Plant and Soil XXX, no. 3 June 1969

NON-PHOTOSYNTHETIC EFFECTS OF RED AND FAR-RED LIGHT ON ROOT-NODULE FORMATION BY LEGUMINOUS PLANTS

by T. A. LIE

Laboratory of Microbiology, Agricultural University, Wageningen, The Netherlands

INTRODUCTION

Root-nodule formation by leguminous plants is dependent on the supply of carbohydrates resulting from photosynthesis. The effect of light on nodulation may therefore be due to its influence on photosynthesis thereby influencing the carbohydrate–nitrogen relationship in the plant [9 11]. However, it has been found recently [5] that leguminous plants, grown under light conditions resulting in the production of the same amount of dry matter, produce more nodules when the shoot is exposed to red light than when exposed to blue light. It was also observed that exposure of the shoot to far-red light (730 mμ) for a few minutes at the end of the photoperiod, reduced the number of nodules formed. The inhibitory action of far-red light on nodule formation was partly reduced by subsequent irradiation with red light (660 mμ), suggesting that the nodulation process, like many other processes in the plant, is controlled by the phytochrome system [1 4 7].

So far, only the shoots have been exposed to light of different wave lengths and the effect of the light treatment on nodulation might have been due to its morphogenetic effect on the shoot, which is usually manifested as an effect on shoot elongation. By using rooted leaves and decapitated plants, *i.e.* plant systems with limited shoot growth, differences due to shoot elongation were virtually reduced [5]. It seemed desirable, however, to investigate if red and far-red light, when applied directly to the roots, also affect nodulation. Such an effect might be anticipated since phytochrome can be detected in the roots of etiolated pea plants [2]. In addition it has been shown that the formation of lateral roots in pea seedlings of a certain age, is controlled by the red/far-red light system [3]. The inhibitory action of daylight on the nodulation of pea plants was demonstrated by Rudin [8] by exposing the roots to light for prolonged periods of time. The inhibitory effect increased proportionally as the exposure time was extended to 12 hours per day. Blue light seemed to be particularly effective in reducing the number of nodules whereas red light was inactive.

In the present investigation the roots of leguminous plants were treated with red and far-red light for a few minutes only. Both normal and etiolated plants were used.

MATERIALS AND METHODS

Seeds of *Pisum sativum* cv. Rondo and *Vicia faba* cv. Niki were surface-sterilized by shaking in 3% H_2O_2 containing a drop of a detergent (Teepol). The seeds were allowed to germinate under aseptic conditions on water agar (1%) at 25°C for 5–7 days, when they were wrapped in sterile cotton wool and transferred to tubes containing 180 ml nutrient solution of the following composition:

K_2HPO_4, 0.36 g; KH_2PO_4, 0.12 g; $MgSO_4.7\,H_2O$, 0.25 g; CaSO4, 0.25 g; Fe (III) citrate, 30 mg; $MnSO_4.4\,H_2O$, 1 mg; $ZnSO_4.7\,H_2O$, 0.25 mg; $CuSO_4.5\,H_2O$, 0.25 mg; H_3BO_3, 0.50 mg; $Na_2MoO_4.2\,H_2O$, 0.05 mg; 1000 ml water.

After a further week the plants were inoculated with *Rhizobium* and transferred to preserving jars (2 plants per jar) of 360 ml capacity, each containing 250 ml nutrient solution. The plants were grown in a light cabinet under fluorescent light (Philips Tl 33, 120 watt, 30,000 ergs/cm^2 sec). The temperature during the light period (8 hours per day) was 24°C and during the dark period (16 hours per day) 18°C. The relative humidity in both instances was kept at *ca* 70–80%.

Red light (R) was supplied directly from Philips Tl 10 fluorescent light (12000 ergs/cm^2 sec) without filtering. To obtain far-red light (I) incandescent light (Comptalux 150 Watt, Philips) was filtered through 10 cm water and then through a red and a blue filter (Röhm and Haas, Darmstadt no. 501 and no. 627, respectively). The light intensity was ca. 50 000 ergs/cm^2 sec.

When the shoots were treated, the jars containing the plants were placed directly in the light. When only the roots were being exposed to light, the plants were removed from the jars, the shoots wrapped in aluminium foil and the roots placed in a shallow tray containing nutrient solution. During exposure to light the roots were turned several times to ensure uniform treatment of the whole root system. The plants were irradiated at the end of the photoperiod and, after the last light treatment they were kept in complete darkness until the following photoperiod. Usually the light treatments were given during the 5 days following inoculation.

Etiolated plants were obtained by sowing the seeds in large tubes (30 × 2.5 cm) containing sterile perlite moistened with nutrient solution. The tubes were placed in wooden boxes wrapped with aluminium foil. After about two weeks the etiolated seedlings were inoculated and the cotton wool removed; these treatments were carried out under white light of low intensity.

Rhizobium strains PRE and P8, effective and ineffective respectively for both pea and broad bean, were maintained on yeast-mannitol-agar slopes. For inoculation a 5-day-old culture was suspended in 10 ml of sterile water and 1 drop of the suspension was added to each jar.

RESULTS

A. PLANTS GROWN IN LIGHT

In this section the results of experiments with plants grown in light cabinets are given. The first experiments in this section are concerned with root-irradiated plants. Subsequently a comparison is made between root and shoot-irradiated plants. Finally an attempt is made to localize the action of far-red light in the nodulation process.

1. *The effect of varying exposure times to red and far-red light on nodulation*

In order to study the effect on nodulation of increasing length of exposure to light, the roots of pea plants were irradiated for 1–30 minutes daily with either red or far-red light during the 5 days following inoculation. The number of nodules, counted 11 days after inoculation, are shown in Fig. 1. Exposure to red light caused only a slight reduction in nodule number and no dose-response relationship was found. On the other hand the number of nodules decreased with increasing exposure to far-red light, up to 15 minutes per day. No further decrease was observed with increasing time of exposure to far-red light; this indicates that treatment of the roots for 15 minutes per day during the 5 successive days, following inoculation, is sufficient to produce a maximal effect.

2. *Interaction of red and far-red light on nodulation*

The interaction of red and far-red light on nodulation was studied by treating the roots of pea plants as followed: C, I, R, I-R, I-R-I,

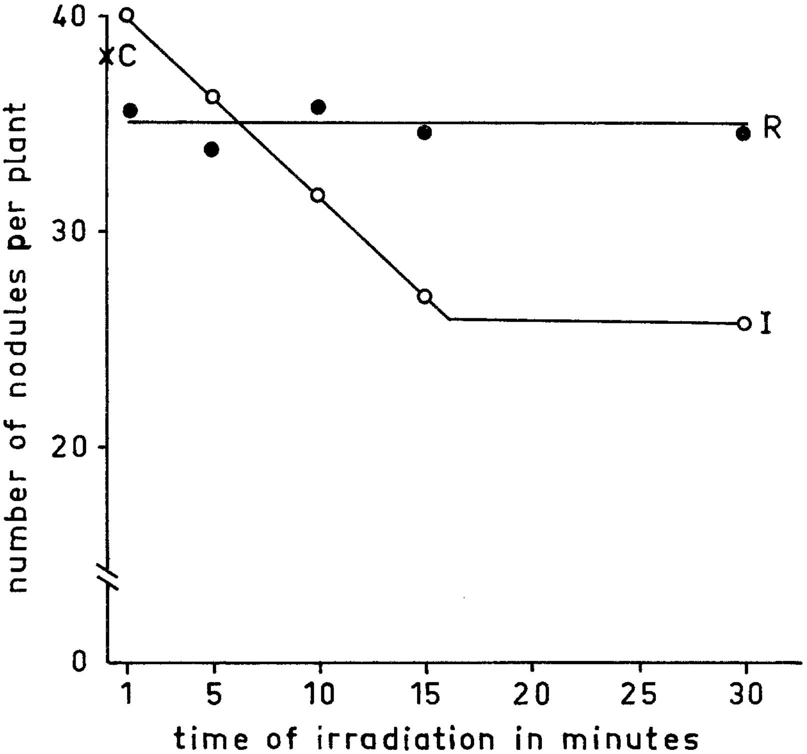


Fig. 1.The effect on nodule formation of increasing length of time of exposure of the roots of pea plants, inoculated with *Rhizobium leguminosarum* strain PRE, to red (R) or far-red (I) light.

I-R-I-R (C = control, roots not irradiated; I = exposure to far-red light for 15 minutes per day; R = exposure to red light for 15 minutes per day).

The number of nodules which had developed 15 days after inoculation, are given in Table 1. As was observed in the experiments with shoot-irradiated plants [5] it was found that when roots were exposed to red light this partly eliminated the inhibitory effect of exposure to far-red light. When after the exposure to red light the roots were then exposed for the second time to far-red light, the numbers of nodules developing dropped to a lower level than occurred after one light treatment. Further treatment with red light again reduced the inhibitory effect of far-red light on nodulation. These observations

TABLE 1

The reversibility of the effect of red (R) and far-red (I) light on nodulation * of pea plants inoculated with *Rhizobium leguminosarum* strain PRE. The roots were exposed to red and/or far-red light for 15 minutes per day at the end of the photoperiod

C (untreated)	I	R	IR	IRI	IRIR
49.1	28.5	46.2	39.2	23.1	27.6

* Mean number of nodules per plant of 10 replicates.

clearly demonstrate the interaction of the effects of application to roots of red and far-red light on nodulation.

3. Comparison of the effect on nodulation of exposure of roots and/or shoots of plants to far-red light

In this experiment a comparison was made between pea plants whose roots had been exposed to far-red light (I_r), with those whose shoot had been exposed (I_s) and with those whose shoot and roots (I_{r+s}) had been exposed. There were two control series, one (C) in which the roots remained undisturbed as in the case of shoot-irradiated plants and a second (C_r) in which the roots were disturbed as in the case of root-irradiated plants except that the light treatment was omitted. The results are given in Table 2. Plants whose roots had been disturbed (C_r) developed considerably fewer nodules than the plants (C) whose roots remained undisturbed. Obviously, merely transferring of the plants to the trays resulted in reduced nodulation. When the first two countings are taken into consideration it will be observed that exposure of the roots to far-red light

TABLE 2

The effect of exposure of root (r) or shoot (s) to far-red light (I) on nodulation * and growth * of pea plants inoculated with *Rhizobium leguminosarum* strain PRE

Treatment	Nodule number at			Dry weight (mg)		Total nitrogen (mg)	
	7 days	10 days	21 days	Shoot	Roots	Shoot	Roots
C, untreated	20.9	72.1	92.3	136	77	5.5	2.4
I_s	10.7	45.1	57.9	136	72	5.8	2.4
C_r, roots disturbed	13.5	40.2	63.7	134	79	5.6	2.3
I_r	0.9	14.0	105.6	110	74	4.6	2.2
I_{r+s}	0.8	17.9	99.0	123	77	5.0	2.4

* Mean values per plant of 10 replicates.

reduced nodulation more markedly than exposure of the shoot. However, the root-irradiated plants recovered from the light treatment and eventually developed a large number of nodules. In contrast, exposure of the shoot to far-red light had a more permanent effect, presumably associated with excessive shoot elongation (Plate IA). Exposure of the roots to far-red light did not result in excessive stem elongation. When the whole plant was exposed to far-red light (I_{r+s}) almost the same results were obtained as in the case of plants whose roots were only irradiated.

Subsequently a comparison was made of nodulation by the roots of broad bean plants whose roots or shoots had been exposed to far-red light. The results, obtained 11 days after inoculation, are given in Table 3. Nodulation of the main root was only slightly affected by

TABLE 3

The effect of exposure of root (r) or shoot (s) to far-red light (I) on nodulation *, lateral root formation * and stem elongation * of *Vicia faba* inoculated with *Rhizobium leguminosarum* strain PRE

Treatment	Number of nodules on			Number of lateral roots	Number of nodules per lateral root	Length of the 4th internode (mm)
	main root	lateral roots	total root system			
C, untreated	8.2	63.6	71.8	35.5	1.79	29.7
I_r	9.7	43.2	52.9	32.7	1.32	28.7
I_s	10.8	43.7	54.5	34.7	1.27	37.8

* Mean values per plant of 6 replicates.

exposure to far-red light. In contrast, nodulation of the lateral roots was significantly reduced by exposure to far-red light. No significant differences were found between the number of nodules developed by plants whose roots or shoots had been exposed to far-red light. As in the case of pea plants it was observed that exposure of the shoot resulted in enhanced stem elongation, whereas exposure of the roots did not.

4. *Interaction between root and shoot irradiation*

So far, the interaction between red and far-red light on nodulation has been studied by irradiation either the shoot or the roots with with both kinds of light. The aim now was to ascertain whether there is any interaction on nodulation when the shoot is exposed to one kind of light and the roots to the other kind. The number of nodules developing 15 days after inoculation are given in Table 4. It will be

TABLE 4

Interaction of red (R) and far-red light (I), when applied to the shoot (s) or to the root (r) on nodulation * of pea plants inoculated with *Rhizobium leguminosarum* strain PRE

C, untreated	I_r	I_rR_r	I_rR_s	I_sR_r	R_r
49.6	28.5	39.2	26.8	30.9	46.2

* Mean number of nodules per plant of 10 replicates.

observed that only when the same kind of plant organ was exposed to both kinds of light was there any interaction. Exposure of the shoot to red light did not reduce the inhibitory effect on nodulation of exposure of the roots to far-red light. In agreement with the results given in Fig. 1 it was found that exposure of the roots to red light results in slightly decreased nodulation.

5. *The effect on nodulation of exposure to far-red light at different intervals of time from inoculation*

In the above-mentioned experiments the plants were exposed to far-red light during the 5 consecutive days following inoculation. Since the first macroscopic nodules were observed on the 5th–7th day after inoculation, it was impossible to distinguish which part of the nodulation process was affected by exposure to the light. To ascertain which part of the nodulation process was being affected, the exposure to light was reduced to a single exposure at different intervals of time from inoculation. At first only the shoots were exposed to light but later on the roots were also irradiated.

Exposure of the shoots to far-red light. The results obtained with shoot-irradiated plants are given in Table 5. From these data it can be concluded that exposure to far-red light before inoculation had no effect on nodule numbers. Obviously the symbiotic system and not the plant is affected by this treatment. Exposure to light on the day of inoculation or on the following day did not reduce the number of nodules formed. However, exposure of the shoots to far-red light on the second day following inoculation or on subsequent days resulted in marked reduction in the number of nodules

TABLE 5

The effect of a single exposure to far-red light (I), applied to the shoot at different days, before (−1, −2 days), during (0) or after (1, 2, 3, 4, 5 days) inoculation, on nodulation * of pea and broad bean plants inoculated with *Rhizobium leguminosarum* strain PRE

Treatment	*Pisum sativum* Number of nodules on total root system		*Vicia faba* Number of nodules on		
	Experiment 1	Experiment 2	main root	lateral roots	total root system
C, untreated	14.1	15.0	8.8	61.5	70.3
I_{-2}	14.7	—	—	—	—
I_{-1}	11.3	14.6	—	—	—
I_0	15.7	15.1	9.5	66.7	76.2
I_1	—	15.3	9.3	69.7	79.0
I_2	6.6	11.6	6.5	55.9	61.4
I_3	—	—	10.6	47.4	58.0
I_4	—	—	10.0	41.6	51.6
I_5	8.9	13.9	—	—	—
I_{0-4} †	—	—	10.8	43.7	54.5

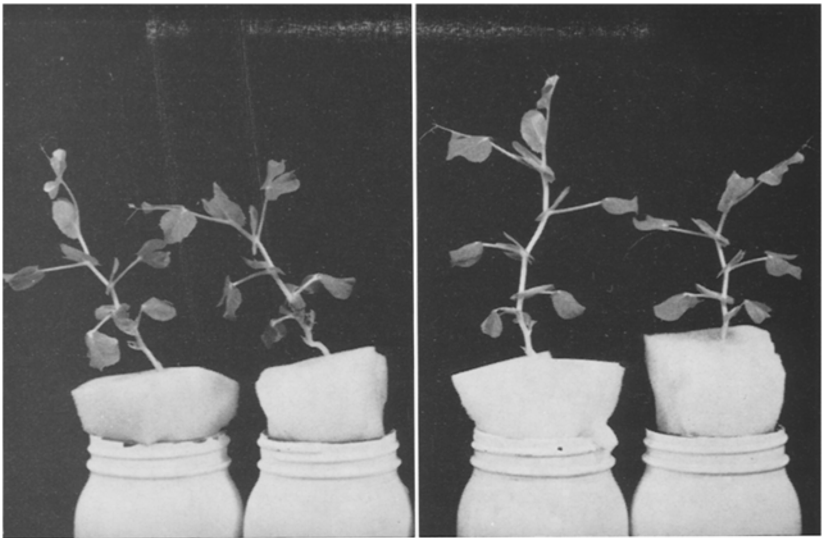
* mean values per plant of 10 pea or 6 broad bean plants.

† I_{0-4}, shoot exposed to far-red light during four days after inoculation

formed. In the case of pea plants exposure to far-red light on the 5th day after inoculation resulted in less pronounced reduction in the number of nodules formed than exposure to light on the second day. From the experiments with *Vicia faba* it is clear that a single exposure to far-red light on the 3rd or 4th day after inoculation had almost the same effect as exposure of the roots during 4 days (I_{0-4}).

Exposure of the roots to far-red light. In Table 6 the effect of exposure of pea roots to a single treatment with far-red light is given. When the numbers of nodules were counted 8 days after inoculation, only slight differences were found between the untreated plants and those whose roots had been exposed to far-red light at 0, 1 and 2 days after inoculation, respectively. Irradiation of the roots at the 3rd day after inoculation resulted in a strong inhibition of nodule formation. When the interval between inoculation and exposure to far-red light was wider (I_4 and I_6) the inhibitory effect on nodulation was decreased. At the second nodule count all the irradiated plants had fewer nodules compared with those which had not been irradiated, but the strongest inhibition of nodulation was again observed in those plants treated on the 3rd and 4th day after

A



B

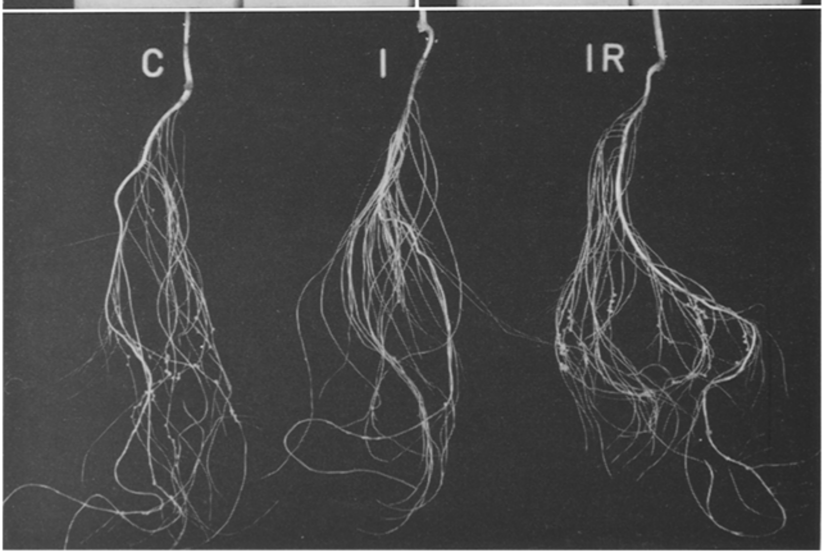


Plate 1**A.** The effect of far-red light, when applied to the roots or to the shoot, on stem elongation of pea plants. From left to right: C, control, untreated; I_r, roots exposed to far-red light; I_s, shoot exposed to far-red light; C_r, roots disturbed but not exposed to far-red light.

B. The inhibitory effect on nodulation of the roots of pea plants, inoculated with *Rhizobium leguminosarum* strain PRE, of exposure to far-red light (**I**) and reversal of this inhibition by subsequent exposure of the roots to red light (**IR**). **C**, control, not exposed to light.

TABLE 6

The effect of a single exposure of the roots to far-red light (I) during the day of inoculation (0) with *Rhizobium leguminosarum* strain PRE and on days 1, 2, 3, 4, and 6 after inoculation, on nodulation of pea plants

Treatment	Number * of nodules at ... days after inoculation			Fresh weight of nodules at harvest (mg per nodule)
	8 days	16 days	28 days (harvest)	
C, untreated	19.9	49.1	79.8	3.1
I_0	17.5	31.1	38.8	3.7
I_1	17.4	32.1	38.9	3.5
I_2	22.9	38.6	42.8	3.3
I_3	7.1	29.0	41.0	1.8
I_4	13.9	22.4	38.7	4.1
I_6	16.8	31.6	40.7	3.5

* Mean number per plant of 10 replicates.

inoculation. The differences in nodule numbers between plants irradiated at different times from inoculation were no longer detectable at the time of harvesting, but from measurements of the weights of the nodules (column 5) it may be seen that plants exposed to far-red light on the 3rd day after inoculation had only small nodules. This is due to the fact that in this case most of the nodules were formed at a later stage, presumably when the roots had recovered from exposure to far-red light.

The results from root-irradiated plants are in general agreement with those from shoot-irradiated plants. The strongest effect of far-red light was observed when the plants were irradiated during the 2–4 day period after inoculation. Only slight effects were observed when the plants were exposed to far-red light before or after this period.

B. ETIOLATED PLANTS

In the previous section the effect of red and far-red light on the nodulation of plants grown in the light was described. It therefore seemed of interest to investigate whether or not small amounts of red and far-red light would influence nodulation of plants otherwise grown in the dark.

Plants grown in the dark usually show little or no capacity to form root nodules [6 8 10]. This may be due to a shortage of carbohydrates, especially in the case of small-seeded plants. The addition of sugar to the culture medium sometimes increased, but usually decreased, nodulation. However, the inhibitory effect on nodulation may have been due to secondary factors *e.g.* high osmotic pressure of the culture medium or excessive multiplication of the nodule bacteria. Favourable results were obtained by McGonagle [6] using etiolated field peas. It is of interest to note that in the latter experiments a rather low temperature (13°C) was used. Furthermore, the plants were exposed to small amounts of red light during the periodic examinations. Therefore a re-examination of this aspect of the problem seemed worthwhile.

1. *Temperature*

Initially the effect of temperature on pea plants inoculated with the effective Rhizobium strain PRE was investigated. The plants were kept in complete darkness at 18, 22 and 25°C, respectively. The results are given in Table 7. Nodulation of plants decreased

TABLE 7

The effect of temperature on nodulation * of etiolated pea plants inoculated with *Rhizobium leguminosarum* strain PRE

Temperature	18°C	22°C	25°C
Percentage nodulated plants	80.0	71.4	28.7
Number of nodules	8.5	5.6	0.57
Shoot dry wt. (mg)	74.5	77.1	65.6
Root dry wt. (mg)	28.0	28.6	23.5

* Mean values per plant of 12–15 replicates.

with rising temperature; this may have been due to a shortage of carbohydrates resulting from a higher respiration rate at high temperatures (see dry-weight data of the plants). It is of interest to note that a similar plant/bacteria system, when grown in the light, had the highest number of nodules at 25°C (unpublished results). The nodules of plants grown in the dark at 18°C had an average diameter of 2 to 3 mm and had a pink colour, whereas those of plants grown at higher temperatures were smaller and were white in colour.

2. *Phytohormones*

The effect of phytohormones was also investigated in dark-grown plants. Indole acetic acid (IAA, 8.5 μg/plant) and 6-furfural-aminopurine (kinetin, 5 μg/plant) either singly or in combination, were given to pea plants inoculated with Rhizobium strain PRE. The plants were grown at 20°C in the dark and harvested after 45 days (Table 8). Instead of stimulation both phytohormones were found to

TABLE 8

The effect of application of IAA (8.5 μg per plant) and kinetin (5 μg per plant) on nodulation * of etiolated pea plants inoculated with *Rhizobium leguminosarum* strain PRE

	Control	IAA	Kinetin	IAA + Kinetin
% Nodulated plants	46.3	41.7	18.8	13.3
Number of nodules per plant	7.4	2.7	1.5	1.7

* Mean values of 12–15 plants.

give rise to a strong inhibition of nodulation at the concentrations used. Kinetin especially appeared to be a strong inhibitor of nodulation in etiolated plants; an observation which is in general agreement with previous experiments with light-grown plants [5]. No interaction between IAA and kinetin, in their effect on nodulation, was observed.

3. *Rhizobium strain*

The influence of the Rhizobium strain on nodulation was also examined. In addition to the effective Rhizobium strain PRE, the ineffective strain P8 was used for inoculation. The latter strain is known to produce a large number of white nodules on pea plants grown in the light. However, when etiolated plants were used there were virtually no differences between the behaviour of these two strains of Rhizobium. For example, in one experiment the percentage of nodulated plants resulting from inoculation with strains PRE and P8 was 36.6 and 39.3 respectively and the number of nodules 3.2 and 3.9 per plant, respectively.

4. *Red and far-red light*

Finally the effect of red and far-red light on nodulation in etiolated plants was studied by exposing the shoots to red and/or far-red light over a period of 5 days after inoculation as followed:

$$C;\ I_{15};\ I_{15}R_{15};\ I_{15}R_{15}I_{15};\ R_{15};\ R_{30}$$

(C = control, untreated; I_{15} = far-red light, 15 minutes per day; R_{15} = red light, 15 minutes per day; R_{30} = red light, 30 minutes per day). The results are shown in Table 9. In general, the differences

TABLE 9

The effect of red (R) and far-red light (I), applied to the shoot, on nodulation * of pea plants inoculated with *Rhizobium leguminosarum* strain PRE

	Control	I_{15}	$I_{15}R_{15}$	$I_{15}R_{15}I_{15}$	R_{15}	R_{30}
% Nodulated plants	80.0	75.0	87.5	78.6	87.5	92.3
Number of nodules per plant	8.9	7.1	7.4	6.2	7.8	5.6

* Mean values of 12–15 plants.

between the plants subjected to the different treatments were rather small. The percentage of nodulated plants increased slightly with exposure to increasing amounts of red light. However, at the same time the number of nodules per plant decreased. Far-red light reduced the percentage of nodulated plants and this effect was counteracted by exposure to red light. A subsequent second treatment with far-red light reduced the number of nodulated plants still more. An interaction between red and far-red light on the number of nodules was also observed but it is less clear.

GENERAL DISCUSSION

The results with root-irradiated plants confirmed the observations made with shoot-irradiated [5] plants that nodulation of leguminous plants is affected not only via photosynthesis by light. The interaction between red and far-red light in the nodulation process, whether these are applied to the shoot or the roots, demonstrates that nodulation is controlled by the phytochrome system – a pigment system present in plants and susceptible to exposure to small amounts of red and far-red light. The possibility that far-red light affects nodulation indirectly through an effect on stem elongation, *i.e.* by way of a differential distribution of carbohydrates to the shoot and the roots, is ruled out by the results of experiments in which the roots were irradiated. Plants with roots exposed to far-red light produced fewer nodules than untreated plants. However, the plants did not show excessive stem elongation and this is characteristic for plants whose shoots have been exposed to far-red light. This is in agreement with the results from earlier experiments with plant material with limited shoot growth. i.e. rooted leaves and decapitated plants [5].

From the experiments in which the plants were exposed to far-red light at different periods of time from inoculation, it can be deduced that far-red light acts directly on the symbiotic system and not indirectly by prior effect on the root system. Since far-red light did not influence nodulation when applied during the first two days following inoculation an effect on the initiation of nodule formation and not root-hair infection is thought to be involved.

The interaction between red and far-red light on nodulation was observed only when the same plant tissue, either the roots or the shoots, was exposed to red and far-red light. No such interaction was found when the light treatments were given to different parts of the plants, suggesting that any effect of the red or far-red light is not translocated. Support for the localized effect of far-red light may be also derived from the fact that the plants exhibited excessive stem elongation when the shoots were exposed to far-red light but not when the roots were so treated.

Pea plants grown in the dark produced nodules provided that the incubation temperature was rather low. Since such etiolated plants had to depend on reserve materials from the seeds, it may be assumed that the lack of nodule formation at high temperatures was due to an exhaustion of the reserve materials resulting from a higher respiration rate at the higher temperatures. The effect of far-red and red light on nodulation in such etiolated plants was discernible but the differences were rather small.

SUMMARY

Nodulation of pea and broad bean plants grown in the light was found to be reduced when the roots were exposed to far-red light for 5–15 minutes daily during 5 consecutive days following inoculation with nodule bacteria. Similar results were obtained following a single exposure to far-red light during a period of 15 minutes at the 3rd or 4th day after inoculation. When the roots were exposed to far-red light either before inoculation or during the first two days afterwards there were either no effects or only slight effects on nodulation The inhibitory effect of far-red light on nodulation was partly reduced by subsequent exposure to red light, provided that the same part of the plant

was exposed to both red and far-red light, *viz* either the root or the shoot. When different parts of the plant were exposed to red and far-red light respectively, there was no interaction between the two kinds of light on nodulation. Plants whose roots were exposed to far-red light did not subsequently show stem elongation.

Nodules were found to develop on the roots of pea plants grown in the dark, provided that the plants were kept at or below 22°C. At 25°C nodulation was almost absent. Nodulation was decreased by addition of kinetin and IAA. In contrast to plants grown in the light pea plants grown in the dark, inoculated with either an effective or ineffective strain of Rhizobium, developed equal numbers of nodules. Exposure to red light slightly increased the percentage of nodulated plants but decreased the number of nodules per plant. Exposure to far-red light slightly decreased both the percentage of nodulated plants and the number of nodules per plant. The effect of far-red light was counteracted by red light and *vice versa.*

ACKNOWLEDGEMENT

Thanks are due to Prof. Dr. E. G. Mulder for correction and critical perusal of the manuscript.

Received June 5, 1967. Revised December 1968

REFERENCES

1 Borthwick, H. A. and Hendricks, S. B., Photoperiodism in plants. Science **132**, 1223–1229 (1960).
2 Furuya, M. and Hillman, W. S., Observations on spectrophotometrically assayable phytochrome in vivo in etiolated *Pisum* seedlings. Planta **63**, 31–42 (1964).
3 Furuya, M. and Torrey, J. G., The reversible inhibition by red and far-red light of auxin-induced lateral root initiation in isolated pea roots. Plant Physiol. **39**, 987–991 (1964).
4 Hillman, W. S., The physiology of phytochrome. Ann. Rev. Plant Physiol. **18**, 301–324 (1967).
5 Lie, T. A., Nodulation of leguminous plants as affected by root secretions and red light. Ph.D. Thesis, Veenman en Zonen, Wageningen (1964).
6 McGonagle, M. P., The effect of certain factors on the formation of root nodules on pea plants in aseptic cultures. Proc. Roy. Soc. Edinburgh B **63**, 219–229 (1949).
7 Mohr, H., Primary effects of light on growth. Ann. Rev. Plant Physiol. **13**, 465–488 (1962).
8 Rudin, P. E., Versuche zur Physiologie der Knölchenbildung bei *Pisum sativum* L. Phytopath. Z. **26**, 57–80 (1956).
9 Schreven, D. A. van, The effect of added sugars and nitrogen on nodulation of legumes. Plant and Soil **11**, 93–112 (1959).
10 Wilson, J. K., Nodule production in etiolated vetch seedlings. Phytopathology **21**, 1083–1085 (1931).
11 Wilson, P. W., The biochemistry of Symbiotic Nitrogen Fixation. Madison (1940).