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Responses of Selected Fibre Hemp Cultivars to *Meloidogyne javanica* under Greenhouse Conditions

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Abstract: Fibre hemp (*Cannabis sativa* L.) is being investigated as an alternative industrial crop for inclusion in crop rotation programmes for the management of various plant-parasitic nematodes. In the Republic of South Africa cropping patterns and cultural practices vary from one area to another, creating a great diversity in the combinations of nematode species present and in the problems they cause. *M. incognita* races 2 and 4 and *M. javanica* are serious problems on most of the major crops grown in South Africa. It was therefore necessary to determine if hemp can be included in the cropping systems of resource poor farmers in areas were root-knot nematodes are problematic. The host-status and host-sensitivity of hemp cultivars Kompolti, Futura 75, Felina 34 and Ferimon to the root-knot nematode (*Meloidogyne javanica*) were tested under greenhouse conditions. A split-plot design with five replications was devised, where main plot factors comprised with or without nematodes and subplot factors the four cultivars. Twelve weeks after initiating treatments, the reproductive factors of *M. javanica* on hemp cultivars were greater than one, without the cultivars suffering damage from the nematode infection. Results of the study suggested that the four cultivars were tolerant to *M. javanica.* Therefore, these cultivars are not suitable for use in crop rotation programmes in the management of *M. javanica* numbers since they would increase nematode build-up for subsequent susceptible crops.

Key words: Cannabis sativa · Hemp · Host-status · Host-sensitivity · Meloidogyne javanica · Nematode

INTRODUCTION

Hemp (*Cannabis sativa* L.) is an industrial crop which is relatively unknown to South African farmers. The crop has been grown in Southern Africa for medicinal purposes for centuries and during the past half-century it has also been cultivated for use as an illegal drug [1]. Due to its high delta-9-tetrahydrocannabinol (THC) content and potential psycho activity, hemp was declared illegal in South Africa in 1928 [2]. Although hemp and marijuana (*Cannabis sativa* L.) are from the same species, they are morphologically different and have also different uses. Generally, hemp refers to the fibre-producing strain of Cannabis species, whereas marijuana *Cannabis* species do not have fibre for industrial purposes [3].

New improved hemp cultivars, characterised by very low levels of THC content and high bast fibre content, have been developed in France and Hungary [1]. Under South African legislation, the hemp plant and its parts may not contain more than 1% THC [4]. For instance, hemp cultivars developed in France, namely, cultivars Futura 75, Felina 34 and Ferimon and the Hungarian entry, cv. Kompolti, have a THC content of less than 0.3%.

Broadly, there are three main groups of hemp cultivars, namely: (i) cultivars primarily cultivated for fibre, (ii) cultivars grown for seed AND (iii) cultivars grown for pharmaceutical or recreational purposes [4]. As an Industrial crop, hemp fibre is being used in a wide range of commodities [5]. Hempseed oil has since attained enormous nutritional and pharmaceutical interests [6-12]. Also, hempseed oil is used during the manufacturing of paints, varnishes, soaps and massage oils [10].

In South Africa, there is renewed interest in the special properties of this industrial crop because it may

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provide a reasonable income for poor-resource farmers [4]. However, for these smallholder farmers, it is preferable that any alternative crop being introduced should not increase the numbers of nematode species that could be problematic to their other crops. In the Republic of South Africa cropping patterns and cultural practices vary from one area to another, creating a great diversity in the combination of nematode species present and in the problems they cause. Root-knot nematodes (*Meloidogyne* spp.) are among the most damaging and economically important pests of subtropical and tropical crops throughout the world [13]. It was therefore necessary to determine if hemp can be included in the cropping systems of resource poor farmers in areas were root-knot nematode are problematic. The objective of this study was to determine the host-status and host-sensitivity of four economically promising hemp cultivars to *M. javanica* in the Republic of South Africa.

MATERIALS AND METHODS

Study Area and Inoculums: The study was carried out at the Agricultural Research Council - Institute for Industrial Crops, Rustenburg, in the North West Province of South Africa (23°53'10"S, 29°44'15"E). The trials were conducted in autumn 2006 (March-May) under greenhouse conditions. A susceptible Kenaf (*Hibiscus cannabinus*) cultivar was planted in the soil for effective reproduction of *M. javanica*. Two months after inoculation the Kenaf plants were removed, the soil was sieved and thoroughly mixed. Forty 30 cm diameter pots were filled with infested soil. Each pot was sampled for nematodes before hemp seeds were sown. Hemp cultivars were sowed directly into the pots.

Growth Conditions: Enough sandy loam soil comprising 81% sand, 6% silt and 13% clay.was prepared to provide for forty 30 cm diameter pots. The soil was steam pasteurized and inoculated with approximately 100 eggs and juveniles of *M. javanica* obtained from a *M. javanica* green house colony. A susceptible kenaf (*Hibiscus cannabinus*) cultivar was planted in the soil for effective reproduction of *M. javanica*. Two months after inoculation the kenaf plants were removed, soil sieved, composited AND mixed thoroughly. Pots refilled with 10l infested soil and each pot was sampled for nematodes before hemp seeds were planted to obtain initial population (Pi). Three days after planting, 3 g 2:3:2 (22) and 2 g 2:1:2 (43) were mixed into the topsoil in each pot. Pots were placed on the greenhouse benches at 0.3 m inter-row and 0.3 m intra-row spacing. At termination, 12 weeks after planting, nematodes were extracted from 250 ml soil using the sugar-centrifugation method [14] for the determination of the final population (Pf). Ambient day/night temperatures and day-length averaged 28/18°C and 14 hours, respectively.

Experimental Design: The experiment was laid out in a randomised split-plot design with five replications. Main plot treatments comprised nematode infested and nematode-free pots, whereas subplot treatments were four cultivars Kompolti, Futura 75, Felina 34 and Ferimon.

Cultural Practices: Plants were irrigated twice a day for 15 minutes using an automated micro irrigation system fitted with filters, which was adjusted to provide for increase in plant size. At seedling emergence 1.5 g LAN (28%) was applied per pot and repeated 10 days later, whereas 2 g 2:3:2 (22) per pot was applied 14 days after emergence. Lights were installed in the greenhouse to extend the day-length, thus, preventing bolting as hemp is a short-day plant.

Data Collection: At harvest, 12 weeks after initiating treatments, shoots were cut at the soil level AND oven-dried for 72 hours at 70°C and weighed. Root systems were removed from the pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density per total roots per plant. Root galling was assessed using the 0 to 5 scale, where 0 = no galls, 1 = 1 - 10 galls, 3 = 11-30 galls, 4 = 31 - 100 galls and 5 = > 100 galls per root system [15].

Nematodes were extracted from 10 g roots per plant by maceration and blending for 1 minute in 1% NaOCl [16], then passed through 38 im sieves, with nematodes being separated from debris of the aliquot through the sugar-floatation and centrifugation method [17]. Soil per pot was thoroughly mixed and a 250-cm^3 soil sample was collected. Nematodes were extracted from soil samples using the sugar-floatation and centrifugation method and eggs and juveniles were counted from a 10-ml aliquot with the use of stereomicroscope. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted nematode per pot.

Statistical Analysis: Reproductive factors (RFs), described as final population/initial population numbers, were computed and measured data were subjected to analysis of variance using SAS software [18].

RESULTS

There were significant differences among cultivars for initial and final nematode numbers (Table 1). Cultivar Futura 75 had the highest number of initial nematodes, with Felina having the lowest initial nematode numbers, whereas cultivars Kompolti and Futura had the highest final nematode numbers. The reproductive factors of *M. javanica* on all four hemp cultivars were greater than one, which suggests that the nematode reproduced on all four cultivars.

There were no significant interaction between nematode application and cultivars for dry root weight and dry shoot weight (Table 2). Also, *M. javanica* did not affect both root and shoot weight of the four cultivars. The observed differences in root and shoot weight were due to cultivar effects (Figure 1). Cultivar Kompolti had significantly the highest dry shoot weight than those of cultivars Futura 75, Felina 34 and Ferimon; whereas the dry shoot weight of these three did not differ significantly.

Table 1: Initial nematode numbers (Pi), final nematode numbers (Pf) and the reproductive factor of *Meloidogyne javanica* on hemp cultivars[a,b]

Cultivar	Pi	Pf	Reproductive factor
Kompolti	247b	1381a	5.04a
Ferimon	200b	959b	4.80a
Futura 75	860a	1256a	1.46c
Felina 34	90c	307c	3.41b
$LSD_{0.05}$	67	263	1.44

[a]Reproductive factor = Pf/Pi

[b] Means followed by the same letter in a column are not significantly different at $P \leq 0.05$.

Table 2: Influence of initial nematode numbers of *Meloidogyne javanica* and four hemp cultivars on dry root weight and dry shoot weight

		Dry root weight (g)		Dry shoot weight (g)	
Source of variation	DF	SS	P-value	SS	P-value
Replication	4	1.38	0.44	220.09	0.08
Nematode (N)	1	9.56	0.42	0.256	0.96
Error (a)	4	0.87		86.87	
Cultivar (C)	3	5.45	0.02	592.71	0.01
N × C	3	3.00	0.13	22.36	0.87
Error (b)	22	1.42		92.18	
Total	37	21.68		1014.46	
R^2	0.56		0.58		

DF = degrees of freedom, MS = means square

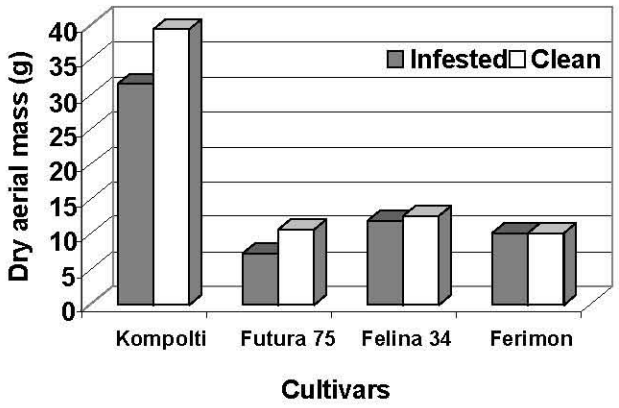

Fig. 1: Dry shoot weight of four hemp cultivars planted in soil infested with and without *Meloidogyne javanica*

DISCUSSION

In plant-parasitic nematodes, nematode-plant relations are described using the concept of host-status and host-sensitivity [19]. Host-status is described using the reproductive factor, which is a measure of the reproductive potential of a nematode on a given host, also referred to as reproductive factor [20]. All reproductive factors below unity suggest that the nematode fails to reproduce on a given host, whereas values above one indicate that the nematode was able to reproduce in the test plant.

The use of reproductive factors needs the determination of the Seinhorst [21] equilibrium point (E), beyond which all reproductive factors are below unity since competition for infection sites becomes intense [22]. In other words, the inoculum level used in determining the host-status must be less than E. Thus, the lowest reproductive factor on cv. Futura 75 should be viewed relative to its high Pi = 860 *M. javanica* juveniles. Also, the reproductive factor is dependent upon the plant and the period allowed after inoculation. In relation to E, Duncan and McSorley [23] argued that for most plants where the reproductive potential is greater than one at Pi below E than above E, these plants are hosts regardless of whether populations increase or decrease at time-related final populations. The argument suggested that even at inoculums levels of Pi lower than E, with increasing infection time, E may be attained, resulting into a situation where the reproductive factors are below unity, with the subsequent inference that the plant is a non-host.

Host-sensitivity is described using both the host-status and the plant's responses to nematode infection [21]. When the host plant allows nematode reproduction and the plant suffers yield loss, the plant is described as a susceptible host, whereas a host that does not incur yield loss is referred to as a tolerant host. However, if reproduction is not allowed and there is, as a result, no yield loss, the test plant is said to be a resistant host [21].

Meloidogyne javanica decreased and increased yield of the hemp cultivars Kompolti and VIR-140, respectively [24]. However, Van Biljon [24] did not report the final nematode population densities (Pf) on both cultivars. Generally, nematodes at densities lower than the damage threshold level have a stimulating effect on yield of various crops [25,26]. In Europe, several hemp cultivars, with claims of resistance to the southern root-knot nematode (*M. incognita*) had since been released [1,27]. Generally, resistance to *M. incognita* is complicated by the existence of races in this nematode species. De Meijer [1,27] did not specify the race used in the hemp-nematode studies. Kok *et al.* [28] demonstrated that cv. Kompolti suppressed *M. chitwood* numbers under both field and laboratory conditions. Also, cv. Kompolti, as reported elsewhere (Brough *et al.*, [4]; Van Biljon, [24]), was more productive than the other three cultivars in this trial.

CONCLUSION

Results of the current study suggested that the four hemp cultivars Kompolti, Futura 75, Felina 34 and Ferimon, were all tolerant to *M. javanica*. Consequently, these cultivars are not suitable for inclusion in crop rotation programmes for the suppression of *M. javanica* numbers since they would result in nematode build-up for subsequent susceptible crops.

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