5000 and 10 000 nematodes per seedling were used, replicated five times. Pots were arranged in a randomised row design in a heated glasshouse. The plants were top-lit by sodium/halogen lights to give a 12-hour day.

The experiment was harvested 22 weeks after inoculation of the nematodes. Measurements were made of the plant heights and lengths of stems plus branches. The number of leaves, number of leaf nodes, total fresh and dry weights of tops, and the fresh weights of each root system were taken. Nematodes were extracted from roots of each plant by cutting the roots into small pieces, macerating in a blender for 20 s, pouring on to a 85 µm aperture sieve in a Petri dish of water and leaving for 48 hours. Nematodes were extracted from 200 cm^3 soil from each pot on a tray modification of the Baermann funnel method (Hooper, 1990) left for 24 hours. Numbers counted were converted to total nematodes per pot. Observations of nematodes within root tissues were obtained by staining roots in lactoglycerol plus 0.05 % acid fuchsin (Bridge *et al.,* 1982). Data was analysed by the one-way ANOVA statistical test.

A second, small experiment was set up to understand further some behavioural aspects of *R. citri* n. sp. Nematodes were cultured and inoculated as in the first experiment. Ten week-old seedlings of citrus rootstock Japanese Citron were transplanted into 11.5 cm diameter plastic pots containing 600 cm^3 soil with the same soil mix as above. Two treatments of 0 and 5000 nematodes with four replicates were used, arranged in a completely randomised design in the heated glasshouse. Plants were harvested 10 weeks after inoculation of nematodes. Heights of seedlings were measured and fresh weights of tops and roots were taken. In addition, lateral roots were removed from the main roots and weighed separately. Numbers of female, male and juvenile nematodes were counted from main and from lateral roots.

Radopholus citri n. sp. = *Radopholus* n. sp. *in* Hahn *et al.* 1994

(Fig. 1)

MEASUREMENTS

Females (n = 20) : L = 0.69 ± 0.05 (0.62-0.81 mm); a = 28.8 ± 2.5 (25.1-32.5); b = 7.62 ± 0.4 (6.9-8.3); b' = 4.4 ± 0.3 (3.8-4.9); c = 14.3 ± 1.2 (12.3-16.7); c' = 3.0 ± 0.4 (2.5-3.6); V = 59.5 ± 2.0 (54.1-62.3); stylet : 18.6 ± 0.6 (17.4-19.4) µm; tail = 48.7 ± 3.4 (43.6-57.0) µm; h* = 7.3 ± 0.9 (5.4-8.7) µm.

Males (n = 20) : L = 0.53 ± 0.03 (0.47-0.58) mm; a = 34.5 ± 3.1 (28.4-41.7); b = 6.6 ± 0.7 (5.4-8.0); c = 14.2 ± 1.1 (12.4-15.9); c' = 3.2 ± 0.5 (2.6-3.9); stylet = 13.5 ± 0.7 (12.7-14.7) µm; tail = 37.8 ± 4.0 (31.5-43.6) µm; h* = 6.7 ± 0.8 (5.4-8.0) µm; spicules = 15.5 ± 1.0 (14.1-17.4) µm; gubernaculum = 8.8 ± 0.9 (7.4-10.7) µm.

Holotype (female) : L = 0.72 mm; a = 32.5; b = 7.7; b' = 4.5; c = 14.7; c' = 2.8; V = 60.7; stylet = 19.4 µm; tail = 48.9 µm; h* = 7.4 µm.

DESCRIPTION

Female : Body vermiform, assuming an almost straight to ventrally arcuate form when heat relaxed. Cuticle annulated, ventral annules 1.3-1.6 µm apart at mid body. Four lateral incisures reducing to three in region of phasmids, outer incisures crenate. Outer bands of lateral fields marked by occasional transverse striae, particularly towards posterior extremity. Head slightly offset, low, rounded and somewhat flattened apically with four or five annules. *En face* view similar to that of type species. Stylet moderately strong. Basal knobs about 5 µm across; distal surface of dorsal knob extending anteriorly; much smaller and indistinct anterior projection usually visible on each subventral knob. Dorsal oesophageal gland opening 4-5 µm behind stylet knobs. Procorpus cylindroid; median bulb round to oval in form and slightly offset from rest of oesophagus. Oesophageal gland well developed, overlapping intestine mostly on dorsal side, with glands in tandem. Nerve ring immediately posterior to median bulb. Excretory pore just posterior to hemizonid and located about two to three bulb lengths posterior to bulb. Vulva postmedian with markedly protuberant lips. Genital branches amphididelphic, outstretched with oval to rod-shaped sperm in axial spermathecae. Uterine egg 20.1 × 58.3 µm. Tail conoid, tapering to rounded terminus which is regularly annulated. Phasmids located at 16 (12-17) annules posterior to anus, about midway along tail.

Male : Body vermiform, showing marked sexual dimorphism in anterior region. Head high, offset, knob-like with four or five annules. Stylet conus highly refringent, relatively strongly developed for males of this genus; shaft and knobs less well developed, but distinct. Oesophagus degenerate and apparently non-functional. Spicules paired, ventrally arcuate. Gubernaculum rod-like, with a dorsally directed process, observed in SEM studies, on dorsal surface; protrusible. Bursa crenate, extending almost to tail tip. Phasmids located about sixteen annules posterior to cloaca, close to midway along tail. Tail conoid, tapering to a finely pointed, partially offset, terminus.

TYPE HOST AND LOCALITY

Collected from the rhizosphere of *Citrus* rootstock cv. Japanese Citron, Tulungagung, East Java, Indonesia.

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* h = hyaline portion of tail.

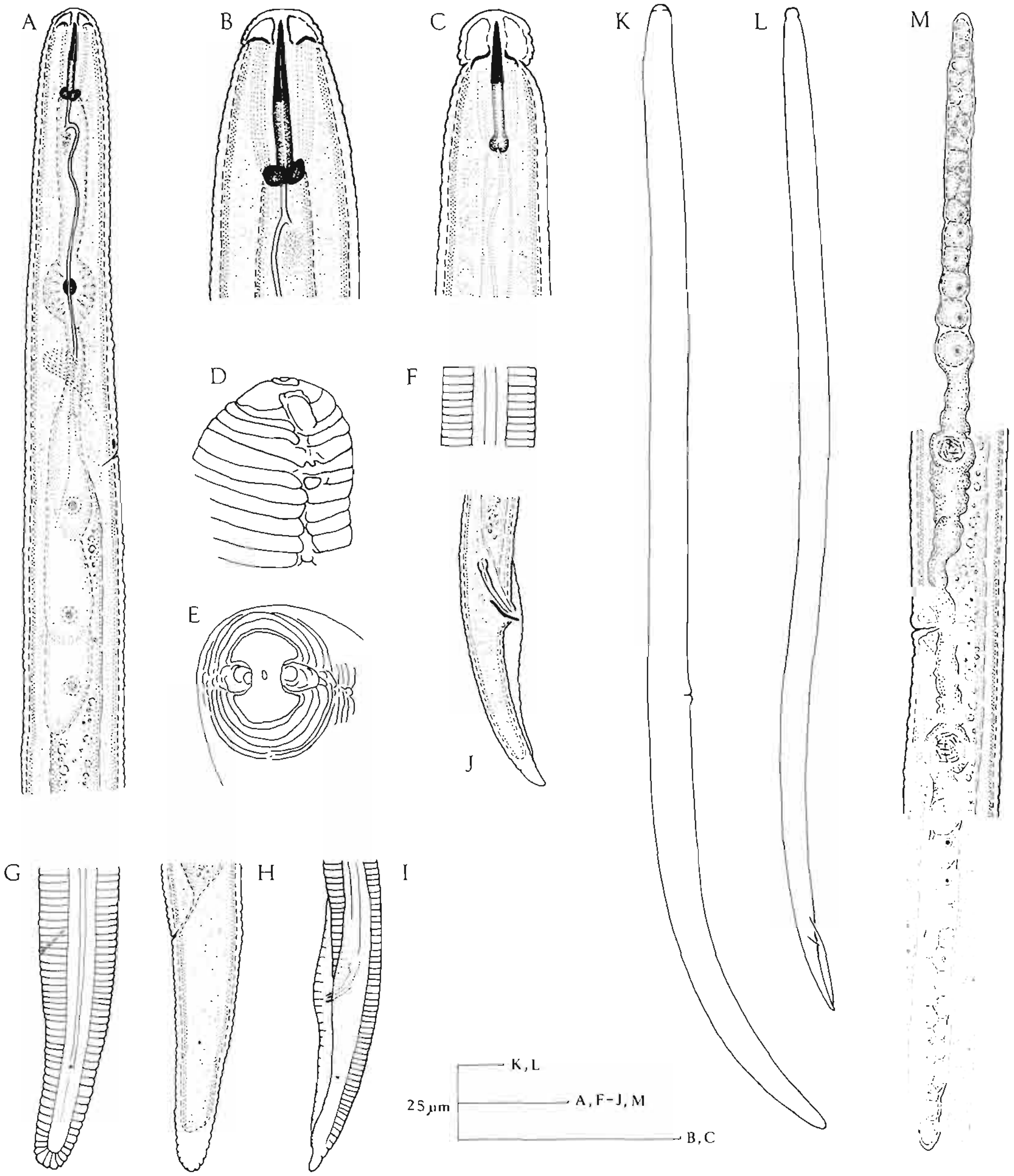

Fig. 1. Radopholus citri *n. sp. A : Female oesophagus; B : Female head; C : Male head; D : Female head, diagramatic from scanning electron micrographs; E : Female head, en face; F : Lateral field, mid-body; G, H : Female tails; I, J : Male tails; K : entire female; L : Entire male; M : Female genital tracts.*

TYPE SPECIMENS

Holotype female and 43 paratypes 24 females and 19 males (IIP Nos. T54/2/1-44 deposited in the collection of the International Institute of Parasitology, St. Albans, Herts, U.K. Four paratypes (two females and two males) deposited at both Rothamsted Experimental Station, Harpenden, Herts, U.K. and Muséum National d'Histoire Naturelle, Paris, France.

DIAGNOSIS AND RELATIONSHIP

R. citri n. sp. is characterized in the male by the relatively well developed stylet the conus of which is unusually heavy, and in the female by the strongly developed anterior projection on the dorsal stylet knob and the conoid tail with rounded, regularly annulated terminus with the phasmid located at 16 (12-17) annules posterior to the anus.

In the combination of general characters such as number of female head annules, number of lateral lines, vulval position, tail length and shape, *R. citri* n. sp. is most similar to *R. vangundyi* Sher, 1968, *R. neosimilis* Sauer, 1958 and *R. nativus* Sher, 1968.

It differs from *R. vangundyi* in : a greater female body length (624-809 µm *vs* 470-650 µm) and a longer hyaline portion to the tail (5.4-8.7 µm *vs* 3-5 µm); stylet with an anteriorly directed dorsal basal knob; more posterior phasmid located at about 16 (12-17) annules from the anus; a knob-like, lower, annulated male lip region and a more extensive bursa. From *R. neosimilis* in : stylet with an anteriorly directed dorsal basal knob; more posterior phasmid located at about 16 (12-17) annules from the anus; a less bluntly rounded female tail terminus and a more robust, knobbed male stylet and a non-enveloping bursa. From *R. nativus* in : stylet with an anteriorly directed dorsal basal knob; more posterior phasmid located at about 16 (12-17) annules from the anus; a shorter female stylet (17.5-19.5 µm *vs* 19-22 µm).

R. citri n. sp. is easily distinguished from *R. similis citrophilus* Huettel *et al.*, 1984, the only other described *Radopholus* pathogenic to *Citrus*, by the presence of a stronger male stylet and the shape and considerably shorter length of the tail in both sexes.

FIELD OBSERVATIONS

Twelve genera and seventeen species of plant parasitic nematodes were extracted and identified from citrus soil and roots, but only *Tylenchulus semipenetrans* and *R. citri* n. sp. were found as root endoparasites. *R. citri* n. sp. occurred in sandy soils of both established citrus orchards and nurseries in the district of Tulungagung, East Java, but not in other parts of Java nor in the areas of Sulawesi and Bali that were sampled. Highest populations of *R. citri* n. sp. that were extracted with the simple techniques used in the survey were 550/dm^3 soil and 320/g root. Samples taken at 0-30 cm and 30-60 cm depths in the same orchards gave higher root populations at the lower depths, but the combined root and soil populations were similar at both depths.

PATHOGENICITY ON CITRUS

In the first experiment, *R. citri* n. sp. invaded roots of all citrus plants inoculated, the nematode causing very severe cortical necrosis and root destruction. All levels of the nematode caused highly significant reductions ($P = < 0.001$) in fresh and dry weights of tops, number of leaf nodes, and growth as measured by length of stems and branches (Table 1, Fig. 2). Fresh root weights were also highly significantly reduced ($P = < 0.001$) in all nematode treatments (Table 1, Fig. 2). Final root populations of *R. citri* n. sp. varied with different initial inoculation levels; most nematodes being found in roots of plants inoculated with 1000 nematodes, and least in roots of plants inoculated with 10 000 nematodes reflecting the amount of root destruction. Final root populations were very variable within treatments, for example exceeding 15 000/g root with a mean of 5407/g root at the 1000 level. Final soil populations in pots showed a similar trend (Table 1). Nematodes observed in stained roots were confined to the cortical tissues often associated with complete destruction of the cortex.

Table 1. *Populations of* Radopholus citri *n. sp. in* Citrus *seedling roots and soil and their effect on root and top growth 22 weeks after inoculation.* (Means of five replicates ± standard error).

Treatment (nematodes per plant)	Height (cm)	Total length of stem & branches (cm)	Nos. leaf nodes	Total fresh wt. of tops (g)	Dry wt. of tops (g)	Fresh wt. of roots (g)	Nos. nematodes/root system	Nos. nematodes/g root	Nos. nematodes in soil/pot
0	29.4	32.0	26	5.05	1.95	5.97	0	0	0
	(± 1.4)	(± 1.0)	(± 1.4)	(± 0.50)	(± 0.19)	(± 0.62)			
1000	14.1	15.2	15.8	1.21	0.49	0.84	4985	5427	2900
	(± 0.7)	(± 0.8)	(± 1.0)	(± 0.12)	(± 0.05)	(± 0.11)	(± 2726)	(± 2716)	(± 781)
5000	15.1	15.9	15.8	1.26	0.54	0.75	2168	2958	1969
	(± 0.8)	(± 0.7)	(± 0.6)	(± 0.33)	(± 0.14)	(± 0.10)	(± 718)	(± 929)	(± 491)
10 000	14.1	14.3	14.6	0.92	0.38	0.63	837	1308	765
	(± 0.8)	(± 0.7)	(± 0.7)	(± 0.22)	(± 0.08)	(± 0.09)	(± 349)	(± 453)	(± 207)
LSD 1 % level	4.08	3.37	3.99	1.35	0.52	1.34			
0.1 % level	5.61	4.63	5.49	1.85	0.71	1.83			

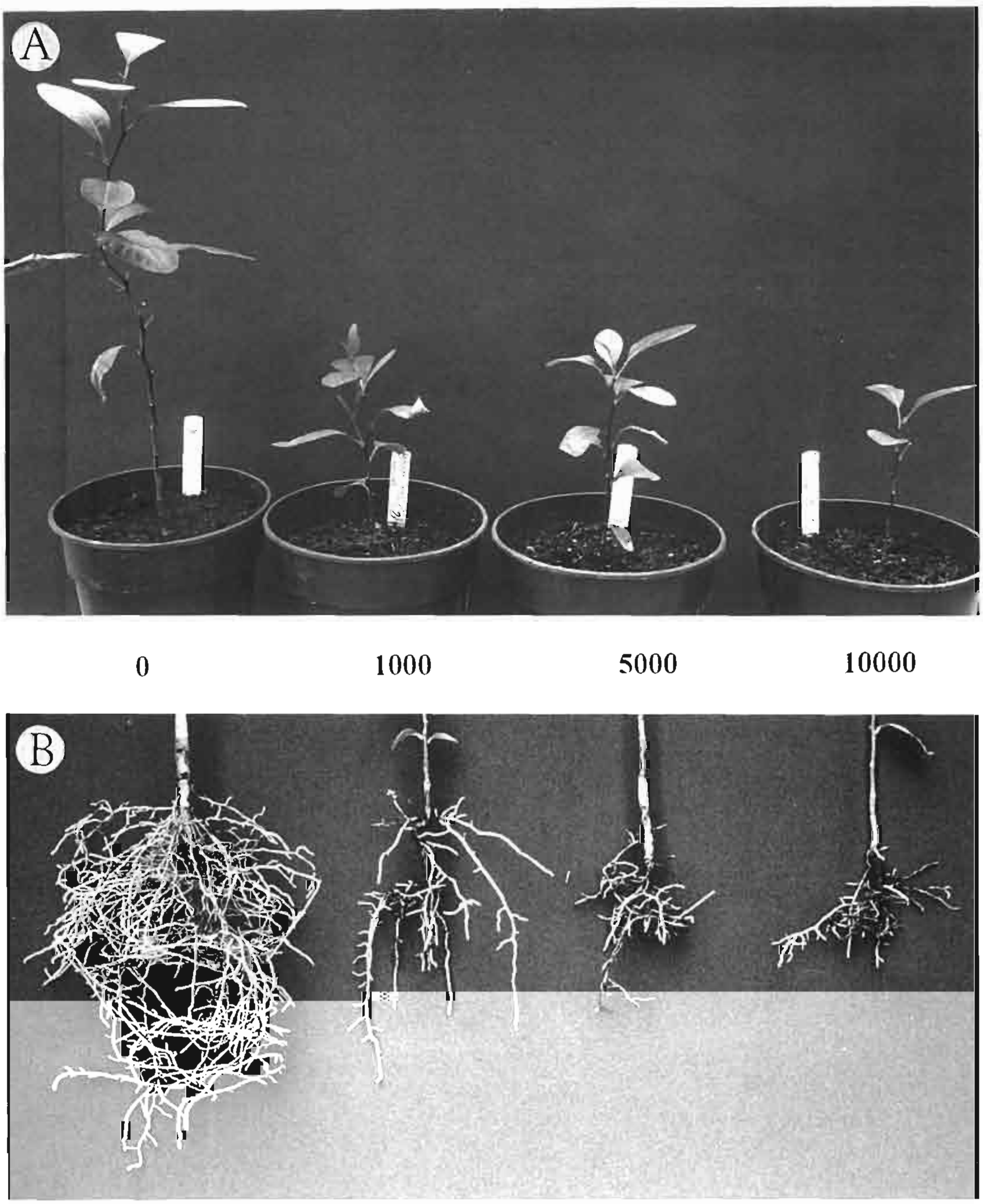

Fig. 2. *Effect on top and root growth of 22 week-old citrus seedlings inoculated with different populations of* Radopholus citri *n. sp. A : In pots; B : Roots removed from pots and washed free of soil.*

Observable root damage associated with *R. citri* n. sp. was less evident in the second experiment after the shorter, 10-week period, but reductions in total fresh root weights were highly significant ($P = < 0.01$) in the presence of the nematodes (Table 2). Populations of the nematode in roots of these younger plants were greater at termination of the experiment after 10 weeks than in the first experiment after 22 weeks. The numbers of nematodes in lateral roots compared to main roots were not significantly different, but the reduction of fresh

Table 2. *Root populations of* Radopholus citri *n. sp. and their effect on growth of Citrus* seedlings 10 weeks after inoculation. (Means of four replicates ± standard error).

Treatment (nematodes per plant)	Height of seedlings (cm)	Total fresh wt. of tops (g)	Total root fresh wt. (g)	No. nematodes/ g root
0	11.3 (±0.7)	1.19 (±0.14)	1.08 (±1.11)	0
5000	9.8 (±0.4)	0.80 (±0.11)	0.48 (±0.07)	8913 (±3088)
LSD 1 % level	2.9	0.66	0.48	
0.1 % level	4.7	1.07	0.77	

weights of lateral roots and of main roots in plants receiving nematodes was highly significant ($P = < 0.01$). The ratio of males to females in roots was 1:4 (Table 3).

Discussion

Radopholus (sensu stricto) and the closely related *Radopholoides* de Guiran, 1967 and *Achlysiella* Hunt, Bridge & Machon, 1989 share a common centre of diversity located in the Australasian/Pacific region (Hunt *et al.,* 1989). Only two species of *Radopholus* have been proposed from countries outside this region *viz. R. nigeriensis* Sher, 1968 from Nigeria and *R. citrophilus* Huettel, Dickson & Kaplan, 1984 from Florida, USA. *R. nigeriensis* is not typical of the genus and was placed in *Zygradus* Siddiqi, 1991 with a similar species, also from Nigeria, by Siddiqi (1991). *R. citrophilus* was formerly the citrus race of *R. similis* which was erected as a sibling species of *R. similis* (Huettel *et al.,* 1984). The validity of *R. citrophilus* has been questioned and is regarded by some authorities as a subspecies, or simply a host race, of *R. similis* (Siddiqi, 1986; Luc, 1987) and in this paper is regarded as a subspecies, *R. s. citrophilus. R. similis* has been widely spread around the world by man on cultivated crops, particularly banana. *R. citri* n. sp. from Java, together with another new species of *Radopholus* from Indonesia currently being described (Siddiqi, pers. comm.), further reinforces this centre of diversity hypothesis.

Radopholoides, which differs from *Radopholus* by the female being monodelphic, is accepted here as a valid genus although it is regarded as a synonym of *Radopholus* by others including Luc (1987). *Radopholoides* has a rather more widespread distribution than *Radopholus,* with species being described from Madagascar and Japan as well as Australia. The genus *Achlysiella* differs from *Radopholus* by having a swollen, sedentary female and different biological characters. The vermiform, immature female is distinct in having few cells in the ovaries and an unusually long oesophageal gland lobe. On the basis of these characters in the immature female, the authors of the genus (Hunt *et al.,* 1989) stated that the following species, in addition to the type species *A. williamsi,* are likely to belong to *Achlysiella : Radopholus brevicaudatus* Colbran, 1971, *R. capitatus* Colbran, 1971, *R. magniglans* Sher, 1968, *R. trilineatus* Sher, 1968 and *R. vacuus* Colbran, 1971. The new combinations were not formally proposed because the authors considered that biological studies needed to be done on the species prior to their inclusion, however Ebsary (1991) has since proposed these combinations without obtaining information on their biology. The genus *Zygradus* lacks sexual dimorphism whereas *R. citri,* which does have a stylet in the male which is stronger than that found in other species of *Radopholus,* retains a marked sexual dimorphism in the anterior region.

Recently, the random amplified polymorphic DNA (RAPD) technique was used to compare genetic variation between *R. citri* n. sp. and fourteen different iso-

Table 3. *Population structure of* Radopholus citri *n. sp. in different roots of* Citrus *seedlings 10 weeks after inoculation.* (Means of 4 replicates ± standard error).

Treatment (nematode per plant)	Main roots					Lateral roots				
	Fresh wt. of roots (g)	Nematodes/g roots				Fresh wt. of roots (g)	Nematodes/g roots			
		♀♀	♂♂	JJ	Total		♀♀	♂♂	JJ	Total
0	0.43 (± 0.05)	0	0	0	0	0.66 (± 0.06)	0	0	0	0
5000	0.22 (± 0.02)	2100 (± 777)	500 (± 178)	5075 (± 1004)	7675 (± 1594)	0.26 (± 0.05)	3150 (± 819)	938 (± 365)	6063 (± 2020)	10150 (± 3088)
LSD 1 % level	0.20					0.30				
0.1 % level	0.33					0.49				