DISSERTATION

SENSORY PHYSIOLOGY OF DODDER (Cuscuta spp) SEEDLINGS PRIOR TO HOST ATTACHMENT

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring, 1992

COLORADO STATE UNIVERSITY

March 26, 1992

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY **MUSTAPHA A. HAIDAR** ENTITLED **SENSORY PHYSIOLOGY OF DODDER (Cuscuta spp) SEEDLINGS PRIOR TO HOST ATTACHMENT** BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

SENSORY PHYSIOLOGY OF DODDER (Cuscuta spp) SEEDLINGS PRIOR TO HOST ATTACHMENT

Dodder is an obligate stem parasite. Characteristic of seedlings of holoparasitic higher plants, early detection of potential hosts by young seedlings is essential for survival. An array of sequential environmental cues mediate dodder development and modify its growth from a non-parasitic higher plant to a parasitic growth mode. Exposure of the apical hook of etiolated seedling to red light stimulates hook opening. Only de-etiolated higher plant seedlings have the potential to transform to the parasitic mode of growth.

In white light dodder begins nutating 'in search' of a host. Neighboring plants (i.e., potential hosts) are detected by dodder initially by changes in light quality. Within the sphere of influence of a neighboring plant, an environment enriched in B and FR light potentiates a change in the state of the thread-like organ of dodder to that of its parasitic growth mode. When a dodder seedling contacts a potential host, it's growth slows. Potentiation for change in growth has now becomes actuated. The seedling

forms tight coils around the host stem. At this stage, dodder seedlings become susceptible to mechanical stimulation (thigmomorphogenesis). Soon prehaustoria develop (an early stage infection structure) on the concave side of coils. Auxin/cytokinin ratios and other chemicals influence development of these structures. Ethylene has no effect at this stage.

De-etiolation of dodder seedlings (hook opening) is controlled by phytochrome. Phytochrome maintains dodder as a non-parasitic higher plant seedling. Parasitic growth of dodder (twining and prehaustoria development) is controlled by two photoreceptors: phytochrome and a specific UV-A/B light photoreceptor (cryptochrome). The involvement of phytochrome was demonstrated by light pulses and R/FR reversibility experiments. Based on chemical and light studies, our results indicate that phytochrome (type I and II) alone cannot mediate parasitic growth of dodder under B light. Blue light acts via a specific UV-A/B photoreceptor, possibly flavin. However, the state of phytochrome is the principal limiting factor that affects B light action. The FR-absorbing form of type II phytochrome (Pfr II) opposes the action of cryptochrome; Pfr II opposes the action of an excited state of cryptochrome on coiling and prehaustoria formation. The data indicate that cryptochrome action is dependent on the state of phytochrome (Φ). A model of interaction between phytochrome and cryptochrome have been proposed. Both photoreceptors are competing for the same

intermediate, X; or same intermediate state, X' in mediating dodder parasitic growth.

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Spring, 1992

ACKNOWLEDGEMENTS

I would like to thank my advisor and academic father, Dr. Gregory Orr, for his scientific insight and immeasurable guidance. His interest, ideas, valuable suggestions, help and delightful sense of humor made my academic accomplishment possible. It was a great privilege to have been a student under his supervision. I also want to give him special thanks for his friendship and trust which will forever be remembered.

I would like also to express my gratitude and thanks to Dr. Philip Westra for his academic advice and for supporting me and my research; to Dr. Penelope Bauer for the use of her laboratory from time to time; and to Dr. Jene Dawson for providing us with dodder seeds.

I would like to express my gratitude, to my brother-in-law Gordon Glazner, for helping me edit my dissertation; and to my colleagues at Weed Science, especially Bill Stump, for helping me draw sketches and photos for my seminars and research dissertation during my academic journey at Colorado State University.

I would like also to express my deep appreciation to my Department and all the people who stood and supported my wife and me during the loss of our son Ali. I am especially

thankful to the Hariri Foundation for the loan provided by them, without which I would not have been able to pursue my graduate studies.

Finally, and most importantly, I would like to express my appreciation to my wife; her love, understanding, devotion and encouragement throughout my graduate studies have made everything possible and worthwhile.

DEDICATION

TO THE MEMORY OF MY SON ALI

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LIST OF ABBREVIATIONS

B	Blue light
BF	Blue + Far-red light
BR	Blue + red light
B/R	Blue followed immediately by red light
Cryp	Cryptochrome
Cryp*	Cryptochrome excited state
DCMU	Diuron [N'-(3,4-dichlorophenyl)-N,N-dimethylurea]
E	Photon energy or irradiance
FR	Far-red light
FR/R	Far-red followed immediately by red light
HIR	High irradiance response
IAA	Indole-3-acetic Acid
IR	Infra-red light (above 700 nm)
KCl	Potassium chloride
KI	Potassium iodide
LFR	Low fluence response
MP	Mechanical perturbation
NF	Norflurazon [4-chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyridazinone]
NFR	Near Far-red light
P	Total phytochrome (Pr + Pfr)
Pfr	Far-red light-absorbing form of phytochrome
Pr	R-absorbing form of phytochrome
Φ	Proportion of P as Pfr form at Photoequilibirium
R	Red light
R/B	Red followed immediately by blue light
R/FR	Red followed immediately by Far-red light
RF	Red + Far-red light
UV-A	Near ultra-violet light (320-400 nm)
VLFR	Very low fluence response
WL	White light
X or X'	Reaction partner for Pfr
z	zeta [Red:Far-red light photon ratio (R:FR)]
Z	Zeatin

INTRODUCTION

Cuscuta, commonly called dodder, is an annual stem holoparasite, occasionally capable of over wintering as dormant haustorial buds in the woody tissue of a host (Dean, 1954). There are more than 3000 species of parasitic angiosperms, distributed among 17 families (Press et al., 1990). Cuscutacae is one of the most agronomically important, although it contains only a single genus, Cuscuta. There are 170 species belonging to this genus, the most important ones are Cuscuta campestris (field dodder), Cuscuta indicora (largeseed dodder) and Cuscuta approximata (smallseed dodder) (Ashton and Santana, 1976).

Dodders are widely distributed in disturbed habitats, but colonize a diversity of ecosystems throughout temperate and tropical zones. Grasses are not attacked, but a wide range of other herbaceous dicot plants are parasitized by dodders (Figs. 1.1a and 1.1b).

Dodders are a serious agricultural problem. In the western United States, onions, tomatoes and alfalfa are the primary commercial crops parasitized by dodders. Dodders are especially troublesome in alfalfa, where they cause large economic losses by reducing seed yields and seed

Figure 1.1a. Dodders on bindweed.

Figure 1.1b. Dodders on onions

quality. They interfere with alfalfa harvest, and add to the cost of seed cleaning.

Quantitative data on losses caused by dodders are scarce and not precise. Ashton and Santana (1976) and Dawson (1987) indicated that in California many farms have been abandoned alfalfa production because of dodder infestations. Furthermore, many third world countries have problems with dodder where it attacks and devastates legume crops and other broadleaf crops. Many weed species, such as field bindweed, pigweed, knotweed, and lambsquarters, are also parasitized by dodders (Abu-Irmaileh, 1987; Ashton and Santana, 1976; Dawson et al., 1965; Parker and Wilson, 1984).

An array of environmental events, or cues, are required by dodders for early morphological and physiological development, and successful host attachment (Fig. 1.2).

Dodder reproduces primarily by producing thousands of hard seeds (although vegetative reproduction is possible with dodder). Some seeds will germinate in the next growing season, while others remain dormant in the soil for many years. Scarification of hard seeds and imbibition results in germination. Germination occurs in the absence of a host. Emerging seedlings are rootless, ecotyledonous, and leafless, with filiform or thread-like, yellow stems. Formation of an apical hook protects the delicate meristem from mechanical injury, as the negatively gravitropic, etiolated, non-parasitic higher plant seedling emerges

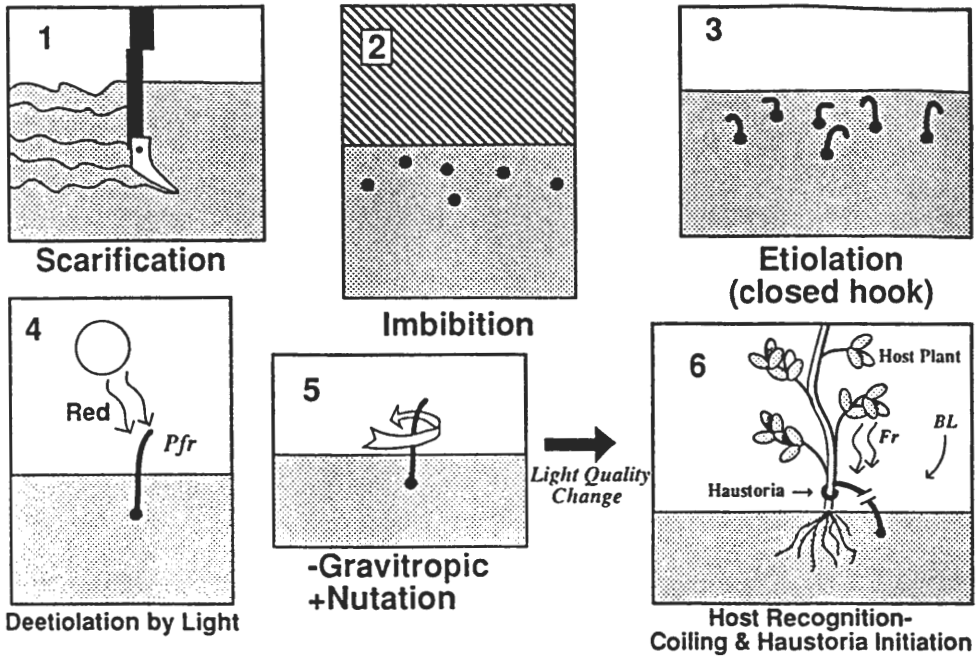


Figure 1.2. Initial characteristics of dodder as affected by biotic and abiotic factors.

through soil. The hook straightens after it emerges from the soil surface. The seedling starts to circumnutate, swinging in a wide counter clockwise circle until it strikes an object. Once it strikes an object, it will immediately start twining around it and haustoria will developed within 48 h on the inner face of the coil and attempt to penetrate the host vascular system. Once host penetration occurs, the dodder seedling structure between the soil and the host withers and dies. If no host attachment occurs, the seedlings will lose its ability to parasitize within one week (Ashton and Santana, 1978; Dawson, 1965; Dawson et al., 1965; Lyshede, 1984, 1985; Parker and Wilson, 1984).

CHAPTER ONE

LITERATURE REVIEW

BIOLOGY OF DODDER

Dodder plants are parasitic angiosperms. They are holoparasite (Bock, 1985), although recent work has shown that dodder seedlings and segments contain a very low chlorophyll concentration (Macleod, 1961; Walzel, 1952) and can fix carbon dioxide (Ciferri and Poma, 1963). Photosynthesis is very low and it cannot support dodder seedlings for long (Ashton and Santana, 1976; Dawson, 1987). Photosynthesis decreases as dodder become parasitic (Ashton and Santana, 1976). Dodder plants are leafless and rootless with twining tendrils and inconspicuous flowers. They attach to a host plant and obtain food nutrients through haustoria (Ashton and Santana, 1976; Dawson and Saghir, 1983; Tsivion, 1978b; Wolswinkel, 1977).

Dodder has un specialized seed biology and germinates spontaneously without the involvement or stimulation of any host plant. The seeds are simple and small with a hard seed coat, that results in a sporadic pattern of germination that may occur over many years after seeds are shed (Ashton and Santana, 1976; Lyshede, 1985). Dormancy can be broken if seeds are subjected to scarification with concentrated sulfuric acid (Abu-Irmaileh, 1987). Seeds germinate in response to moisture and temperature, independent of light

(Dawson, 1965a). But seeds emerge because the tiny seedling coiled within the seed, enlarges and elongates (Dawson, 1987). However, survival of dodder seedlings depends on initiating parasitic connections with the host within one week following germination (Parker and Wilson, 1984; Wolswinkel, 1977).

The shoot grows out very quickly as an etiolated slender yellow stem with a closed hook (Dorr, 1987). The seedling lacks cotyledons, but it has a 'hook' which is analogous to the hypocotyl or epicotyl hook found in dicot plants (Sitkin, 1976). The seedling apex emerges, reaches the soil surface and receives sunlight which causes the seedling hook to open. Lane and Kasperbaver (1965) indicated that R light is the main light responsible for hook opening via phytochrome (Pfr).

The de-etiolated, dodder seedling starts to circumnutate, 'counter-clock wise' as viewed from above, swinging in a wide circle until it strikes any object. Then under suitable light conditions, circumnutation slows down and the seedling forms a tight coil around the object. Thus, the dodder seedling is transformed into a parasitic mode of growth and is no longer gravitropic. As a result, haustoria will begin to develop after 24 h on the concave side (Nagar et al., 1984) of the dodder. The part of the dodder seedling between the soil and the host will wither and die. Dodder is then completely dependent on its host for water and organic materials (Abu-Irmaileh, 1987; Parker

and Wilson, 1984; Sitkin, 1976). If dodder fails to couple to a suitable host within a few days, it begins to creep over the ground, growing at the apex and wilting from the base. A dodder seedling may live up to one month on moistened soil (Lyshede, 1985).

Circumnutation.

Circumnutation is a rhythmic movement of a plant organ such as twining shoots and tendrils of climbing plants or hypocotyl of the seedlings in space (Johnsson and Heathcote, 1973; Johnsson, 1979). Circumnutation is light mediated. Etiolated seedlings show little or no circumnutation, while it increases in light treated ones (Britz and Galston, 1982). The period of circumnutation depends on the plant organ, i.e, leaflet move within minutes while shoots move within many hours (Johnsson, 1979).

The mechanism of twining shoots involve variations in rhythmic turgor resulting from changes in cell membrane permeability (Badot and Millet, 1990; Jaffe and Galston, 1968). It also involves modification of electrical potential and changes in K^+ distribution along the free-moving parts (Badot and Millet, 1990). However, Johnsson (1979) indicated that there are three major models of circumnutation: (a) the geotropic 'overshoot' model, in which circumnutation is controlled by gravity; (b) the hormone oscillations geotropic reactions model, in which circumnutation is due to a hormone gradient across the stem

as well as geotropic stimulations and reactions; (c) the hormone oscillations model, in which circumnutation is a result of a hormone gradient and gravity is not involved.

Parasitism.

The relationship between dodder and its host is a good example of a parasitic relationship between two plants and can be looked upon as a disturbance of phloem and xylem translocation in the host. The mechanism of parasitism is not well understood. The organ of parasitism is the haustorium, which forms the morphological and physiological bridge between the host and dodder plants (Kuijt, 1969; Kuijt and Toth, 1976; Wolswinkel, 1977).

Parasitism by dodder is restricted to the vegetative part of the host plant. Young seedlings less than three weeks old are not attacked by dodder seedlings (Lyshede, 1985). In many cases dodder seedlings parasitize other dodder plants or itself, "self parasitism", by means of haustoria (Jacob et al., 1985: Wellman, 1964).

Dodder seedlings consist of two modes of growth: (a) normal growth including, de-etiolation and circumnutation; (b) parasitic growth including, coiling and haustoria development.

Coiling development.

Coiling is the initiation of the parasitic appearance of dodder and is vital as a host recognition event. Coiling

of dodder tendrils around a potential host is associated with a specific light condition (Lane et al., 1964). Dawson (1965b) indicated that shading by alfalfa suppressed dodder twining by 90%.

Many scientists have indicated that hormones such as cytokinin induced coiling and haustoria formation on free hanging dodder tendrils (Paliyath et al., 1978, 1989; Rajagopal et al., 1988; Ramasubramanian et al., 1988; Tsivion, 1978a). Paliyath et al. (1989) stated that "...application of cytokinin evoked cell division events needed for its survival, namely the induction of coiling growth (to twine around a host) and formation of haustoria (for penetration and absorbing nutrients)."

Haustoria Development.

The haustorium is a highly modified cone-like tip (Dorr, 1987), and its evolutionary origin is unknown (Kuijt and Toth, 1976). Kuijt (1969) was the first scientist who studied the structure of dodder haustoria. He defined it as a physiological bridge through which nutrients move from one plant to another. Wolswinkel (1977) defined it as a highly modified adventitious root. Whatever the definition, it is important to indicate that dodder is a highly advanced parasite and that dodder constantly produces new contact with the host plant (Wolswinkel, 1977). Thus the development of the haustorium from endogenously arising

meristematic tissue to a highly differentiated organ occurs quickly and repeatedly within a few days (Dorr, 1987).

The main functions of haustoria are: (a) attachment, by compounds secreted in a very low amount that may contain adhesive materials or cell wall digestive enzymes (Fer and Capdepon, 1985); (b) penetration, which is achieved by a combination of turgor pressure in the developing haustoria and enzymes such as, phosphatases (Gupta et al., 1979), pectin esterases, transaminase enzymes and glutamic dehydrogenase (Macleod, 1963; Nagar et al., 1984), formed by the parasitism processes; (c) absorption and transfer of nutrients from host to parasite (Gupta and Singh, 1985; Press et al., 1990; Wolswinkel, 1977).

The haustorial structures varies enormously among the parasitic angiosperms. In dodder, haustoria structures are very different from that of parasitic fungi (Kuijt, 1977). Tsivion (1979) indicated that haustoria development in dodder has four successive stages. These are: first, induction of haustoria (prehaustoria); second, emergence of haustoria; third, production of hyphae; and fourth, differentiation of hyphae within the host vascular system. He stated that" haustoria induction does not require the presence of a host, but they (haustoria) are formed as a result of events caused by the curvature of the stem". Nagar at al. (1984) indicated that haustoria production is a result of the rotation of dodder seedlings around the host plant. This rotation activates cell division,

differentiation, and cytokinin accumulation in the concave site of dodder seedlings.

A general feature of mature functional dodder haustoria, is the presence of continuous xylem and phloem routes between host and parasite (Dorr, 1987; Kuijt and Toth, 1976). Vascular continuity is achieved by the growth of hyphae from the epidermis of the parasite, which grow either inter- or intra-cellularly within the host tissue (Press et al., 1990). The finger like-hyphae that grow in and among host cells (Kuijt and Toth, 1976) are described by having a dense cytoplasm and abundance of organelles, such as mitochondria, dictyosomes and ribosomes (Gupta and Singh, 1983). According to Dorr (1987) the hyphae change in structure and content from the time of penetration to the time of transport nutrients to other dodder parts.

Wolswinkel (1979, 1982) indicated that phloem transport in plants parasitized by dodder is interrupted dramatically and has a negative impact on the physiology of the host. Furthermore, he and Press et al.(1990) showed that an enhanced unloading rate of the host phloem appears crucial for the transfer of nutrients from host to parasite.

Mechanisms of Coiling and Prehaustoria Initiation.

The Role of light.

Light is the ultimate source of energy for all life on earth. For the plant, light is not only a source of energy but also an information medium. Therefore, plants acquire

information about light's direction, spectral composition and duration. In the natural environment there are vast fluctuations in the quantity and quality of light above and below the plant canopy.

Photobiology of Dodder Growth.

Dodder requires full sunlight to grow. Effects vary with the light quality, quantity, and duration as well as with the growth and developmental stages of dodder plants. In general, we know that there are two modes by which light mediates plant growth. The first is via photosynthetic pigments that may not be important aspects in the biology of dodder. The second is via photomorphogenetic pigments. These pigments provide neither the energy nor the carbon used in dodder growth, but they recognize light quality, quantity, duration and direction, and mediate dodder growth in response to these conditions (Cosgrove, 1986).

Photomorphogenesis is crucial to all terrestrial plant life. Normal development in higher plants is photomorphogenesis. Mancinelli (1989) stated that "photomorphogenesis is a result of an interaction between light and living matter in which light is used primarily as a signal and not as an energy". Signal perception, transduction, and induction are the basic processes in photomorphogenesis (Mancinelli, 1989).

The photomorphogentic light effect is detected at all major stages of plant growth and development; i.e., seed

germination, later stages of vegetation, reproductive development, and senescence (Mohr and Shropshire, 1983).

In dodder there are two main photo-responses that control its parasitic mode of growth. The first one is called low fluence response (LFR) and is controlled by phytochrome, and the other photo-response is called high fluence response (HIR) and is controlled by phytochrome and a BF light photoreceptor (Lane and Kasperbaver, 1965). Dodder responses to HIR are much more striking than that of LFR. Zimmermann (1962) observed that continuous FR light induced inter-twining and haustoria initiation and that R light inhibited such responses. Lane and Kasperbaver (1965) indicated that coiling is controlled by B or FR light applied separately, and that haustoria will form if FR light is applied after an HIR requirement has been established. Pizzolongo (1966) found that B light was most effective for coiling and haustoria formation. However, its not clear if such responses are due to phytochrome as the sole photoreceptor involved.

In general, plants respond to light in different ways. However, responses can be put under three categories based on their light requirements: (a) phototropic responses, (b) induction responses, and (c) high irradiance responses (HIR) (Cosgrove, 1986). Induction responses are divided into very low fluence responses (VLFR) and low fluence responses (LFR) (Attridge, 1990; Salisbury and Ross, 1992; Smith and Whitelam, 1990).

Very low fluence responses (VLFR) are saturated at extraordinarily low fluences rates. Energy requirements varies from 10^{-1} to 10^{-4} $\mu mol \cdot m^{-2} \cdot s^{-1}$ R light (Attridge, 1990). FR reversibility is unknown (Salisbury and Ross, 1992). Rapid chlorophyll synthesis and studies on coleoptiles of cereals is a good example of VLFR responses in plants.

Low fluence responses (LFR) are mediated by phytochrome, and are designated as an inductive type response induced by small flashes of R or B light (Holmes and Schafer, 1981), showing full or nearly full reversibility upon subsequent exposure to FR light. These responses require low energy (10^{-3} to 10^{-6} $\mu mol \cdot m^{-2} \cdot s^{-1}$) R light and obey the Bunsen-Roscoe reciprocity law (the response to a light treatment is a function of the total number of photons in treatment and is dependent on the time over they are delivered)(Smith and Whitelam, 1990). The LFR is used as a standard to demonstrate phytochrome commitment (Mancinelli and Rabino, 1978) and it can be easily demonstrated in germination of light-sensitive lettuce seeds, and in etiolated seedlings of many plants (See Kendricks and Kronenberg, 1986).

High fluence responses are defined as a steady-state type response, requiring continuous, long term exposure to high irradiances of light. It is dependent on fluence rate. These responses lack photoreversibility with subsequent R or FR light treatment, and do not obey Bunsen-Roscoe law (Salisbury and Ross, 1992; Smith and Whitelam, 1990). High

fluence responses require at least 100 X more energy than LFR (Salisbury and Ross, 1992). Plant responses to these light conditions are much more striking than in the case of LFR's and VLFR's (Cosgrove, 1986). HIR is observed in imbibed seeds, etiolated seedlings and in de-etiolated seedlings (Smith and Whitelam, 1990).

Plants in the natural environment are exposed to long periods of irradiation. So what is the role of HIR in the natural environment and what is the nature of the photoreceptors? For HIR to occur, Pfr should exist at a low concentration and for a long time. Rapid destruction of phytochrome in light due to degradation of Pfr is considered to be the basis of HIR. Therefore, Pfr concentration should be held low enough to reduce degradation, but high enough to cause action (Smith and Whitelam, 1990).

Not all HIR can be explained in terms of Pfr; after prolonged exposure to light the amount of Pfr becomes independent of light quantity and quality. Therefore, HIR is determined by the ratio of Pfr to Ptot (Frankland, 1986).

The identity of photorecptors is not completely clear. Many candidates were proposed: chlorophyll, phytochrome and cryptochrome. Evidence suggests that chlorophyll is not involved. However, most studies illustrate HIR on the basis of phytochrome and can't explain the high activity of B light, suggesting that there is an interaction between two types of photoreceptors, cryptochrome(s) and phytochrome(s) (Mohr, 1972; Hartmann, 1966; Mancinelli and Rabino, 1978).

Most models that explain HIR on the basis of phytochrome speculate a strong FR, a weak B and a poor R action under a prolonged irradiation conditions (Hartmann, 1966; Mancinelli and Rabino, 1978; Schafer, 1975, 1976).

A typical HIR action in etiolated higher plants requires prolonged irradiation with FR or B but not R light (Cosgrove, 1986). Unlike light grown plants, the action spectra exhibit slight or no effect in B and FR (Jose, 1977; Salisbury and Ross, 1992), and a large shoulder in R at 640 nm and not 655 nm (Attridge, 1990; Beggs et al., 1980). The FR peak is explained on the basis of the sustained Φ value which results in the highest Pfr over the experimental period. Spectral sensitivity depends on plant species, age, organ type, light pre-treatments, and light post-treatment (Cosgrove, 1986).

Three models have been used to explained HIR on the basis of phytochrome: (a) Pfr is controlled by light and dark reactions of phytochrome; (b) cycling rate and (c) phytochrome acts through multiple induction. The first and third models assume that Pfr is the active form while the second one is based on the different rates of interconversion of Pr and Pfr; i. e., different cycling rates (Cosgrove, 1986).

Properties and Mechanisms of the Photomorphogenic Photoreceptors.

Perception of specific photomorphogenetic light stimuli signifies the operation of specific sensor pigments. Four

photoreceptors are known to exist in plants: (a) phytochrome, (b) cryptochrome, (c) a UV-B photoreceptor and (d) protochlorophyllide (a pigment that reduces to chlorophyll-a after exposure to R or B light (Horwitz and Gressel, 1986; Salisbury and Ross, 1992). The BR photoreceptor for plastid development in Euglena is protochlorophyllide (Horwitz and Gressel, 1986). The function of photomorphogenetic photoreceptors is to acquire information about the radiation environment (Smith, 1983).

Light and Phytochrome(s).

Many features of plant growth and development are mediated by phytochrome. Phototropism of the fern Adiantum capillus veneris (Iino et al., 1990) and maize coleoptile (Iino et al., 1984). Biosynthesis of chlorophyll, carotenoids, and anthocyanins are good examples of responses controlled by phytochrome (for reviews, see Attridge, 1990; Kendrick and Kronenberg, 1986; Pratt, 1987; Rudiger, 1986).

Between 1945-1960 a group of researchers in the United States Department of Agriculture research station, Beltsville, MD determined and isolated a photomorphogenetic pigment. This pigment was named "phytochrome" (Salisbury and Ross, 1992). Phytochrome is a bluish chromoprotein with an open-chain tetrapyrrole that absorbs light at 600-800 nm (Mohr and Shropshire, 1983; Smith and Whitelam, 1990). It exists in two relatively stable, photointerconvertable

forms: an R absorbing form (Pr) and a FR absorbing form (Pfr) (Casal and Smith, 1989; Vierstra and Quail, 1983). The reversible photoconversion between Pr and Pfr is the fundamental process underlying phytochrome action in photomorphogenesis (Mancinelli and Lim, 1989). The state of phytochrome in vivo is determined by the association between photochemical (Pr and Pfr photoconversion) and nonphotochemical (dark reversion, destruction, synthesis) reactions (Mancinelli, 1988).

Conversion of Pr to Pfr and vice versa passes through a number of intermediates. Most of them have very short half lives with unknown physiological significance (Attridge, 1990). Pfr is usually deemed to be the physiologically active form (Colbert, 1988; Vierstra and Quail, 1983). The absorption maxima for Pr are 666, 379, and 280 nm and for Pfr are 730, 673, 400 and 280 nm (Vierstra and Quail, 1983). The spectral characteristic of phytochrome is a result of the interactions between the apoprotein and the chromophore (Colbert, 1988).

Light is absorbed by the chromophore and not by the protein, and it's likely that the excited chromophore undergoes photochemical reactions before any protein changes. The sum of Pr and Pfr is called 'total phytochrome" or Ptot. Phytochrome (Pfr) is not stable, it disappears through a first-order destruction process which is independent of light.

Phytochrome responses were demonstrated in gymnosperms, liverworts, mosses, ferns, certain green, red and brown algae (Salisbury and Ross, 1992), and recently in many marine macroalgae including Corallina (Lopez-Figueroa et al., 1989). Phytochrome is located in most plant organs including roots (Salisbury and Ross, 1992; Tanada, 1968). A high concentration of phytochrome is formed in the meristematic tissue of shoots and roots (Haupt and Schonbohm, 1970). Within cells, phytochrome is located in the nucleus and in the cytosol (Salisbury and Ross, 1992).

The function, action, and ecological significance of phytochrome in light-grown plants have been addressed by a number of reviews on phytochrome (Attridge, 1990; Furuya, 1987; Kendrick and Kronenberg, 1986; Shropshire and Mohr, 1983; and Smith and Holmes, 1984).

Phytochrome has multiple functions at all stages in the life cycle of a plant (Smith and Whitelam, 1990). The fundamental one is to detect the R/FR ratio of natural radiation, empowering the plant to sense vegetation shade from competing plants (Smith;, 1981, 1982, 1986; Smith and Morgan, 1983). Phytochrome also provides the plant with the capacity to adjust to oscillations in irradiation (Smith, 1982) and operates as a clue for seeds if they are covered by vegetation or soil. Lastly, it detects the presence or absence of light and the spectral quality of light (Holmes and Smith, 1975).

Phytochrome is synthesized in the Pr form in dark grown seedlings (Quail et al., 1973). Synthesis is enhanced by FR and inhibited by R irradiation (Salisbury and Ross, 1992). Phytochrome synthesis is regulated by environmental and genetic factors (Colbert, 1988). The rate of phytochrome synthesis decreases quickly after photoconversion from Pr to Pfr (exposure to light) (Vierstra and Quail, 1986). In darkness only the physiological inactive form Pr exists (Mohr and Shropshire, 1983). The rates of phytochrome accumulation and destruction changes with age of the plant, wavelength of light and irradiance (Frankland, 1986; Schafer, 1978).

Phytochrome consists of a linear tetrapyrrole chromophore covalently attached by a thioether linkage to cysteine-321 of a dimer protein (Vierstra and Quail, 1986). Recently Smith and Whitelam (1990) indicated that phytochrome consists of a group of related proteins, attached to a chromophore with multiple physiological roles and with dissimilar spectrophotometric, biochemical and physiological characteristics. Phytochrome found in de-etiolated tissue of oat (Tokuhisa et al., 1985) and pea (Abe et al., 1985) is immunologically and spectrally different from that found in etiolated tissue (Nagatani et al., 1989). Phytochrome that predominates in etiolated seedlings has been designated as type I (PI) and is light-labile, and that which predominates in green plants and seeds as type II (PII) and is light-stable (Salisbury and Ross, 1992). Both

types vary in their molecular weight, protein and spectral properties (Salisbury and Ross, 1992). Nagatani et al. (1989, 1990) indicated that etiolated seedlings contain type I at 100 X more than de-etiolated seedlings. Thus, etiolated seedlings contain more Ptot than de-etiolated ones (Salisbury and Ross, 1992).

The mechanism of action and the transduction processes of phytochrome action remain obscure (Furuya, 1987). However, multiple modes of phytochrome action are proposed: (i) Pfr is an active enzyme (Hendricks, 1964), (ii) phytochrome may have protein kinases activity (Salisbury and Ross, 1992), (iii) phytochrome activates and deactivates specific genes (Mohr, 1966). Song (1983) indicated that R light triggers transcription of dormant genes thus, inducing synthesis of enzymes essential for the morphogenetic and development responses in plants.

A popular hypothesis on phytochrome action is based on variation in cellular membranes. Hendricks and Borthwick (1967) indicated that phytochrome action is associated with changes in cell membranes. Tanada (1968) indicated that phytochrome regulates cell membrane potential by mediating rapid changes in the surface charges of barley root tips. Phytochrome has been found to control ion fluxes and change membrane permeability to certain hormones (Brownlee et al., 1979). Cytokinin level is affected by R/FR reversibility (Smith, 1976). Red light induces Ca^{++} fluxes into the cytosol via phytochrome (Pfr), and this Ca^{++} binds to a

protein called "calmodulin". Calcium-calmodulin activates various enzymes, such as, protein kinases (Salisbury and Ross, 1992). Phytochrome (Pfr) inhibits influx and efflux of K^+ across the plasma membrane in mung bean sub-hook segment and enhances efflux and influx in apical mung bean hypocotyl hook segments (Brownlee et al., 1979).

Photoequilibirium.

Below 700 nm the absorption spectra of Pr and Pfr overlap thus, a photo-equilibrium is established (Vierstra and Quail, 1986). The Pfr/Ptot ratio is termed "photoequilibrium". It is abbreviated by Φ and can be estimated by measuring the Pfr/Ptot-ratio in tissue (Jabben et al., 1982). The Pfr/Ptot ratio at photoequilibrium is a function of light quality and quantity (Mancinelli and Lim, 1989). Kronenberg and Kendricks (1986) and Smith (1981, 1983) proposed that Pr and the ratio of Pfr to Pr or Pfr to Ptot is active and not Pfr.

Values of Φ varies with light quality and intensity. The highest value of Φ (0.8-0.87) is produced under R light, the lowest (0.02 or less) under FR (Mancinelli and Lim, 1989). Values of Φ are large at 660 nm, but small at the 400-460 nm region and at wavelengths longer than 700 nm. This difference in the B spectral region is important to distinguish between phytochrome and cryptochrome in the mediation of B light action (Mancinelli, 1988).

The relationship of plant growth to Pfr/Ptot has been reported for many plants. Stem extension in de-etiolated Sinapis alba (Morgan et al., 1980, 1981) and Cucumis sativus (Gaba and Black, 1985) were stimulated by a decreasing Pfr/Ptot. Apical dominance in dicotyledonous (Kasperbauer, 1971) and monocotyledonous plants (Casal and Smith, 1989) was increased by a decreasing Pfr/Ptot (Morgan et al., 1981). In certain plants, chlorophyll content is reduced by low Pfr/Ptot (Kasperbauer, 1971).

Light and Cryptochrome(s).

Blue light controls a wide variety of physiological and morphological changes in plants organs (see Briggs, 1981; Gressel, 1979; Kowallik, 1982; Presti et al., 1977; Schmidt, 1980, 1981, 1987; Senger and Briggs, 1981; Senger, 1982, 1987). Senger and Schmidt (1986) indicated that there are 16 different effects under B, violet and near UV radiation controlled by a group of photoreceptors called "cryptochrome". Responses mediated by B light can be described as metabolic, morphological and directional responses.

Directional responses include phototaxis in Euglena and phototropism (Lipson, 1980) and photomovement (Nultsch, 1980). Metabolic effects include stimulation of respiration (Kowallik, 1982), carotenogenesis (Rau, 1980), chlorophyll biosynthesis (Kamiya et al., 1981), anthocyanin formation (Drumm-Herrel and Mohr, 1982) and regulation of

photosynthetic and carbohydrate-degrading enzymes (Ruyters, 1987), carbohydrate degradation, protein and RNA synthesis and metabolism (Kowallik, 1987; Ruyters, 1987; Senger and Schmidt, 1986), pigmentation and carotenogenesis in fungi (Duran, 1987). Morphological effects include protonemal development of ferns (Furuya et al.,1980), chloroplast development in algae (Brinkmann and Senger, 1980), seed germination, flowering and growth promotion and inhibition (Senger and Schmidt, 1986) and stomatal opening (Zeiger, 1983, 1984).

These responses are mediated through a B light photoreceptor(s) "cryptochrome(s)". Cryptochrome(s) include all the photoreceptors that have an action spectra with peaks or shoulders near 370, 420, and 480 nm with the largest at 450 nm (Horwitz and Gressel, 1986; Senger, 1984). However, cryptochrome(s) is not the only pigment that absorbs in the B light region. Chlorophylls (chlorophyll a near 450 nm and chlorophyll b and phytochrome 378 nm for Pr and 392 nm for Pfr) have peaks in the B light region (Attridge, 1990).

The chemistry and transduction chain of cryptochrome is obscure (Horwitz and Gressel, 1986; Vierstra, 1981). No definite B light photoreceptor has been unequivocally distinguished or isolated (Senger, 1987), unlike phytochrome which has been identified and the gene sequenced and the amino acid sequence presumed, and its structure and photochemistry extensively examined (Horwitz and Gressel,

1986). Furthermore, Jayaram et al. (1979) indicated that the action of cryptochrome diminished rapidly after light cessation.

Several hypotheses have been proposed for B light action: (i) blue light may act as an initial warning system for many stresses i.e., phototropism could be a slowing down of growth on the stressed side (irradiated) in plants; (ii) the membrane/ion hypothesis which indicates that the photoreceptors are bound to membranes, where they control ion fluxes or enzyme function; and (iii) the electrical effect. The best relationship between B light and electrical changes and morphogenesis are found in the alga Vaucharia (Horwitz and Gressel, 1986). However, the most popular one is that B light enhances depolarization of the membrane potential (Nishizaki, 1987). A good example is in the stomatal guard cells where B light causes hyperpolarization of the membrane potential and H^+ extrusion resulting in K^+ uptake, osmotic swelling and enlarged stomatal pore apertures (Zeiger, 1983).

The studying of cryptochrome physiology is not an easy task. The concentration of B light photoreceptor(s) is low and there are different chromophores or proteins (Senger and Schmidt, 1986). However, determining the action spectrum for B light is the only assay (Vierstra, 1981). Based on this action spectra, flavin(s)/flavoprotein(s) and carotenoid(s)/ carotenoprotein(s) are possible candidates for cryptochrome(s) (Senger, 1987; Vierstra, 1981).

Absorption spectra of flavoprotein(s) and carotenoid(s) differ mainly in the UV region. Support for carotenoids as the photoreceptor pigment came from experiments on Phycomyces (Shropshire and Whitaker, 1981) and on phototropism in corn seedlings (Vierstra, 1981). However, the overwhelming majority of scientists favor flavoprotein as a blue light receptor, mainly for phototropism (Salisbury and Ross, 1992; Song-P, 1987). Biochemically, flavins participate in a wide variety of redox reactions. Certain flavoenzymes are light activated such as nitrate reductase (Aparicio et al., 1976). Furthermore, carotenoids are poor candidates because of their short excited lifetime, i.e. trans-ß-carotene has a singlet excited state lifetime of 10^{-13} to 10^{-15} seconds while flavin posses excited state lifetimes long enough to be efficient at 10^{-8} and 10^{-4} seconds for the singlet and triplet excited states (Oster et al., 1962).

Flavin(s).

Flavins are potentially photochemically active act in transferring electrons from electron donors. Flavins also have a long-lived triplet state (Horwitz and Gressel, 1986). For many B light responses, flavin is the photoreceptor pigment, bound to a protein or plasma membrane (Salisbury and Ross, 1992) with the redox reaction the fundamental reaction (Schmidt, 1984, 1987). Membrane bound flavins mediate membrane transport of electron and protons resulting

in redox and pH-gradients. Thus B light depolarizes the plasma membrane by inhibition of the ion pump or by opening ion channels (Spalding and Cosgrove, 1990).

Carotenoids.

Carotenoids are yellow-orange pigments consisting of C40 tetraterpenes. Carotenoids are lipophilic. About 500 different carotenoids have been identified in the plant kingdom and these are divided into two groups, the oxygen free carotenes and the oxygen containing xanthophylls. ß-carotene and lutein are the most common in higher plants. All carotenoids are coupled by noncovalent bonds to protein complexes and absorb blue light (Attridge, 1990; Salisbury and Ross, 1992).

Carotenoids are widely distributed among living organisms. In higher plants roots, leaves, flowers and various plant parts contain carotenoids (Goodwin and Britton, 1988; Salisbury and Ross, 1992). At the cellular level carotenoids are located together with chlorophylls in the thylakoid membranes of chloroplasts (Salisbury and Ross, 1992).

Carotenoids have many functions. The dominant function of is that they act as antenna pigments, absorbing light and transporting energy to chlorophyll. They p otect chlorophyll from photo-destruction by quenching potentially dangerous triplet chlorophyll and singlet oxygen (Burnnet, 1976).

Carotenoids have been considered as a B light photoreceptor since the early 1930's. Carotenoids are considered to be the chromophore of cryptochrome for two reasons: The action spectra in blue light coincides with the absorption spectra of carotenoids and plants contain a large amount of carotenoids (Drumm-Herrel and Mohr, 1982). However, are carotenoids the only photoreceptor? Accumulative evidence indicates that carotenoids are photoreceptors for phototropism (Rau, 1988). However, for other responses it has been debated that the excited state of carotenoids is too short-lived to initiate responses (Vierstra, 1981).

Biosynthesis of carotenoids is restricted to plastids in higher plants. Although carotenoids are formed in darkness, production is stimulated by light (Britton, 1988). Red light acting through phytochrome and chlorophyll is the primary stimulus (Oelmuller and Mohr, 1985).

The Role of UV Light.

Ultra-violet light was detected in 1801 by Johann Wilhelm Ritter. The UV region of the electromagnetic spectrum covered all the wavelength band from 100 to 400 nm. The UV can be classified into UV-C (Below 280 nm), UV-B (280-320 nm) and UV-A (320-400) (Klein, 1978; Senger and Schmidt, 1986). UV-C is extremely harmful to organisms. UV-B is interesting because it causes certain damaging effects in plants and it also causes skin tanning and

cancer. The most destructive action of UV-C and UV-B involves the changes in DNA or RNA.

UV-A is the least hazardous part of UV radiation. UV-A effects on coiling and haustoria initiation in dodder has not been reported. It is well known that UV-A affects pigment induction, and reduction in various plants. It is also involved in reduction in photosynthesis and leaf expansion, destruction of plastoquinone (Mantai and Bishop, 1967), inhibition of electron transport in PSII (Brandle et al., 1977), inhibition of nonpathogenic tumors in tomato plants (Morrow and Tibbitts, 1988), and induction of conidia formation in certain fungi (Kumagai, 1983).

UV is absorbed by proteins, nucleic acids, flavinoids, phenylpropanoids, lipid components of plant membranes, and several plant growth regulators such as, indole acetic acid (IAA) and abscisic acid (ABA) (Wellmann, 1983). Furthermore, a large number of organic compounds absorb in the UV, making the search for photoreceptors to be even more difficult than for the B light (Horwitz and Gressel, 1986).

UV effects are controlled by cryptochrome, phytochrome or both. Little is known about the UV-B photoreceptor. Induction of flavonoid synthesis by UV-A in seedlings and culture cells of parsley as well as effects on root growth of parsley and carrot seedlings are controlled by phytochrome (Wellmann, 1983). The molecular mechanism of UV light is summarized by Fraikin and Rubin (1979) and Jagger (1976).

Photoreceptor(s) Interaction.

For plants to respond properly, a plant should sense the light conditions in its environment continuously and precisely. This information is obtained by three different sensor pigments in higher plants, phytochrome, cryptochrome(s) and UV-B (Mohr, 1987; Sponga et al., 1986). These photoreceptors collaborate with each other to cause photomorphogenesis (Salisbury and Ross, 1992). The cooperation or interaction between each other depends on the response, species and age of the plant (Mancinelli, 1989). Phytochrome (Pfr) is required for the final response expression in most interactions studied. In some conditions, action of cryptochrome(s) can be expressed only in the presence of phytochrome. In other cases cryptochrome and/or UV-B is required to establish/enhance/maintain responsiveness toward Pfr (Mancinelli, 1989).

It is hard to prove action of cryptochrome in responses where phytochrome also is active. There is no specific test for cryptochrome action (Sponga et al., 1986). Plant responses to B light could be mediated by cryptochrome(s), phytochrome or both either interacting or acting dependently (Schafer and Haupt, 1983; Sponga et al., 1986). However, phytochrome has a low absorbance in B light. According to the light-equivalent principle the state of phytochrome under B should be similar to that under RF light, with about one-tenth of the photon fluence rate of B light. Under these conditions, if the rate of response is different, then

we theorize that cryptochrome is involved in the B light response (Sponga et al., 1986). Laskowski and Briggs (1989) indicated that quantum efficiency of R light for transformation of Pr to Pfr is 50 X greater than the quantum efficiency of B light. However, in a number of plant tissues Φ-value at 450 nm is = 0.4, where Φ at 660 nm is = 0.8 whereas medium FR is = 0.03 and less than 0.01 at long-wavelength FR (Mohr, 1987).

Little is known about the mechanisms of interactions. There are four major hypotheses for the mechanism of interaction: (a) direct interaction between photoreceptors, (b) interaction at the level of the signal transduction chain, (c) interaction at the level of post-signal transduction processes and, (d) coaction (Mancinelli, 1989). There are at least two modes of coaction between phytochrome and cryptochrome, (a) independent coaction and (b) interdependent coaction; the latter is the most common (Mohr, 1987). Fernbach and Mohr (1990) indicated that there is coaction between phytochrome and cryptochrome in controlling growth of pine seedlings. Hypocotyl elongation is controlled by R light; but, it requires B or UV-A to become fully responsive to Pfr.

The Role of Plant Growth Regulators.

Cytokinins.

Cytokinins are among the most active plant hormones discovered in plant morphogenesis. Cytokinins were

discovered in the USA in the 1950's (Miller et al., 1956). Cytokinins have high cell division-stimulatory activity (Thomas and Blakesley, 1987). Most cytokinins that occur naturally in plants are purine compounds with a five carbon N^6-substitution (McCaw, 1986; Miller, 1974). The methyl group(s) and its position in the side chain of N^6-substituted adenines determine cytokinin activity (Matsubara, 1990). Kinetin was the first cytokinin discovered, and zeatin and zeatin riboside were the first free natural widely occurring cytokinins found in plants (Letham and Palni, 1983). Since then over 30 free cytokinins have been identified in plant tissues (McCaw, 1986). Their activity varies from almost inactive to highly active. The most effective natural cytokinin is zeatin (Salisbury and Ross, 1992).

Cytokinins are found in higher plants in diverse forms including free "unbound" bases, ribosides and ribotides or as a component nucleoside in tRNA of plants, animals and microorganisms and in plant viral RNA (Stuchbury and Burch, 1987; Sziraki and Balazs, 1979). Unbound cytokinins are physiologically active in plants (Salisbury and Ross, 1992).

Cytokinins have been identified in a wide range of plant organs at very low levels (0.01 to 1 μM) (Zhao, 1987). Young organs such as, seeds, fruits, leaves, root and meristems are rich in cytokinins (Salisbury and Ross, 1992; Thomas and Blakesley, 1987). However, many scientists believe that root meristems are the major site of cytokinin

synthesis (Incoll and Jewer, 1987). Cytokinins were found in the phloem and the possibility of bio-directed transport was suggested (Palmer et al., 1981).

Cytokinins have been implicated to elicit a diversity of physiological and biochemical processes. These include stimulation of cell division and organ formation, lateral bud development and chlorophyll synthesis (Salisbury and Ross, 1992); enhanced bud formation of Funaria and Caulonemata branching (Bopp and Jacob, 1986); delay senescence of mature leaves (Salisbury and Ross, 1992); and promotion of seed germination and dormancy (Skinner et al., 1957; other effects include stomatal function, flowering and fruit set, and fruit ripening (Thomas and Blakesley, 1987); tumorigenesis in tobacco hybrids (Nandi et al., 1990); and control of assimilate movement in plants, particularly by altering phloem unloading and loading (Hayes and Patrick, 1985).

In cooperation with auxins, cytokinins enhance callus development (Salisbury and Ross, 1992). Skoog and Miller (1957) reported that bud or root formation are determined by the auxin/cytokinin balance present in the culture medium. Ratios of these two classes of hormones are speculated to control gall and tumor morphology (Akiyoshi et al., 1983).

Cytokinins stimulate in darkness a number of processes usually controlled by light, such as amaranthine synthesis, chloroplast division, chlorophyll synthesis and photomorphogenesis (Bianco-Colomas et al., 1988; Tong et

al., 1983). How light and cytokinin operate together is unknown. However, in mustard seedlings, kinetin and R light act together in many ways such as: enzyme induction and carotenoid accumulation (Tong et al., 1983). A synergistic effect was observed with R light and cytokinin on the expansion of cucumber cotyledons (Cohen et al., 1988). The first data concerning effects of cytokinins on cell expansion were reported by Letham (1971) with radish. He indicated that several cytokinins are effective in light and darkness.

Little is known about regulation of cytokinin biosynthesis. However, metabolic processes, both anabolic, (i.e., conjugation) and catabolic (i.e., oxidative N^6 side chain cleavage) may be important in controlling endogenous levels of biologically active cytokinins (McCaw, 1986). Biosynthesis of cytokinins in plants involves formation of ($\blacktriangleleft^1$-isopentyl)adenosine monophosphate ([9R-5'P]ip), derived from 5'-AMP and $\blacktriangle^1$-isopentylpyrophosphate (Horgan, 1986). [9R-5'p]ip is rapidly hydroxylated to yield zeatin and trans-zeatin cytokinins which predominate in plant tissue (Letham and Palni, 1983; McCaw, 1986).

Plant tissues convert exogenous supplied cytokinin bases into a great diversity of metabolites. Adenine and its derivatives are the major metabolites of zeatin. The principal metabolite formed after application of cytokinin bases to plant tissue initially is cytokinin riboside 5-'phosphate (Letham and Palni, 1983). The significance of

cytokinin metabolites is obscure but they could be storage forms of cytokinins (Stuchbury and Burch, 1987). Letham and Palni (1983) indicated that cytokinin metabolites are important in the biosynthetic pathway and in transport of cytokinins.

The molecular mechanism of cytokinin action remains vague. Cytokinins affect ion fluxes, particularly K^+ (Elliott, 1983). Cytokinin may stimulate production or activity of particular enzymes via protein receptors (Zhao, 1987).

A tremendous body of experimental evidence suggests that exogenous application of cytokinin induces formation and differentiation of haustoria in dodder. Tsivion (1978a) indicated that exogenous application of cytokinin induces haustoria formation and differentiation, while GA3, IAA, and ABA, or their various combinations, did not cause these effects. Haustoria induction by BA (benzyl adenine) was also observed by Paliyath et al. (1978), and high cytokinin levels were found in the haustoria-bearing region (Gupta and Singh, 1985). The involvement of GA3 and its interaction with IAA have been described by Maheshwari et al. (1980). Both hormones induced growth but not haustoria formation in dodder. Paliyath (1979) and Ramasubramanian et al. (1988) found that GA3, IAA, ethylene and ABA inhibit cytokinin action, thereby inhibiting haustoria formation.

Auxins.

Auxins are defined classically by their capacity to enhance growth by its influence on cell elongation as well as cell division (Key, 1989). There are four natural auxins found in plants. The most important one is indole-3-acetic acid (IAA) (Napier and Venis, 1991), followed by PAA which is widespread among plants and more abundant than IAA but less active; 4-chloro IAA is found in many young legumes seedlings and recently indolebutyric acid (IBA) has been identified in leaves of maize and some dicot plants. Furthermore, there are three additional compounds that have auxin activity. Many scientists consider them as auxin precursors. These are indoleethanol, indoleacetaldehyde and indoleacetonitrile (Salisbury and Ross, 1992).

Auxins are found in most organs of the plant. Synthesis occurs mainly in meristematic tissues; i.e., shoots, roots, young leaves, flowers and fruits (Salisbury and Ross, 1992). The concentration of free IAA in plants is around 1 to 100 μg/kg weight fresh tissue (Schneider and Wightman, 1974).

IAA moves laterally (Sembdner at al., 1980) or longitudinally from its site of synthesis to its site of action (Salisbury and Ross, 1992). IAA does not move in xylem or phloem, but mainly via parenchyma cells in contact with vascular bundles (Aloni, 1987). Lateral movement was reported by Cholodny-Went. They suggested that IAA is transferred laterally from the upper to the lower side of

the cell in response to light and gravity (Hatfield and Lamotte, 1984). Longitudinal movement is polarized and involves movement from cell to cell as described by Raven (1975), and Rubery and Sheldrake (1974). According to later theory, uptake and accumulation of auxins are a function of pH and electrical gradients at membranes as well as the permeability of both the auxin anion and the undissociated acid. Polar movement comprises uptake and efflux, both of which may be partly by diffusion and partly by a carrier (Sabatar and Sabatar, 1986). This movement requires metabolic energy (Salisbury and Ross, 1992).

Light affects auxin transport and metabolism (O'brian et al., 1985). Briggs (1963) first indicated that light might affect auxin transport. Light effect on auxin transport is an important factor in the regulation of photomorphogenesis. Rajagopal and Bulard (1975) indicated that R light through phytochrome modifies auxin transport, while UV-A and B light act by enhancing auxin degradation. Red light inhibits auxin production in rice coleoptiles (Sherwin and Furuya, 1973), bean seedlings (Tillberg, 1974) and corn seedlings (Iino, 1982).

The primary action of auxin requires a specific receptor protein (Salisbury and Ross, 1992). The auxin-binding protein group is best known receptor system in plants. Classes of auxin-binding proteins have been characterized. These include binding sites for auxin transport inhibitors, membrane-bound auxin-binding sites,

and soluble/nuclear bound auxin-binding sites (Roberts and Hooley, 1988).

There are three major hypotheses regarding auxin activity. Auxin regulates gene activity (Sembdner et al., 1980), auxin induces cell wall plasticity as explained by the acid growth hypothesis (Rayle and Cleland, 1979), or auxin-induces ethylene synthesis through induction of 1-aminocyclopropane-1-carboxylic acid (ACC) (Chadwick and Burg, 1967; Yang and Hoffman, 1984). Auxin stimulates ethylene formation in stems (Abeles and Rubinstein, 1964), roots (Andrea et al., 1968; Chadwick and Burg 1967), leaves and fruits (Abeles and Rubinstein, 1964).

Ethylene.

Ethylene is a simple, unique type of hormone that regulates plant growth and development. The species, environment, stage of growth, type of cell and tissue, concentration level of ethylene, and interactions with other plant hormones all influence the effect of ethylene on plants (Lieberman, 1979).

Ethylene regulates a variety of plant responses. The most notable effect is ripening of fleshy fruits and stimulation of abscission (Salisbury and Ross, 1992). Other effects include growth and differentiation of cells and tissues in culture (Reid et al., 1985), cell elongation (Sanchez-Calle et al., 1989), increased seed germination

(Abeles, 1973, 1986) induction of flowering in pineapple and induction of leaf abscission (Salisbury and Ross, 1992).

Ethylene is produced in trace amounts by all higher plants and a few bacteria (Salisbury and Ross, 1992). Ethylene is synthesized in all plant parts (Leopold, 1971; Yang and Hoffman, 1984). However, the shoot apex of plant seedlings is an important site of production (Salisbury and Ross, 1992). Ethylene production is regulated by a diversity of developmental and environmental factors. Ethylene production increases during certain stages of growth such as germination, ripening of fruits, abscission of leaves and senescence of flowers (Yang and Hoffman, 1984). However, ethylene production can be enhanced by mechanical wounding, environmental stress (light, drought), auxin and by chemicals including herbicides, metals and others (Abeles, 1973).

Light can promote or suppress ethylene evolution. Many scientists suggest that light inhibits ethylene production in many green leaf tissue (Grodzinski et al., 1982). Gepstein and Thimann (1980) were the first to report that light inhibits ethylene production. Wright (1981) observed that ethylene production was inhibited by light in the plumular hook of wheat leaves. Light inhibits ethylene synthesis mainly by interfering with conversion of ACC to ethylene (Gepstein and Thimann, 1980).

Light effects on ethylene production are mediated by photosynthetic (Laat et al., 1981) or by photomorphgenetic

pigments (phytochrome) (Kang and Ray, 1969). The interaction between phytochrome and ethylene has been known for a long time. In Oryza sativa coleoptile, Glycine max hypocotyl, R light reduces ethylene production and is FR reversible. However, in cotyledons of Cucumis sativus and pea epicotyls, R light stimulates ethylene production while it has no effect in Lactuca sativa (Attridge, 1990). Erez (1977) indicated that, in de-etiolated plants, ethylene production is affected by the R:FR ratio of incident light; low ratios stimulate ethylene synthesis.

Ethylene is synthesized from carbon 3 and 4 of the amino acid methionine via S-adenosyl methionine (SAM) and 1-amino-cyclopropane-1-carboxylic acid (ACC) (Salisbury and Ross, 1992; Yang and Hoffman, 1984). Carbon dioxide, ethylene oxide and ethylene glycol are major ethylene metabolites (Salisbury and Ross, 1992). Ethylene action and metabolism might be coupled. The conversion of SAM to ACC via ACC synthase is the key reaction controlling production of ethylene (Kende, 1989).

Several theories have been used regarding ethylene action. One theory is that ethylene binds reversibly to a metal-containing receptor site (Burg and Burg, 1967). It could be Zn^{++} or Cu^{+} (Beyer, 1976). Ethylene binding sites have been identified in a variety of plant tissue such as tobacco leaves (Sisler, 1979) and rice seedlings (Sanders et al., 1990). Another theory is that ethylene attaches to membrane surfaces and increases permeability (Pratt and

Goeschl, 1969). Liebermann and Kunishi (1971) indicated that ethylene induces membrane permeability of cucumber tendrils. The last theory indicates that ethylene regulates growth processes by controlling gene transcription (Salisbury and Ross, 1992).

The Role of Mechanical Perturbation (MP).

Mechanical stimulants for coiling and haustoria induction in dodder have not been reported. Physical disturbance has been shown to retard the growth rate of herbaceous plants (Hammer et al., 1974; Jaffe, 1973) as well as woody plants (Harris et al., 1972; Larson, 1965; Neel and Harris, 1971). Plant responses to mechanical stimulation include reduction in stem or root elongation, and increase in stem diameter. These responses are called "thigmomorphogenesis" (Giridhar and Jaffe, 1988; Jaffe, 1973).

Thigmomorphogenesis responses are typically stunting with concurrent thickening and often reduction in leaf growth, fruit yield, fresh and dry weights (Jaffe, 1973; Mitchell, 1977; Wheeler and Salisbury, 1979). Boyer (1967) indicated that rubbing the internodes of young Bryonia dioica plants thrice daily caused a 58% reduction in length. Neel and Harris (1971) indicated that vibrating or shaking the trunks of Liquidamber styraciflua trees for 30 s daily reduced elongation by 80%. The responses to MP, have also been documented in beans (Biro et al., 1980; Erner et al.,

1980) which are characterized by an increase in radial growth, increase in production of secondary xylem and decrease in cell elongation (Biro et al., 1980). Mechanical stimulation can also induce differential growth in Phycomyces sporangiophore (Dennison and Roth, 1967), the dandelion peduncle (Clifford et al., 1982), and the bean stem (Jaffe, 1985).

There are multiple mechanisms of action proposed for the mechanical stressed responses. The effect of mechanical perturbation on plants has been demonstrated to be mediated by a change in action potentials such as a change in bioelectrical properties, whether changes in electrical resistance, (Jaffe, 1976), or in action potentials (Asher, 1968). This suggests that ions such as K^+ move rapidly through membranes from the symplast into the apoplast after mechanical stimulation (Salisbury and Ross, 1992).

The effect of mechanical perturbation on plants also was demonstrated to be mediated by plant hormones. Ethylene (Hiraki and Ota, 1975; Jaffe, 1985) and IAA (Erner and Jaffe, 1982; Mitchell, 1977) are the possible candidates. Auxin stimulates ethylene production (Burg and Burg, 1966). Mechanical stress inhibits auxin polar transport, but not synthesis. Therefore, auxins may accumulate in tissue near the site of stress thus, stimulating ethylene production (Burg and Burg, 1966; Erner and Jaffe, 1982).

The Role of Potassium.

Chemical stimulation for coiling and haustoria induction in dodder has not been reported. A few studies have indicated that dodder has a low calcium content and a high potassium content (Wolswinkel, 1977). Hoch et al. (1987), Kaminskyi and Day (1984), and Staples et al. (1983, 1984, 1985) were first to indicate that potassium, calcium, and sucrose induced appressoria formation in bean and sunflower rust fungi. Calcium alone has no effect, but with low level of K^+, Ca^{++} greatly enhances induction. Opening of K^+ channels in plasma membrane of Chara corallina are mediated by changes in cytoplasmic Ca^{++}. Increasing the Ca^{++} concentration results in closure of K^+ channels (Tester and Macrobie, 1990).

Potassium concentration in higher plants is 100 mM or more (Outlaw Jr, 1983). Potassium moves apoplastically (Outlaw Jr, 1983) and symplastically (Satter and Galston, 1981). However, the most important one is through membranes channels (Outlaw Jr, 1983).

Potassium is an activator of many enzymes that are essential for photosynthesis and respiration (Salisbury and Ross, 1992). Potassium is known to play a major role in turgor regulation and it was proposed that changes in cell water content are related to changes in K^+ distribution along twining shoots (Badot and Millet, 1990). Movement of K^+ in and out of plant cells results in changes in cell volume which leads to diverse responses such as thigmonastic

leaf folding in sensitive plants, and stomatal opening and closing (Salisbury and Ross, 1992). Satter and Galston (1971) indicated that there is an interaction between an endogenous rhythm and phytochrome in control of potassium flux and leaflet movement of Albizzia julibrissin. Phytochrome (Pfr) inhibits K^+ movement either into ventral motor cells or out of dorsal motor cells or both, during leaflet opening as well as during nyctinastic closure. K+ flux in motor cells is the basis for rhythmic leaflet movement.

Potassium has been found to stimulate cytokinin effects. Expansion of cucumber cotyledons in response to cytokinin was enhanced by K^+ (Ross et al., 1983). Greeen and Muir (1978) reported that 40 mM KCl enhanced kinetin effects on cotyledons detached from 5-day-old cucumber seedlings.

THE SIGNIFICANCE AND OBJECTIVES OF THE DISSERTATION RESEARCH

This dissertation addresses a problem in weed science that was given a very high priority by the Weed Science Society of America (Riggleman, 1987); namely, the ecophysiology of weed-crop interactions. This dissertation provides basic information about the sensory physiology and response biology of weed-crop canopy interactions, and specifically, how light conveys useful information to plants concerning their immediate environment.

Understanding the molecular events controlling the response of plants to the 'nearness of others,' will almost surely, aid in the identification [and development] of novel [weed management] strategies that are effective and compatible with social concern(s). We studied how stressful interactions are established between plants, and how such interactions are influenced by environmental factors inherent to the interacting organisms. Furthermore, we added information about the basic biology of stress-causing organism(s).

We also explored the behavior of dodders as influenced by light conditions expected in environments enjoined with neighboring plant canopies (i.e., potential hosts). It is reasonable to assume this research has a significance beyond

the specific weed-crop canopy interaction(s) of dodders and their host(s). Therefore, the main objectives of this research included:

1. determination of the characteristics of dodder parasitic mode of growth;

2. identification of environmental cues signaling morphological changes leading to parasitism;

3. studying the sensory physiology involved in early detection of potential hosts;

4. investigating the possible photoreceptors involved in perception of light that are responsible for initiating coiling and prehaustoria formation;

5. investigating the interaction between cryptochrome and phytochrome.

CHAPTER TWO

CHARACTERISTICS OF DODDER PARASITIC GROWTH: EFFECTS OF LIGHT, AND OTHER FACTORS ON COILING AND PREHAUSTORIA DEVELOPMENT

INTRODUCTION

The developmental regulation of coiling and haustoria initiation in dodder (Cuscuta spp) is not well understood. Signals such as light and hormones may control these responses (Lane and Kasperbaver, 1965; Paliyath et al., 1979, 1989; Pizzolongo, 1966; Ramasubramanian et al., 1988; Tsivion, 1978a; Zimmermann, 1962). The effect of light on the parasitic growth of dodder is one aspect of photomorphogenesis that has received little attention. Effects vary with light quality, quantity and duration as well as growth and developmental stages of dodder plants. A few scientists reported that hook opening, twining and haustoria initiation in dodder are controlled in part by light (Lane and Kasperbaver, 1965; Pizzolongo, 1966; Zimmermann, 1962). Zimmermann (1962) observed that continuous FR light induces inter-twining and haustoria formation, while R light is inhibitory. Lane and Kasperbaver (1965) indicated that B or FR light control dodder twining. Pizzolongo (1966) indicated that coiling and haustoria are mediated by B light.

It is well documented that mechanical plant stimulation (MP) (thigmomorphogenesis), retards the growth rate of many herbaceous and woody plants (Hammer et al., 1974; Harris et

al., 1972; Salisbury and Ross 1992). Plant hormones have been implicated in mediating the effects of mechanical perturbation. Ethylene (Hiraki and Ota, 1975; Jaffe, 1985) and IAA (Erner and Jaffe, 1982; Mitchell, 1977) are possible candidates.

An enormous body of experimental evidence indicates that exogenous application of cytokinin induces coiling and haustoria formation in dodder tendrils, while IAA is inhibitory regardless of light treatments (Paliyath et al., 1979, 1989; Ramasubranian et al., 1988; Tsivion, 1978a).

Most of these earlier and recent studies ignored the role of light and mechanical stimulation on haustoria development. Therefore, the first chapter of this dissertation attempts (1) to determine the initial characteristics of dodder parasitic mode of growth, (2) to identify environmental cues signaling morphological changes leading to parasitism, and (3) to better understand the sensory physiology involved in early detection of potential hosts.

MATERIALS AND METHODS

Seed Source.

Seeds of two dodder species, *Cuscuta campestris* and *Cuscuta indicora* were obtained from Dr. J. Dawson (USDA, Prosser, WA) and stored in darkness at room temperature until used for experimental purposes.

Scarification.

Seeds were soaked in concentrated sulfuric acid and stirred for 30 min to enhance germination. Seeds then were caught on a wire screen, rinsed several times with tap and distilled water, and air dried before use.

Imbibition.

Approximately 100 hundred scarified seeds consisting of both species were placed in 11 x 11 cm plastic boxes, each containing one blue blotter germination paper and 10 ml of distilled water. Twelve replications of each germination trial were used daily. Boxes were wrapped with aluminum foil and incubated in the dark at 28°C for 96 h.

Chemicals.

Zeatin (trans-isomer) and indole-3-acetic acid (IAA), were purchased from Sigma Chemical Company. Chemicals were

applied as described in detail by Zhao and Ross (1989). Ethylene (Floral™) was purchased from Lawn and Garden Products, Inc. Ethylene application will be described later. Potassium salts, and sucrose were dissolved in distilled water.

Characterization of Light Treatments.

To determine the interaction between hormones and different sequences of short flashes of light (low fluence rate [LFR]) on coiling and prehaustoria development, 96 h old seedlings received 1 min R, 2 min FR and their sequence (e.g. 1 min R followed by 2 min FR). Seedlings were returned to dark (DK) incubation for another 24 h at 28°C. At 120 h after sowing and under green safe light (2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$), plants were selected for uniformity, excised (the upper 4 cm were used in all experiments, these segments are called threads) and placed horizontally on 5.5 cm plastic petri dishes (Fisher brand) each containing one layer of glass fiber filter paper (G6-Fisher brand) and 2 ml of water or chemical solution. All petri dishes were put in darkness and threads were allowed to grow for another 48 h at 28 ± 2°C.

To determine the effect of prolonged exposure (high fluence rate [HIR]) of different light treatments on coiling and prehaustoria development, 96 h-old seedlings received 24 h WL (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as a pretreatment to induce hook opening. White light was provided in a growth chamber from

cool-white fluorescent lamps (F36 T12-CW-Ho, GE). At 120 h after sowing and in the laboratory under WL (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$), de-etiolated seedlings were selected for uniformity and excised as previously described. Petri dishes were exposed to experimental light treatments consisting of monochromatic light such as UV-A, B, R, and FR or dichromatic light such as UV-A/FR, BF, RF, RB applied simultaneously. Threads were left to grow for another 48 h at 30 ± 2°C.

Light Sources.

The R and FR sources used for LFR were provided to dodder seedlings using a cabinet that had, for each source, four 150-watt flood lamps that were air cooled. This cabinet contained compartments for heat removal through water with the following filters: R light one sheet, 3.18 mm thick, Rohm and Haas no. 2444 red plexiglass: FR light one sheet, 3.18 mm thick, Westlake Plastics Co., Lenni, PA no. FRF 700 Black plexiglass.

Light sources used for HIR were provided using special chambers that have been designed in our laboratory for many types of light treatments. Lamps and filters were air cooled and were separated from the petri dishes by a water heat trap to eliminate possible heat damage to the threads. For monochromatic light studies, B, R and UV-A light were provided from above the petri dishes. Blue light treatment was obtained by filtering light from B fluorescent lamps (F40B, GE) through one layer, 3.18 mm thick, Rohm and Haas

blue plexiglass no. 2424. Red light treatment was provided by filtering light from cool-white fluorescent lamps (F40-CW-RS-EW-II, PH) through one layer of R filter (described before). UV-A light treatment was obtained by filtering light from long wave ultra-violet (Black-Ray, model B-100A) through one layer of UV filter (Kodak) and 2.5 cm of a 1.5% $CuSo_4.7H_2O$ solution. The lateral FR irradiation was provided by one or more 100-watt incandescent lamps filtered through FR filter (described above).

For dichromatic light studies, all possible light combinations were provided. Blue or UV-A was provided from above the petri dishes while FR irradiation was provided simultaneously from below or from the side. For the RF light combination, one or more 100-watt incandescent lamps filtered through the red filter (described before) was provided from above or from the side of the petri dishes. Fluence rates at the dish height were measured with a Licor radiometer (model No. LI-185B) except for UV-A where a UV meter (model No. J-221) was used. Fluence rates were varied with distance and lamp voltage (100W or 250W). Transmission spectra of UV-A, B, R, FR and RF light combination, were measured with an ISCO model spectroradiometer (Figs. 2.1, 2.2, 2.3).

Experimental Measurements.

The number of coiled segments per dish and number of prehaustoria per segment were determined using a dissecting

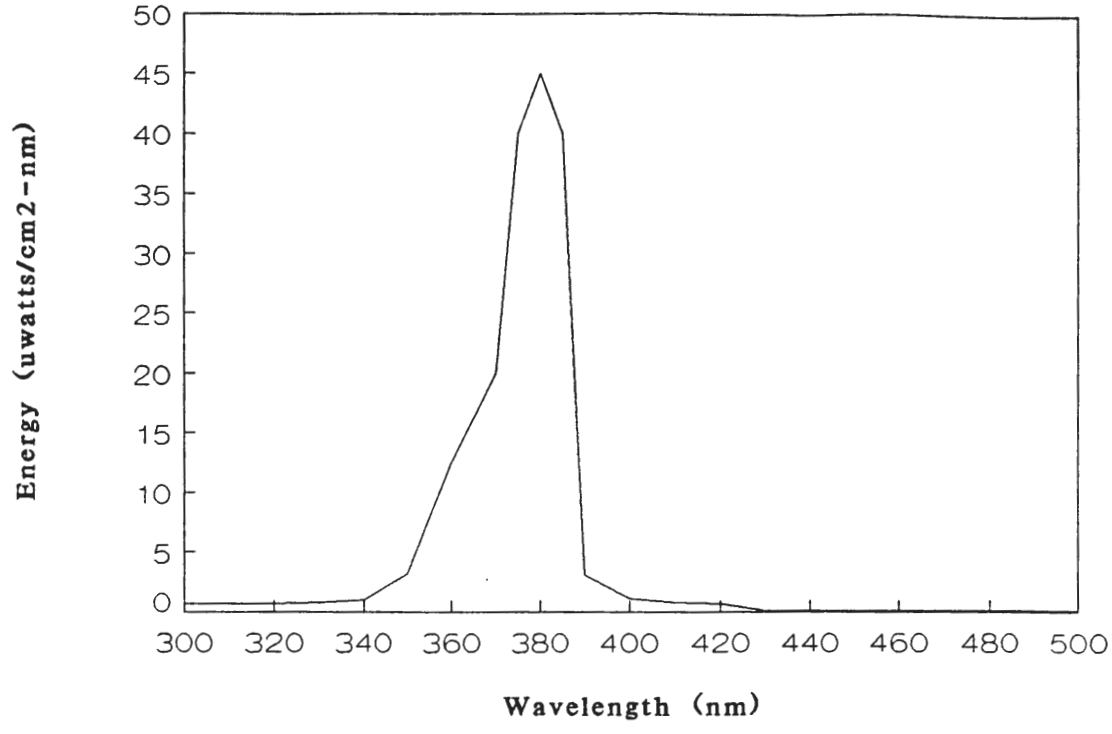


Figure 2.1. Transmission spectrum for the UV-A light source. Near ultra-violet (UV-A).

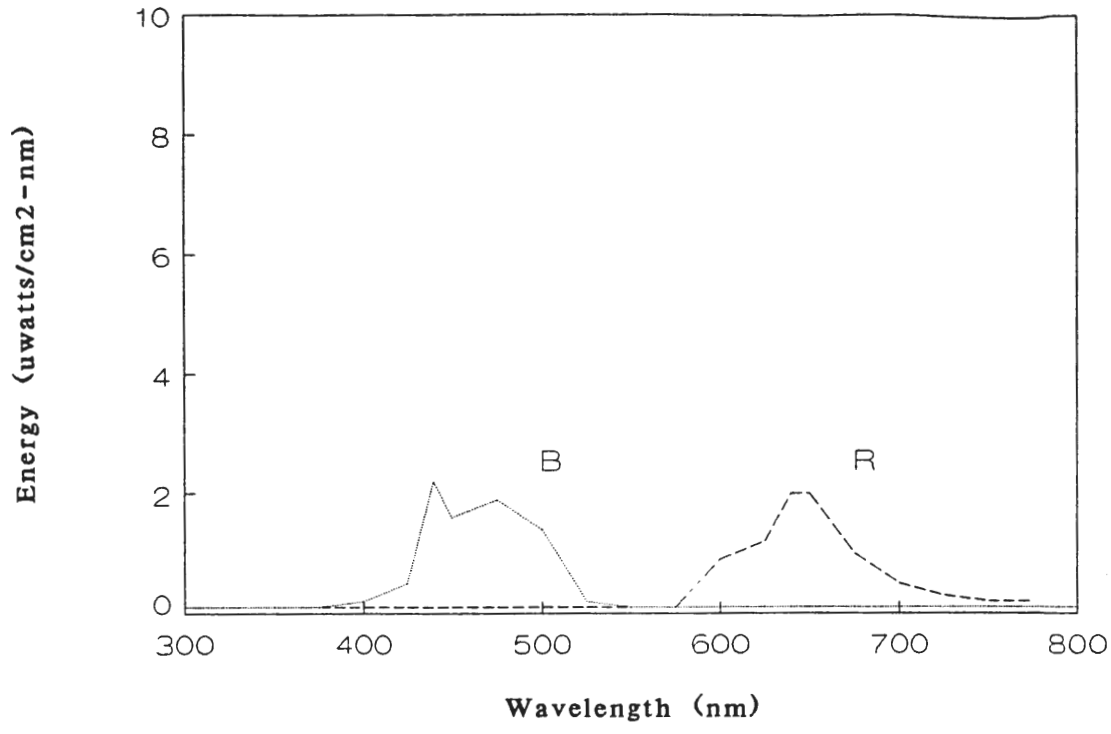


Figure 2.2. Transmission spectrum for the B and R light sources. Blue light (B); Red light (R).

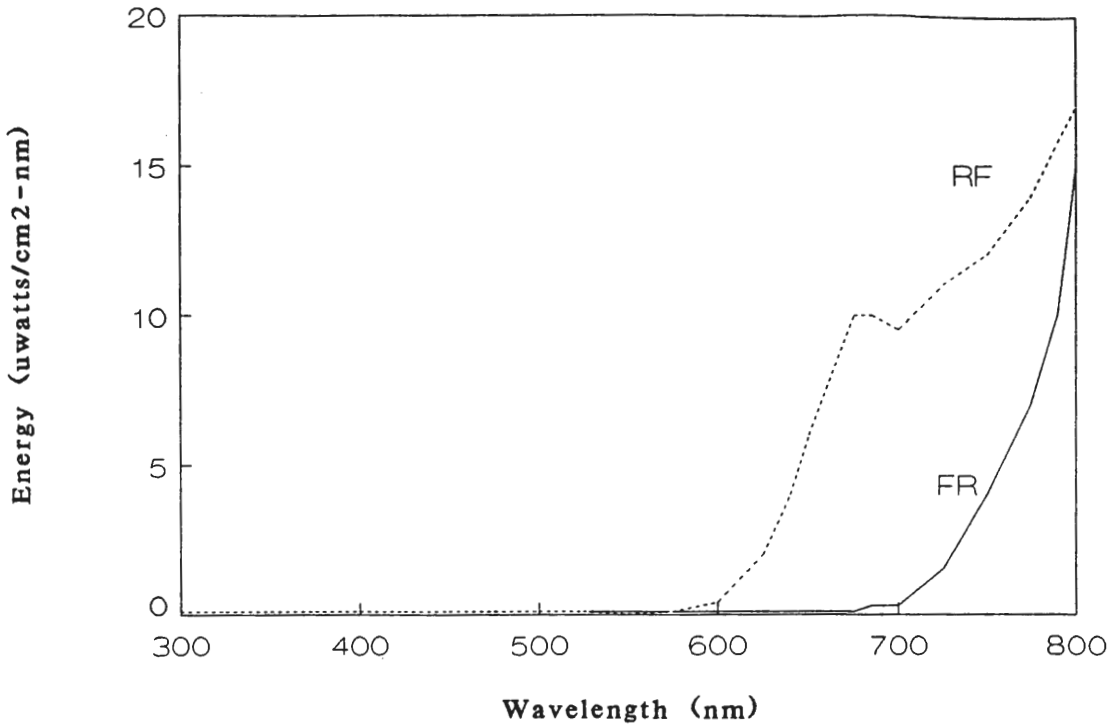


Figure 2.3. Transmission spectrum for the RF and FR light sources. Red + Far-red light (RF); Far-red light (FR).

microscope. Threads with 360° curvature were considered coiled. Threads with any apparent sign of mounding or any sign of saucer-shaped bell on the top of the mound were counted as pre-haustoria (see Figures, 2.4a and 2.4b). However, Tsivion (1978) and Ramasubramanian at al. (1988) named the structure described above the "haustorium".

Statistical Analysis.

Unless otherwise stated, three petri dishes, 5 threads each were used for each light treatment. Experiments were repeated more than three times. Data reported are averages of treatment means with standard errors (SE) of three experiments.

Figure 2.4a. Coiled dodder thread with prehaustoria after 24 h under BF light. Blue + Far-red light (BF).

Figure 2.4b. Coiled dodder thread with prehaustoria after 48 h under BF light. Blue + Far-red light (BF).

RESULTS

Effects of Light Pre-treatment.

Experiments were performed to test the hypothesis that etiolated dodder seedlings cannot display parasitic behavior (cannot form coils or prehaustoria). If that is true then de-etiolation (hook opening) of dodder seedlings is the initial sign of plants characteristic with potential to transform to the parasitic mode of growth.

Hook opening is the first stage toward developing parasitism in dodder. One minute of R light induces hook opening during a subsequent 24 h DK period. The effect was nullified by immediate irradiation with 2 min FR light (data not shown). The same pattern was observed during parasitic growth. Irradiation of dark-grown seedlings with 1 min R light resulted in a significant stimulation in coiling and prehaustoria number in threads over a 48 h DK period when zeatin was applied (Fig. 2.5). This stimulation was completely reversed by irradiation with 2 min FR applied directly after R light. Two major conclusions can be drawn from this; (1) R light is required to stimulate hook opening, and (2) zeatin significantly stimulates coiling and prehaustoria once a R light effect has occurred.

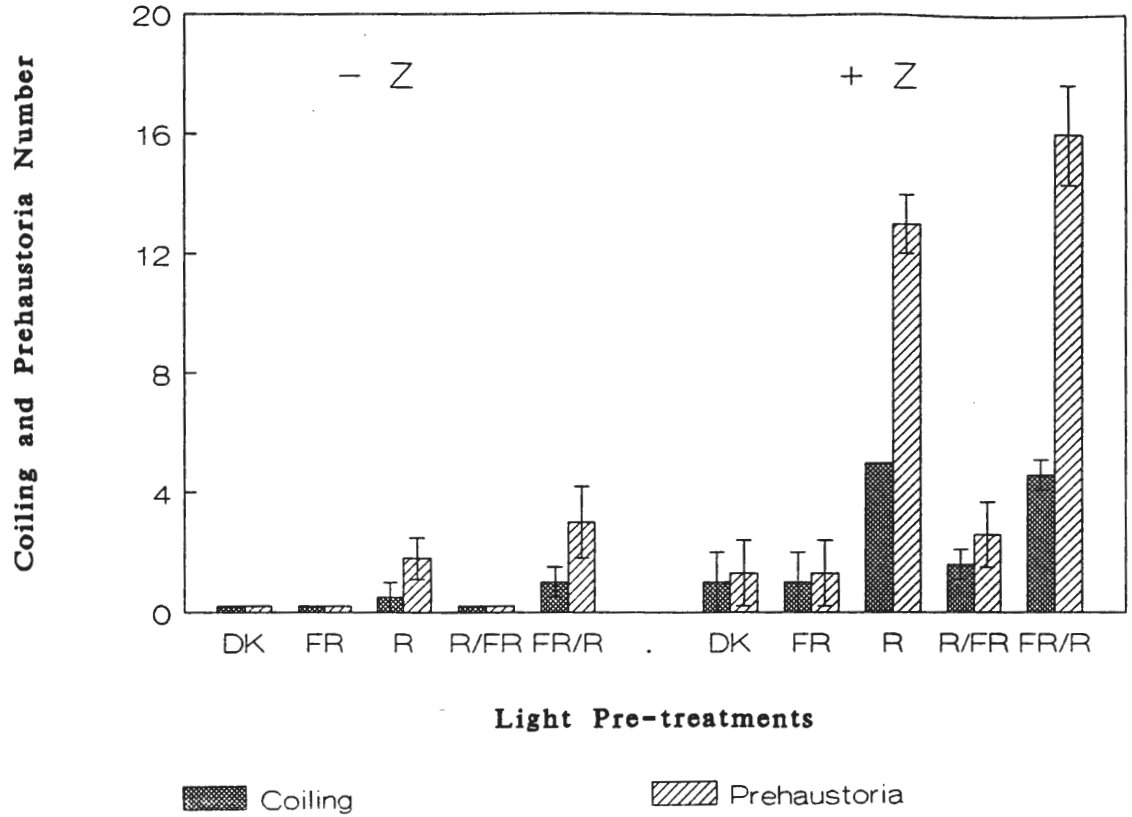


Figure 2.5. Coiling and prehaustoria number after alternating exposure to R and FR radiation with or without 10 μM zeatin (see materials and methods, LFR section). Each point represents the mean of three experiments ± SE. Dark (DK); Far-red light (FR); Red light (R); Red light followed immediately by Far-red light (R/FR); Far-red light followed immediately by Red light (FR/R).

Another experiment was conducted to study the effect of different periods of light pretreatment. Four-day-old etiolated seedlings received 0 or 10 min WL followed by 24 h DK or WL. Seedlings were excised and transferred into different light treatments for another 48 h. Table 2.1 shows that no coiling and prehaustoria can be induced in dark green grown seedlings by any monochromatic light. Coiling and prehaustoria were observed only under a mixture of BF light (BF effect will be described later). However, coiling and prehaustoria were similar in de-etiolated seedlings that received 10 min or 24 h WL light pretreatment.

Effects of Monochromatic Light.

Comparisons made between UV-A, B, R and FR wavebands each applied separately, demonstrated that B light (400-500 nm) was the only effective light treatment in promoting coiling and prehaustoria development in de-etiolated dodder seedlings (Fig. 2.6). Coiling occurred within 6 h while prehaustoria developed after 22 h of light application (data not shown).

An experiment was conducted to study if coiling and prehaustoria formation under B light are fluence rate dependent. Figure 2.7, shows that B light has no effect at photon fluxes lower than 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Above this rate coiling and prehaustoria development increase linearly with increasing B fluence. Maximum coiling and prehaustoria

Table 2.1. Effect of light pre-treatments on coiling and prehaustoria development. Threads were then transferred into different light treatments for 48 h. Each point represents the mean of three experiments ± SE. White light (WL); Blue light (B); Far-red light (FR); Red + Far-red light (RF); Blue + Far-red light (BF).

WL pre-treatments (hours)	Light post-Treatments Continuous for 48 h	Coiling Number	Prehaustoria Number	
		Time (hours)		
		24	24	48
0.0	B	0.5 ± 0.5	0.0 ± 0.0	2.0 ± 1.0
	FR	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	RF	0.5 ± 0.5	0.0 ± 0.0	1.0 ± 1.0
	BF	2.6 ± 1.1	2.7 ± 0.9	16.0 ± 2.6
0.16	B	4.3 ± 0.5	0.0 ± 0.0	19.0 ± 1.3
	FR	0.8 ± 0.5	0.2 ± 0.3	1.6 ± 1.0
	RF	4.0 ± 0.5	7.0 ± 2.0	21.0 ± 1.1
	BF	4.6 ± 0.5	23.0 ± 2.6	29.5 ± 1.1
24	B	4.6 ± 0.5	0.0 ± 0.0	22.0 ± 1.8
	FR	1.0 ± 0.5	0.3 ± 0.3	2.1 ± 0.7
	RF	4.0 ± 0.5	7.6 ± 2.3	21.0 ± 1.8
	BF	4.6 ± 0.5	26.0 ± 2.0	33.0 ± 2.1

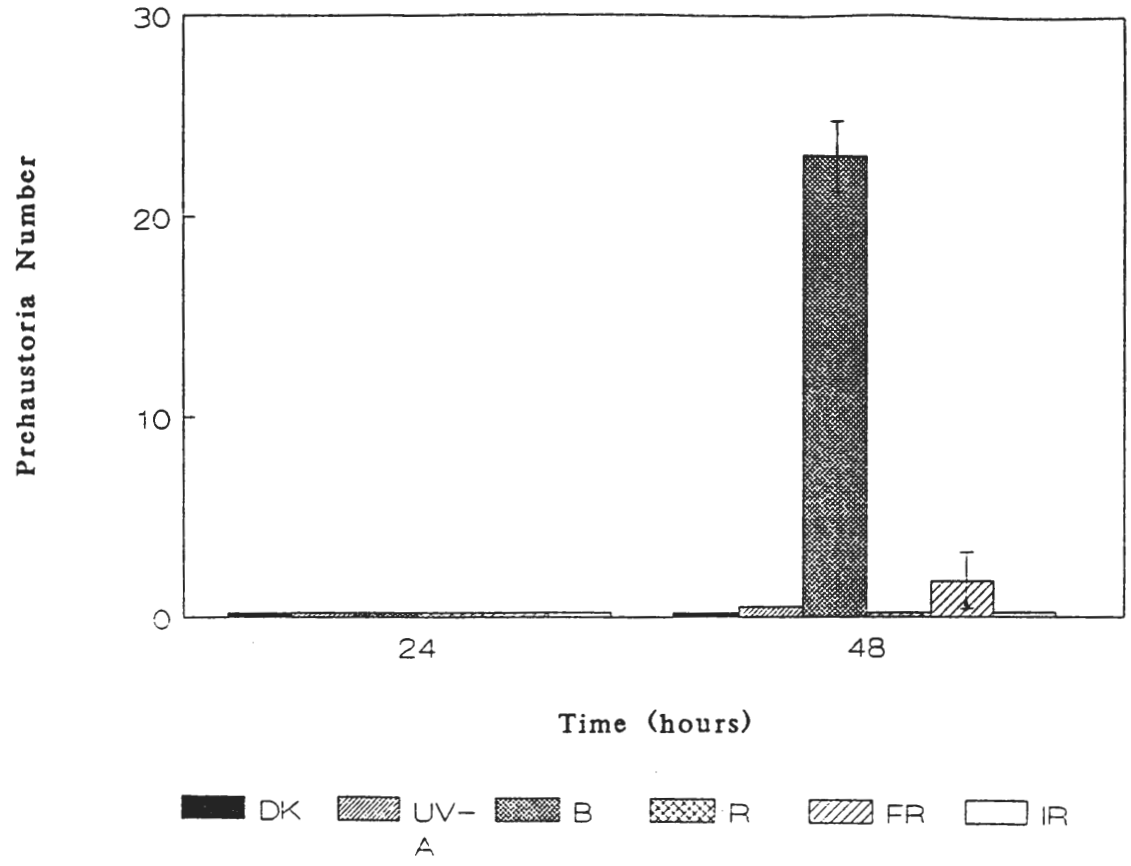


Figure 2.6. Effect of different monochromatic lights on prehaustoria development. Each point represents the mean of 3 experiments ± SE. Dark (DK); Near ultraviolet light (UV-A); Blue light (B); Red light (R); Far-red light (FR); Infrared light (IR).

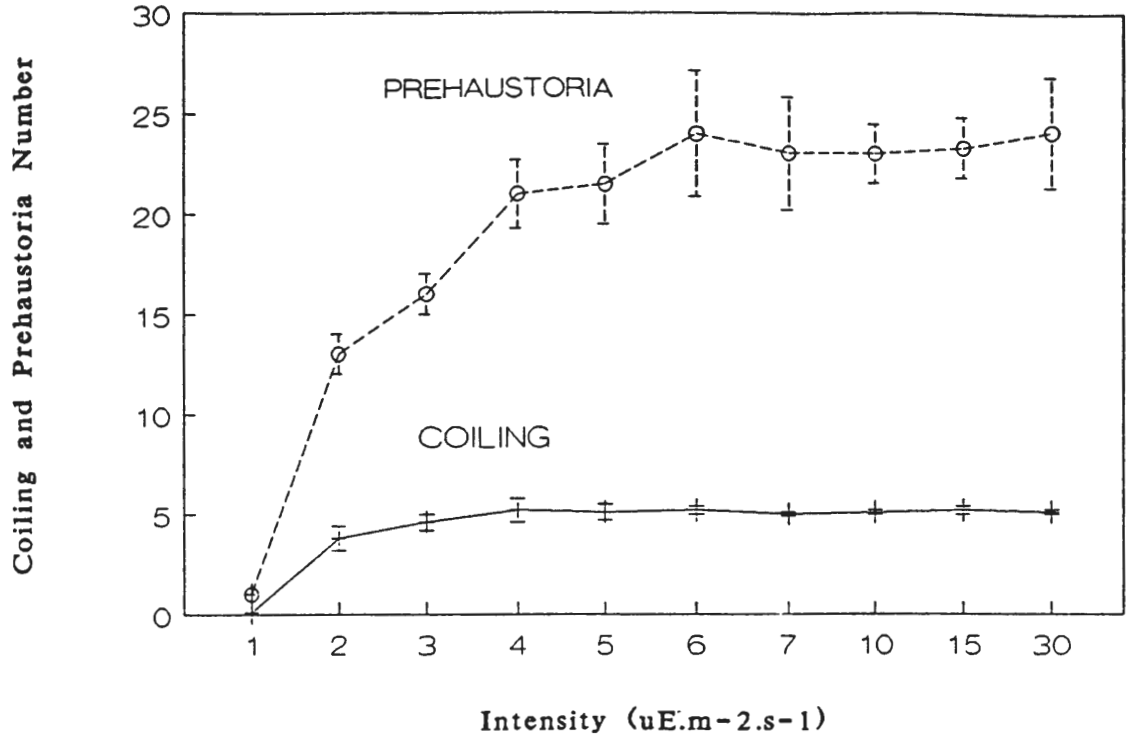


Figure 2.7. Blue-light fluence response curves for coiling and prehaustoria development. Light was provided from above. Forty eight hours after B light treatment, coiling and prehaustoria were measured. Each point represents the mean of 3 experiments ± SE. Blue light (B).

development occurred at a fluence rate of approximately 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ respectively. At this level (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$), prehaustoria covered approximately 80% of the dodder thread. Increasing B light fluence above this level did not cause additional stimulation of either response.

Effect of Dichromatic Light.

Further characterization of coiling and prehaustoria induction were studied. Responses are fully expressed under prolonged exposure to simultaneous application of different light treatments. Prehaustoria were most evident whenever FR light was applied simultaneously with any other light treatment (Fig. 2.8). A mixture of BF light was the most effective in inducing coiling and prehaustoria after 24 and 48 h of light application. Prehaustoria number under this light environment reached a maximum, producing a mean of 6 to 7 prehaustoria on the inner surface of each thread.

No responses were observed whenever R light was applied simultaneously with any light except FR light. However, the latter effect depended on the ratio of R:FR (which will be described in the following chapter) and not B:FR ratio. Under a BF light combination, addition of a small amounts of B (0.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or a high amount of B light (15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to any level of FR light resulted in a similar stimulating effect on coiling and prehaustoria number (data not shown). Furthermore, responses were strongly enhanced by continuous

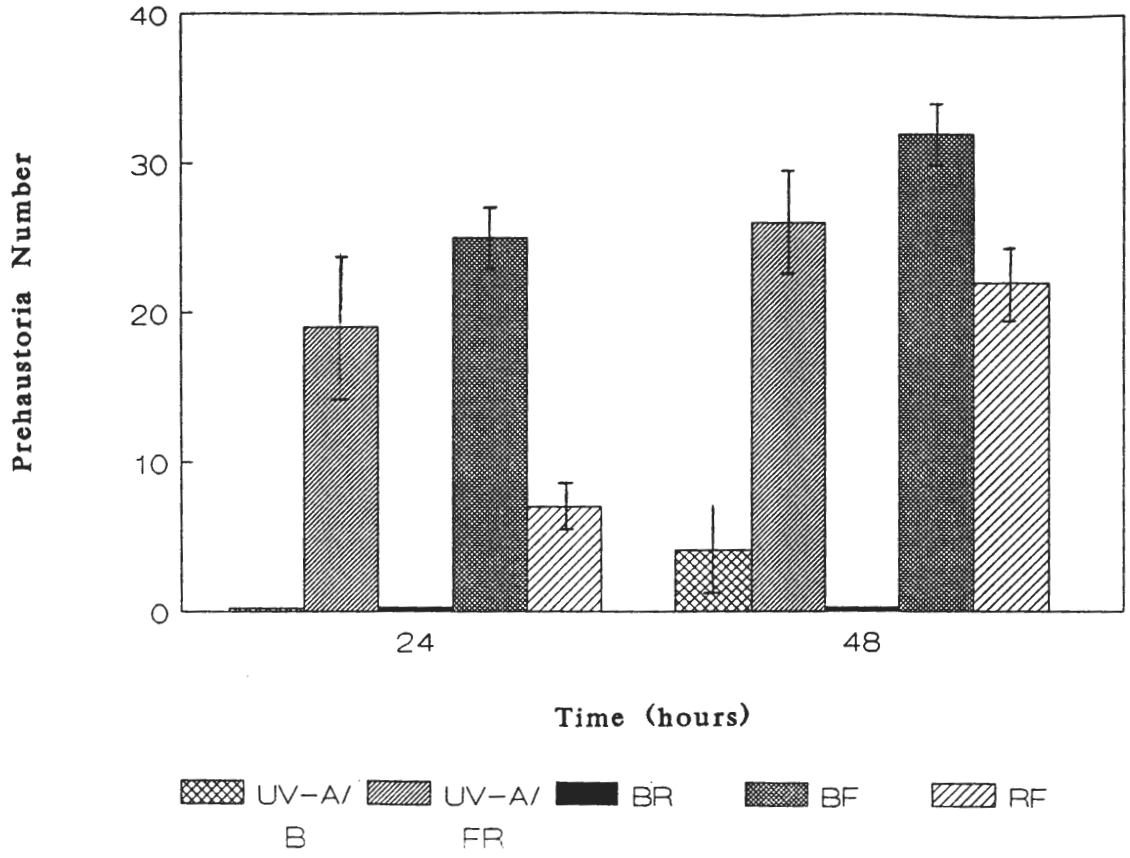


Figure 2.8. Effect of different dichromatic lights on prehaustoria development. Each point represents the mean of 3 experiments ± SE. Near ultra-violet + Blue (UV-A/B); Near ultra-violet + Far-red light (UV-A/FR); Blue + Red light (BR); Blue + Far-red light (BF); Red + Far-red light (RF).

irradiation and duration but they were not fluence rate dependent.

Experiments were conducted to study the time course and duration of coiling and prehaustoria formation. Because the BF light combination was most effective in inducing the above responses, threads were put under this light combination. Figure 2.9, shows that coiling occurred within a period of 2 to 6 h of BF light application, while prehaustoria initiation occurred within a period of 16 to 18 h and continued to increase reaching a maximum at 36 to 48 h of light application. Additional experiments were carried out to determine the duration of coiling and prehaustoria development. Figures 2.10 and 2.11 show that prehaustoria production under 1 h BF light followed by a subsequent 23 or 47 h darkness is equivalent to a continuous application of 18 h BF light (Fig. 2.9). The same pattern was observed with a continuous application of 20 h BF followed by a subsequent 28 h darkness (Fig. 2.11), and is equivalent to a continuous application of 36 or 48 h BF light (Fig. 2.9).

Reciprocity.

To separate the effects of illumination period from total fluence, experiments were conducted in which B and a mixture of RF light treatments of constant total fluence were delivered over several periods of time. The results, shown in Figure 2.12, demonstrated that hourly 5 min light pulses could not substitute for continuous B or RF

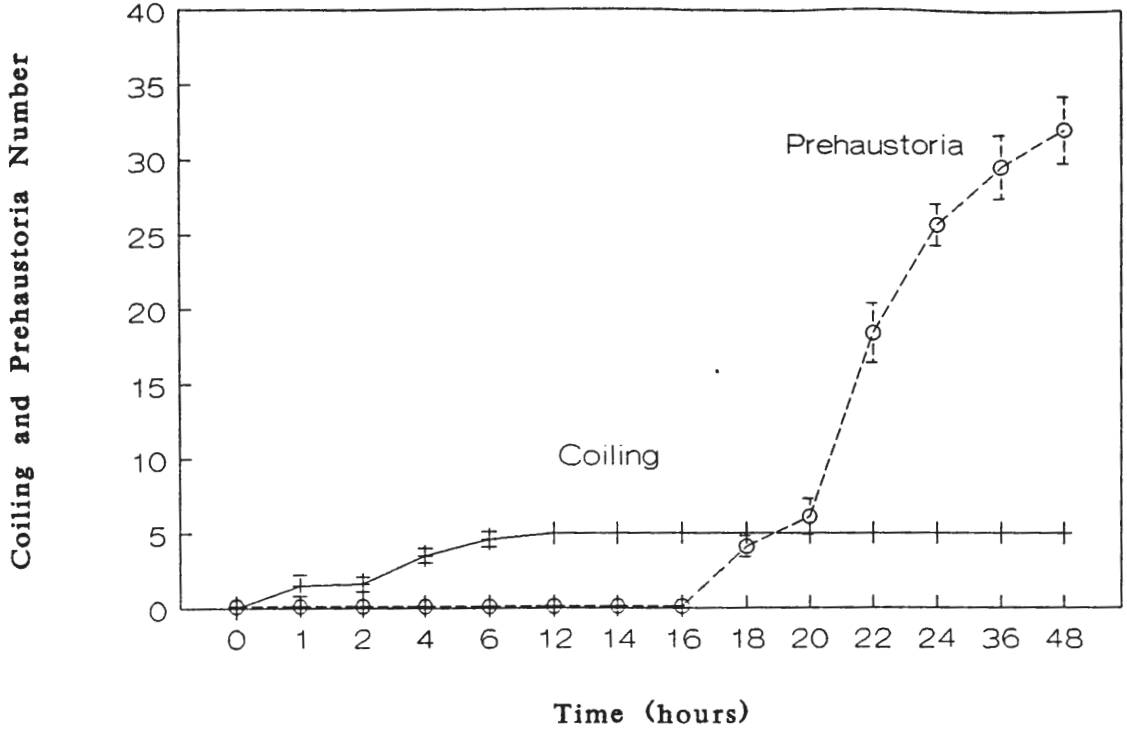


Figure 2.9. Time course of coiling and prehaustoria development under BF light. Each point represents the mean of 3 experiments ± SE. Blue + Far-red light (BF).

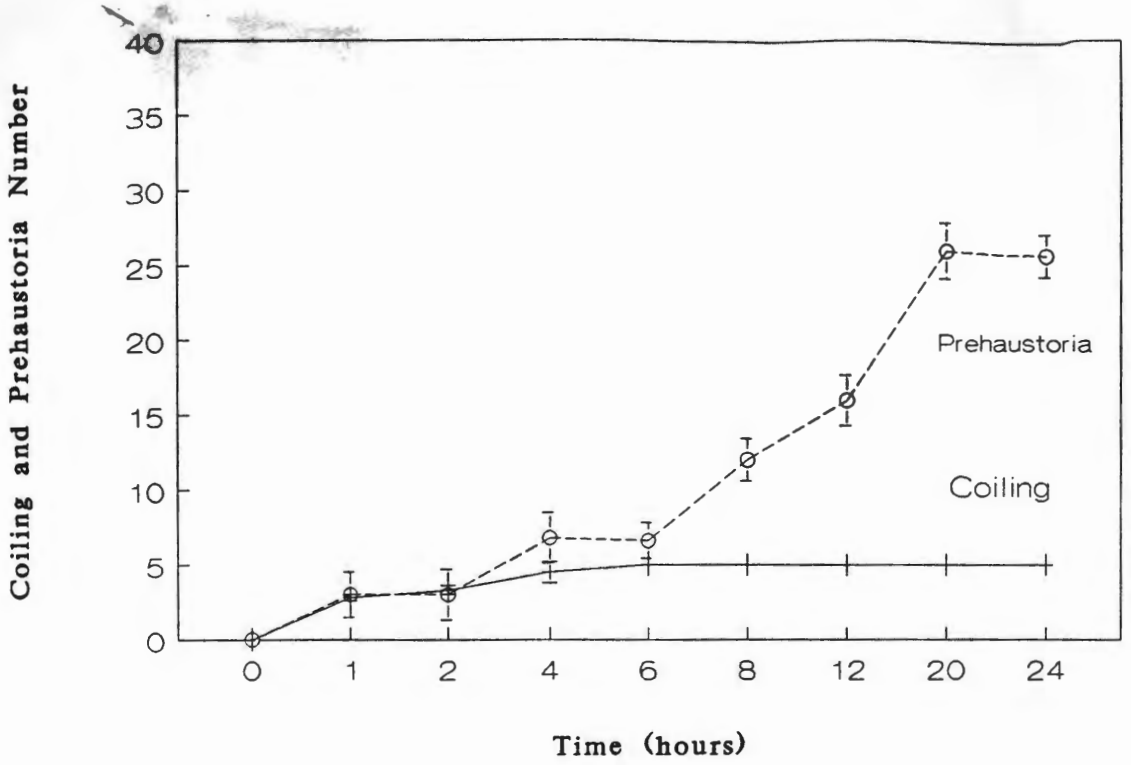


Figure 2.10. Duration course of coiling and prehaustoria development under BF light. BF light was applied for 1, 2, 3, 4...24 h, then threads were placed in darkness; e.g. 1 h BF then 23 h DK. Coiling and prehaustoria were measured at the end of this period. Each point represents the mean of 3 experiments ± SE. Blue + Far-red light (BF).

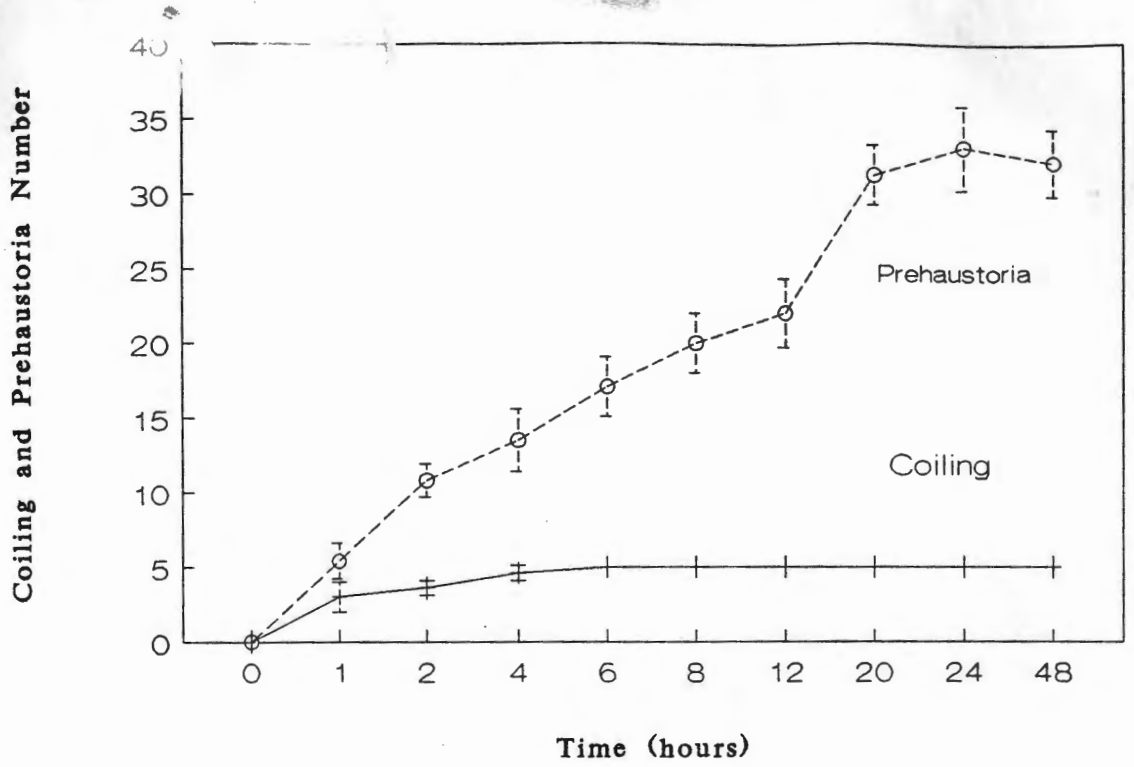


Figure 2.11. Duration course of coiling and prehaustoria development under BF light. BF light was applied for 1, 2, 3, 4...48 h, then threads were placed in darkness; e.g. 1 h BF light then 47 h DK. Coiling and prehaustoria were measured at the end of this period. Each point represents the mean of 3 experiments ± SE. Blue + Far-red light (BF).

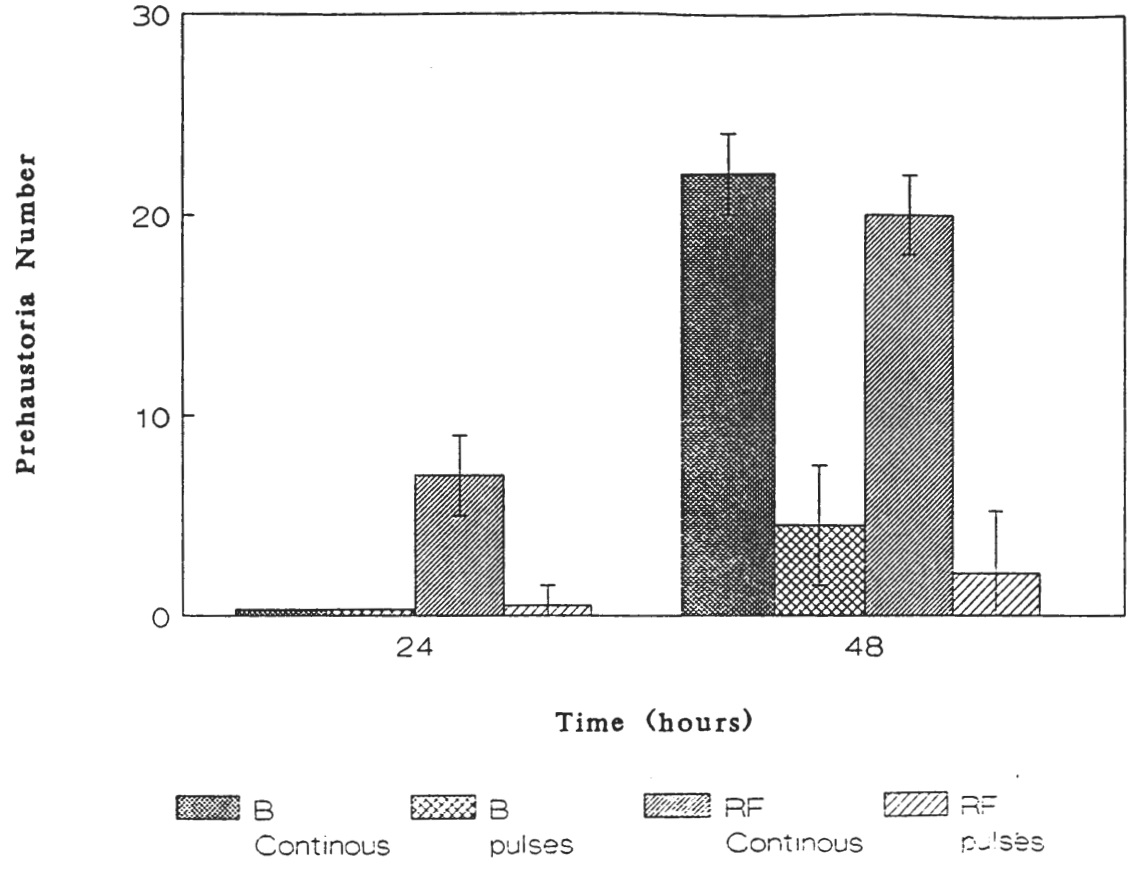


Figure 2.12. Reciprocity. Threads were either irradiated with continuous B (4.0 μmol·m^{-2}s^{-1}) or RF (35 μmol·m^{-2}·s^{-1}) light for 48 h, or irradiated with 5 min pulse/hour of B (150 μmol·m^{-2}s^{-1}) or RF light (280 μmol·m^{-2}s^{-1}) for 48 h. Each point represents the mean of 3 experiments ± SE. Blue light (B); Red + Far-red light (RF).

combination for 48 h, even if the total fluence of the two different light regimes over the irradiation period was the same. The number of prehaustoria formed is in part a function of length of irradiation periods and not the total number of photons delivered (total fluence). Thus the Bunsen-Roscoe reciprocity law does not hold.

Effect of Mechanical Stimulation and Ethylene.

Results in Figure 2.13 show that dodder threads did not coil and form prehaustoria when threads were floated on water. This was true regardless of the light conditions, although if they were mechanically stimulated or zeatin was applied they were capable of coiling and forming prehaustoria.

It is well documented that effects of mechanical perturbation (gentle rubbing) on plants is mediated by ethylene (Hiraki and Ota, 1975). So a hypotheses was formed that when dodder encounters a host plant, ethylene will be evolved thus inhibiting dodder parasitic growth. However, this was not the case. Results in Figure 2.14, showed that ethylene significantly reduces prehaustoria number at 22 h of light application. But prehaustoria overcame ethylene's effect over time. Moreover, ethylene has no effect on prehaustoria development if added or removed after 22 h of BF light application (Fig. 2.15).

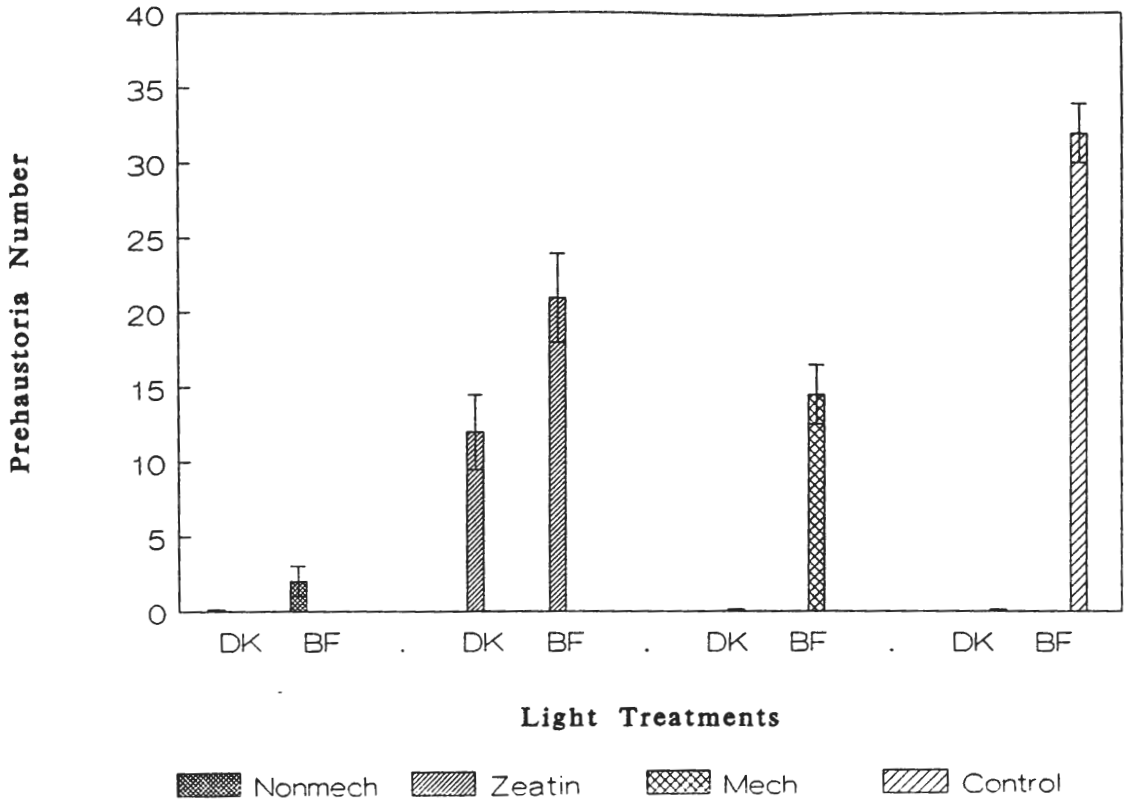


Figure 2.13. Effect of mechanical perturbation on prehaustoria development in DK and under BF light. De-etiolated 96 h old threads were mechanically stimulated by moving each thread gently from one side 10 times over a wet glass fiber filter. At the end of the period of the stimulation, mechanically and non-mechanically stimulated threads were floated on H_2O or zeatin (40 μM) solution in petri dishes. Dishes, each containing one thread (15 threads/trt) were transferred into DK or BF light for 48 h. A temperature of 30 $\pm$ 2°C was maintained during incubation. Prehaustoria formed at the end of this period were determined. Each point represents the mean of 3 experiments $\pm$ SE. Dark (DK); Blue + Far-red light (BF).

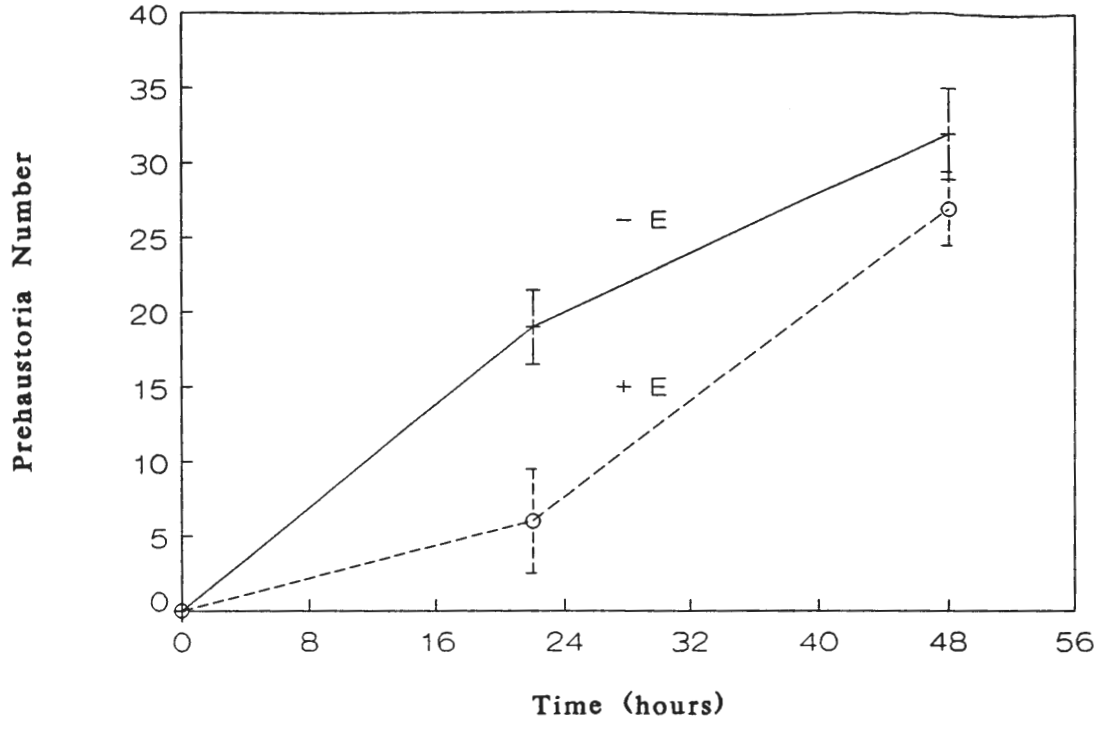


Figure 2.14. Effect of ethylene (E) on prehaustoria development under BF light. De-etiolated 96 h old dodder threads were placed horizontally on 5.5 cm plastic petri dishes each containing one layer of glass fiber filter paper and 2 ml of water. Petri dishes were left open, and placed in a plastic boxes (3/box) containing the test solution consisting of 10 mM phosphate buffer(KH_2PO_4), pH 7.0 with or without 0.11 μL ethylene (floral™) (equivalent to 100 μM). The boxes were sealed with plastic lids, each was then wrapped with a clear cellophane and transferred into BF light. Prehaustoria were measured after 48 h. Each point represents the mean of 3 experiments ± SE. Blue + Far-red light (BF).

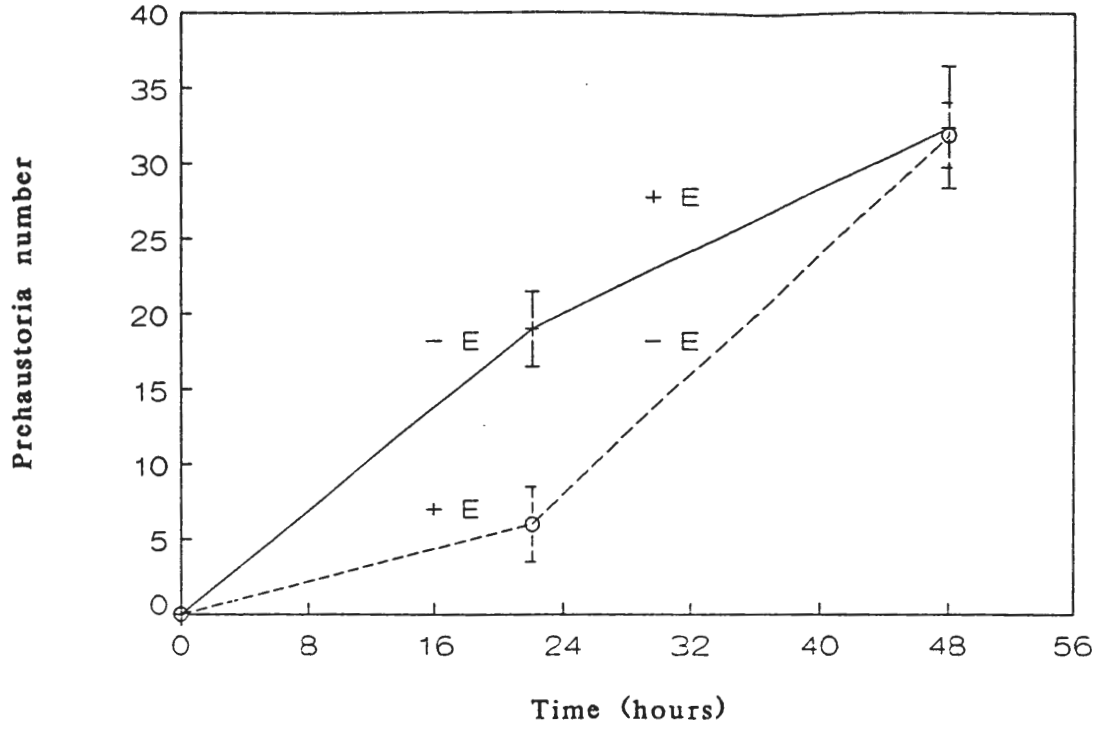


Figure 2.15. Effect of ethylene (E) on prehaustoria development under BF light. Sealed boxes ± ethylene solution (see Fig. 2.14 for more details) were transferred into BF light for 22 h. Prehaustoria were measured, then ethylene was either added or removed by transferring the petri dishes into new boxes containing a new buffer solution ± ethylene. Boxes were transferred into BF light for another 24 h. Prehaustoria were measured again at the end of this period. Each point represents the mean of 3 experiments ± SE. Blue + Far-red light (BF).

Effect of Zeatin.

Zeatin at a concentration of 10 μM significantly induced coiling and prehaustoria initiation under different light regimes, except under BF light combination (Tables 2.2 and 2.3). Increasing zeatin concentration did not result in further stimulation under BF light. However, zeatin has greater effect under the FR or RF light combinations.

Effect of IAA.

Auxins inhibited light and zeatin-induced coiling and prehaustoria formation. The inhibition by auxin depended on light quality and IAA concentration. Prehaustoria number were significantly reduced at 50 to 100 μM IAA applied alone (Table 2.4) or with 50 μM zeatin (Table 2.5) for most of the light treatments. However, under BF light combinations zeatin treated or untreated dodder threads were able to overcome the low concentration of IAA (50 μM) 48 h after light and hormonal application (Table 2.4 and 2.5).

Effect of Potassium Salts and Sucrose.

Following these initial studies, an experiment was conducted to identify additional potential sources of stimulation. As shown in Figures 2.16a and 2.16b, KCl was effective in inducing coiling and prehaustoria under prolonged exposure to FR and RF light. The same pattern was observed with KI (Figs. 2.17a and 2.17b). Both chemicals were unable to cause an additional stimulation under BF

Table 2.2. Effect of zeatin (10 μM) ± 20 mM KCl on coiling and prehaustoria development under different monochromatic light treatments. Each point represents the mean of three experiments ± SE. Dark (DK); Far-red light (FR); Red light (R); Blue light (B).

Light Treatments	Chemicals.	Coiling Number	Prehaustoria Number	
		Time (hours)		
		24	24	48
DK	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	KCl	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Zeatin	4.0 ± 0.5	3.6 ± 1.0	7.0 ± 1.6
	Zeatin + KCl	4.0 ± 0.5	4.1 ± 1.1	6.3 ± 1.8
FR	Control	1.0 ± 0.5	0.3 ± 0.3	2.1 ± 0.7
	KCl	3.0 ± 0.3	0.8 ± 0.3	8.6 ± 0.5
	Zeatin	4.6 ± 0.5	8.5 ± 1.1	17.0 ± 1.0
	Zeatin + KCl	4.3 ± 0.5	11.0 ± 2.3	16.5 ± 1.0
R	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	KCl	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Zeatin	3.0 ± 1.0	5.3 ± 2.0	8.0 ± 1.0
	Zeatin + KCl	4.3 ± 0.5	5.0 ± 1.0	9.0 ± 1.1
B	Control	4.6 ± 0.5	0.0 ± 0.0	22.0 ± 2.0
	KCl	4.6 ± 0.5	1.2 ± 1.0	23.0 ± 1.0
	Zeatin	5.0 ± 0.0	5.0 ± 1.0	23.5 ± 1.1
	Zeatin + KCl	5.0 ± 0.0	6.0 ± 1.8	25.0 ± 1.7

Table 2.3. Effect of zeatin (10 μM) ± 20 mM KCl on coiling and prehaustoria development under different dichromatic light treatments. Each point represents the mean of three experiments ± SE. Blue + Red light (BR); Blue + Far-red light (BF); Red + Far-red light (RF).

Light Treatments	Chemicals	Coiling Number	Prehaustoria Number	
		Time (hours)		
		24	24	48
BR	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	KCl	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Zeatin	3.3 ± 0.4	3.3 ± 1.0	5.5 ± 0.5
	Zeatin + KCl	3.3 ± 0.5	4.0 ± 1.0	5.8 ± 0.7
BF	Control	4.6 ± 0.5	25.0 ± 1.5	32.0 ± 2.0
	KCl	5.0 ± 0.0	28.0 ± 3.0	32.0 ± 2.0
	Zeatin	5.0 ± 0.0	27.0 ± 1.0	31.0 ± 1.0
	Zeatin + KCl	5.0 ± 0.0	26.0 ± 2.1	30.0 ± 3.0
RF	Control	3.8 ± 0.5	7.0 ± 1.6	21.0 ± 2.0
	KCl	4.5 ± 0.3	13.2 ± 2.5	28.5 ± 2.8
	Zeatin	5.0 ± 0.0	16.0 ± 2.0	26.0 ± 3.0
	Zeatin + KCl	4.6 ± 0.5	22.0 ± 2.1	29.5 ± 2.8

Table 2.4. Effect of IAA on coiling and prehaustoria development under different light treatments. Each point represents the mean of three experiments ± SE. Blue light (B); Blue + Far-red light (BF); Red + Far-red light (RF).

Light Treatments	IAA (μM)	Coiling Number	Prehaustoria Number	
		Time (hours)		
		24	24	48
B	0	4.6 ± 0.5	0.0 ± 0.0	22.0 ± 2.0
	50	4.0 ± 0.5	0.0 ± 0.0	13.0 ± 1.7
	100	4.0 ± 0.5	0.0 ± 0.0	8.0 ± 2.0
BF	0	4.6 ± 0.5	25.3 ± 2.0	33.0 ± 2.6
	50	3.6 ± 0.5	9.0 ± 3.0	28.0 ± 3.6
	100	3.5 ± 0.6	6.0 ± 3.0	17.0 ± 2.6
RF	0	3.8 ± 0.2	7.0 ± 1.6	21.0 ± 2.0
	50	1.8 ± 0.5	0.0 ± 0.0	8.0 ± 2.0
	100	1.5 ± 0.4	0.0 ± 0.0	5.0 ± 3.0

Table 2.5. Effect of zeatin (50 μM) ± IAA on coiling and prehaustoria development under different light treatments. Each point represents the mean of three experiments ± SE. Blue light (B); Blue + Far-red light (BF); Red + Far-red light (RF).

Light Treatments	Hormones (μM)	Coiling Number	Prehaustoria Number	
		Time (hours)		
		24	24	48
B	Control	4.6 ± 0.5	0.0 ± 0.0	22.0 ± 2.0
	Zeatin	5.0 ± 0.0	6.0 ± 1.0	23.0 ± 1.0
	Zeatin + IAA (50)	3.6 ± 1.0	0.0 ± 0.0	15.5 ± 3.6
	Zeatin + IAA (100)	4.0 ± 1.0	0.0 ± 0.0	10.0 ± 1.7
BF	Control	4.6 ± 0.5	25.3 ± 2.0	33.0 ± 2.6
	Zeatin	5.0 ± 0.0	25.0 ± 1.4	31.5 ± 2.6
	Zeatin + IAA (50)	4.0 ± 0.5	17.0 ± 3.0	26.0 ± 2.0
	Zeatin + IAA (100)	4.0 ± 0.5	12.0 ± 2.8	18.0 ± 3.0
RF	Control	3.8 ± 0.2	7.0 ± 1.6	21.0 ± 2.0
	Zeatin	4.6 ± 0.5	17.0 ± 1.5	26.0 ± 1.0
	Zeatin + IAA (50)	4.3 ± 0.5	12.0 ± 2.0	14.0 ± 1.0
	Zeatin + IAA (100)	4.3 ± 0.5	10.0 ± 2.0	11.0 ± 1.3

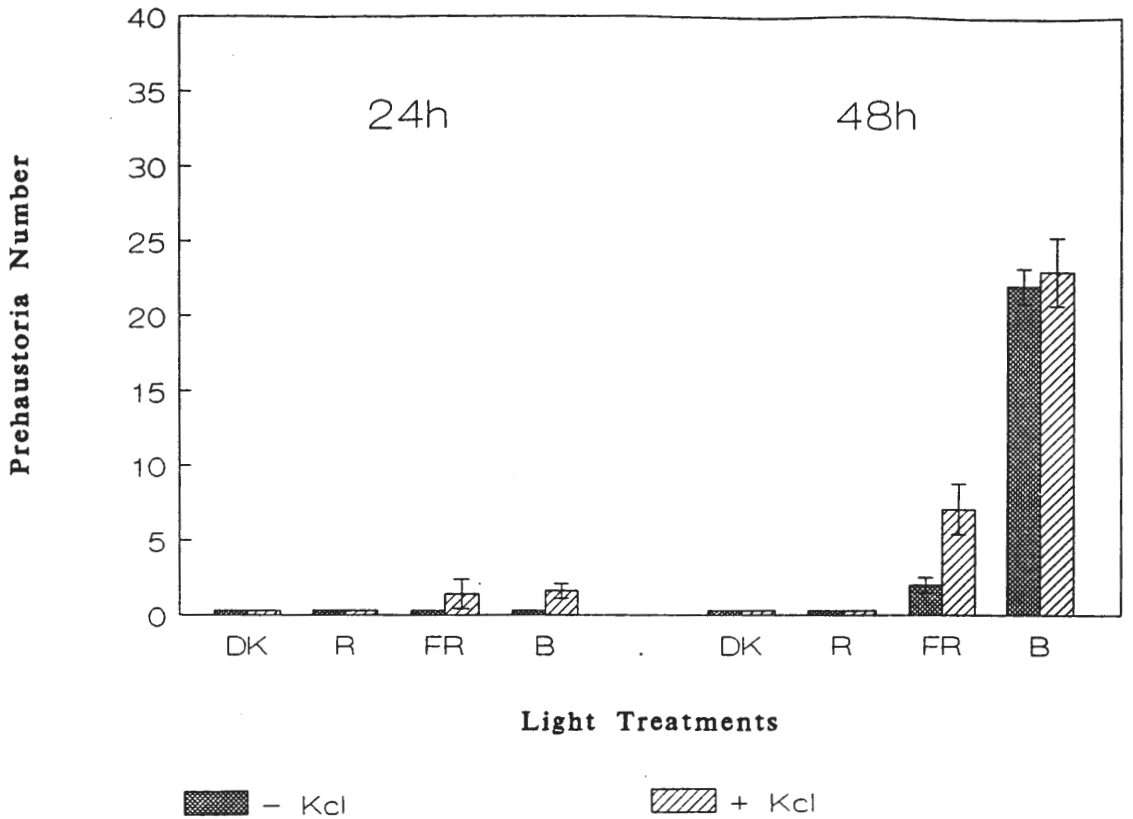


Figure 2.16a. Effect of 20 mM KCl on prehaustoria formation under different monochromatic light treatments. Each point represents the mean of 3 experiments ± SE. Dark (DK); Red light (R); Far-red light (FR); Blue light (B).

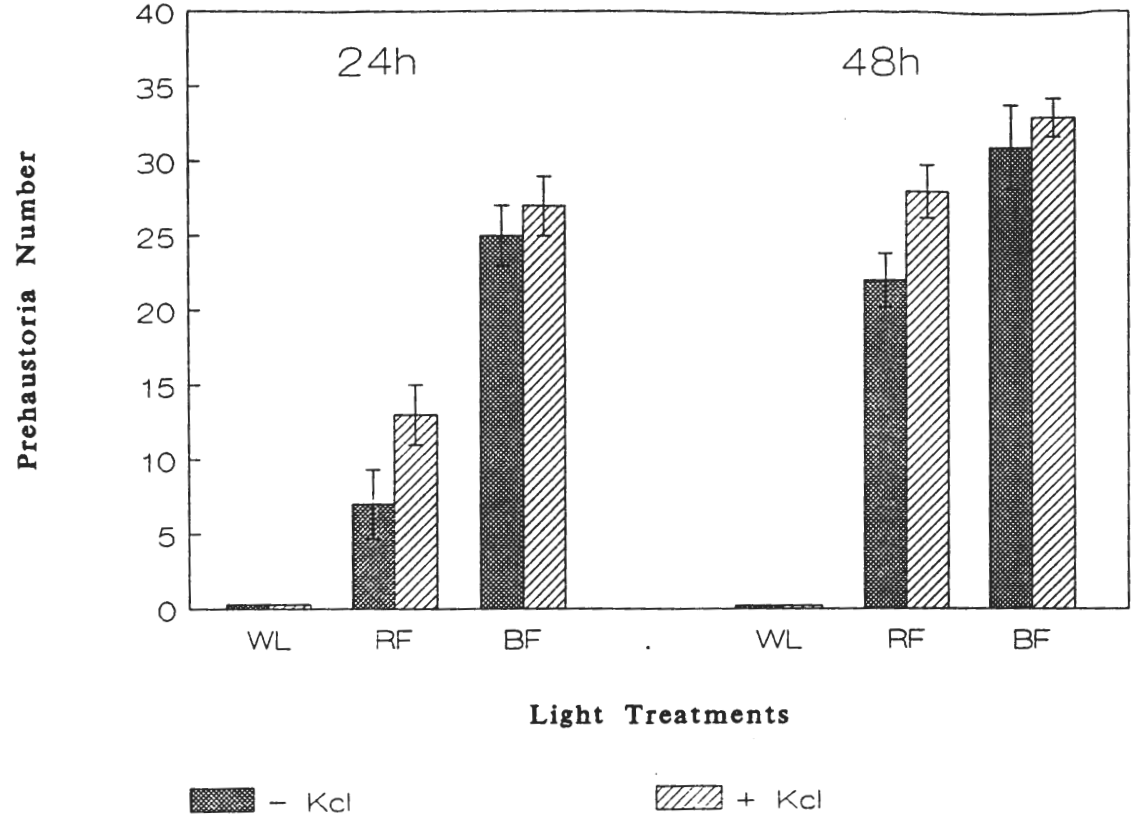


Figure 2.16b. Effect of 20 mM KCl on prehaustoria formation under different dichromatic light treatments. Each point represents the mean of 3 experiments ± SE. White light (WL); Red +Far-red light (RF); Blue + Far-red light (BF).

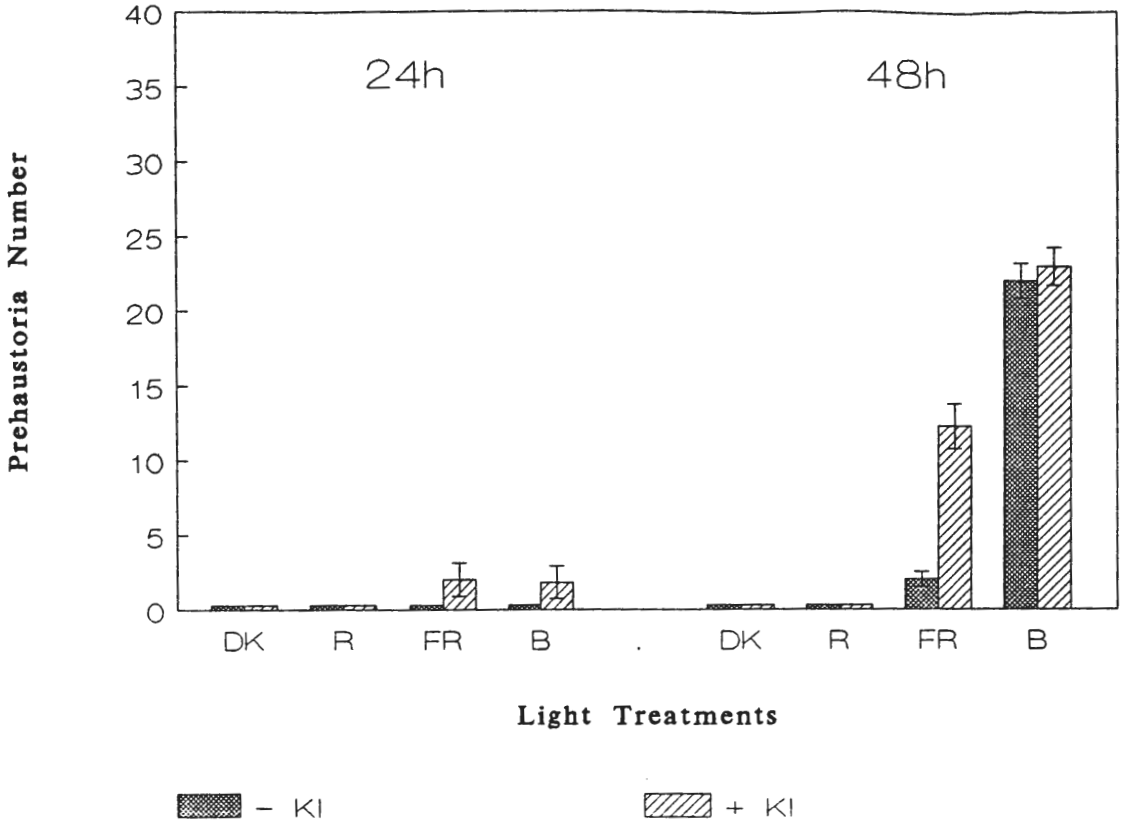


Figure 2.17a. Effect of 20 mM KI on prehaustoria formation under different monochromatic light treatments. Each point represents the mean of 3 experiments ± SE. Dark (DK); Red light (R); Far-red light (FR); Blue light (B).

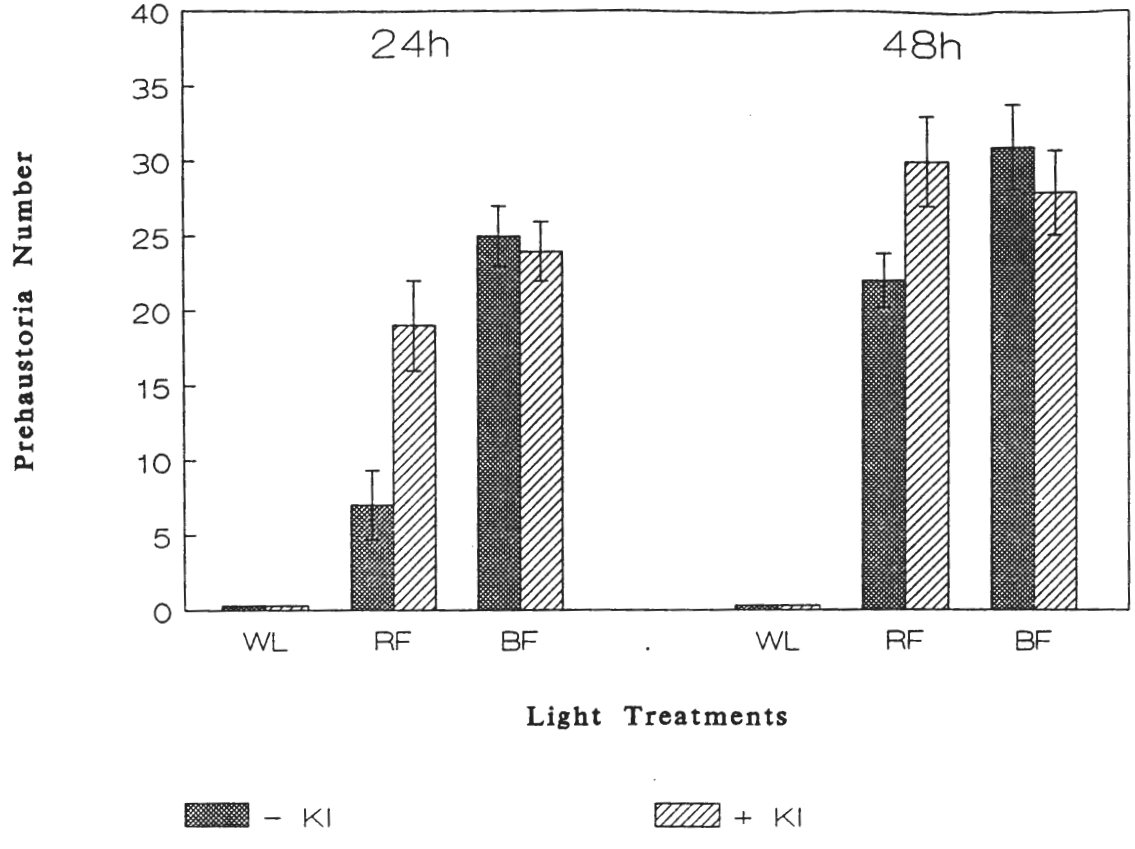


Figure 2.17b. Effect of 20 mM KI on prehaustoria formation under different dichromatic light treatments. Each point represents the mean of 3 experiments ± SE. White light (WL); Red + Far-red light (RF); Blue + Far-red light (BF).

light. However, KI significantly stimulated more prehaustoria than KCl under most of the light treatments. Neither $CaCl_2$ nor $MgCl_2$ had any effect on prehaustoria development (data not shown).

An experiment was conducted to study the effects of organic sources on prehaustoria under certain light regimes. As shown in Table 2.6, 20 mM sucrose enhanced prehaustoria development 24 h after B and 48 h after FR light applications.

Table 2.6. Effect of sucrose on prehaustoria development under different light treatments. Each point represents the mean of three experiments ± SE. Dark (DK); Blue light (B); Red light (R); Far-red light (FR); Red + Far-red light (RF).

Light Treatments	Sucrose (mM)	Prehaustoria Number Time (hours)	
		24	48
DK	0	0.0 ± 0.0	0.0 ± 0.0
	20	0.8 ± 0.5	2.1 ± 1.1
B	0	0.0 ± 0.0	23.0 ± 2.3
	20	5.1 ± 2.0	23.0 ± 2.0
R	0	0.0 ± 0.0	0.0 ± 0.0
	20	0.0 ± 0.0	0.0 ± 0.0
FR	0	0.0 ± 0.0	1.8 ± 1.1
	20	0.6 ± 1.0	9.0 ± 2.0
RF	0	7.0 ± 2.0	19.0 ± 2.6
	20	9.1 ± 1.8	24.0 ± 4.0

DISCUSSION

De-etiolation of dodder seedlings is the first outwardly visible characteristic of the imminent, true higher plant seedling of dodder that has the capacity to transform to its parasitic mode of growth. Dodder plants required a light pretreatment for light or zeatin-induce coiling and prehaustoria development becomes effective (Fig. 2.5 and Table 2.1). Physical and chemical signals can act once de-etiolation occurs.

From the results in Figure 2.5, it can be concluded that light pretreatment (de-etiolation) will affect parasitic growth of dodder. Etiolated dodder seedlings cannot transform to the parasitic mode of growth without passing through the de-etiolation procedure. The lack of any significant induction in coiling and prehaustoria development in dark grown dodder seedlings is consistent with the results obtained by Mohr (1957) on hypocotyl elongation of Sinapis alba.

In dodder, light pretreatment was necessary for the full prolonged effect of light or zeatin to be manifested, and experiments were performed to investigate the nature of the required pretreatment. Results in Table 2.1 show that whether dodder seedlings received 10 min or 24 h WL pre-

irradiation, coiling and prehaustoria responded similarly under all the light treatments tested. It is worth mentioning that with no WL pretreatment, BF light stimulated prehaustoria development at 48 h.

Prolonged Exposure to Monochromatic Light.

Plants in a natural environment are usually exposed to long periods of radiation at relatively a high fluence rate. The effects of different wavelengths on parasitic growth of dodder show that a fairly narrow band of the visible spectrum is most effective in eliciting coiling and prehaustoria formation (Fig. 2.6). Of the wavelength spectrum of 300-800 nm, only the B wavelengths (440 through 480 nm) strongly promote coiling and prehaustoria development. It is worth noting that these effects occur in seedlings that have been de-etiolated by R or WL.

Blue light effects on plants have been well documented. Action spectra for many different phenomenon have been determined, and are essentially similar to the spectrum for the light activation effect presented here. Among the B light effects are phototropism and phototaxis, photomorphogenesis, carotenogenesis and the light growth reaction in Phycomyces (Briggs, 1976). Most of these spectra show a shoulder near 420 nm with a maximum between 445 and 460 nm and another shoulder between 470 and 490 nm in the visible B light region, as well as a single broad peak near 370 nm in the UV-A region. In dodder the action

spectrum exhibits peaks at 440 and 480 nm but no shoulder at 380 nm (UV-A) unless FR was added.

The results in Table 2.1, show that the 'classical' HIR, as defined by peaks of action in the FR and B range of the spectrum, does not exist in etiolated dodder seedlings. The effectiveness of B and FR light does not exist. However, HIR 'classical' response does exist in de-etiolated dodder seedlings where a peak in B light action appears (Fig. 2.6).

An obvious feature of the response to B light is that fluence rates less than 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ fail to stimulate coiling and prehaustoria (Fig. 2.7). This type of response has been reported in de-etiolated Cucumis sativus (Gaba and Black, 1983) and etiolated Sinapis alba (Holmes and Schafer, 1981) where photo-inhibition of hypocotyl elongation by B light occurred only when a threshold was of the order of 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for Cucumis and 0.1 to 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for Sinapis, above which B becomes more inhibitory.

It is not clear in dodder why prolonged exposure of low fluence rate of B light elicits no stimulating effect on coiling and prehaustoria development. One possible explanation is that some conversion of phytochrome is required. This possibility is supported by the finding that FR increases the effect of B fluence rate below 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (data not shown) or above 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2.8). This is an indication that reduction of Pfr is required for B light to be effective. Furthermore, if a certain amount of

Pfr/Ptot (Φ) is required, this may not be established at fluence rates less than 1.5 $\mu M \cdot m^{-2} \cdot s^{-1}$, as photo-conversion of phytochrome by B light is strongly fluence rate dependent (Jabben et al., 1982) (See Chapter Three for further discussion).

Prolonged Exposure to Dichromatic Light.

The major results, shown in Figure 2.8, indicated that the addition of FR light enhances UV-A, R, and B light in eliciting coiling and prehaustoria formation. This suggests that a low Pfr over Ptot is required during prolonged irradiation of the above lights.

The close similarities in prehaustoria kinetics in response to B (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and mixture of RF light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) is an interesting observation that will be addressed in chapter three. However, it is important to note that the responses to the RF mixture has a relatively weak fluence rate dependence over the range tested. The increase in prehaustoria rate induced by supplementary FR light is reversed by an increase in the fluence rate of R light. Increasing the fluence rate of an RF mixture results in statistically significant reductions in coiling and prehaustoria development (data not shown). However, whatever the fluence rate of BF mixture, coiling and prehaustoria did not significantly change (data not shown). It appears that the physiological response to a reduction in the fluence rate of RF radiation is in the same direction as

the response to a reduction in the Pfr/Ptot (Φ). These data indicate that the responses are controlled by phytochrome photo-equilibrium (Φ).

The ratio of R:FR may serve as a signal to indicate canopy cover or leaf density. This signal interacts with other resources (water, etc.) to determine the rate of parasitic mode of dodder growth. The modification in light quality reaching the base of plants results in a change in R:F ratio (zeta) which appears to be a mechanism of importance for regulating dodder growth. During the summer the fully developed canopy causes a reduction in the R:FR that may be sufficient to stimulate parasitic mode of dodder growth.

Early detection of potential hosts by dodder seedlings growing under plant canopies or in simulated conditions, such as those used in Figures 2.6 and 2.8 is controlled initially by the change in spectral quality in the RF spectrum and the change in the quantity of B light. Both of them appear to be the major factors for controlling coiling and prehaustoria development in dodder seedlings. In situations where shading is caused by objects which have only a small influence on spectral quality (Ritter et al., 1981), prehaustoria development might be controlled primarily by changes in the quantity of the B light, with changes in the amount of RF radiation playing a smaller role.

Coiling and prehaustoria development under B and a mixture of RF light is not solely a function of the total number of photons delivered (i.e. total fluence), but is due in part to the length of the irradiation period. The results, shown in Figure 2.12, demonstrate that the number of prehaustoria varied as a function of length of irradiation periods for total fluence rate. Thus the Bunsen-Roscoe Law of reciprocity does not hold.

Many studies indicated that prehaustoria development occurred within a few days. An attempt was made to further validate these reports. The time course of coiling and prehaustoria development was determined as shown in Figure 2.9. The presented study exhibited two phases; a large increase in coiling rate occurred within 2 to 6 h, peaking at about 5 h after onset of added BF light (phase I). Prehaustoria development occurred within 16 to 18 h followed by a sudden increase at 20 h of light application (phase II).

The kinetics of coiling and prehaustoria formation to short-term BF light were identical to those of long term BF. Figures 2.10 and 2.11, show that, the effect of continuous 48 h BF on coiling development could be replaced by 1 h BF light. The same pattern was observed in prehaustoria development. Prehaustoria number under continuous 18 h BF light (Fig. 2.9) were identical to those under 1 h BF light followed by a subsequent 23 or 47 h DK (Figs. 2.10 and 2.11).

Under field conditions there is a variation in light quality and quantity (Smith 1982). Dodder plants must have a strategy to respond rapidly to such changes. Thus, from the results obtained in Figure 2.10 and 2.11, one conclusion can be made, that dodder seedlings do not require a prolonged exposure to sunlight to develop their parasitic mode of growth. Exposure of dodder seedlings to a short period of BF light during the day is sufficient for dodder to change its growth from a higher plant to a parasitic mode of growth.

Mechanical Perturbation and Ethylene Effects.

One of the new findings reported in this chapter is that MP stimulated prehaustoria formation in the presence of BF light. The results suggest that mechanical stimulation is not sufficient alone to induce prehaustoria, but also requires the effects of light. Light is one of the determining factors in the capacity of dodder threads to coil and produce prehaustoria in response to MP. When MP threads were placed in DK no prehaustoria were observed (Fig. 2.13).

One interpretation of this effect is that MP causes redistribution of certain hormones or ions in the perturbed thread. Cytokinins stimulate chlorophyll biosynthesis (Salisbury and Ross, 1992). In dodder, we observed that chlorophyll accumulates to higher visible levels on the concave sides of MP coils (data not shown).

Our results are not consistent with the earlier data of Ramasubramanian et al., (1988), where they stated that mechanical stimulus is not required for haustoria initiation, and that ethylene (ethrel) inhibits BA-induced haustoria development. Results obtained here suggest that ethylene has a dramatic effect on dodder as a higher plant but not as a parasite (Figs. 2.14 and 2.15).

A possible explanation for the lack of ethylene to inhibits prehaustoria relates to potential effect on IAA transport. Many studies indicated that ethylene causes leaf epinasty by affecting auxin transport. The data indicates that IAA is not essential for ethylene production, emphasized by the relationship between MP, ethylene and IAA.

Zeatin and IAA Effects.

Cytokinin and IAA have been implicated in haustoria development in dodder plants. Cytokinin induces while IAA inhibits haustoria initiation (Paliyath, 1979; Rajagopal et al., 1988 and Ramasubramanian et al., 1988). In this study, the effects of zeatin and IAA and their interaction under different light conditions on coiling and prehaustoria formation were examined. The results indicate that the activity of cytokinin differs between etiolated and de-etiolated dodder seedlings. As shown in Figure 2.5, etiolated dodder seedlings (0 R light) did not coil or form prehaustoria in darkness when zeatin was present during the incubation.

The ecological significance of this finding is that etiolated dodder seedlings under the soil cannot transform into the parasitic mode of growth even if they encounter a root of a host plant. Plant roots are rich in cytokinin (Incoll and Jewer, 1987). Our results indicate that the first signal for dodder seedlings to respond to phytohormones is light.

The de-etiolated dodder seedlings respond to zeatin in darkness or under any light environment. As shown in Table 2.2 and 2.3, zeatin stimulated coiling and prehaustoria development under different light treatments.

The effect of zeatin on prehaustoria development was similar under R and darkness and differs from the effects of other light treatments (Table 2.2). Zeatin exhibited greater effect under prolonged exposure to FR light as compared to control.

The reason zeatin had a greater effect under FR compared to DK or R light should be examined. One possible hypothesis is that FR light inhibits Ca^{++} efflux from certain vesicles in plant cells. Many studies have indicated that cytokinin action is mediated by Ca^{++}. Red light (Pfr) activates Ca^{++} influx in many plant cells and FR reverses it. Dieter and Marme (1983), indicated that continuous irradiation of corn coleoptiles by FR light inhibits the Ca^{++} transport activity of mitochondria and of microsome vesicles. They concluded that FR light results in a higher Ca^{++} concentration in the cytoplasm and that this

concentration is enough to allow Ca^{++} to combine with calmodulin and activate certain enzymes. So it is possible that fluxes of Ca^{++} in the cytoplasm mediate prehaustoria development. However, it is important to note that addition of Ca^{++}, calcium ionophore (A23187), calcium chelator (EGTA) and calcium channel inhibitors (La^{3+}) were without effect on prehaustoria formation whatever the light or zeatin application used (data not shown).

Auxins decreased light except BF light at 48 h exposure and zeatin induced prehaustorial formation (Table 2.4 and 2.5). The decrease varied with IAA concentration and the light application. It was less suppressive under a mixture of BF light. This suggests that endogenous levels of the above hormones and their ratios may similarly influence <u>in vivo</u> prehaustoria induction. Therefore, it seems likely that both hormones might play an important role in the parasitic growth of dodder plants.

These experiments elucidate the crucial role of cytokinin and auxin in the later stages of parasitism (i.e., haustoria differentiation). From these observations it seems possible that dodder meristimatic cells are induced to transform into prehaustoria with increasing endogenous cytokinin-to-auxin ratios. However, determination of endogenous levels of the above hormone concentrations during the initial and later stages of dodder growth with or without involvement of a host plant, may further help to

define the role of cytokinins and auxins in dodder life cycle.

Potassium and Sucrose.

Chemical stimulation of coiling and prehaustoria induction in dodder has not been reported. It is well documented that K^+, Ca^{++} and sucrose stimulate infection structures in bean rust fungi (Staples et al., 1983, 1984;1985). Our results are unique in demonstrating that prehaustoria in dodder threads were induced by exogenous application of K^+ salts (Figs. 2.16a,b and 2.17a,b) and sucrose (Table 2.6) under FR light.

Both KCl and KI stimulated zeatin effects on prehaustoria development (Table. 2.2). In most cases KI was more effective in initiating prehaustoria than KCl (Figs. 2.17a and 2.17b). Effects of K^+ salts and sucrose on prehaustoria formation under FR light raises the question of their role and mechanisms in dodder plants.

CHAPTER THREE

ROLE OF PHYTOCHROME, CRYPTOCHROME AND THEIR POSSIBLE INTERACTIONS IN MEDIATING COILING AND PREHAUSTORIA DEVELOPMENT

INTRODUCTION

The identities of the photoreceptors which mediate coiling and prehaustoria development in dodder seedlings have not been determined. Phytochrome and BF light photoreceptors have been proposed (Lane and Kasperbaver, 1965). Evidence suggests no direct role for photosynthesis (Lane et al., 1964; Lane and Kasperbaver, 1965).

Demonstrating the involvement of phytochrome and cryptochrome in B-mediating coiling and prehaustoria development in dodder threads, and their possible interactions is not simple. The possibility that more than one photoreceptor absorbing in the B part of the spectrum is involved in controlling plant growth has long been a source of controversy. Much of this controversy centers around the fact that Pr and Pfr absorb light in the B region of the spectrum.

In higher plants, B light effects may be mediated by cryptochrome, phytochrome, or both photoreceptors, either interacting or acting independently (Schafer and Haupt, 1983). There is no specific test for cryptochrome and the chemical identity of cryptochrome is not known. In addition, even in those cases in which the action of B light is mediated exclusively through cryptochrome, the final

expression of the response might be affected by the state of phytochrome (Mohr and Drumm-Herrel, 1983).

To demonstrate the B light response in dodder independent of phytochrome, it is necessary to use chemical and light treatments. Chemical treatments include compounds known to affect photosynthesis (i.e. diuron and atrazine) as described by Ashton and Craft (1982), Janardhanarao et al., (1984), and Lane et al., (1964), carotenoids (i.e. norflurazon) as described by Feldman (1985), Frosch et al. (1979), and Jabben and Dietzer (1979) and phytochrome (i.e gabaculine) as described by Gardner and Gorton (1985), and Konomi and Furuya (1986).

Light treatments include studies presented by Meijer (1968), Thomas and Dickinson (1979) and Schafer et al., (1981). Both chemical and light studies were tested for their capacity to specifically inhibit or stimulate prehaustoria development in dodder excised seedlings under certain light conditions. Therefore, the main objective of this chapter was to investigate (1) what are the possible photoreceptors involved in light perception that are responsible for initiating coiling and prehaustoria formation, and (2) is there an interaction between cryptochrome and phytochrome in mediating the above responses?

MATERIALS AND METHODS

The experimental procedure, light sources, and measurements used in this study were the same as described in chapter two, except for chemicals and light protocols.

Chemicals.

SAN 9789 (norflurazon), technical grade, was obtained from Sandoz Wander, Inc. DCMU (diuron), atrazine and gabaculine were purchased from Sigma Chemical Company.

Norflurazon (NF) Application.

Scarified dodder seeds were allowed to imbibe for 3 h in 10^{-4} M NF. Norflurazon is a potent inhibitor of carotenoid synthesis and chlorophyll accumulation (Bartels and Hyde, 1970; Hilton et al., 1969). The herbicide was dissolved in 1 ml ethanol, then after the ethanol evaporated, D.H_2O was added producing a 10^{-4} M concentration. The solution containing seeds was continuously oxygenated. Seeds were sown on blue paper dampened with the same NF solution. Trays were kept in darkness for 3 days at 27 ± 2°C, then exposed to WL for 48 h. Bleached seedlings were selected, excised and transferred into petri-dishes containing 2 ml D.H_2O. These were exposed to different

light treatments for another 48 h (see materials and methods in Chapter Two).

Gabaculine Application.

Gabaculine (5-amino-1,3 cyclohexadienylcarboxylic acid) application was based on the protocol described by Gardner and Gorton (1985). Briefly, four-day-old etiolated dodder seedlings were excised under a green safe light, and placed horizontally on 5.5 cm plastic petri dishes, each containing one layer of glass fiber filter paper and 3 ml of water or gabaculine. This chemical is also a potent chlorophyll inhibitor (Gardner and Gorton, 1985). Gabaculine was dissolved in KCl to a concentration of 1 mM gabaculine and 10 mM KCl, pH 7.0. All petri dishes were put in darkness and threads were allowed to grow for another 12 h at 28 ± 2 °C. During this period, threads were treated with successive 15 min exposures to R light, separated by 105 min of darkness. After the fourth R light period, dishes containing threads were returned to darkness for 4 h. After this period dishes were exposed to WL for another 12 h. At the end of this light treatment, 1 cm from the base of each thread was eliminated. Threads in dishes were allowed to grow under RF and BF light combination for another 48 h.

Light Studies.

To demonstrate the involvement of phytochrome, cryptochrome, and their interaction, various irradiation

protocols and criteria have been used (see, Mancinelli, 1989; Gaba and Black, 1987; Mohr, 1984, 1987). Estimates of Φ (the ratio of Pfr/Ptot) at different wavelengths were based on Mancinelli (1989). However, since we were interested in order of magnitude differences only, Φ was not judged precisely (Table 3.1).

Table 3.1. Expected Φ-values *in vivo*, photoexcitation of cryptochrome and relative numbers of prehaustoria formed. Blue + Red light (BR); Near ultra-violet (UV-A); Red light (R); White light (WL); Near ultra-violet + Blue light (UV-A/B); Blue light (B); Near ultra-violet + Far-red light (UV-A/FR); Blue + Far-red light (BF); Red + Far-red light (RF); Near Far-red light (NFR); Far-red light (FR).

Light Sources	Φ-values	Cryptochrome Activation	Prehaustoria
BR	≥ 0.8	+	-
UV-A	≥ 0.8	+	-
R	≥ 0.8	-	-
WL	≥ 0.8	+	-
UV-A/B	high	+	-
B	0.45	+	+ +
UV-A/FR	Low ?	+	+ +
BF	≤ 0.2	+	+ + + +
RF	0.55	-	+ +
NFR	≤ 0.1	-	-
FR	≤ 0.1	-	-

RESULTS

Chemical Studies.

Photosynthesis and Carotenoid Involvement.

The photosynthetic inhibitors diuron and atrazine were examined for their effect on prehaustoria development under different light treatments. Figure 3.1 shows that both chemicals reduced prehaustoria number under B and RF light. However, under BF light neither diuron nor atrazine had any effect. To validate the above results, more dodder threads were completely bleached by the application of 10^{-4} M NF (Fig. 3.2). The results shown in Figure 3.3 indicated that prehaustoria development in NF treated threads were the same as control under B and BF light; i.e., the effectiveness of B light in mediating coiling and prehaustoria was not reduced in the presence of NF. Thus carotenoid and chlorophyll were excluded as the photoreceptor chromophore mediating prehaustoria development under B light.

A confusing feature in Figure 3.3, is that NF reduces the effectiveness of RF light. The inhibitory effect of NF on prehaustoria development under RF could be attributed to its effect on phytochrome, since at wavelengths above 500 nm, phytochrome is the only photoreceptor activated.

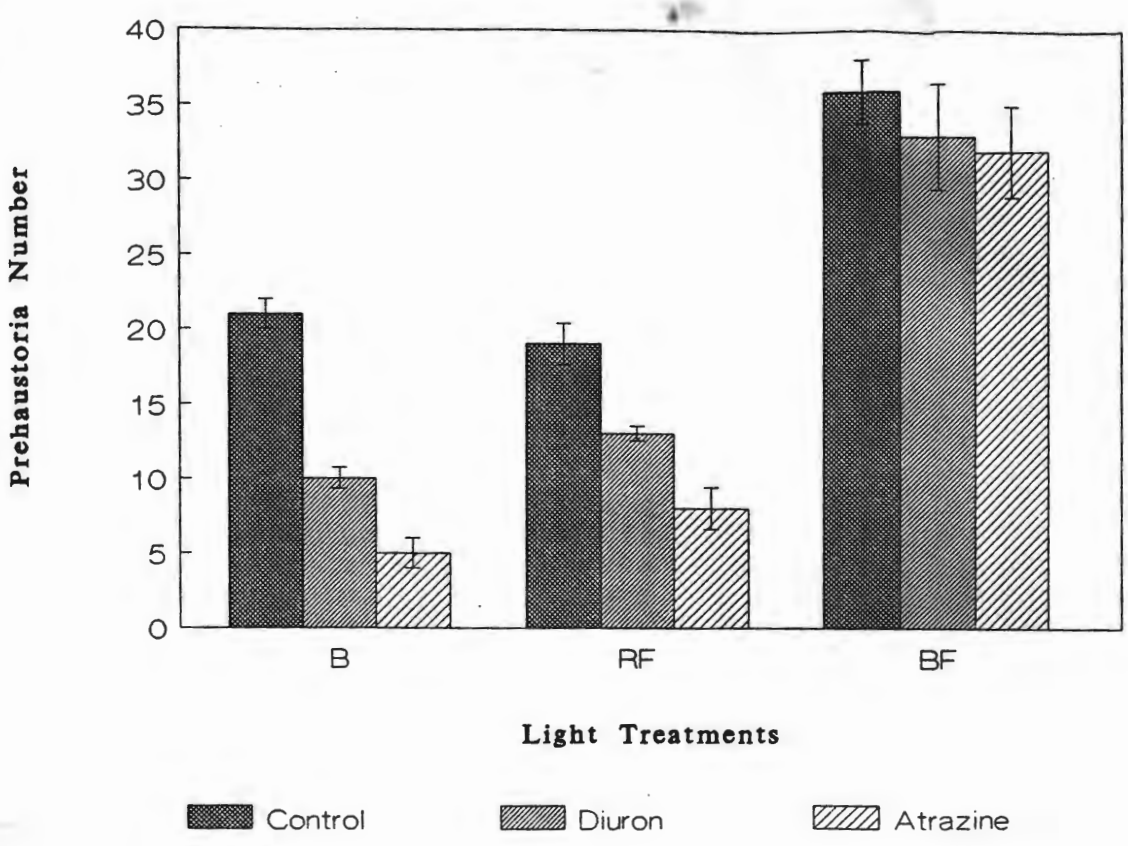


Figure 3.1. Effects of the herbicides diuron and atrazine on prehaustoria development. De-etiolated dodder threads were treated with 10^{-4} M diuron or atrazine and put in darkness for 3 h. Dishes, each containing 6 threads, were then transferred into different light treatments for another 48 h. Each point represents the mean of 3 experiments ± SE. Blue light (B); Red + Far-red light (RF); Blue + Far-red light (BF).

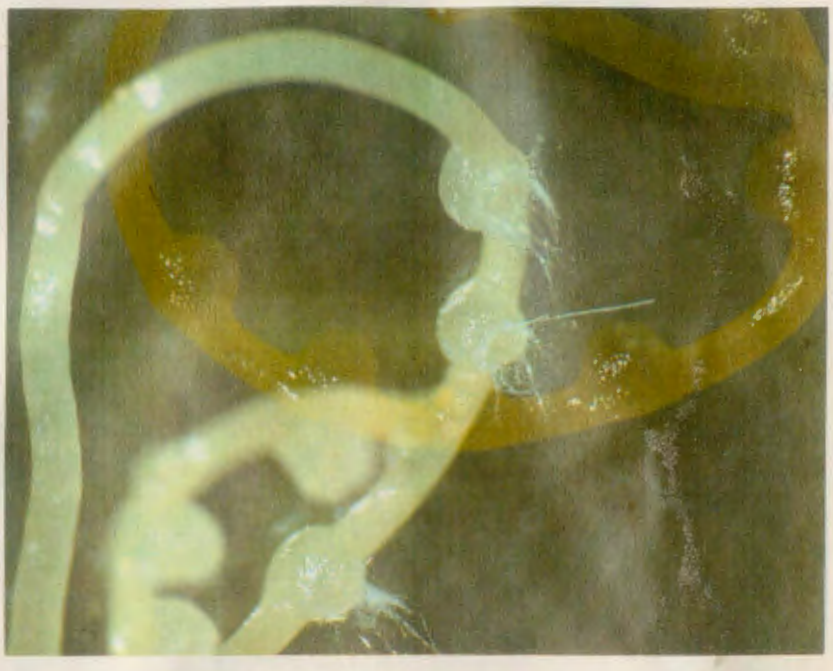


Figure 3.2. Norflurazon-treated dodder thread with prehaustoria under B or BF light, completely bleached. Blue light (B); Blue + Far-red light (BF).

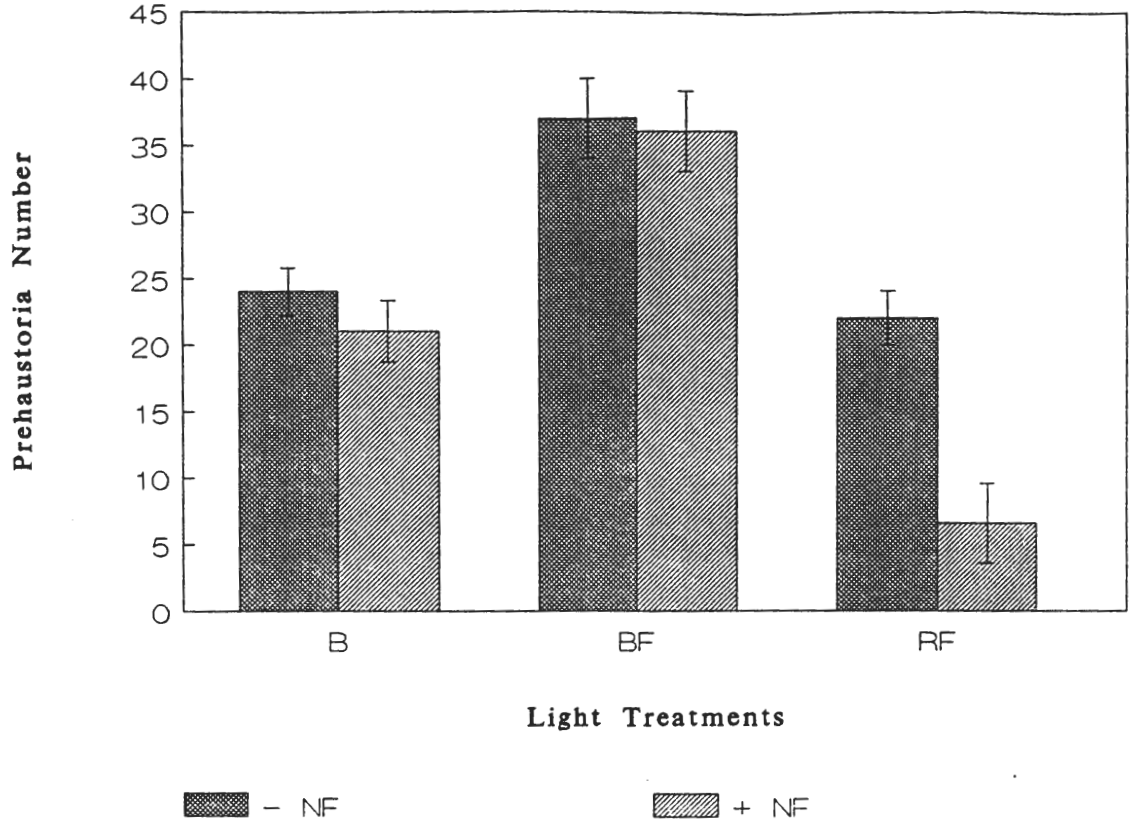


Figure 3.3. Effects of norflurazon (NF) on prehaustoria development. Dishes, each containing 6 threads, were transferred into different light treatments for 48 h. Each point represents the mean of 3 experiments ± SE. Blue light (B); Blue + Far-red light (BF); Red + Far-red light (RF).

To test that NF affects phytochrome, an R/FR light reversibility experiment was conducted using light pulse treatments following a prolonged B light exposure. Figure 3.4 shows that a R light pulse caused an inhibition in prehaustoria development in untreated and NF treated threads stimulated by B light of 70% and 33% respectively. Far-red light pulse had no effect. This suggests that phytochrome mediated prehaustoria development is affected by NF.

Phytochrome Involvement.

An experiment was conducted to determine whether gabaculine would inhibit prehaustoria development under RF light by affecting phytochrome. The results in Figure 3.5 show that gabaculine at 1 mM caused approximately a 40% reduction in prehaustoria development after 48 h of RF light applications, while it had no effect under BF light.

Light Studies.

Phytochrome Involvement.

Data presented in the first chapter implicates phytochrome involvement in mediating coiling and prehaustoria development, since prehaustoria induction under continuous RF light has been demonstrated (see Chapter Two, Fig. 2.8).

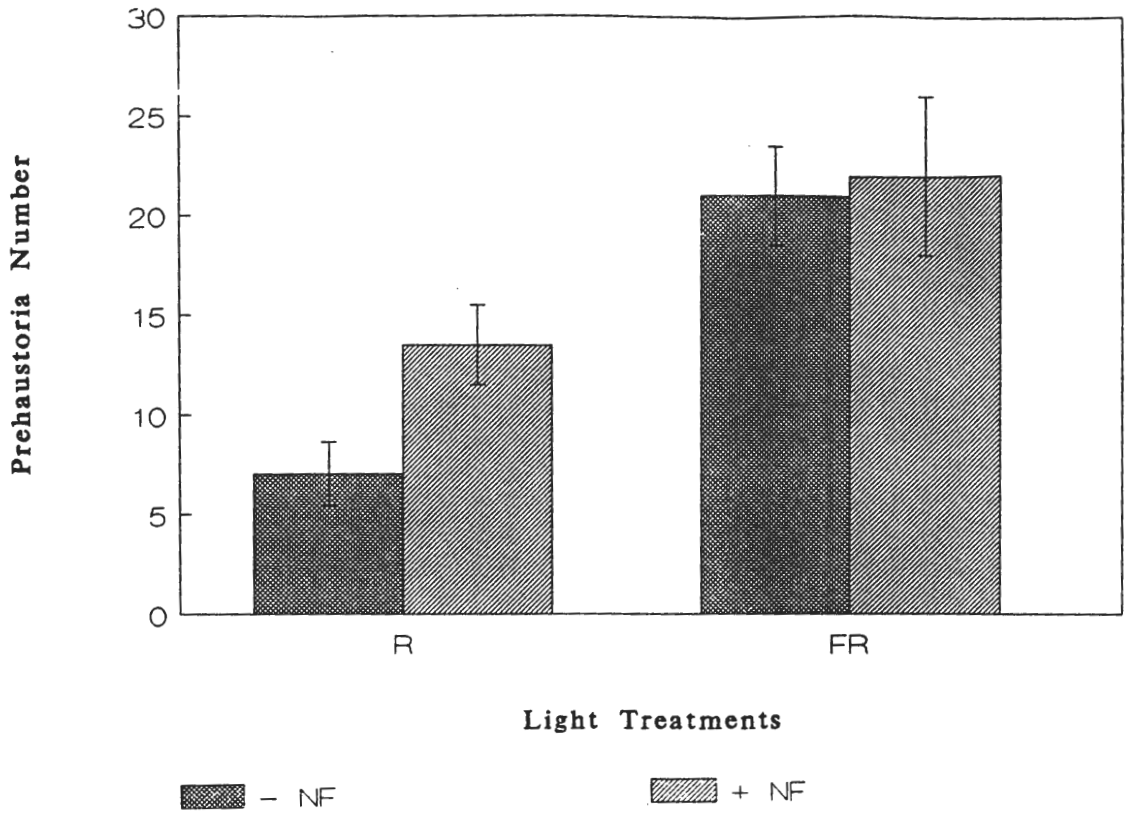


Figure 3.4. Effect of R/FR reversible experiment on prehaustoria development with norflurazon (NF) treated dodder threads. The NF treated and untreated threads were exposed to B light for 17 h. The B light treatment was terminated with a saturating 10 min R or FR light pulse and put in DK. Prehaustoria were counted 31 h after the end of the light treatment. Each point represents the mean of 3 experiments ± SE. Blue light (B); Red light (R); Far-red light (FR); Dark (DK).

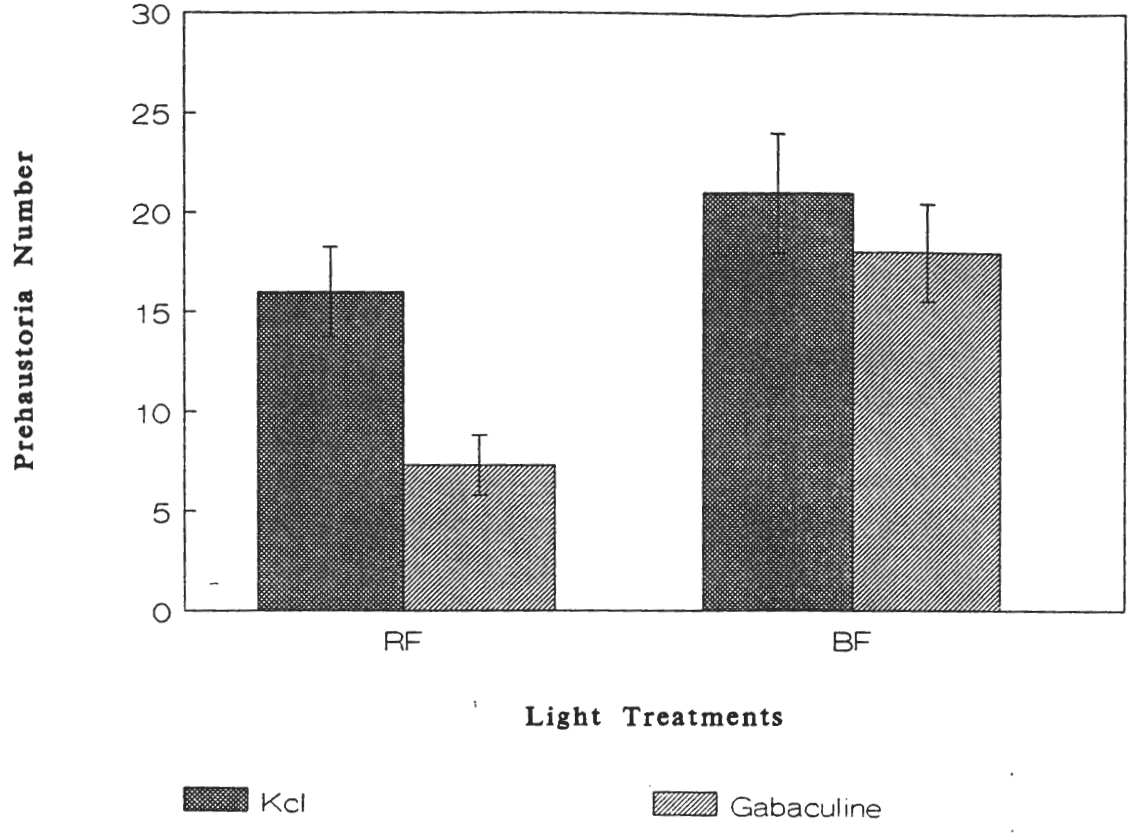


Figure 3.5. Effect of gabaculine on prehaustoria development. Dishes, each containing 6 threads, were transferred into different light treatments for 48 h. Each point represents the mean of 3 experiments ± SE. Red + Far-red light (RF); Blue + Far-red light (BF).

Cryptochrome Involvement.

Mohr (1984) indicated that there are three criteria that can be used to test for cryptochrome involvement during B irradiation:

1. Criteria of Meijer (1968).

Coiling and prehaustoria were measured in darkness and under UV-A, B, R and FR irradiations. Results, in Figure 2.6 (see Chapter Two), show that B light stimulates coiling and prehaustoria whereas R and FR were without effect. This indicates that parasitic growth of dodder under B light is not mediated by phytochrome (see Meijer, 1968). However, coiling and prehaustoria development under B light is affected by the presence or absence of R or FR light (Fig. 2.8) and thus it's dependent on the state of phytochrome (see Gaba and Black 1979).

2. Criteria of Thomas and Dickinson (1979).

Results in Figure 3.6 show that the addition of B light to a high fluence rate of FR light caused a significant stimulation in coiling and prehaustoria development unlike the treatment without B light. To validate this study, further experiments were conducted in which de-etiolated dodder threads were kept under high fluence rate of RF (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Again, prehaustoria development was higher when B was added, unlike treatment without B light (Fig. 3.7).

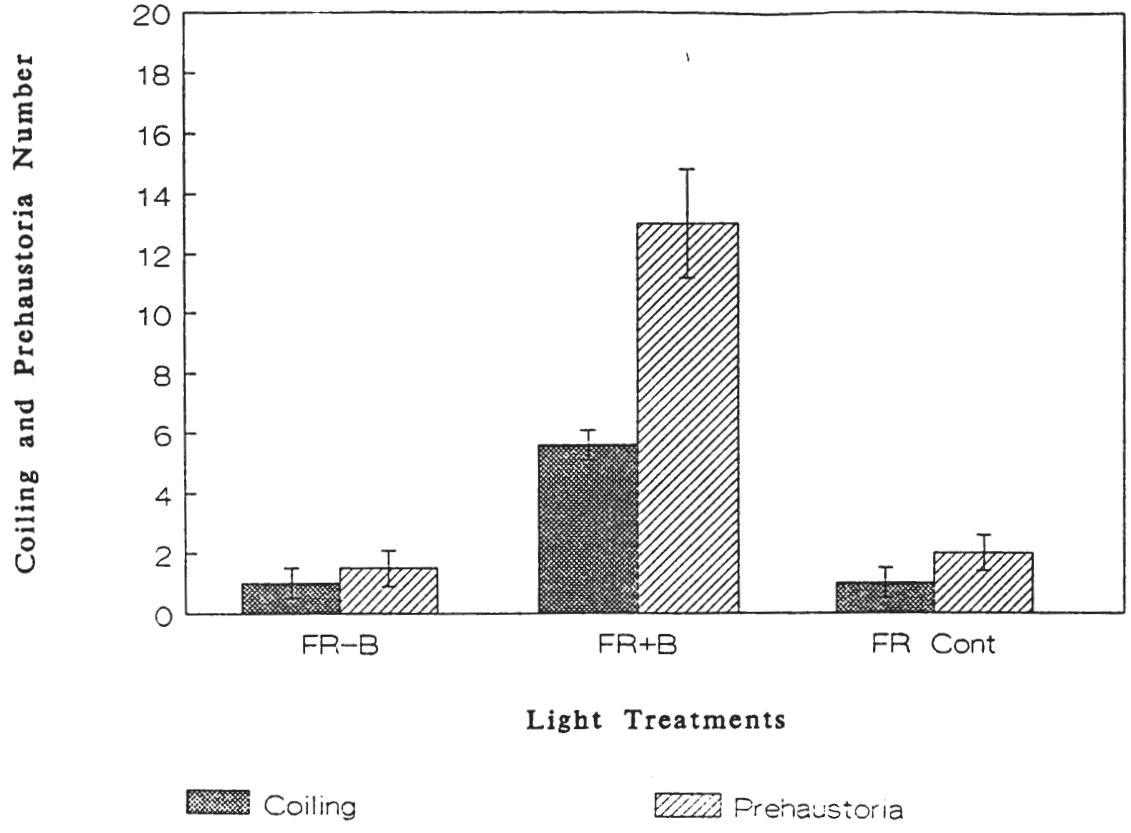


Figure 3.6. De-etiolated dodder threads (6/Dish) were kept under high fluence rate of FR for 12 h. In the middle of this period a small amount of B light (4 $\mu mol \cdot m^{-2} s^{-1}$) was added for 6 h followed by 24 h DK. Each point represents the mean of 3 experiments $\pm$ SE. Far-red light (FR); Blue light (B); Intermediate fluence rate of FR light for 24 h, followed by 24 h DK (FR Cont).

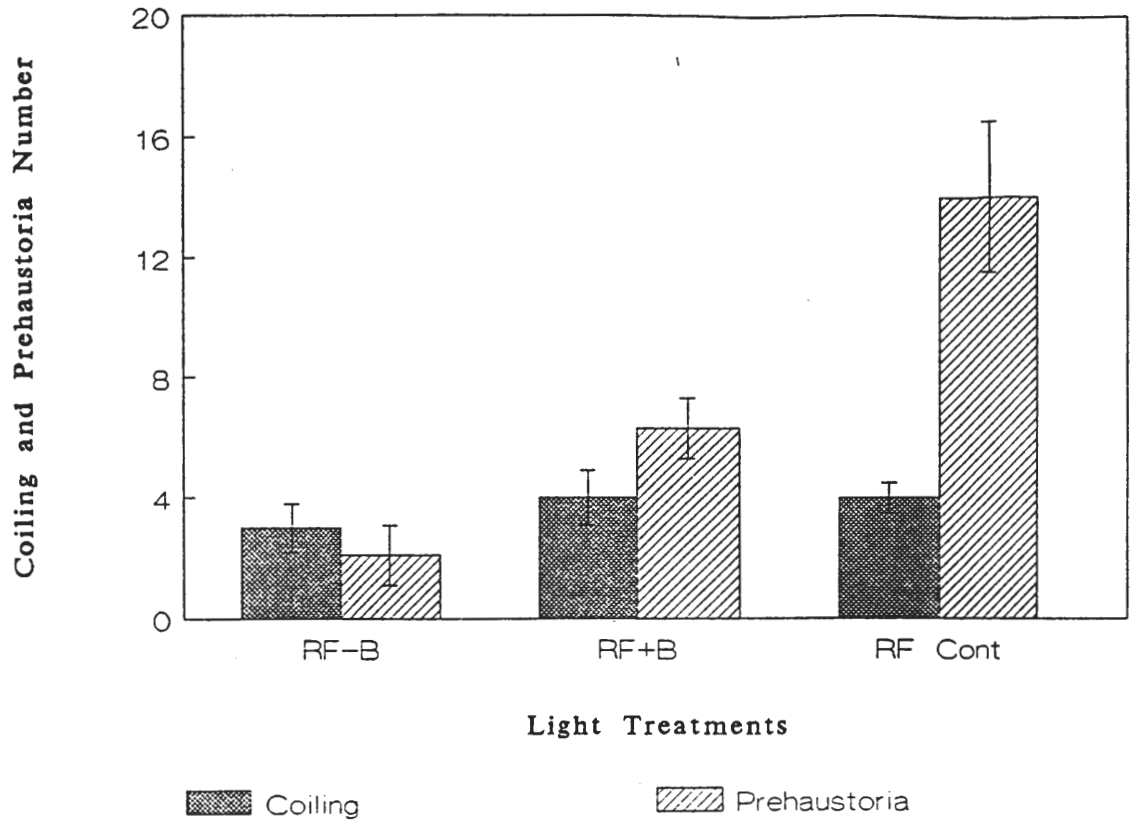


Figure 3.7. De-etiolated dodder threads (6/Dish) were kept under high fluence rate of RF for 12 h. In the middle of this period a small amount of B light (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was added for 6 h followed by 6 h DK. This cycle was repeated one time. Each point represents the mean of 3 experiments ± SE. Red + Far-red light (RF); Blue light (B); Intermediate fluence rate of RF (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 24 h, followed by 24 h DK (RF Cont).

3. Light Equivalent Studies (Schafer et al., 1983; Sponga et al., 1986).

Further light applications to determine the involvement of cryptochrome in the mediation of the action of B light on prehaustoria development were conducted. The possibility that B-induced prehaustoria development are mediated by phytochrome was tested, where the effect of Φ value was compared. If only phytochrome is involved, wavebands of equal Φ should result in the same rate of prehaustoria development (Fig. 3.8). The results indicate that B light is more effective than RF in inducing prehaustoria formation. These results indicate cryptochrome involvement in mediation of B light action.

Interaction Between Phytochrome and Cryptochrome.

End-of-Day Pulse.

Application of R or FR light pulses to establish a high Φ or very low Φ after a prolonged exposure to B light affects coiling and prehaustoria development. The data shown in Table 3.2 indicates that dodder seedlings require low Pfr for B light to be effective. This table shows that prehaustoria development after 18 h B light exposure continues in darkness as long as the rate of Pfr is low. When most of the Pr returned to Pfr by a saturating R light pulse, prehaustoria development was reduced by about 50%.

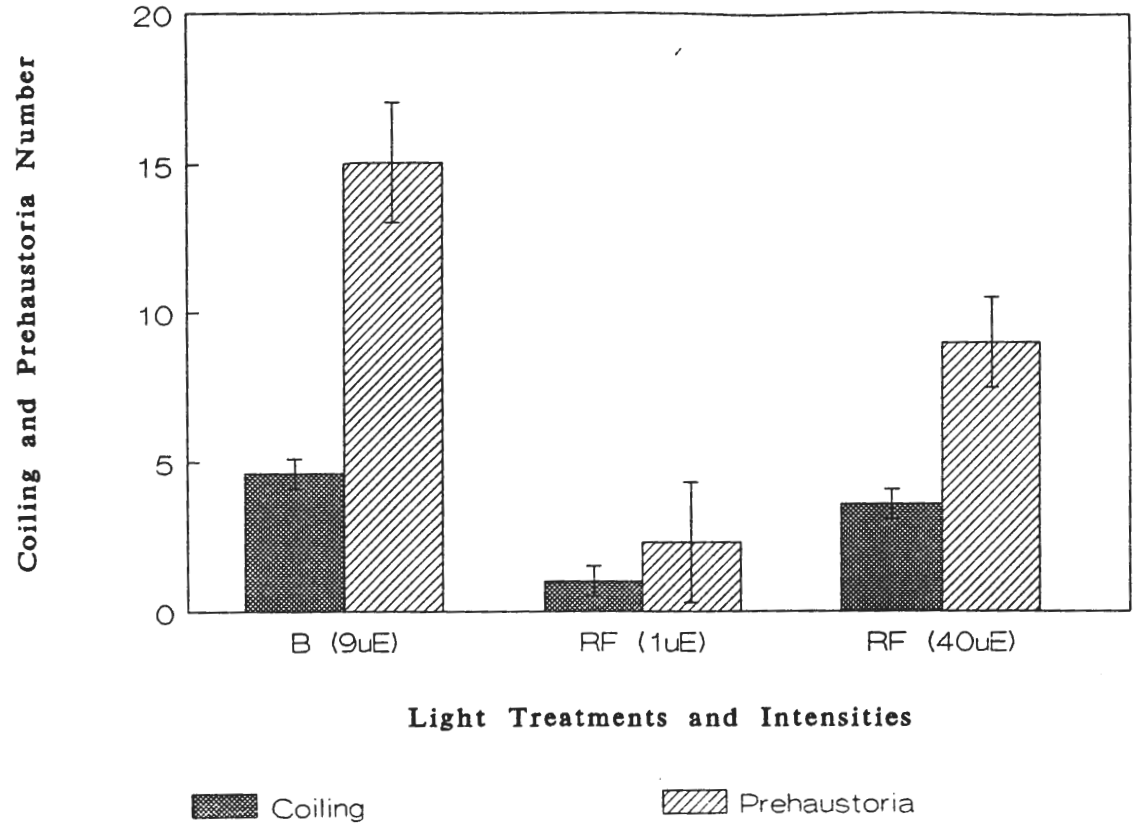


Figure 3.8. Prehaustoria development under B and RF light have the same Φ value based on Light Equivalent Studies. De-etiolated dodder threads received 16 h B or RF light followed by 26 h DK. Each point represents the mean of 3 experiments ± SE. Blue light (B); Red + Far-red light (RF).

Table 3.2. Conspicuous changes in coiling and prehaustoria development. De-etiolated dodder threads were put under B light for 18 h then terminated with a different saturating 10 min light pulses and put in DK. Coiling and prehaustoria were counted 30 h after the end of the light treatment. Each point represents the mean of 3 experiments ± SE. Dark (DK); Far-red light (FR); Red light (R); Near ultra-violet light (UV-A); Far-red treatment followed immediately by Red light (FR/R); Far-red treatment followed immediately by Blue light (FR/B); Red treatment followed immediately by Far-red light (R/FR); Red treatment followed immediately by Blue light (R/B); Red treatment followed immediately by Far-red light followed by Red light (R/FR/R); Far-red treatment followed immediately by Red light followed by Far-red light (FR/R/FR).

Light Treatment	Coiling Number	Prehaustoria Number
DK	4.5 ± 0.3	19.2 ± 0.9
FR	4.6 ± 0.4	20.6 ± 1.6
R	3.8 ± 0.3	9.5 ± 1.5
UV-A	4.3 ± 0.3	10.0 ± 1.4
FR/R	3.9 ± 0.2	10.3 ± 1.5
FR/B	4.6 ± 0.2	18.0 ± 2.0
R/FR	4.7 ± 0.3	19.4 ± 1.5
R/B	4.3 ± 0.7	14.0 ± 1.4
R/FR/R	3.8 ± 0.3	11.5 ± 1.5
FR/R/FR	4.6 ± 0.5	20.2 ± 0.8

Light Pulses.

Another series of experiments were conducted to determine if phytochrome excitation affects the rate of prehaustoria development. Repeated pulses (10 min/50 min DK, cycled for 48 h) with R, FR, UV-A or B light alone were totally ineffective in stimulating coiling and prehaustoria formation (data not shown), although, long exposure to B light induces coiling and prehaustoria development (see Chapter Two, Fig. 2.6). However, when FR light pulse is applied immediately before, during or after a B light pulse, it caused higher prehaustoria formation when measured 48 h after the beginning of these light pulses (Figs. 3.9, 3.10, 3.11 and 3.12). In addition, these results show that if a B light pulse was replaced by a R (5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or UV-A (7 $\mu W \cdot cm^2$ x 100) light pulse, little or no prehaustoria were observed. Furthermore, repeated sequences of different light pulses cycled for 48 h affect prehaustoria development. Results shown in Table 3.3 show that a sequence of B and FR light pulses, regardless of order, induced prehaustoria development. A B light pulse for 1 min or 5 min followed directly by 1 min or 5 min FR light respectively (cycled for 48 h) stimulates prehaustoria development, while sequences of R and FR light pulses stimulated fewer prehaustoria to develop.

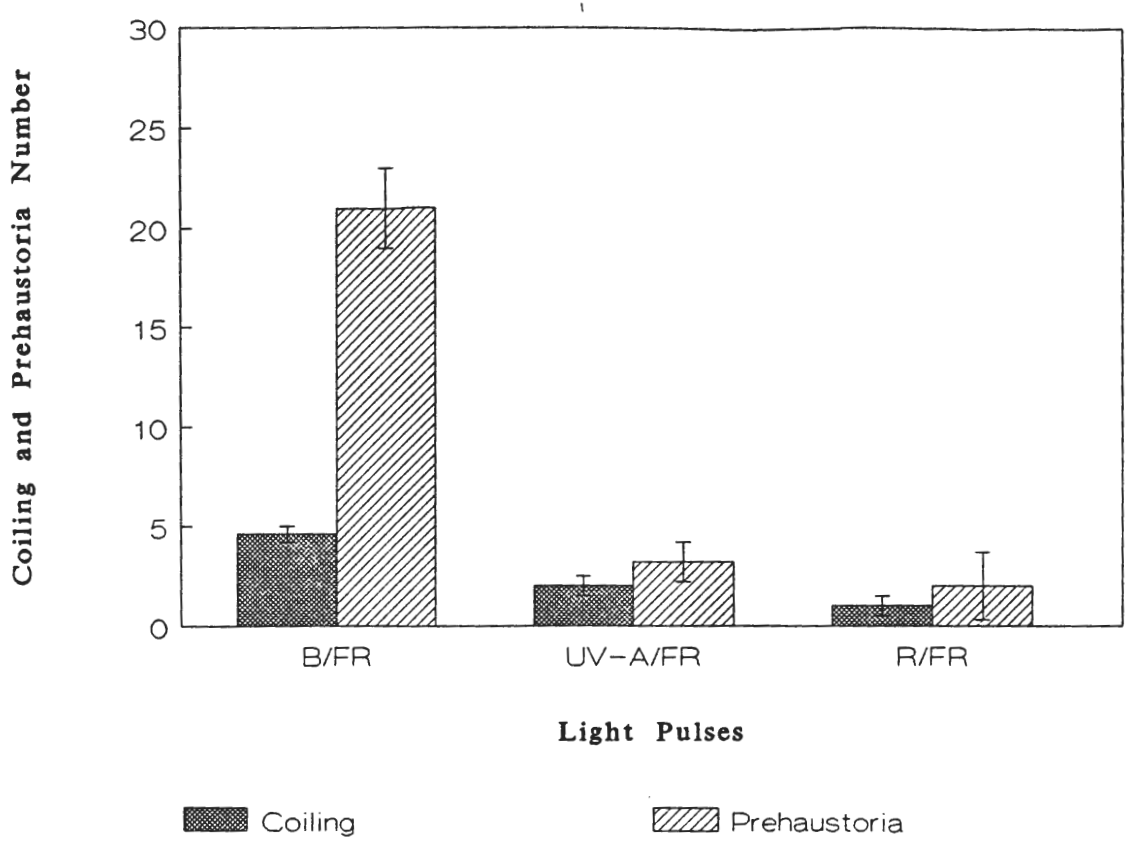


Figure 3.9. The potentiating effect of light pulses on coiling and prehaustoria development. Dishes, each containing 6 threads, received 10 min B, UV-A or R light. FR light was followed directly for 2 min and then by 50 min DK. The cycle was repeated for 48 h. Each point represents the mean of 3 experiments ± SE. Blue light (B); Far-red light (FR); Near ultra-violet light (UV-A); Red light (R).

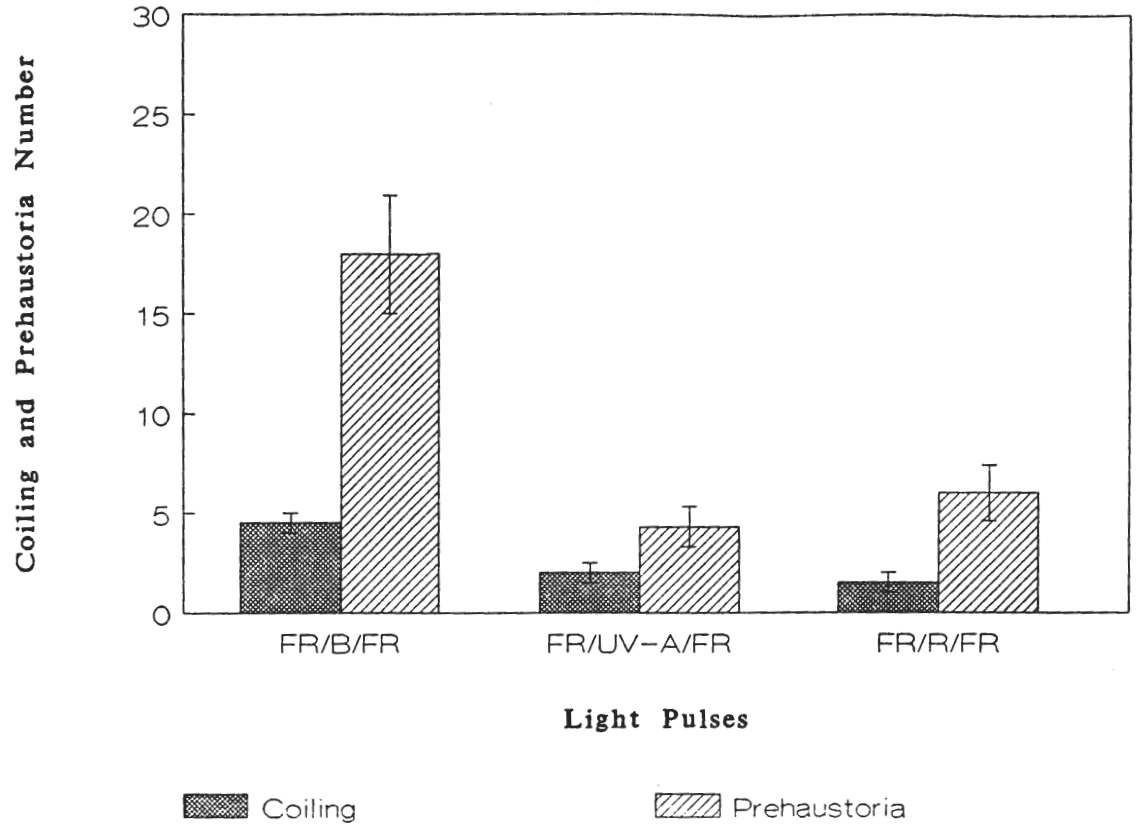


Figure 3.10. The potentiating effect of light pulses on coiling and prehaustoria development. Dishes, each containing 6 threads, received 10 min FR light. In the middle of FR light, B, UV-A or R light was added for 3 min followed by 50 min DK. The cycle was repeated for 48 h. Each point represents the mean of 3 experiments ± SE. Far-red light (FR); Blue light (B); Near ultra-violet light (UV-A); Red light (R).

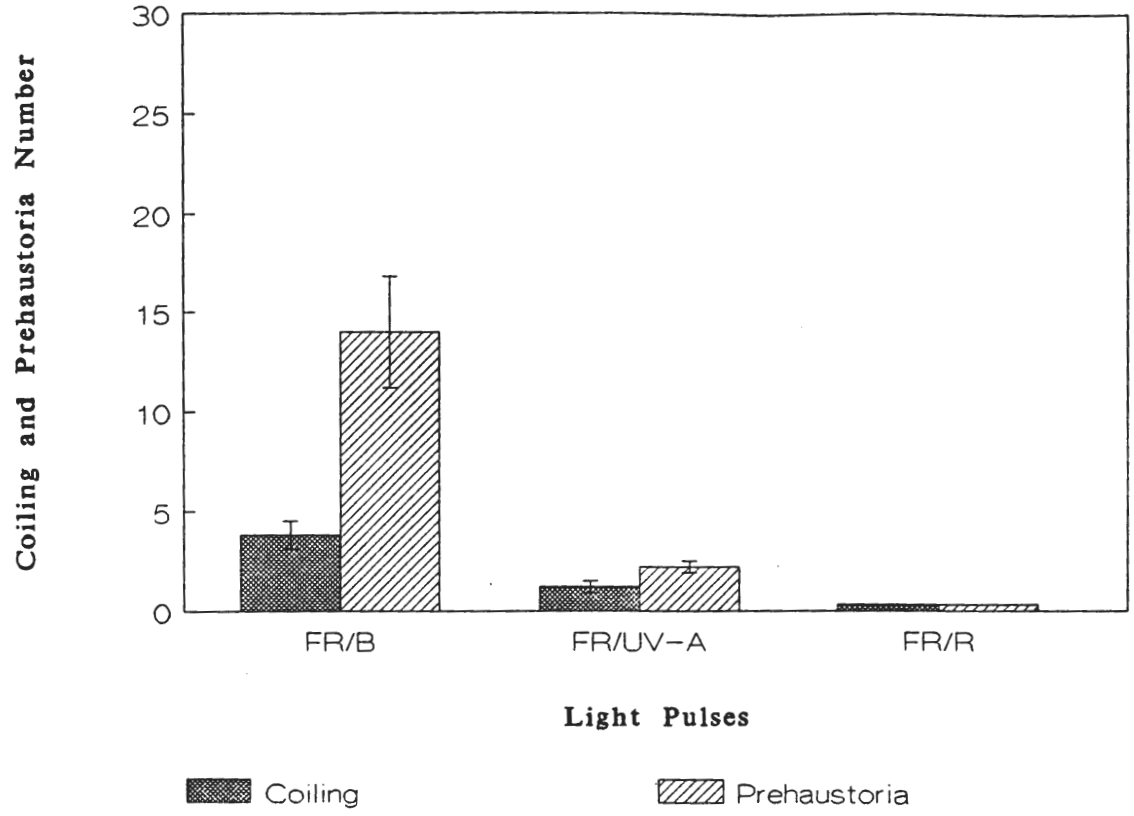


Figure 3.11. The potentiating effect of light pulses on coiling and prehaustoria development. Dishes, each containing 6 threads, received 10 min FR light. After 2 min FR light, B, UV-A or R light was added for 10 min followed by 48 min DK. The cycle was repeated for 48 h. Each point represents the mean of 3 experiments ± SE. Far-red light (FR); Blue light (B); Near ultra-violet light (UV-A); Red light (R).

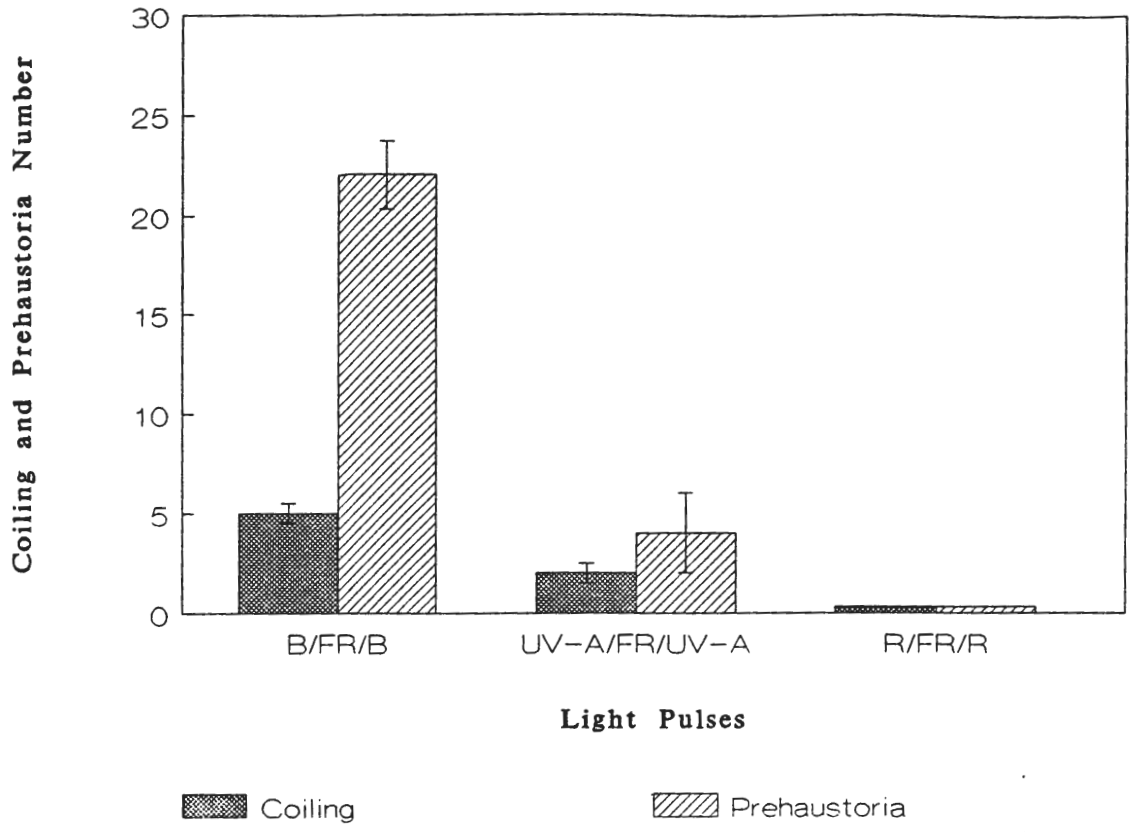


Figure 3.12. The potentiating effect of light pulses on coiling and prehaustoria development. Dishes, each containing 6 threads, received 10 min B, UV-A or R light. In the middle of each light treatment FR light was added for 2 min followed by 50 min DK. The cycle was repeated for 48 h. Each point represents the mean of 3 experiments ± SE. Blue light (B); Far-red light (FR); Near ultra-violet light (UV-A); Red light (R).

Table 3.3. Effect of continuous sequential application with B and FR or R and FR light on prehaustoria development for 48 h. Each point represents the mean of three experiments ± SE. Blue light (B); Far-red light (FR); Red light (R).

Light Treatment	Duration of Light Treatment (min)	Prehaustoria Number Time (hours)	
		24	48
B x FR			
	1x1	18.0 ± 2.0	27.6 ± 4.0
	5x5	25.6 ± 4.2	30.5 ± 5.0
	15x15	8.3 ± 3.5	14.0 ± 2.6
FR x B			
	1x1	16.5 ± 2.1	25.1 ± 3.1
	5x5	26.1 ± 3.2	27.0 ± 4.0
	15x15	10.0 ± 2.6	13.7 ± 2.6
R x FR			
	1x1	3.0 ± 2.0	5.1 ± 2.3
	5x5	2.0 ± 2.0	3.0 ± 2.0
	15x15	2.0 ± 2.0	2.0 ± 2.0

DISCUSSION

It is widely agreed that the effect of R and FR light on stem elongation in etiolated seedlings is exerted through phytochrome. However, the pigment involved in the action of long term B light is still under debate. Hartmann (1966) attempted to explain the action of long term B light in terms of phytochrome. Others are still convinced that continuous B light is mediated by another photoreceptor.

Chemical Studies.

Effect of Diuron and Atrazine.

Data presented in Figure 3.1 do not agree with other studies where diuron has no effect on twining (Lane and Kasperbaver, 1965). However, it is consistent with the theory that photosynthesis is not involved in mediating coiling or prehaustoria development, even though diuron inhibited photosynthesis in dodder (Lane et al., 1964). It might be that both chemicals exhibit their effect by retarding seedling growth and development and not through photosynthesis.

It is well documented that the role of photosynthesis under a prolonged exposure to these lights is solely to provide energy and maintenance of plant growth. Therefore,

if photosynthesis is involved in mediation of prehaustoria development addition of sucrose would stimulate prehaustoria development under R or B light, which is not the case here (Table 2.6). Furthermore, it is well known that R light is the most efficient in driving photosynthesis. The inability of R as well as UV-A light to provide any significant prehaustoria development indicates that photosynthesis is not involved (Fig. 2.6). Rather, the stimulatory effect by B and BF light indicates that induction of prehaustoria is strictly controlled by non-photosynthetic (and non-carotenoid) photoreceptors, presumably cryptochrome and phytochrome.

Effect of Norflurazon.

Data presented in Figures 3.2 and 3.3 demonstrated that the photosynthetic pigments (chlorophyll and carotenoids) are not involved in mediating B-induced coiling and prehaustoria development, since synthesis of these compounds are inhibited by NF. Treatments with NF did, however, inhibit prehaustoria under RF (Fig. 3.3), and significantly interfered with R, FR light reversible experiments (Fig. 3.4). This suggests that NF is interfering with the phytochrome system. These results agree with Shimazaki et al., (1983), who reported that NF affected the phytochrome system, but contradicts many other studies conducted by Bartels and Hyde, 1970; Hilton et al., 1969 and Jabben and Dietzer, 1979).

Many conclusions can be made from these results. First, 'bulk' carotenoids, as a possible photoreceptor chromophore for B mediated coiling and prehaustoria development can be excluded. It is possible that dodder threads contain traces of carotenoids, and one cannot rule out the possibility that carotenoids are involved. Therefore, positive evidence is required to show that flavin is the photoreceptor chromophore in B light mediated coiling and prehaustoria development. Second, the present data indicated that the phytochrome system was affected by NF. Prehaustoria number were stimulated to a greater extent by R/FR reversibility experiments in herbicide treated threads as compared to the control (Fig. 3.4).

Effect of Gabaculine.

The fundamental significance of these experiments is that phytochrome is the sole photoreceptor active under RF light (Fig. 3.5), since gabaculine inhibits phytochrome synthesis and re-synthesis (Gardner and Gorton, 1985). However, under BF light gabaculine had no effect on prehaustoria development, suggesting that more than one photoreceptor is involved in mediating parasitic growth of dodder under the latter light condition.

Light studies.

The involvement of phytochrome in photo-regulation of coiling and prehaustoria development could be demonstrated

through the usual R/FR photo-reversibility tests if light pulses were applied at the end of a prolonged exposure to B light, or by continuous dichromatic irradiation with RF as a main test (see, Mohr, 1983). The results presented in the second chapter indicate that hook opening is controlled by phytochrome. The stimulating effect of R light on hook opening and the inhibiting effect of R on coiling and prehaustoria (Figure 2.5 and 2.6) are standard phytochrome reactions. Therefore, prehaustoria development in dodder was induced by RF; it is a phytochrome response.

To determine if Pfr or Pfr/Ptot (Φ) is the active form in eliciting coiling and prehaustoria development, experiments using de-etiolated dodder seedlings to investigate long-term effects of light on prehaustoria formation suggest that phytochrome photo-equilibrium (Φ) regulates these infection structures (Table 3.1). Evidence that Φ controls extension growth in many de-etiolated plants came from Holmes and Smith (1975, 1977) and Morgan and Smith (1978).

Data presented in Chapter Two indicate that the effect of long term light is correlated with the Φ-values of phytochrome. As an example, Φ UV-A and Φ R are similar (see Table 3.1) and the effect of continuous UV-A and continuous R on prehaustoria development is the same. An increase in R:FR ratio causes a reduction in coiling and prehaustoria development (data not shown). Coiling and prehaustoria were inhibited or reduced by addition of R light. Red light

was ineffective in inducing coils and prehaustoria (Fig. 2.6). If the fluence rate of FR is kept constant and the fluence rate of R or UV-A varied, prehaustoria number changes correspondly. Increasing R or UV-A fluence rate completely suppresses prehaustoria development (data not shown). Results indicate that there is a certain fluence rate ratio of these wavelengths which leads to an optimum response.

Furthermore, prehaustoria were promoted when FR was added to a background of B ,UV-A or WL light, while addition of R light inhibited prehaustoria development (see Chapter Two, Fig. 2.8). The highest response was under BF light. These results suggest that prehaustoria development is controlled by Pfr/Ptot.

Cryptochrome Involvement.

The involvement of cryptochrome has been presented by Meijer (1968), who showed that B, unlike R or FR light, inhibits the growth of etiolated gherkin hypocotyls. Data presented in Chapter Two show that the comparison of the UV-A, B, R and FR effects in inducing coils and prehaustoria indicates that B is clearly mediated by a photoreceptor other than phytochrome, since R and FR irradiation each applied alone are ineffective in inducing the above responses (Fig. 2.6). Furthermore, prehaustoria induction by B light is similar to the stimulation produced by RF mixture of different Φ, but only 48 h after light

applications. In addition, phytochrome can respond to B light, especially in long-term irradiation studies (Mancinelli and Rabino, 1978).

Furthermore, prehaustoria developmental patterns under B, FR and BF were compared (see Chapter Two, Figs. 2.6 and 2.8). If phytochrome played a major role in the B light response, the dichromatic irradiation treatments would have produced a similar effect to that of FR light alone, due to the similarity of photo-equilibria produced by FR and BF light (Ritter et al., 1981)(also see Table 3.1). However, it was observed that the addition of FR to B light resulted in a greater (synergism) prehaustoria development when compared to B alone. Even a small amount of B added to background FR light is highly effective in stimulating prehaustoria initiation (data not shown). It seems that this synergism can occur once Pfr is reduced to a certain level until an optimum Pfr/Ptot is established. Clearly, both phytochrome and a separate B light photoreceptor are implicated in mediating prehaustoria development in dodder seedlings.

Presented evidence indicates that prehaustoria development is related to B photoreceptor and phytochrome photo-equilibrium (Φ). Addition of B light to high fluence rate of FR (Fig. 3.6) or RF (Fig. 3.7) light has no effect on Φ, based on Thomas and Dickinson (1979). Therefore the effectiveness of B light is independent of FR or RF light. Thus, the evidence is consistent with the existence of a

specific B light photoreceptor in mediating prehaustoria formation under B light.

In another series of experiments based on the light-equivalent principle, data presented in Figure 3.8, indicate that B light is more effective than RF even though both produce similar Φ (according to Sponga et al., 1986). Presented evidence suggest that a specific B light photoreceptor is involved in mediation of prehaustoria development under B light. Furthermore, if prehaustoria production is expressed as a function of the photon fluence rate, B light is more effective than RF. About 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of RF are required for the production of similar amounts of prehaustoria (see chapter two, Fig. 2.6 and 2.8); thus, in terms of quantum efficiency, B light is about 10 times as effective as RF light in initiation of prehaustoria.

It is well documented that B and R light mediated by phytochrome are acting in the same direction; resulting in a high Pfr. However, in the case of dodder the fact that B light was the only light that induced prehaustoria, strongly points to the possibility that B light acts via a separate photoreceptor rather than via phytochrome. Furthermore, under light pulse conditions, B light pulses stimulated prehaustoria in the presence of a subsequent FR light pulse with no reversibility, unlike R light pulses (Figs. 3,9, 3.10, 3.11 and 3.12). These data suggest that prehaustoria development is due solely to a B light and phytochrome

receptor interacting with each other. Both phytochrome and cryptochrome elicit a significant action (i.e., B and RF), but they act together for the final response.

Study of Interaction Between Phytochrome and Cryptochrome.

The involvement of more than one photoreceptor in the photo-control of coiling and prehaustoria formation rather than phytochrome alone, increases the number of possible responses to changes in incident light which plants may exhibit. Dodder apparently is sensitive to phytochrome as well as cryptochrome.

Many studies have indicated that the conclusive expression of cryptochrome-mediating responses to B light count on the state of the phytochrome system (Schafer and Haupt, 1983; Mohr and Drumm-Herrel, 1983). The results obtained in this research indicate that Pfr is required for the onset of the light-induced coiling and prehaustoria development; apparently both phytochrome and cryptochrome mediated action can be expressed once Pfr is available. As an example, the B and RF light effects are not apparent in etiolated dodder seedlings, but are apparent in de-etiolated seedlings (see Chapter Two, Table 2.1).

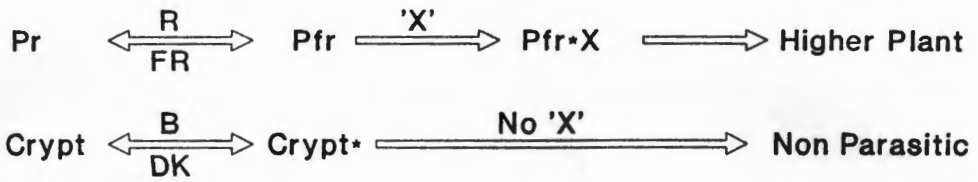
If the level of Pfr was high under B and R (mixture), neither coils nor prehaustoria were observed, while when Pfr was low under B and FR (Fig. 2.8), responses were much higher than B light applied alone (Fig. 2.6). Evidently R light opposes the B light's effect in mediating coils and

prehaustoria. Two possibilities can be described for the R light in reducing B light's effectiveness: 1. Pfr must be present before and at the onset of B light irradiation but not after, or, 2. seedlings must be in a specific developmental state at the time of B irradiation. To test the theory that R light specifically provides Pfr, de-etiolated dodder threads were irradiated with a single pulse of R or FR immediately after a prolonged exposure of B light. The results in Table 3.2 show that a R light pulse inhibited the stimulating effect of B light, whereas a FR light pulse had no effect on B-induce-prehaustoria formation. This suggests that the role of R light is simply to produce Pfr which affects the responses mediated by B irradiation. More evidence was provided by applying R or FR light given in sequence with B light instead of simultaneous application. Indeed, similar results were obtained. As documented in Table 3.3, prehaustoria development after the end of a R period can be attributed to the action of Pfr.

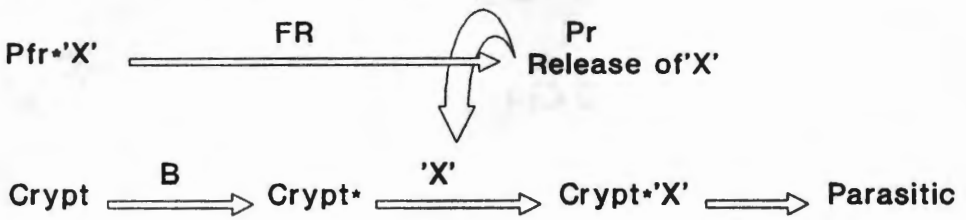
These results suggest one possible interpretation: cryptochrome is involved in the mediation of B-induced prehaustoria formation, and that the state of phytochrome is the main limiting factor for the final expression of the response. Therefore, a model of interaction is proposed to explore role(s) of cryptochrome and phytochrome, in the intra-conversion of growth habit of Dodders:

MODEL OF INTERACTION

HIGH Pfr/Ptot (Direct Sunlight)



LOW Pfr/Ptot (Cool Shade)



Phytochrome (Pfr) is required to maintain dodder as a higher plant seedling (de-etiolation), and also potentiates seedlings for parasitism. However, Pfr is not required for dodder parasitic growth. Prehaustoria do not develop when there's too much R light; when Φ-values in vivo are too high by limited but broad spectrum light source(s); when threads are placed under cool white (WL) fluorescent light or BR, or R light, or when placed under UV-A, or UV-A/B. This latter, possibly confounding effect(s) because cryptochrome in dodder is proposed as a UV-A/B light photoreceptor. However, UV-A alone, and UV-A/B light do not permit formation of prehaustoria ($\Phi > 0.7$). When UV-A was supplemented with FR light to lower Pfr II levels, prehaustoria developed; cryp* is active, and Φ is low.

In fact, Pfr opposes the action of an excited state of cryptochrome, which suggests that two photoreceptors may compete for a common intermediate, X; or common intermediate state, X'. Binding of cryptochrome to X will not affect phytochrome, but binding of phytochrome to the same X will inhibit cryptochrome action. Cryptochrome can be activated once it interacts with an X. According to this model, the effect of R light is explained by its action on two systems simultaneously, 1) establishing a Pfr gradient via phytochrome and, 2) operating as a switch for cryptochrome action.

Prehaustoria are developed in greatest numbers in BF; i.e., when FR (in FR Pfr II form is converted to Pr I), is added to B light (and, cryp is photo-excited to cryp*). Blue light alone yields Φ-value ≤ 0.5 (Pfr II/Ptot). Blue light is absorbed by cryp; and cryp* competes better for X when Pfr II is low. Blue light supplemented with FR light favors most, prehaustoria formation; because, cryp* is active, and Φ ≤ 0.2 FR (near-FR, or far-FR, or both) is insufficient, alone, to cause prehaustoria formation; Φ ≤ 0.1, but there is no cryp*.

A mixture of RF (Φ ≤ 0.5) cause prehaustoria to form; and, as well as B light (Φ ≤ 0.45). A mixture of BF light displays the greatest stimulatory effect (cryp*, and Φ ≤ 0.2). Pfr II, at specified, and set Φ-values, can, in a pinch, stimulate prehaustoria; 'deep under' the canopy (or, late in the day). But Dodders 'want' to climb. They

'prefer' the juicy, and succulent members, of their host(s). Far-red light alone ($\Phi \leq 0.1$) is mostly insufficient; no cryp*.

Placement of dodders in cool shade, where the environment is enriched in FR may not be sufficient to potentiate a change in growth habit. Removal of Pfr merely results in release of x. The addition of B to FR light activates the cryptochrome and captures x from phytochrome. This potentiates a change in the state of growth from a higher plant to a parasite.

CHAPTER FOUR

GENERAL DISCUSSION AND CONCLUSIONS

Dodders are holoparasitic higher plants. Thus, early detection of potential hosts, by their young seedlings, is essential for survival. Experiments reported herein have convinced us that there are environmental cues useful to dodders for control of their early morphological and physiological development that leads to successful host attachment.

The data in Chapter Two is concerned mainly with defining the initial characteristics of dodder's parasitic mode of growth. Biotic and abiotic factors that affect coiling and prehaustoria development have been studied. Until this study there had been no available methodology to study this parasitic growth away from a host plant. The results show that light is the most important factor in mediating coiling and prehaustoria development. The etiolated seedlings cannot attack encountered objects 'whilst still writhing in the dirt' beneath the soil surface (Fig. 4.1). Etiolated seedlings must yet participate in a second rite of passage. It is the de-etiolated, higher plant seedling that has the potential for transformation to the parasitic mode of growth. Hook opening is the first outwardly visible characteristic of the soon-to-be, true higher plant seedling of dodders. Exposure of the apical hook of the de-etiolated seedling to R light initiates by

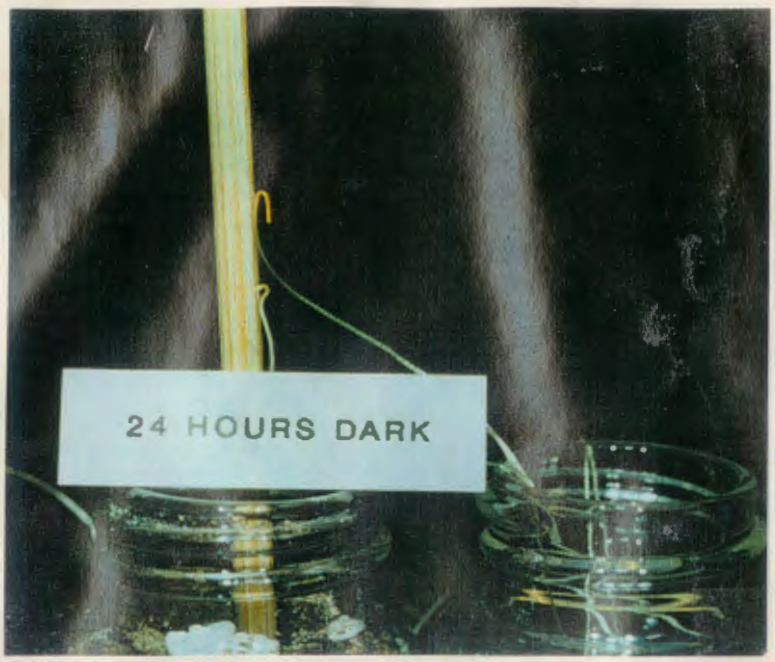


Figure 4.1. Etiolated dodder seedlings. Dodder never twined or form prehaustoria in DK. Dark (DK).

action of phytochrome (Pfr I), a complex sequence of developmental changes. This is a classical inductive phytochrome effect; it is photoreversible with FR.

The de-etiolated, higher plant seedlings nutate widely (counter-clockwise, as viewed from above), searching for a host(s). Dodders in sunlight open environments, which have high zeta value, 'seek' the shade of neighboring green plants. Dodders initially detect nearness of others (i.e., potential hosts) by changes in light quality. Within the sphere of influence of a neighboring plant (e.g. alfalfa), an environment, illuminated actinically with B and FR, potentiates a change in the state of the higher plant, thread-like organs of dodders, to that of their parasitic mode of growth. Dodders in alfalfa are exposed to B skylight from the side and FR light from above. This FR light is transmitted through chlorophyllous foliage above and reflected from (potential host) plants nearby and below. Actuation of that change to a parasite awaits only mechanical stimulation such as, contact with a support or with the stem of a host. In our experimental system and with threads excised from de-etiolated dodder seedlings, we placed them onto glass fiber filters in closed petri dishes. We were thus able to induce this parasitism by exposing the dodder threads to B alone, B plus FR, UV-A plus FR, or (lowered zeta of) R plus FR but not with cool white (WL) fluorescent light, R alone, UV-A alone, UV-A plus B, or FR alone.

Mechanical stimulation, resulting from contact by dodders with a potential host, causes dodders' threads to form tight coils around the object that is contacted, such as host stem, or in our experimental model an 'artefactuated', physical support (Fig. 4.2 a and 4.2b). Development of prehaustoria on the concave side of the coils is visible within 18 h.

Interestingly, ethylene inhibits growth of dodders during non-parasitic life period (higher plant). But exogenously supplied ethylene, has no effect on the parasitic phase. The significance: Wounding of a potential host stem by the parasitic dodder thread likely increases host biosynthesis of ethylene, a typical wound response in higher plants. It is not surprising that the parasite is insensitive to ethylene. After we observed this, an attempt was made to examine the role of other plant hormones and chemicals on dodder parasitic growth. Results show that manipulation of auxin:cytokinin ratios exogenously applied across the thread likely determines the developmental distribution of prehaustoria on coiled dodders. Zeatin induces while IAA inhibits development of prehaustoria on the de-etiolated dodder threads. Asymmetrical auxin distribution here, resulting in unequal growth rates, across threads, can likely account, in part, for coiling of threads in B light. Signal transduction(s) within tendrils likely involve ions, K^+ and proton(s), since application of K^+, and

Figure 4.2a. Dodder as a parasite under BF light. Dodder seedlings twined and prehaustoria formed on wood stick. Blue + Far-red light (BF).

Figure 4.2b. Dodder as a parasite under BF light. Dodder seedlings twined and prehaustoria formed on glass rod. Blue +Far-red light (BF).

sucrose, stimulated prehaustoria formation mainly under FR light.

Other series of experiments were conducted to determine the photoreceptors involved in mediating dodder parasitic growth. Data in Chapter Three shows that phytochrome (at least phytochrome; types I and II) alone can account for parasitic growth under RF light but not under B light. The results implicate a specific UV-A/B photoreceptor, possibly a flavin, signalling changes in growth habit; i.e., stimulation of coiling, and initiation of prehaustoria by B (400 to 500 nm). We believe the mechanism of this stimulation is mediated by the electrochemical state of the plasma membrane.

Seedlings of dodders, in environments with high z-value (R:FR), 'seek' the shade of neighboring green plants. Changes in light quality are translated into changes in Φ-values of type II phytochrome. Far-red-enriched areas are 'sensed' as lowered Φ-value(s), indicating the location of potential host(s).

Prehaustoria initiation requires low Pfr. If Pfr is high no coiling or prehaustoria were observed. Prehaustoria can be induced by B light. The question arises whether this B light effect is also mediated by phytochrome. Results that were presented support the hypothesis that there is a separate photoreceptor for B light. Evidence of a true interaction of two photoreceptors has been obtained in the light-equivalent, high fluence, and light pulse studies.

Red and/or FR light given in combination with B light can change prehaustoria number substantially. By analyzing this effect further we found that cryptochrome action depends on phytochrome status in mediating dodder parasitic growth.

We suggest that in dodders the FR-absorbing form of type II phytochrome (Pfr II) opposes the action of an excited state of cryptochrome (cryp*). We believe the two photoreceptors may compete for a common intermediate, X; or common intermediate state, X'.

In light of high z-value, dodders have relatively high Φ-values. It is Pfr II that maintains dodders as higher plant seedlings. In white light, the UV-A/B photoreceptor absorbs photons. We account for its inactivity if we assume that Pfr II more effectively competes with cryp* for X.

To our knowledge, our proposed mechanism(s) is not typical of HIR responses, and is not quite like the specific B responses of 'truer' higher plants in which B and R often act in 'the same direction'; i.e., for those response systems studied within plants which continue only in a higher plant mode of growth. If both B and R are active, treatment of 'higher' plants with B or R can have qualitatively similar, although not necessarily quantitatively similar, morphological consequences.

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