematode populations* on fourteen** cultivars of forage legumes.

Cultivar number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.0671	0.0269	0.1662	0.6789	0.1298	0.0300	0.5080	0.2437	0.5424	0.6976	0.3325	0.2883	0.5632
2	0.0671	0	0.0202	0.6230	0.0277	0.7367	0.7251	0.2387	0.0045	0.0194	0.1511	0.1373	0.3111	0.0223
3	0.0269	0.0002	0	0.0006	0.0680	0.0004	0.0001	0.0053	0.2813	0.1965	0.0110	0.0123	0.0031	0.1046
4	0.1662	0.6330	0.0006	0	0.0757	0.8863	0.8063	0.4677	0.0137	0.0522	0.3201	0.2065	0.5526	0.0577
5	0.6789	0.0277	0.0680	0.0757	0	0.0576	0.0111	0.2836	0.4433	0.6405	0.4232	0.9816	0.2106	0.8621
6	0.1298	0.7367	0.0004	0.0863	0.0576	0	0.4925	0.3906	0.0103	0.0405	0.2620	0.2413	0.4881	0.0455
7	0.0300	0.7251	0.0001	0.4068	0.0111	0.4925	0	0.1251	0.0015	0.0072	0.0733	0.0658	0.1719	0.0083
8	0.5080	0.2387	0.0053	0.4677	0.2836	0.3906	0.1251	0	0.0695	0.2042	0.7829	0.6452	0.0984	0.2182
9	0.2427	0.0045	0.2813	0.0137	0.4433	0.0193	0.0015	0.0695	0	0.5792	0.1292	0.1519	0.0475	0.5536
10	0.5424	0.0194	0.1065	0.0522	0.8405	0.0405	0.0072	0.2042	0.5702	0	0.0166	0.3599	0.1887	0.9789
11	0.6576	0.1511	0.0110	0.3201	0.4232	0.2620	0.0733	0.4829	0.1202	0.3166	0	0.9696	0.6440	0.3305
12	0.7339	0.1371	0.0122	0.2965	0.4516	0.2413	0.0658	0.8453	0.1319	0.3400	0.0606	0	0.6128	0.3561
13	0.3983	0.3111	0.0031	0.8826	0.2106	0.4921	0.1419	0.8554	0.0578	0.1497	0.6499	0.6128	0	0.1597
14	0.6632	0.0222	0.1046	0.0577	0.0621	0.0455	0.0096	0.8152	0.5536	0.9789	0.3209	0.3861	0.1597	0

* The nematode populations included the total individuals of *Criconebella*, *Tylenchorhynchus*, *Tylenchus* and *Pratylenchus*.

** The fourteen cultivars as numbered in the matrix are as follows; 1 = 'Florida' red clover, 2 = 'Nolin' red clover, 3 = 'Kenland' red clover, 4 = 'Kenstar' red clover, 5 = 'Tibbee' crimson clover, 6 = 'Dixie' crimson clover, 7 = 'Mt. Barker' Subterranean clover, 8 = 'Amclo' arrowleaf clover, 9 = 'FL - 77' alfalfa, 10 = 'Abon' persian clover, 11 = 'Wood ford' big flower vetch, 12 = 'Seqrest' ball clover, 13 = 'Kondinin' rose clover, and 14 = 'Chief' crimson clover.

Discussion

Data on nematode populations on the legume cultivars supported the phenomenon of uneven distribution of nematodes in the field as previously reported (**Goodell and Ferris, 1980; Abd-Elgawad, 1992, 2016; Abd-Elgawad and Hasabo, 1995; Been and Schomaker, 2013**). Using analysis of covariance, we adjusted for the error resulting from uneven distribution. **Seinhorst (1967)** stated that some authors consider the rate of multiplication as the only important parameter ($P_f = a P_i$) where P_i and P_f are initial and final nematode densities, respectively and a is the rate of nematode multiplication. **Seinhorst (1970)** noted that those authors seem to be unaware of the possibility that the value of a may not be independent of P_i . Therefore, we adjusted for the rate of multiplication by using analysis of covariance. However, we could not use one of the multiple comparison methods because each treatment had different mean of squares for the experimental error. Consequently, a matrix was designed to test the significance of nematode populations on the cultivars using the t-test. Using a computer program, the exact level of significance for nematode densities among the cultivars could be established (Tables 2-4).

Data showed that 'FL-77' alfalfa had relatively higher levels of nematode resistance than did the other cultivars and so it could be used perhaps to incorporate resistance into other varieties either by hybridization or by recurrent selection within alfalfa populations. This cultivar may be better adapted to *Meloidogyne* spp.-infested soils than the other studied forage legumes. Since the tested cultivars differed as hosts for nematode development, additional genetic material should be examined to search for higher levels of resistance. Therefore, **Hamdi (1982)** and **Quesenberry et al. (2014)** recommended further promotion of research, development, application and dissemination of information available on various aspects of biological nitrogen-fixation, including symbiotic systems of rhizobia/legume and Azolla/blue-green algae, and free-living nitrogen-fixing bacteria and blue-green algae. The latter authors presented prospects for continued genetic improvement in resistance for *Meloidogyne* spp. using conventional and modern molecular methods.

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الملخص العربي

تطور النيماطودا المتطفلة نباتيا على محاصيل العلف في الحقل

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لقد أدت الزيادة المستمرة في تكلفة وندرة الأسمدة المعدنية الناتجة عن استخدام مصادر طاقة عالية التكلفة إلى تجديد الاهتمام بإعادة التدوير العضوي وتثبيت النتروجين بيولوجيا لتحسين خصوبة التربة وإنتاجيتها. بيد أن للبقوليات العلفية ميزة تثبيت النتروجين من الغلاف الجوي وتسخيروه لإنتاج المحاصيل وخصوبة التربة. ومع ذلك، يمكن أن تتدخل النيماطودا المتطفلة نباتياً لتلحق أضراراً بهذه البقوليات العلفية في ظل ظروف مختلفة. تناولت هذه الدراسة تطور تعداد عشائر النيماطودا المتطفلة نباتياً التابعة لأجناس ميلودوجين، باراتريكودوروس، كريكونيميلا، براتيلينكس، تيلنكورينكس، وتيلينكس على أربعة عشر نوعاً من البقوليات. تفاوتت أعداد النيماطودا الأولية والنهائية تفاوتاً كبيراً بين أصناف هذه البقوليات. لذلك، قمنا باستخدام تحليل التباين المتغاير لضبط معدل تكاثر النيماطودا، ولكن لم تتمكن من استخدام أيّاً من طرق المقارنات الإحصائية بين متوسطات تعداد هذه النيماطودا لأن كل معاملة لها متوسط مختلف خاص بمربع الخطأ التجريبي. بناء على ذلك، تم تصميم مصفوفة لكل مجتمع نيماطودي لتحديد مدي معنوية الفرق بين متوسطات تعداد هذه النيماطودا على أصناف العلف باستخدام اختبار (ت) ومن خلال برنامج حاسوبي، إذ تم تحديد مستوى معنوية الفرق بين متوسطي تعداد هذه النيماطودا بدقة (رقمياً) على كل صنفين من الأعلاف المختبرة. كانت متوسطات تعداد عشائر هذه النيماطودا قليلة نسبياً على البرسيم الحجازي 'فل-٧٧'.

الكلمات الدالة: تحليل التباين المتغاير، البقوليات العلفية، تطور عشائر النيماطودا.