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Establishment and Distribution of Pea Primary Root Nodules (*Pisum sativum L.*) as Affected by Shading*

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Abstract

In order to investigate the determinism of nodules establishment on the primary root, pea plants (*Pisum sativum L.*) were grown in the field at two irradiance levels (50% shading–0% shading). It appeared that complete establishment of visible nodule took place during a short period between the two-leaf and six-leaf stages (250° and 500° days from sowing) in both treatments. Aerial dry matter (DM) and nodule DM were decreased significantly by shading after the 4-leaf-stage. In contrast, the number of nodules was equal in both treatments. In both treatments, the uppermost nodules (95%) were located in the first 10 cm of the root, although its elongation was over 40 cm. The distribution of the nodules presented two characteristics: (1) nodules were clustered in some peaks. (2) the number of nodules per peak decreased with the increasing distance below the seed. Those characteristics suggested a regulation of nodules distribution in field and may be compared with those obtained on hydroponically grown seedlings.

Introduction

Nodulation is a necessary step for the biological nitrogen fixation of legumes. Plant nodulation characteristics are useful indicators of strain population (Damirgi et al., 1967) and/or environmental factors such as soil pH (Evans et al., 1988), aluminium content, nitrate content and temperature (Oghogorie and Pate, 1971; Munevar and Wollum, 1981). Plant nodulation may also provide an index of N_2 fixation (Herridge, 1988) especially during the nodule growth period.

A broad range of evidence indicates that nodulation is self regulated and optimized by the host. However, most studies on the distribution of nodules have been carried

*Reviewed

out on the primary root of seedlings grown hydroponically in plastic pouches (Bhuvaneswari et al., 1980). When soil and strain-cultivar variation were added, Pueppke (1986) showed that the distribution of nodules was modified.

The present study reports a part of the results from a research program dealing with nodulation of field-grown pea under various irradiance levels and soil structure. Under French soil conditions, natural *Rhizobium leguminosarum* (biovar viceae) populations form nodules on both primary and lateral roots. Nodulation takes place, first on the primary root (Pate, 1958). In the present experiment our objective was to study how photosynthetic levels (i.e. growth rate) affect nodule establishment and distribution.

Materials and Methods

Plant culture

Pea plants (*Pisum sativum* v. Solara) were sown at 90p/m^2 on 30 march 1989. Soil structure, water content, soil fertility (P.K. low N mineral) were controlled in order to limit nodulation as little.

Irradiance levels

Two levels were used, 0 and 50% of total light intercepted. Cages (height = 1.50 m area = 3 m × 1.5 m) were installed before emergence and left during the whole cycle.

Sampling procedure

Groups of 15 plants with their primary roots were sampled at 2-8-day intervals between pre-emergence and 7-leaf-stage. On the whole 7 dates were observed. Roots were sampled with a spade and washed. In addition, at each date, several roots were observed, *in situ*, in a trench-profile (Shuurmann and Goedewaagen, 1971). The trajectory of the root was reported on a transparent sheet applied on the smooth vertical surface of the soil profile. The position of the seed, the soil surface, the seed-bed and the ploughed layer were also indicated.

Recordings from samples

Shoot. Developmental stages (Maurer et al., 1965) and Dry Matter (DM).

Nodule establishment. Number and DM/root. Nodules with several lobes were counted as single nodules.

Nodule distribution along the root. Among the 15 plants sampled, subsamples were made to quantify the number of nodules per cm of primary root. Distribution was also studied on the roots which were observed *in situ*.

Root. DM and length were measured. Surface was considered as a cylinder and calculated per cm of root.

Nitrogenase activity. It was estimated by the acetylene reduction assay (ARA) as described by Hardy et al. (1973). Total and specific ARA were estimated at 7-8 leaf-stage, on 10 plants-3 replicates.

Results

Number

Establishment of primary root nodules was fast and took place in a short period between the 2- and 6-leaf-stages (250 and 500° days from sowing). Maximum number was reached at 6-leaf-stage. After this stage, it began to decrease (Fig. 1). The number was identical in both treatments until the 6-leaf-stage. Nodules with several lobes 20% of the total number) were present in the non-shaded treatment only. If multiheaded nodules were counted as single nodules, the number was equal in both treatments during the whole establishment period.

Lateral root nodules occured as nodule number on the primary root was maximum.

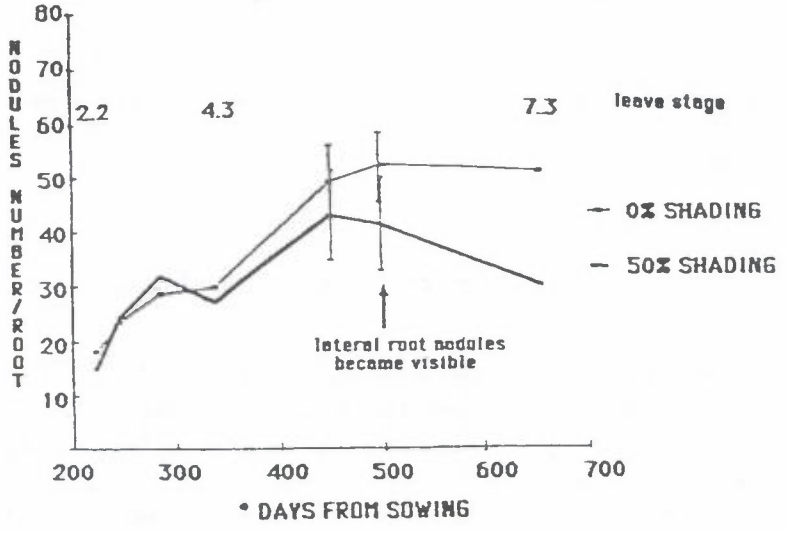

Figure 1. Effect of shading on evolution of nodules number per primary root. At each date, 15 plants were sampled. Nodules with several lobes were only present in the non-shaded treatment. They were counted as single nodules.

Nodule growth

Shading strongly affected nodule growth (Fig. 2). DM was significantly affected by shading at each date. The effect of shading on nodules DM became important at the 6-leaf-stage, when aerial DM became also affected (Fig. 2).

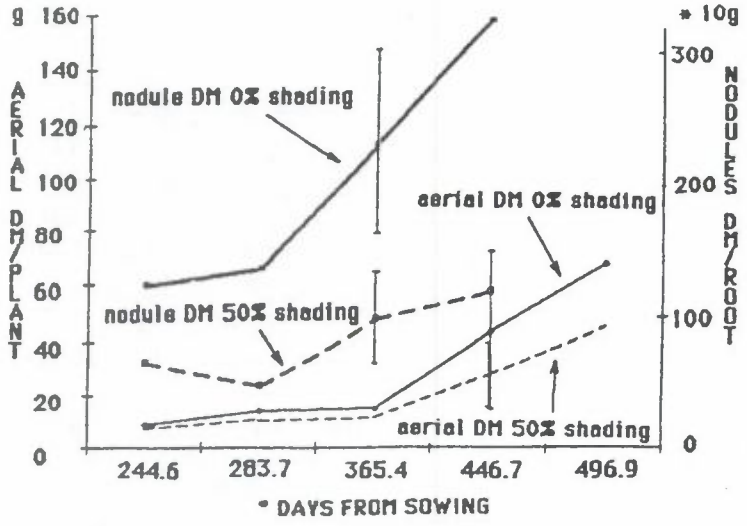

Figure 2. Effect of shading on nodules and shoot growth. Each value is the mean of 15 measures. Nodules DM is only given from the 2-3-leaf stage onwards, as they are too small to be excised before this state.

Nodule functioning

Total and specific ARA were significantly decreased by shading (Table 1.).

Nodule distribution along the primary root

The uppermost (90 to 100%) nodules were located in the first 10 cm of the root although the total primary root length may reach 40 cm. Observations made *in situ* showed that this 10 cm length was reached before emergence. Distribution curves in both treatments were typical:

(1) nodules were clustered in some peaks with a period that seems constant (2-3 cm); see Table 2.

(2) the number of nodules per peak decreased with the increasing distance below the seed. It appeared that neither change of soil structure between the seed-bed and ploughed layer nor change of root surface could explain this decrease. See Fig. 3 for example of this distribution on a primary root.

Table 1. Effect of shading on nitrogenase activity. Nitrogenase activity is estimated at 7-8-leaf-stage on excaved primary roots (10×3 replicates) by the acetylene reduction assay (ARA). Specific ARA is reported as umoles of C_2H_4/h.g of nodule DM, total ARA as umoles of C_2H_4/h. plant.

ARA	0% shading	50% shading
total		
mean	479	34
standard error	68	12
specific		
mean	11	2.8
standard error	1.5	0.6

Table 2. Types of repartition from 10 primary roots.

number of primary root observed	number of peaks	mean period (cm)
4	2	$3{,}1 \pm 1{,}2$
2	3	$3{,}0 \pm 1{,}0$
2	4	$3{,}1 \pm 0{,}9$
2	6	$2{,}7 \pm 0{,}7$

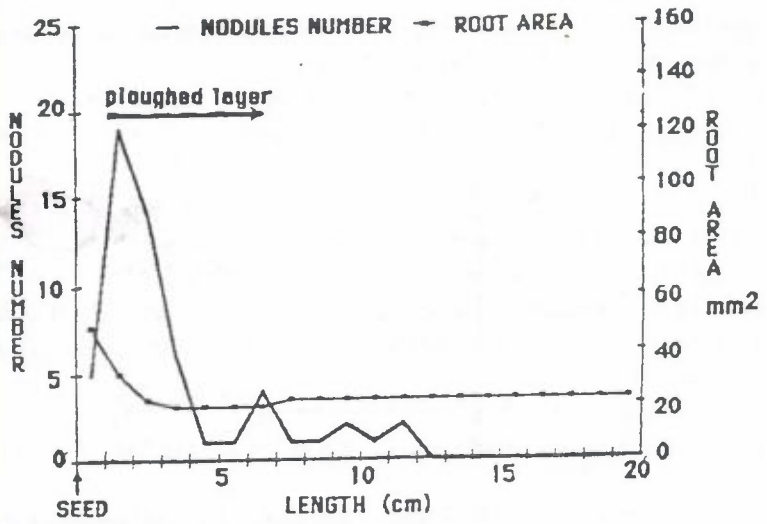

Figure 3. Example of a typical distribution of nodule on a primary root. The root is divised into segment of 1 cm length and nodules number is given per segment. Area of each segment is calculated. Positions of the seed and of the ploughed layer are also reported.

Discussion

Establishment of primary root nodules occurred during a short period and the maximum number was fixed very early in the cycle. The number is independent from the growth of the shoot, on the contrary, nodule growth as well as specific activity were strongly affected by shading. The shape of the nodules seems to be affected by the shoot growth. The 10 cm root length where uppermost nodules appeared were elongated and probably infected before emergence. It suggests that nodule number establishment depends on the seed reserves whereas growth and functioning are subsequently affected by the growth of the shoot. Distribution curves however suggest a mechanism of regulation dependent on the plant as described by Pierce and Bauer (1983). Data confirm that regulation also exists in field conditions. A further study would show which factors or conditions stop nodule primary root establishment. The establishment of lateral root nodules should also be taken further in consideration.

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