Original article

Resistance mechanisms of *Prunus* rootstocks to root-knot nematode, *Meloidogyne incognita*

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Resistance mechanisms of *Prunus* rootstocks to root-knot nematode, *Meloidogyne incognita*.

Abstract — **Introduction**. Root-knot nematodes (*Meloidogyne* sp.) cause significant economic damage to *Prunus* species in China. One of the most economical and environmentally sustainable methods to reduce the impact of root-knot nematodes is the use of resistant rootstock cultivars. Our aim was to examine resistance to *M. incognita* and its mechanisms. **Materials and methods**. Four rootstocks were assessed: Tsukuba-4 (*P. persica*), Tsukuba-5 (*P. persica*), Nanking cherry (*P. tomentosa*) and wild peach (*P. persica*). The susceptible tomato (*Lycopersicon esculentum* Mill.) cultivar 'Baiguoqiangfeng' was used as a positive control. **Results**. Nematodes did not penetrate roots of Tsukuba-4 and Tsukuba-5, which were considered to be immune varieties. Nanking cherry was highly resistant to *M. incognita,* whereas wild peach was susceptible. **Conclusion**. The differences in resistance among the rootstocks were not attributed to differences in effects of root diffusates, but were related to the different structural organizations of the root tips. The epidermal structure of Tsukuba-4 and Tsukuba-5 completely prevented the penetration of second-stage juveniles of *M. incognita* (J2). In Nanking cherry, penetration of J2 juveniles was reduced, and the development of nematodes from the J2 to female stage was delayed.

China / *Prunus* / pest resistance / *Meloidogyne incognita* / rootstocks / variety trials / plant tissues

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Mécanismes de résistance des porte-greffes de *Prunus* au nématode des racines, *Meloidogyne incognita*.

Résumé — **Introduction**. Les nématodes des racines (*Meloidogyne* sp.) causent des dommages économiques significatifs aux espèces de *Prunus* en Chine. L'une des méthodes la plus économique et la plus durable pour l'environnement pour réduire des impacts des nématodes des racines est l'utilisation de porte-greffes résistants. Notre but a été d'étudier la résistance à *M. incognita* et à ses mécanismes. **Matériel et méthodes**. Les quatre porte-greffes évalués ont été Tsukuba-4 (*P. persica*), Tsukuba-5 (*P. persica*), le cerisier de Nanking (*P. tomentosa*) et un pêcher sauvage (*P. persica*). Le cultivar de tomate (*Lycopersicon esculentum* Mill.) 'Baiguoqiangfeng' sensible a été utilisé comme témoin. **Résultats**. Les nématodes n'ont pas pénétré les racines des cultivars Tsukuba-4 et Tsukuba-5 qui ont été considérés comme des variétés immunes. Le cerisier de Nanking a montré une haute résistance à *M. incognita*, tandis que le pêcher sauvage a été sensible. **Conclusion**. Les différences de résistance parmi les porte-greffes n'ont pas été attribuées à des différences de diffusats de racine, mais à des différences de structure des pointes de racine. La structure épidermique de Tsukuba-4 et de Tsukuba-5 a complètement empêché la pénétration des nématodes juvéniles du deuxième stade de *M. incognita* (J2). Dans le cerisier de Nanking, la pénétration des juvéniles J2 a été réduite et le développement des nématodes, du stade J2 à celui de la femelle, a été retardé.

Chine / *Prunus* / résistance aux organismes nuisibles / *Meloidogyne incognita* / porte greffe / essai de variété / tissu végétal

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1. Introduction

Root-knot nematodes (*Meloidogyne* sp.) cause significant economic damage to *Prunus* species in China, with *Meloidogyne incognita* Chitwood and *M. javanica* Chitwood as the predominant species [1, 2]. One of the most economical and environmentally sustainable methods to reduce the impact of root-knot nematodes is the use of resistant rootstock cultivars. In other countries, there has been extensive research on selection of *Prunus* rootstocks for resistance to root-knot nematode [3–5], but there is little information on resistance of *Prunus* rootstocks to root-knot nematode in China. Root-knot nematode-resistant rootstocks, such as Guardian, Nemaguard and Nemared, have not been introduced into China.

Plants' resistance to root-knot nematode can be divided into three stages; pre-penetration, penetration and penetrated [6]. There is only limited information on resistance mechanisms of *Prunus* to root-knot nematode, especially at the pre-penetration and penetration stages. Root diffusates and structural organization of the root are both factors that can affect nematode infection [7–9].

The major objectives of our study were to evaluate resistance of *Prunus* rootstocks to root-knot nematode. Resistance to *M. incognita* was determined at the pre-penetration and penetration stages.

2. Materials and methods

2.1. Plant material

Four *Prunus* rootstocks were assessed: Tsukuba-4 (*Prunus persica*), Tsukuba-5 (*P. persica*), Nanking cherry (*P. tomentosa*) and peach (*P. persica*). Tomato (*Lycopersicon esculentum* Mill.) was used as the susceptible control. Tsukuba-4 and Tsukuba-5 were bred in Japan between 1967 and 1980, and result from crosses between a F2 seedling of dwarf peach (*P. persica* var.·*densa*) and a redleaf variety. Tsukuba-4 and Tsukuba-5 were introduced into Beijing, China, in 2001 [10]. Nanking cherry was collected from China Agricultural University. Wild peach was collected from Fangshan, Beijing. The tomato cultivar 'Baiguoqiangfeng' was collected from China Agricultural University. For *P. persica* cv. Tsukuba-4, cv. Tsukuba-5, peach and tomato, plantlets were obtained from seeds. Nanking cherry was propagated from softwood cuttings. Both germinated seeds and wood cuttings were planted in 10-cm-diameter plastic pots containing a mixture of [2:2:1] (v:v:v) soil, vermiculite and peat moss previously sterilized at 120 °C; then, they were moved to a greenhouse at a mean temperature of 25 °C according to already implemented methodologies [11, 12].

2.2. Nematode species

Meloidigyne incognita was obtained from the tomato cultivar 'Jiafen' in TongZhou, Beijing. Nematode populations were propagated on the tomato 'Baiguoqiangfeng' from single-egg-mass cultures.

2.3. Penetration and development of *M. incognita*

Prunus plantlets with uniform growth were inoculated on the same date (D), 60 d after transplanting. The inoculum consisted of a suspension of 2000 freshly hatched (24–72 hours old) second-stage *M. incognita* juveniles (J2) per plant. The positive control to verify inoculum viability and infectivity of J2 nematodes was the susceptible tomato cultivar 'Baiguoqiangfeng'. It was inoculated at the 3-leaf stage on the same date with a suspension of 1000 J2 nematodes per plantlet. Penetration and development were assessed during a 31-d period following inoculation. Three replicates per *Prunus* rootstock were harvested for root staining on days D+3, D+7, D+11, D+15, D+19, D+23, D+27 and D+31. Nematodes were stained using the acid fuchsin-lactophenol method described by Hooper [13]. Nematode development (J2, swollen J2, J3–J4, female) and the day of nematode emergence were determined in each plant by examining the whole root system under a stereomicroscope [11].

2.4. Resistance of *Prunus* rootstocks to *M. incognita*

Sixty days after transplanting, *Prunus* plantlets with uniform growth were inoculated on the same date with a suspension of 2000 freshly hatched *M. incognita* juveniles (24–72 hours old) per plantlet. The experiment was set up in a completely randomized design with 10 replications per rootstock. Gall number was recorded 120 d after inoculation. The gall index represents a 0–5 scale, in which 0 = no galls; 1 = 1–10 galls; 2 = 11–30 galls; 3 = 31–70 galls; 4 = 71–150 galls; and 5 = more than 150 galls per plant [6, 14]. A mean gall index was calculated using the formula described by Liu [6]: Gall index = {[∑ (scale class number) × number of plants in this class)] / [(total number of plants) × 5]} × 100.

Resistance of each rootstock was estimated by the gall index, where I = immune (gall index = 0); HR = highly resistant (0 < gall index ≤ 10%); R = resistant (10.0% < gall index ≤ 50.0%); S = susceptible (gall index > 50.0%) [6, 14].

2.5. Effects of root diffusates on hatching of *M. incognita* eggs

The method described by Yuan [9] was used to test the effects of root diffusates on hatching of nematode eggs. The root diffusate solution was prepared as follows: 1-year-old *Prunus* rootstock was removed from the growth medium, the roots were washed clean, and then soaked in 100 mL distilled water for 24 h. To prepare root diffusate from tomato, 30-day-old plants were removed from the growth medium; the roots were washed clean, and then soaked in 20 mL distilled water for 24 h. A smaller volume of water was used to prepare the tomato root diffusate as the mass of the tomato roots was approximately one-fifth that of the *Prunus* roots.

Egg masses of similar size were selected, then a single egg mass was placed in a Petri dish filled with 5 mL of diffusate solution. Distilled water was used as control. The Petri dish was kept in the dark at 25 °C for 12 d. The number of hatched J2 nematodes was recorded daily for 10 d, and the ratio of dead J2 individuals to all hatched J2 nematodes was evaluated to assess J2 mortality on day 12. Three replicates of each plant were used in this experiment.

2.6. Influence of roots on *M. incognita* J2 taxis

Two trials were carried out.

Trial 1: One-year-old *Prunus* rootstocks and 30-d-old tomato plants were removed from their growth media. Roots were cut into 1-cm segments, including the root apex, after being washed. A sample of (20 ± 5) J2 nematodes suspended in 0.05 mL H_2O were deposited at the center of a Petri dish containing 0.5% agar medium. Four root segments of one of the four *Prunus* rootstocks and one tomato root segment were placed in a circle around the J2 suspended droplet (radius = 1 cm). The Petri dishes were kept in the dark at 25 °C for 24 h and the number of J2 nematodes near each root segment was recorded every 2 h. Four replicates were used for each root type [9].

Trial 2: A root segment of the four *Prunus* rootstocks and 30-d-old tomato plants was placed at the center of a Petri dish containing 0.5% agar medium, and approximately 40 J2 nematodes suspended in 0.1 mL H_2O were deposited on the root segment. The Petri dishes were kept in the dark at 25 °C for 24 h, and distribution of J2 nematodes was observed under a stereoscopic microscope. Treatments were replicated three times [9].

2.7. Relationship between structure of root tip and resistance to nematode penetration

One-year-old *Prunus* rootstocks and 30-d-old tomato plants were removed from their growth media, and roots were sectioned by hand to examine the cell structure of the cortex under a microscope.

Table I.
Dates (in days after inoculation) of the first observations in roots of different development stages of *Meloidogyne incognita* on four *Prunus* rootstock cultivars compared with observations in roots of tomato.

Plant species	Development stages of *M. incognita*			
	J2	Swollen J2	J3–J4	female
Tomato cv . 'Baiguoqiangfeng'	D+3	D+7	D+11	D+19
P. persica cv. Tsukuba-4	None	None	None	None
P. persica cv. Tsukuba-5	None	None	None	None
Peach (*P. persica*)	D+3	D+11	D+15	D+27
Nanking cherry (*P. tomentosa*)	D+7	D+15	None	None

3. Results

3.1. Penetration and development of *M. incognita*

J2 nematodes were observed from 3 d after inoculation in tomato and peach.

In tomato, the first swollen J2 nematodes were observed at D+7 and the first female was observed at D+19 (*table I*).

In peach, the first swollen J2 nematodes were observed at D+11 and the first female was observed at day D+27. J2 nematodes were observed in Nanking cherry from D+7; swollen J2 nematodes were observed in Nanking cherry at D+15, but no nematodes were observed in plantlets of Nanking cherry after 7 d and no J3–J4 nematodes were observed during the experimental period, indicating that the Nanking cherry affected the penetration of nematodes, and the nematode development from the J2 stage to female stage was delayed or aborted. In our experiment, J2 nematodes were unable to penetrate roots of Tsukuba-4 and Tsukuba-5 (*table I*).

3.2. Resistance of *Prunus* rootstocks to *M. incognita*

Tsukuba-4 and Tsukuba-5 were immune to *M. incognita* infection. No galls were observed in the root even 120 days after inoculation (*table II*). Nanking cherry was resistant to *M. incognita*, but resistance varied among individuals; no galls were observed in the roots for 3/10 plantlets. Wild peach was susceptible to *M. incognita*.

3.3. Effects of root diffusates on hatching of *M. incognita* eggs

Root diffusates from Tsukuba-4 promoted hatching of *M. incognita* eggs from D+3 (*figure 1*). After 6 d, the lowest number of J2

Table II.
Classification and comparison for resistance to *Meloidogine incognita* of four *Prunus* rootstock cultivars. Resistance level was rated by assessing the root gall index 120 days after inoculation of 2000 *M. incognita* J2 nematodes on 60-day-old plantlets.

Rootstock	N° of plants by gall index classes (scale: 0–5[a])						Gall index[b]	Resistance rating[c]
	0	1	2	3	4	5		
P. persica Tsukuba-4	10	0	0	0	0	0	0	Immune
P. persica Tsukuba-5	10	0	0	0	0	0	0	Immune
Wild Peach (*P. persica*)	0	0	0	3	6	1	76	Susceptible
Nanking cherry (*P. tomentosa*)	3	4	2	1	0	0	22	Resistant

[a] 0 = no galls; 1 = 1–10 galls; 2 = 11–30 galls; 3 = 31–70 galls; 4 = 71–150 galls; and 5 >150 galls

[b] Gall index was calculated of ten replications, according to Liu [6], by the formula: Gall index = {[∑ (scale class number) × number of plants in this class)] / [(total number of plants) × 5]} × 100.

[c] Resistance rating of each rootstock was estimated by the gall index, with immune (gall index = 0), resistant (10.0% < gall index ≤ 50.0%), susceptible (gall index > 50.0%) rootstocks.

nematodes was observed in the presence of tomato diffusates. The numbers of hatched J2 nematodes were more than 300 in the presence of distilled water and all four *Prunus* rootstocks. These results indicate that resistance of the tomato cultivar "Baiguoqiangfeng" and *Prunus* rootstocks is not related to the effects of root diffusates on hatching of *M. incognita* eggs.

Approximately 3% mortality was observed among J2 nematodes treated with root diffusates of Tsukuba-4, Tsukuba-5 and Nanking cherry, and 2.25% and 1.36% mortality was observed after treatment with diffusates from peach and tomato, respectively (*figure 2*). A mortality rate of less than 1.00% was observed among J2 nematodes treated with distilled water. Because mortality was lower than 3.5% among J2 nematodes, even for those treated with root diffusates of the resistant cultivars Tsukuba-4, Tsukuba-5 and Nanking cherry, these results indicate that resistance of *Prunus* rootstocks and the tomato cultivar "Baiguoqiangfeng" is not related to lethal effects of root diffusates on nematode juveniles.

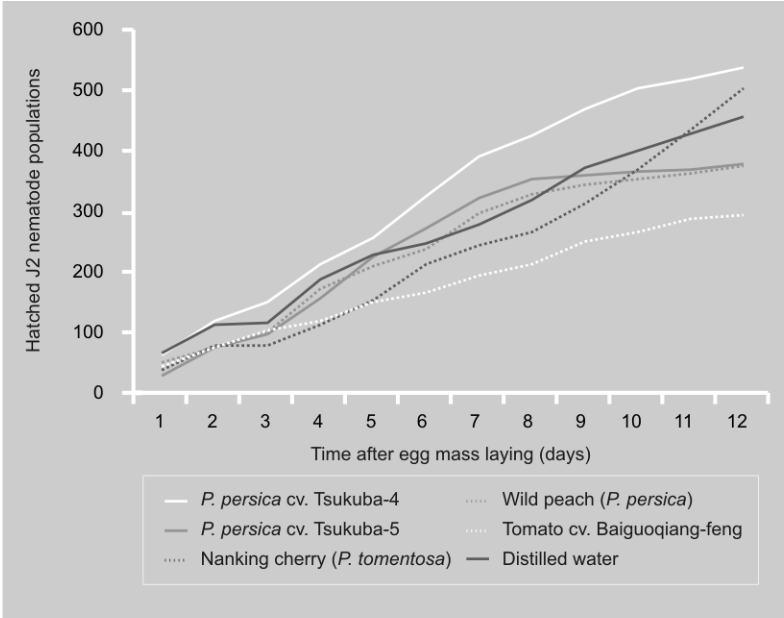


Figure 1.
Effect of four *Prunus* rootstock cultivars and tomato cv. Baiguoqiangfeng root diffusates on *Meloidogyne incognita* egg mass hatching in a Petri dish assay. Data are means of three replications.

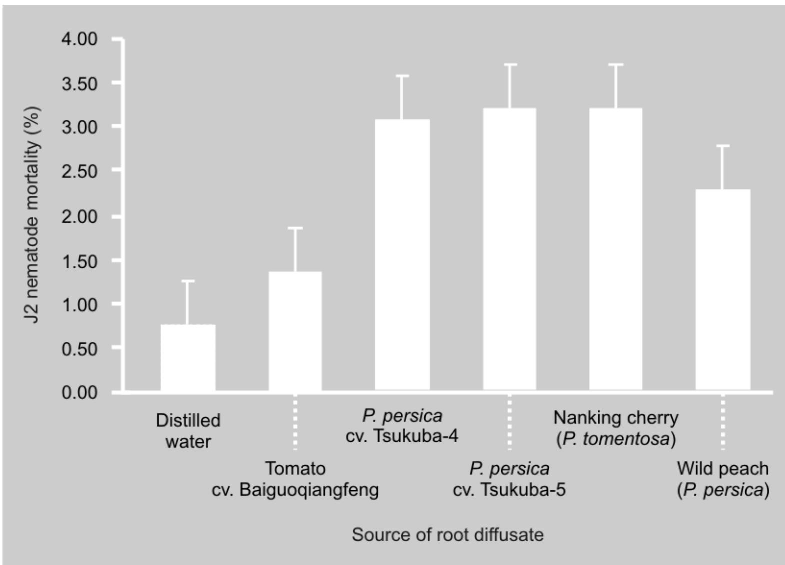
3.4. Influence of roots on J2 taxis

We examined whether a J2 nematode taxis induced by root segments was an indicator of plant nematode resistance level. In trial 1, J2 nematodes were observed close to root segments of all *Prunus* rootstocks and tomato after 4 h (*figure 3*). After 18 h, more J2 nematodes were observed close to root segments of Tsukuba-4 and Tsukuba-5 than around the other *Prunus* rootstocks and tomato. In trial 2, J2 nematodes were observed around all rootstock and tomato roots after 24 h (*figure 4*). This result indicates that the taxis of *M. incognita* J2 is not related to the plant nematode resistance.

3.5. Relationship between organization structure of root tip and penetration resistance

The resistant *P. persica* cvs. Tsukuba-4, Tsukuba-5 and Nanking cherry had small epidermal root cells, the susceptible peach cultivar had larger epidermal cells, and

Figure 2.
Effect of four *Prunus* rootstock cultivars and tomato cv. Baiguoqiangfeng root diffusates on *Meloidogyne incognita* egg mass hatching in a Petri dish assay. For each bar, the standard error is represented.



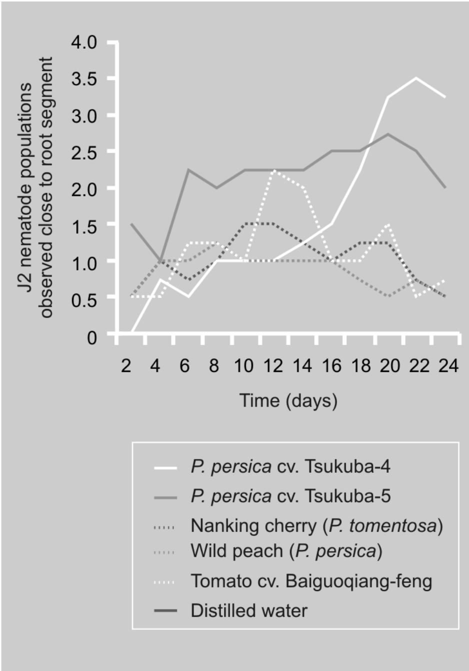


Figure 3.
Influence of four *Prunus* rootstock cultivars and tomato cv. Baiguoqiangfeng root diffusates on *Meloidogyne incognita* J2 taxis in a Petri dish assay. Data are means of four replications.

tomato had the largest cells (*figure 5*). Cells were tightly arranged in root tips of the resistant rootstocks Tsukuba-4, Tsukuba-5 and Nanking cherry. Cells were more loosely arranged in peach and tomato. In Tsukuba-4, Tsukuba-5 and Nanking cherry, a tightly arranged two-cell layer was located beneath the epidermal cell layer. This layer was also present in peach, but the cells were larger and more loosely arranged. This layer was absent in tomato. These observations indicate that the size and arrangement of epidermal cells and the presence of the sub-epidermal layer were related to resistance to *M. incognita*.

4. Discussion

In the penetration and development experiment, no J2 nematode was observed in roots of Tsukuba-4 and Tsukuba-5. In the experiment testing resistance of *Prunus* rootstocks, no galls were observed in the roots of *P. persica* cv. Tsukuba-4 and cv. Tsukuba-5. This indicates that these two cultivars seem to be immune. No J3–J4 nematodes were observed in Nanking cherry from D+3 to D+31. Moreover, *M. incognita* development from the J2 stage to female stage was delayed on Nanking cherry, so it

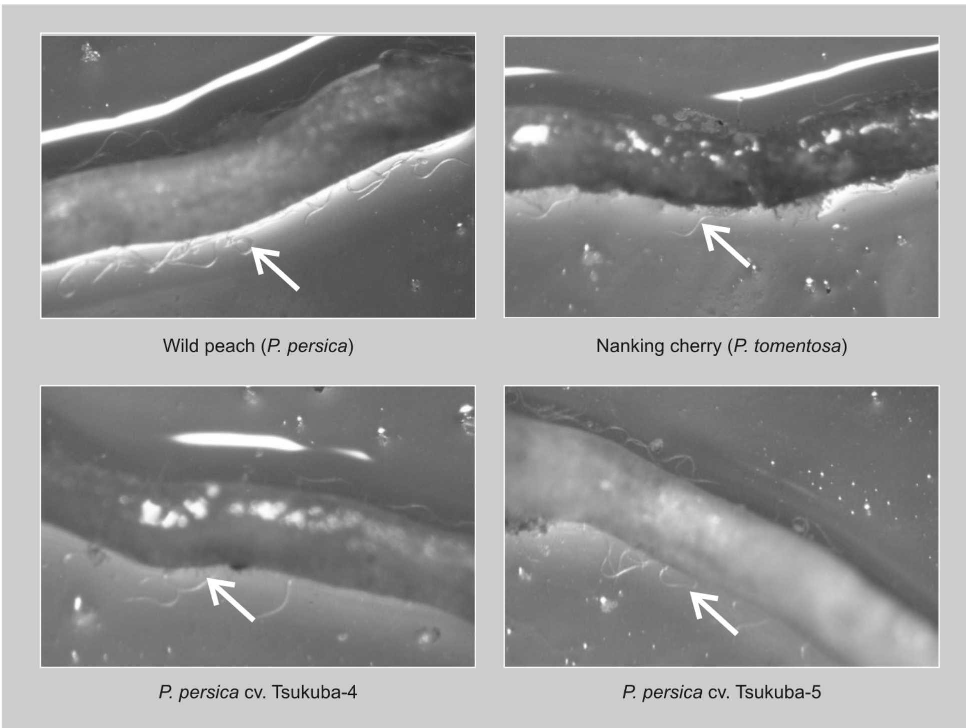


Figure 4.
Observation under a stereomicroscope of root tips of four *Prunus* rootstock cultivars and peach (*P. persica*) root diffusates on *Meloidogyne incognita* J2 taxis in a Petri dish assay. Arrows show the presence of *M. incognita* juveniles close to the root segment (50×).

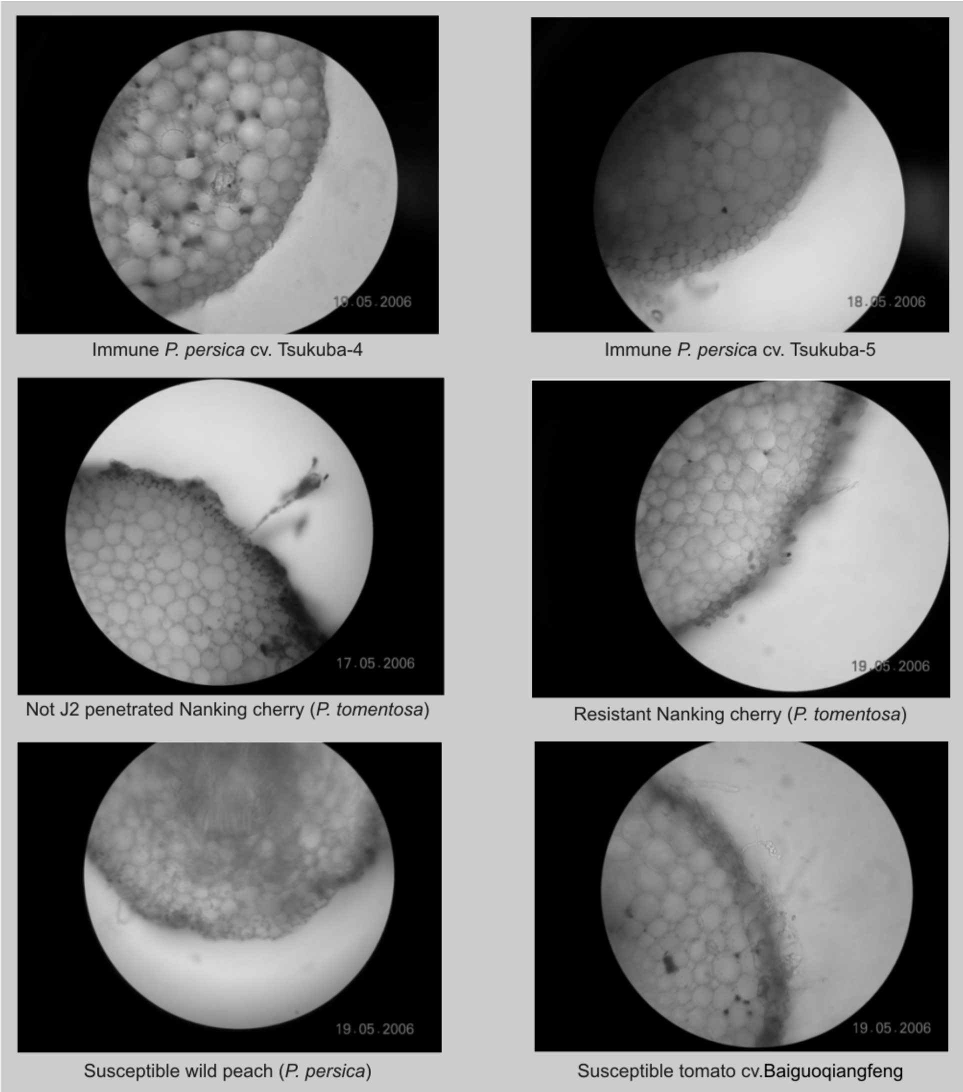


Figure 5.
Observation under a stereomicroscope of transverse sections of root tips of four *Prunus* rootstock cultivars and of tomato cv. Baiguoqiangfeng (400×).

can be considered as highly resistant to this nematode. Wild peach is susceptible to *M. incognita.*

The key factor in resistance at the pre-penetration stage is the proximity of J2 nematodes to the root [6]. Our data on root diffusate effects on *M. incognita* egg hatching showed that root diffusates of the resistant cultivar Tsukuba-4 promoted hatching, whereas diffusates from tomato inhibited hatching. After 12 days, the mortality rates of J2 nematodes treated with diffusates from all roots were less than 3.5%. Taken together, our results suggest that the effects of root diffusates on hatching and/or mortality were not significant factors in resistance.

We examined the influence of roots on J2 taxis. Our data showed that J2 remained alive even when close to root segments; thus, the resistance of the different plants was not determined by differences at the pre-penetration stage.

Resistance of Tsukuba-4 and Tsukuba-5 to *M. incognita* was due to the inability of J2 nematodes to penetrate the root. The major penetration points for J2 nematodes are the root-hair zone and the elongation zone of the root tip. The structural organization of these zones can affect J2 penetration [9, 15, 16]. Observation of root tip sections showed that epidermal cells in the resistant cultivars Tsukuba-4, Tsukuba-5 and Nanking cherry were small and tightly arranged. Beneath the epidermal cell layer there was another two-cell layer of tightly arranged cells, which were smaller than phloem parenchyma cells.

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Mecanismos de resistencia de los porta-injertos de *Prunus* en el nematodo de las raíces, *Meloidogyne incognita*.

Resumen — Introducción. Los nematodos de las raíces (*Meloidogyne* sp.) causan daños económicos significantes en las especies de *Prunus* en China. Uno de los métodos más económicos y más duraderos para el medioambiente para reducir los impactos de nematodos de las raíces, es el empleo de porta-injertos resistentes. Nuestro objetivo fue el de estudiar la resistencia a *M. incognita* y a sus mecanismos. **Material y métodos**. Los cuatro porta-injertos evaluados fueron Tsukuba-4 (*P. persica*), Tsukuba-5 (*P. persica*), el cerezo de Nanking (*P. tomentosa*) y un melocotonero silvestre (*P. persica*). Se empleó como testigo el cultivar de tomate (*Lycopersicon esculentum* Mill.) 'Baiguoqiangfeng' sensible. **Resultados**. Los nematodos no penetraron las raíces de los cultivares Tsukuba-4 y Tsukuba-5, los cuales se consideraron como variedades inmunes. El cerezo de Nanking mostró una gran resistencia a *M. incognita*, mientras que el melocotonero silvestre sí fue sensible. **Conclusión**. Las diferencias de la resistencia entre los porta-injertos no se atribuyeron a las diferencias de las sustancias segregadas por la raíz, sino a las diferencias de la estructura de las puntas de raíz. La estructura epidérmica de Tsukuba-4 y de Tsukuba-5 impidió completamente la penetración de los nematodos juveniles del segundo estadio de *M. incognita* (J2). En el cerezo de Nanking, la penetración de los juveniles J2 se redujo y el desarrollo de los nematodos, del estadio J2 al de la hembra, se retrasó.

China / *Prunus* / resistencia a las plagas / *Meloidogyne incognita* / portainjertos / ensayos de variedades / tejidos vegetales