Nematol. medit. (1996), 24: 37-42

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THE INFLUENCE OF DARKNESS ON THE RESISTANCE OF PEACH ROOTSTOCK 'NEMAGUARD' TO *MELOIDOGYNE INCOGNITA*

by
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Summary. A study was carried out to investigate the effect of light and darkness on the resistance of the peach rootstock 'Nemaguard' to *Meloidogyne incognita.* Varying lengths of darkness were applied at different times after nematode inoculation to establish the effect of increasing levels of root-knot infestation as well as the effect of increasing periods of darkness. When the nematode inoculated plants were completely shaded for four days or more, gall formation on the roots was observed; phenylalanine-ammonia-lyase (PAL) level in the roots decreased significantly and the content of soluble phenols decreased in the roots but increased in the leaves. If the plants were subsequently uncovered, the small galls previously formed during the darkening time disappeared; the PAL level increased greatly in comparison with that in natural condition; the content of soluble phenols did not change evidently. The peroxidase isozyme pattern of the roots did not show clear difference between the two treatments: a specific anionic band observed in the leaves of the shaded plants is postulated to be related to hydrolysis from insoluble phenols to soluble ones. The mechanism of the plant resistance to nematode is discussed.

The peach rootstock 'Nemaguard' is highly resistant to the root-knot nematode *Meloidogyne incognita.* This resistance is due to the walling-off of the giant cells initially induced by the nematode and which in susceptible roots provide a continuous source of nutrients for the nematode. In the resistant rootstock the giant cells gradually degenerate and as a consequence the nematodes die (Malo, 1967).

Plant response to pathogen attack includes the production of enzymes (Zacheo *et al.*, 1982, 1983) which may degrade the pathogen (chitinases or glucanases), or those whose products form a physiological barrier (lignin, hydroxyproline-rich glycoproteins, callose) or lead to the synthesis of defensive compounds (phytoalexins) (Sharon *et al.*, 1991) or to the breakdown of the root-knot induced giant cells (Graham and Graham, 1991). Some of the enzymes involved in the plant response to pathogen attack are dependent on light for their activation; for example phenylalanine-ammonia-lyase (PAL), which is the key enzyme of phenolic phenilpropanoid metabolism, is light-induced, being activated by light through phytochrome and deactivated in the dark (Harborne, 1980).

There are several report which closely relate PAL to resistance to plant pathogens (Ouyang and Xue, 1988) but only a few refer to nematodes.

The present study explores the possibility that lack of natural light induces susceptibility to root-knot nematode attack in 'Nemaguard' by deactivating the genes that require light for phenilpropanoid metabolism.

Materials and methods

One-year old seedlings of peach rootstock 'Nemaguard' (*Prunus persica* x *Prunus dasicarpa*) were cut off 20 cm above soil level in the spring, while the plant were still dormant. They were then transplanted to plastic pots containing sterilized soil and each pot was inoculated with 10,000 juveniles of *Meloidogyne incognita* (Kofoid *et* White) Chitw.

The experiment consisted of covering batches of seedlings with thick black paper for 72, 96 and 120 hours at 5, 8, and 15 days after nematode inoculation, this latter to establish increasing levels of *M. incognita* infestation in relation to the increasing period of darkness (Table I). Seedlings grown naturally acted as control. There were three replicates of each treatment.

At the end of the treatments the following data were collected: 1. number of galls on the roots per plant; 2. PAL activity: rootlets were washed thoroughly with distilled water and homogenized in ten volumes (w/v) of 0.2 M borate buffer, pH 8.8, with mercaptethanol and 0.2-0.3 mg of polyvinilpirrilidone (PVP) and quartz sand with mortar at 4 °C. This material was centrifuged at 20,000 g for 10 min, at 4 °C; the supernatant was used immediately for enzyme assay; the reaction mixture contained 1 ml of enzyme extract, 1 ml of distilled water and 1 ml of 0.02 M L-phenylalanine; the rate of enzymatic reaction was measured spectrophotometrically at 30 °C by following the increase in absorbancy at 290 nm after a period of 5 hrs; 3. protein content (by Lowrry); 4. soluble phenols: 20 mg of dried samples were extracted in 2 ml 80% aqueous methanol for 1 hr stirring, and reiterating it four times; after centrifugation at 10,000 g the pellet was re-extracted twice with 2 ml of the same solvent for 10 min; the combined extract was used for spectrophotometric determination of soluble phenols with Folin-Ciocateu solution at 650 nm; 5. electrophoretic analysis of peroxidase: starch gel electrophoresis was performed as described by Quarta and Arnone (1987). All data were statistically analyzed for differences between averages.

TABLE I - *Periods of darkness and light applied to 'Negmaguard' seedlings.*

Treatment	N° days after inoculation with *M. incognita*	Lengths of dark period (hours)	Lengths of uncovered period (hours)
1	5	72	96
	5	72	0
2	8	96	192
	8	96	0
3	15	120	168
	15	120	0
Control	5	0	72
	8	0	96
	15	0	120

Results

'Nemaguard' seedlings subjected to extensive periods of darkness developed small galls on the white fibrous roots, the number of galls increasing as the dark period was extended (Table II). However, there were very few galls on those plants that were exposed to natural light for 168 or 192 hours after the dark period. No galls were found on roots of plants that were kept in the dark for 72 hours (Table II).

PAL activity was significantly reduced when the plants were subjected to darkness, but there was a rapid recovery of activity when the plants were then exposed to light for about one week (Fig. 1).

The leaves and roots of treated plants differed with respect to their phenol content. Phenol decreased in the roots of plants exposed to periods of darkness and did not increase when the plants were re-exposed to light (Fig. 2). However, exposure to darkness increased the soluble phenols content in the leaves (Fig. 3)

TABLE II - *Number of galls recorded on* Meloidogyne incognita *inoculated 'Nemaguard' trees grown in different light conditions.*

Treatment		Number of galls
Darkness	Light	
72	–	0
96	–	3
120	–	10.3
72	96	0
96	192	0
120	168	1

which was then reduced when the plants were re-exposed to light; the main differences were found between dark and light at 72 and 120 hours.

Peroxidase isozyme patterns (Fig. 4) showed three anionic bands, designated A1, A2 and A3, and one cationic band, C1, in the roots. There were no differences among the three treatments. On the contrary, the leaves showed four anionic bands, designated from A1 to A4, with one visible cationic band. A1 was constitutive, stronger staining, and its level increased in the leaves of the plants grown in darkness. A2 only appeared in the leaves of these treatment. A3

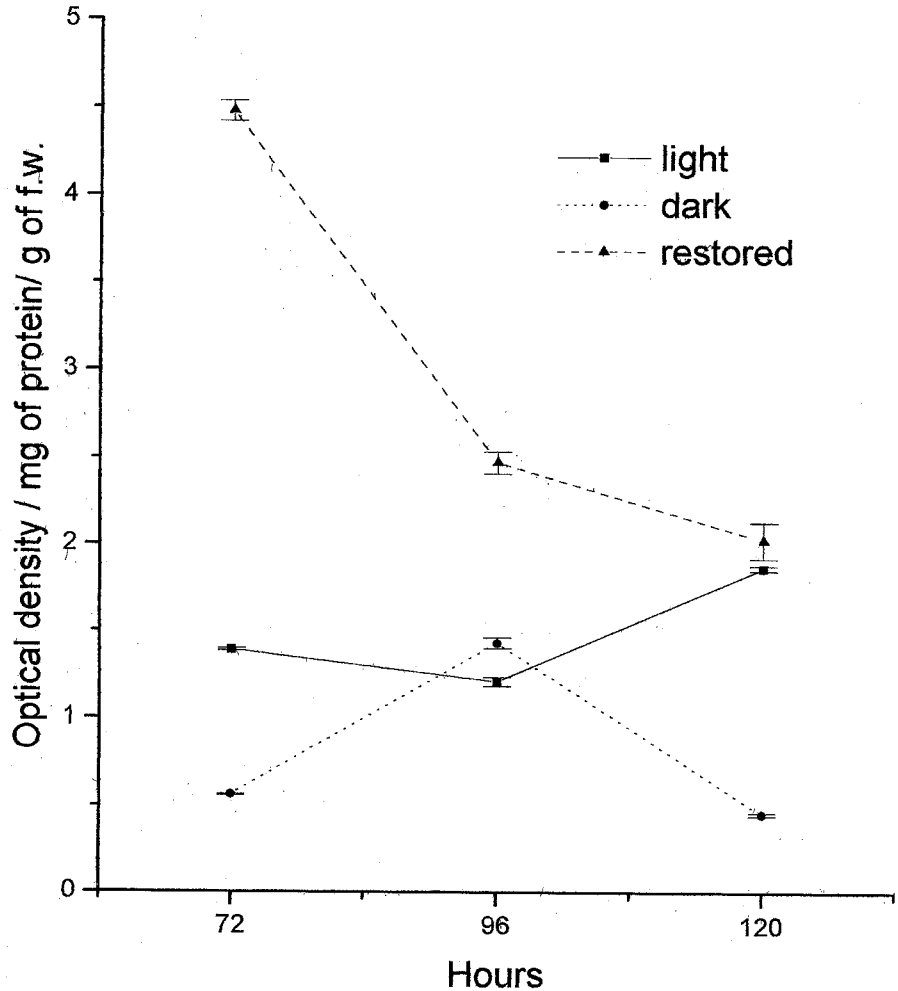

Fig. 1 - PAL activity in 'Nemaguard' plants grown in different light conditions for increasing time after inoculation with *Meloidogyne incognita.*

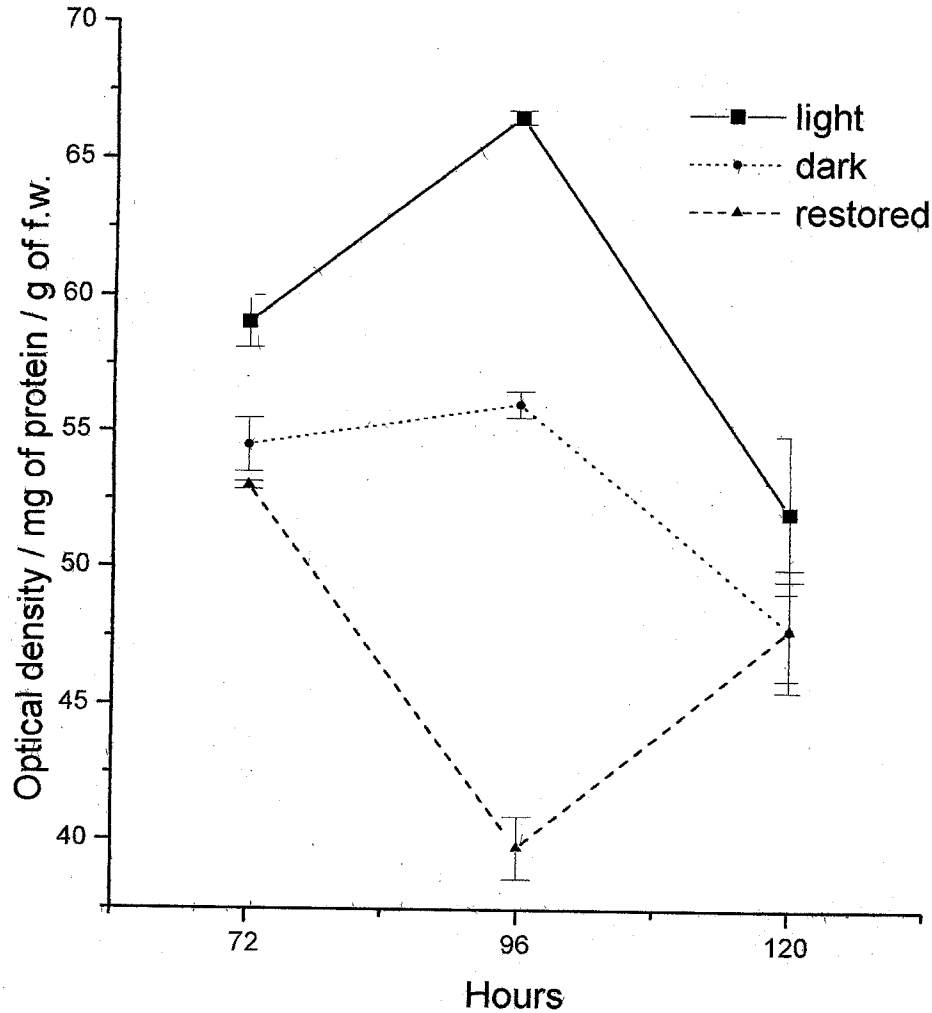

Fig. 2 - Soluble phenols in the roots of *M. incognita* inoculated 'Nemaguard' plants grown in different light conditions for increasing times.

and A4 were more weakly staining and there were no differences among treatments, although A3 was somewhat stronger in the plants grown in darkness.

Discussion

When *M. incognita* inoculated 'Nemaguard' were maintained in darkness for a long period, a few galls appeared in the roots and PAL activity decreased significantly. It is postulated that the decrease of PAL activity is linked with the decline in plant resistance, as already indicated with resistance to other pathogens (Dixon and Lamb, 1990; Ouyang and Xue, 1988). The products of the PAL reaction are phenolic compounds, and our experiment shows that in darkness they did not decrease as the PAL level in the roots, suggesting a mechanism of action of the light on the resistance reaction; it is postulated that in such a defensive process the peroxidases play a key role, causing the polymerization of phenolic substances (with thickening of cell walls) by the peroxidase isozymes, promoted by light, while the PAL is a prerequisite

for the subsequent event. Thus, when the plants were kept in darkness, PAL and peroxidase were inhibited, leading to the decline of nematode resistance. This can explain why, in our experiment, the soluble phenols content did not evidently decrease when PAL level dramatically decreased in the roots and why, when light conditions were restored, PAL level increased but phenols content did not.

It is also hypothesized that the 'Nemaguard' resistance to nematode infestation is directly related to the content of soluble phenols in the plants.

Literature cited

DIXON R. A. and LAMB C. J., 1990. Molecular communication in interaction between plants and microbial pathogens. *Ann. Rev. Plant Physiol. Plant Mol. Biol., 41*: 339-367.

GRAHAM M. Y. and GRAHAM T. L., 1991. Rapid accumulation of anionic peroxidases and phenolic polymers in soybean cotyledon tissues following treatment with *Phytophotora megasperma* f. sp. *glycinea* wall glucan. *Plant Physiol., 97*: 1445-1455.

HARBORNE J. B., 1980. Plant phenolics, pp. 329-331. In: *Secondary plant products* (Bell E. A. and Charlwood B. V. eds.) Springer-Verlag, Berlin.

MALO S. E., 1967. Nature of resistance of 'Okinawa' and 'Nemaguard' peach to root-knot nematode *Meloidogyne javanica*. *Proc. Amer. Soc. Hort. Sci., 90*: 39-46.

OUYANG G. C., and XUE Y. L., 1988. Physiological role and

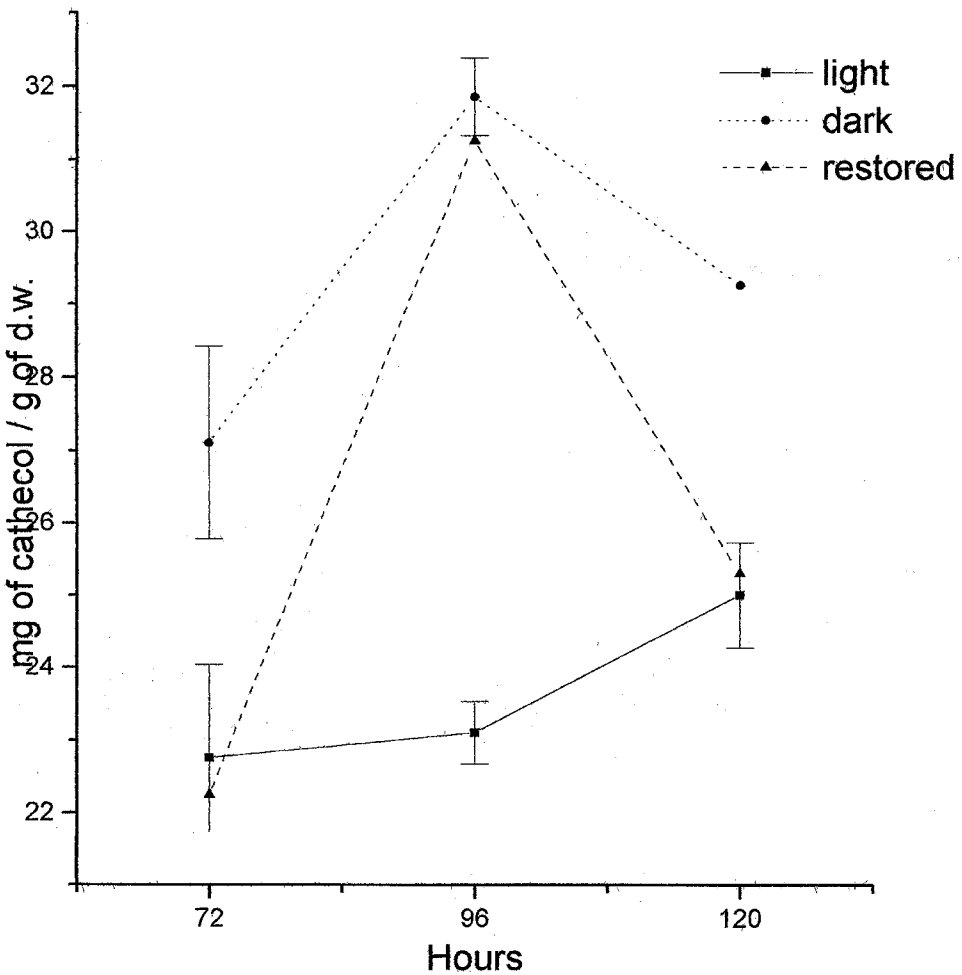

Fig. 3 - Soluble phenols in the leaves of *M. incognita* inoculated 'Nemaguard' plants grown in different light conditions for increasing times.

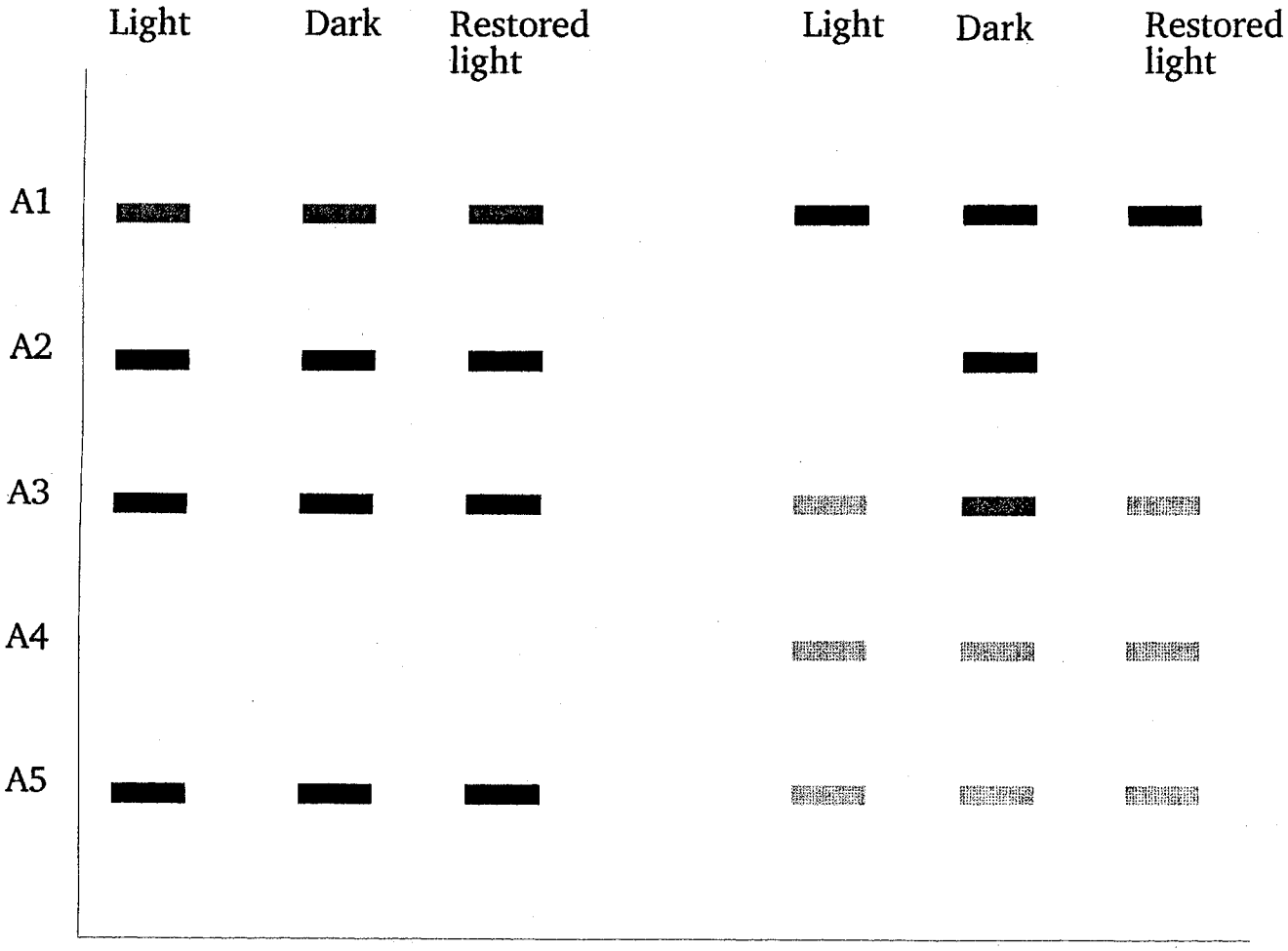

Fig. 4 - Peroxidasic isozymes pattern from roots and leaves of 'Nemaguard' peach rootstock; the plants were completely darkned for 72 hours five days after inoculation with *M. incognita*, then uncovered for 96 hours and analyzed.

regulation of phenylpropanoid metabolism in plant. *Plant Physiol. Commun.*, 1988-(3): 9-16.

QUARTA R. and ARNONE S., 1987. Peroxidase polymorphism in apple cultivars. *Advances in Hort. Sci., 1*: 83-86.

SHARON A., AMSELLEM Z. and GRESSEL J., 1991. Glyphosate suppression of an elicited defense response. *Plant Physiol., 98*: 654-659.

ZACHEO G., ARRIGONI-LISO R., BLEVE-ZACHEO T., LAMBERTI F. and ARRIGONI O., 1983. Mitochondrial peroxidase and superoxide dismutase activities during the infection by *Meloidogyne incognita* of susceptible and resistant tomato plants. *Nematol. medit., 11*: 107-114.

ZACHEO G., BLEVE-ZACHEO T. and LAMBERTI F., 1982. Role of peroxidase and superoxide dismutase activity in resistant and susceptible tomato cultivars infested by *Meloidogyne incognita*. *Nematol. medit., 10*: 75-80.

Accepted for publication on 29 September 1995.