

Effects of the root-knot nematodes *Meloidogyne incognita* and *M. javanica* on olive plants growth in glasshouse conditions

F. JAHANSHAH AFSHAR¹, N. SASANELLI², S.A. HOSSEININEJAD¹, Z. TANHA MAAFI¹

¹Iranian Research Institute of Plant Protection, P.O. Box 1454-19396, Tehran, Iran, E-mail: afshar_ff@yahoo.com;

²Institute for Sustainable Plant Protection (IPSP), U.O.S. of Bari, Via G. Amendola 122/D, 70126 Bari, Italy

Summary

The influence of ten initial population levels (0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 second stage juveniles/cm³ soil) of *Meloidogyne incognita* and *M. javanica* on olive cultivar Zard growth was studied in two pot trials. Ten month old self-rooted olive cuttings were individually transplanted into 2 000 cm³ pot and inoculated with the defined initial populations, of both nematode species. Plants were grown in glasshouse for 13 months, then they were uprooted and plant growth (percent growth increase of main shoot length, number of nodes on main shoot, top and root fresh and dry weights and root length) and nematode parameters (root gall index, J₂/g root, final nematode population density and reproduction rate Pf/Pi) were recorded. Results showed that cv. Zard was more susceptible to *M. javanica* than to *M. incognita*. A significant reduction of main shoot length growth 37.6 % and 10.7 % was observed at 0.1 and 12.8 juveniles/cm³ soil of *M. javanica* and *M. incognita*, respectively, in comparison to uninfested plants. Root systems of olive plants grown in *M. incognita* or *M. javanica* infested soils were galled within the gall index range 1.4 – 6. No significant differences were observed in the number of nodes on main shoot, top and root fresh weights and root dry weight at high levels of *M. incognita* Pi. A tolerance limit (*T*) of 0.4 juveniles/cm³ soil was estimated for olive plants cv. Zard to *M. javanica*. The use of resistant olive rootstock or selected cultivars is recommended to minimize or to limit damage of nematode infections in nurseries and to prevent secondary attacks of soil borne plant pathogens especially *Verticillium dahliae*.

Keywords: *Olea europaea*; pathogenicity; phytoparasitic nematodes; resistance; susceptibility

Introduction.

Olive trees (*Olea europaea* L., subsp. *Europaea*), are essentially grown in sub tropical climatic conditions (Argen-

tina, Australia, California, Chile, China, Mexico, Perú, Iran, Iraq, Jordan and South Africa) and especially in the countries of the Mediterranean Basin where about 85 % of the world olive production is concentrated. Over one hundred species of phytoparasitic nematodes have been reported in association with olive (Lamberti & Vovlas, 1993; Nico *et al.*, 2002; Sasanelli, 2009). However, only a few genera and species can affect growth of olive trees such as the root-lesion nematodes *Pratylenchus vulnus* and *P. penetrans*, the citrus nematode *Tylenchulus semipenetrans*, *Gracilacus peratica*, *Rotylenchulus macrorhatus*, the longidorids *Xiphinema elongatum* and *X. index*, and the root-knot nematodes *Meloidogyne* spp. (Diab & El-Eraki, 1968; Lamberti & Baines, 1969; 1970; Abrantes *et al.*, 1992; Lamberti & Vovlas, 1993; Sasanelli *et al.*, 1997; 1999; Sasanelli & D'Addabbo, 2002). Among all, root-knot nematodes, being polyphagous, are the most destructive plant parasitic nematodes causing severe damage to olive seedlings and groves. Mechanical injury caused by feeding mechanism of second stage juvenile of *Meloidogyne* on the roots of young olive tree favours penetration and infection of the root tissue by soil borne pathogen especially *Verticillium dahliae* (Lamberti *et al.*, 2001). Root-knot nematodes often damaged young olive plants in pots in nurseries. The symptoms are often overlooked by nurseryman and confused with nutrient deficiencies or other causes (Sasanelli *et al.*, 2002).

The main areas of olive production are principally restricted to the north of Iran. Since 1990s decade a strong expansion of new olive plantations, especially in regions with different environmental conditions compared to the north, was developed in order to supply the increasing demand for oil. Simultaneously in a survey carried out in the north of Iran the most common plant parasitic nematodes associated with olive plantations were *Helicotylenchus pseudorobustus*, *H. dihystra*, *H. digonicus*, *Nothocriconema mutabilae*, *Macroposthonia macrolobota*,

Meloidogyne javanica, *Pratylenchus thornei*, *Xiphinema pachtaicum* and *X. index* (Hosseininejad *et al.*, 1997). On the base of this survey, the most widespread phytoparasitic nematode specie was *M. javanica*

Subsequently nineteen olive cultivars were screened for their resistance to the root-knot nematode under glasshouse condition, the cultivars Coratina, Manzanillo and Leccino were found to be resistant, whereas Amigdalifolia and Frangionato showed a moderately susceptible reaction and cultivars Marii, Fishemi, Shengeh, Kalamata, Amphis, Clonaris, Blady, Conservallia, Mission, Sevillano Zard-e-Jonoub 1, Zard-e-Jonoub 2, and Roghani were found to be susceptible or highly susceptible (Hosseininejad & Ramezani Malakrodi, 2005).

Considering that the adoption of good sanitation practices in olive nurseries is imperative to obtain good quality reproduction material which is expected by national and international olive tree industries, two experiments were carried out on one of the most olive diffuse cultivar (Cv. Zard) in Iran to determine the quantitative relationship between the root-knot nematodes *M. incognita* and *M. javanica* population densities and growth reduction of olive plants.

Materials and methods

Root-knot nematodes populations of *M. javanica* and *M. incognita* were reared on susceptible tomato cultivar after obtaining pure culture from single egg mass. For each nematode species, infected tomato roots, with large egg-masses, were chopped, then macerated in a Warren blender for 45 seconds at high speed (8 000 rpm). The mixture was then added to the 75 ml centrifuge tubes which contained 20 g kaolin according to Coolen and D'Herde's method (1972). The suspensions were then incubated at room temperature to obtain active second stage juveniles using the modified Baermann's funnel method. The suspensions containing the second stage juveniles were poured through 500 mesh sieve and recollected into 100 mL becker flasks. Juveniles were quantified by counting them under light microscope and the nematode suspensions for inoculation were calibrated to the 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 juveniles/cm³ of soil. Ten month old self-rooted cuttings of olive cv. Zard were then individually transplanted into clay pots containing 2 000 cm³ sterilized soil. Seedlings were of the same size and the main shoot length, diameter at the insertion at the stem level and numbers of nodes were recorded at transplanting time. Seedlings were inoculated with each of the considered levels of initial populations (*Pi*) of *M. javanica* and *M. incognita*.

Experiments were arranged in a completely randomized design with five replications. During the experiments olive plants were maintained in the glasshouse randomizing the position of the blocks and at the same time repositioning each plant within a block every month, to avoid a block position effect and at the same time the factor position of the plant within the block. Plants received all the necessary maintenance (irrigation, fertilization, etc).

Thirteen months later inoculation plants were uprooted, soil removed from roots, and fresh top and root weights were recorded. Tops were then oven dried at 60 °C per 48 hours and weighted again.

The effect of each nematode species on plant growth parameters was assessed by calculating the percentage increase of main shoot length with respect to their initial values at transplanting and its diameter at its insertion with the stem and the number of nodes on the main shoot.

Final population densities of each nematode species were determined from soil and roots. Nematodes were extracted from the soil processing two soil samples of 100 cm³ from each pot by Jenkins's method (1964). The root system of each plant was chopped into small pieces and mixed thoroughly and then 5 g were macerated in a Warren blender in a 1 % NaOCl aqueous solution for three periods of 20 sec (Marull & Pinochet, 1991). The mixtures were subjected to the centrifugal sugar flotation technique. Second stage juveniles in the obtained water suspension were then counted and final nematode population density (*Pf*) determined.

Root gall index (RGI) on each olive plant was assessed according to a scale from 1 to 6 (Marull & Pinochet, 1991), where 1 = no galls, 2 = 1 – 10 galls, 3 = 11 – 30 galls, 4 = 31 – 70 galls, 5 = 71 – 90 galls and 6 = more than 91 galls/root system.

All data from the experiment were subjected to analysis of variance (ANOVA) and means compared by Least Significant Difference's test or Student's *t* test. All statistical analyses were performed using the PlotIT program (v3.2). Table Curve program (v1.0) was used to analyze the relationship between different nematode population densities and olive plant growth.

Results

Plant growth parameters

Most of the root systems of the olive plants grown in *M. incognita* or *M. javanica* infested soils were galled within the gall index range 1.4 – 6. Main shoot length was significantly (*P* = 0.05) suppressed by *M. incognita* at 12.8 J₂/ml soil (Table 1). However, olive plants cv Zard were more susceptible to *M. javanica* than *M. incognita* because of a significant reduction of main shoot length growth (about 37.6 %) was observed just at 0.1 J₂/ml soil (Table 2). No significant difference was observed in main shoot length of *M. incognita* infested plants in the range of nematode population density between 0.1 and 12.8 J₂/ml soil (Table 1). In *M. javanica* infested plants, at all tested nematode population densities (0.1 – 25.6 J₂/ml soil), the growth reduction was significant in comparison to uninfested plants ranging between 37.6 % and 79.8 % (Table 2). In the range between 0.8 and 25.6 J₂/ml soil no difference was observed in main shoot length among the infested plants and the growth reduction was significantly higher than those observed in olive plants with an initial population density between 0.1 and 0.4 J₂/ml soil (Table 2).

According to the light susceptibility of cv. Zard olive plants

Table 1. Effect of different *Meloidogyne incognita* initial population densities (*Pi*) on the growth of olive plants (cv. Zard)

<i>Pi</i> (eggs and J ₂ /ml soil)	Main shoot length (cm)	% Growth increase of main shoot length	Shoot diam (cm)	N° nodes of main shoot	Top fresh weight (g)	Top dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
0	104.8 [*] a ^{**}	147.8 [*] a	1.27	49	125.8	84.8	21.6	119.1	40.5
0.1	95.2	132.0	1.03	47	129.0	84.9	25.4	107.7	40.4
0.2	96.2	125.8	1.04	47	130.4	86.4	23.4	96.9	33.1
0.4	96.0	123.9	1.01	49	127.8	83.6	21.0	97.4	35.1
0.8	94.0	120.6	1.00	46	118.3	75.6	19.0	89.1	32.7
1.6	84.0	96.5	1.02	46	125.4	82.5	27.4	100.5	34.7
3.2	81.2	91.1	1.01	45	116.8	76.1	22.2	106.1	36.0
6.4	81.0	88.1	1.04	44	131.9	86.1	24.6	95.6	36.3
12.8	69.0	62.7	1.01	41	120.6	77.3	26.8	107.8	37.9
25.6	65.0	52.5	0.99	41	117.5	75.1	25.6	107.7	31.3

* Each value is an average of five replications;

** Data flanked in each column by the same letter are not statistically different according to Least Significant Difference's test ($P = 0.05$)Table 2. Effect of different *Meloidogyne javanica* initial population densities (*Pi*) on the growth of olive plants (cv. Zard)

<i>Pi</i> (eggs and J ₂ /ml soil)	Main shoot length (cm)	% Growth increase of main shoot length	Shoot diam (cm)	N° nodes of main shoot	Top fresh weight (g)	Top dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
0	118.8 [*] a ^{**}	171.3 [*] a	1.08	45	119.0	69.6	21.0	100.4	29.5
0.1	92.8	106.9	1.02	45	105.5	61.9	15.0	101.0	33.8
0.2	76.8	86.4	1.00	42	112.5	65.9	14.6	102.1	31.8
0.4	78.2	77.4	0.98	43	107.3	62.0	13.4	97.6	30.3
0.8	62.8	50.9	0.97	39	99.5	57.4	10.0	100.1	30.5
1.6	61.6	50.4	0.95	37	101.0	58.6	11.8	79.8	26.9
3.2	62.4	45.4	0.93	42	109.4	62.5	15.8	97.4	30.5
6.4	62.2	42.7	0.94	39	113.2	64.1	11.0	90.8	29.5
12.8	60.2	38.8	0.93	35	105.3	59.0	9.6	93.6	27.9
25.6	58.4	34.6	0.89	36	96.5	56.6	10.2	81.4	28.9

* Each value is an average of five replications;

** Data flanked in each column by the same letter are not statistically different according to Least Significant Difference's test ($P = 0.05$)

to *M. incognita* shoot diameter was significantly reduced compared to the uninfested control plants, however, there was not evidence of statistical differences among the tested population levels (Table 1). The maximum reduction (22 %) of shoot diameter respect to the control was observed at the highest tested nematode population density (25.6 J₂/ml soil). Conversely, although the higher susceptibility of cv Zard olive plants to *M. javanica* a significant reduction of shoot diameter was observed in plants transplanted in infested soil with 0.4 J₂/ml soil (Table 2).

The number of nodes recorded on main shoot of olive plants was significantly influenced only by *M. javanica* with values ranging between 45 and 35. No difference was observed in number of nodes on main shoots of plants transplanted in soil infested with nematode population densities varying from 0.1 to 6.4 J₂/ml soil, with the exception of 1.6 J₂/ml soil, in comparison to non infected olive plants (Table 1, 2).

Both nematode species significantly influenced top fresh and dry weights. In *M. incognita* infected plants top dry weight was significantly lower than that observed in the control at population densities of 0.8, 3.2 and 25.6 J₂/ml soil (Table 1). Because of the higher susceptibility of cv. Zard olive plants to *M. javanica* top dry weight in control plants was significantly higher than those observed in plants transplanted in infested soil at all nematode population densities excluding 0.2 J₂/ml soil (Table 2).

Root length in *M. incognita* infested plants was extremely variable and it did not differ from that recorded in the control plants with the exception of 1.6 and 12.8 J₂/ml soil. At these nematode population densities root length resulted higher than that observed in the control (Table 1). Dry root weight was not influenced by *M. incognita* attack with no statistical difference among the different levels of nematode population density (Table 1). In *M. javanica* infected olive plants root length was significantly reduced at all nematode population density in comparison to the uninfected control. The highest root length reduction was recorded at 12.8 J₂/ml soil although this value was not statistically different from those recorded at 6.4 and 25.6 J₂/ml soil (Table 2). On the contrary, as before indicated for *M. incognita*, dry root weight was not affected by nematode attack with the exception of that at 0.1 J₂/ml soil which was significantly higher than those recorded in the control and in the three higher tested nematode population densities (Table 2).

Nematological parameters

Root gall index of olive plants infected with *M. incognita* significantly increased with increasing of nematode population density and ranged between 1 and 5.6, from 0.4 to 25.6 J₂/ml soil. A similar trend was observed in plants infected with *M. javanica*. The maximum value of root gall index (6.0) was recorded on roots of plants transplanted in soil with at least 0.4 J₂/ml to 12.8 J₂/ml soil. The comparison between the two nematode species was significant at the same nematode population density in all the range of tested nematode population densities (0.1 – 25.6) but not at 12.8 J₂/ml soil (Table 3).

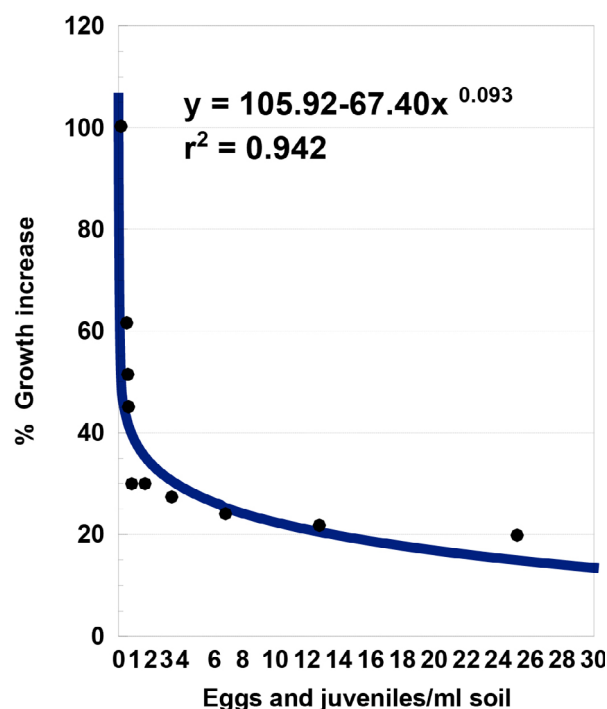
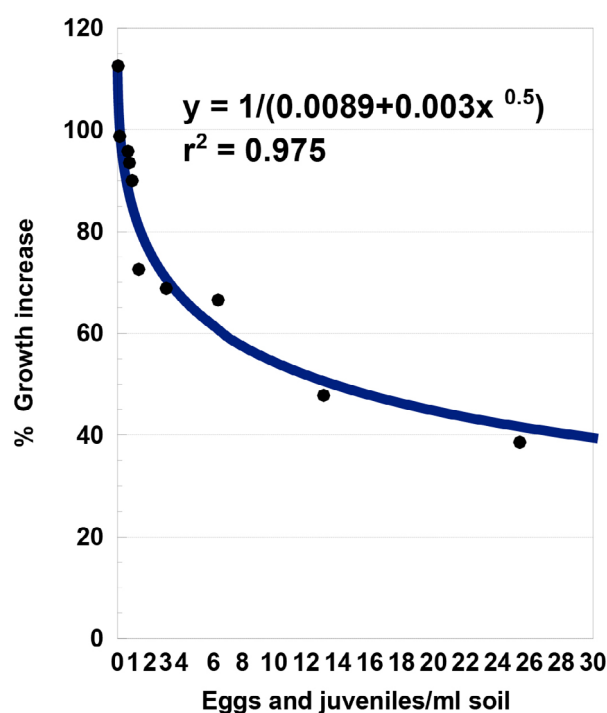


Fig. 1. Relationship between initial nematode population density and relative percentage growth increase of main shoot length of olive cultivar Zard infested by *Meloidogyne incognita* (A) and *M. javanica* (B)

Table 3. Reproduction of *Meloidogyne incognita* and *M. javanica* on olive plants cv. Zard

<i>Pi</i> (eggs and J ₂ /ml soil)	Root gall index (1-6)		<i>t</i> [*]	Final population (roots+soil) (eggs and juveniles/ml soil)		<i>t</i>	<i>Pf/Pi</i>		<i>t</i>
	<i>M. incognita</i>	<i>M. javanica</i>		<i>M. incognita</i>	<i>M. javanica</i>		<i>M. incognita</i>	<i>M. javanica</i>	
0	1.0 ^{***}	a	—	0	0	—	—	—	—
0.1	1.6	ab	**	3 020	a	*	15.1	a	**
0.2	1.4	ab	**	1 161	a	**	2.9	b	**
0.4	2.0	b	**	2 934	a	**	3.7	b	**
0.8	3.6	cd	**	4 027	ab	*	2.5	b	*
1.6	4.4	de	**	5 641	bc	**	1.8	b	**
3.2	3.0	c	**	4 324	ab	**	0.7	b	**
6.4	4.4	de	**	5 980	bc	**	0.5	b	**
12.8	5.0	ef	—	9 653	cde	**	0.4	b	**
25.6	5.6	f	*	10 869	d	—	0.2	b	—

* For each nematode population density significance of differences according to Student's *t* Test (* for P=0.05; ** for P=0.01);

** Each value is an average of five replications;

*** Data flanked in each column by the same letter are not statistically different according to Least Significant Difference's test (P=0.05)

The final population density of *M. incognita* (calculated summing nematodes from roots and soil) ranged between 1 161 and 10 869 J₂/ml soil. The highest nematode population density was observed at the initial population density (*Pi*) of 25.6 J₂/ml soil. However, this value was not significantly different from that at 12.8 *Pi*. A significant higher final nematode population density (*Pf*) was observed in *M. javanica* infected plants according to Student's *t* test ($P = 0.01$) with the exception of *Pi* 25.6 J₂/ml soil. At this *Pi*, the lowest final nematode population density (22 219 J₂/ml soil) was calculated and it was no statistical different from that calculated at *Pi* 0.1 J₂/ml soil (Table 3). No significant difference in *Pf* was observed in the range 3.2 and 12.8 J₂/ml soil.

According to the highest reproduction factors, $r = Pf/Pi$ corresponding to 15.1 and 151.4 for *M. incognita* and *M. javanica*, respectively, the host reaction of the olive cv. Zard was rated as highly susceptible because of more than 10 (Di Vito *et al.*, 1996; Sasanelli *et al.*, 1997). In *M. incognita* olive infected plants the reproduction rate calculated at *Pi* 0.1 J₂/ml soil was significantly higher than those of all other *Pi* which were no statistically different each other (Table 3). Significantly higher Pf/Pi were calculated at the lower *Pi* (0.1 and 0.2 J₂/ml soil), in comparison to the others *Pi* from 0.4 to 25.6 J₂/ml soil, for olive plants infected with *M. javanica*. As for final nematode population density a significant difference in the reproduction rate was found between the two nematode species (Table 3).

The relationship between initial nematode population density and relative percentage growth increase of main shoot length of olive cv Zard infested by *Meloidogyne incognita* and *M. javanica* showed a significant negative correlation between the two parameters, as indicated by the high values of the correlation coefficient r^2 . ($P = 0.01$) (Fig. 1).

Discussion and conclusions

The effect of different *M. incognita* initial population densities (*Pi*) on the growth of olive plants (cv. Zard) was of difficult interpretation. Also at high nematode infestation levels no difference was found in number of nodes on main shoot, top and root fresh weights and root dry weight. Final nematode population density and nematode reproduction rate were significantly lower than those assessed on olive plants for *M. javanica* attack.

On the contrary, the pathogenicity trial revealed a strong susceptibility of olive plants cv. Zard to *M. javanica*. Significant differences in comparison to non infested olive plants were evident in *M. javanica* infested plants for main shoot and root lengths and top fresh and dry weights already at 0.1 J₂/ml soil *Pi*, showing the high susceptibility of olive cv. Zard to the nematode as confirmed also by the high reproduction rate (151.4). Considering that it was not possible to apply to our data the Seinhorst's equation $y = m + (1-m) z^{(P-T)}$ for the different used initial population densities (Seinhort, 1965), on the base of results, it is reasonably possible to assume a tolerance limit *T* of 0.1 J₂/ml soil for olive plants cv. Zard to *M. javanica* (Seinhort, 1965;

1979; Sasanelli, 1994). This tolerance limit is lower than those found for the same root-knot nematode specie in pathogenicity tests on the olive cv. FS17 and on the rootstock DA 12 I, 0.61 and 0.49 J₂/ml soil (Sasanelli *et al.*, 2002). Moreover, the trial confirmed data from previous experiments with olive plants infested by *M. javanica* (Sasanelli *et al.*, 1997). The reproduction rate of the nematode, at an initial population density of 12 J₂/ml soil, on eight olive cultivars, Cellina di Nardò, Cima di Bitonto, Coratina, Frantoio, FS17, Leccino, Yusti and the rootstock DA 12 I varied from 0.4 to 13.2 with an average of 4.5. In our trial at about the same level of initial population density (12.8 J₂/ml soil) the reproduction rate of *M. javanica* on the cv. Zard was 4 so not far from the previous findings. Based on the equation of the relationship between *Pi* and relative percentage growth increase of main shoot length and on the consideration that in the Seinhorst's equation the minimum relative yield (*m*) is reached at 64 J₂/ml soil it is possible to calculate the value *m* for the cv. Zard at high *Pi* of *M. javanica* ($m = 6.7\%$). For *M. incognita* it was possible to calculate only the value of *m* (30 %) and no the value of the tolerance limit because of no significant difference was evident in percent growth increase in the range of initial population density between 0 and 6.4 J₂/ml soil (Table 1).

The low tolerance limit found for the cv. Zard to *M. javanica* indicates that growth of young olive plant can be strongly suppressed by the presence of the nematode in the soil. A possible quick estimation of plant growth reduction with the help of the Tables of Nematode-Pathogenicity (Sasanelli, 1994) might be possible from the *T* and *m* values of the experiment. In every case our data show that to obtain good quality reproduction material as expected by international olive tree industries it is necessary to apply good sanitation practices in olive tree nurseries. The use of resistant olive rootstock or cultivars selected by rapid screening trials for the resistance to the root-knot nematodes (Sasanelli *et al.*, 2000) or the use of olive residues derived from pruning or olive oil extraction (Renčo, 2013) can minimize or limit the damage of nematode infections in nurseries although it may cause a selection of more virulent root-knot nematode populations capable of circumventing resistance to olive plants grown in poor sanitation conditions.

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