

THESIS

TRICHODERMA, TRUBAN[®], PYTHIUM, AND LIGHTING

INFLUENCE ON CARNATION DEVELOPMENT AND FLOWERING

Submitted by

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In partial fulfillment of the requirements

for the degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 1985

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COLORADO STATE UNIVERSITY

August 29, 1985

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DAVID F. GRAPER ENTITLED, TRICHODERMA, TRUBAN[®], PYTHIUM AND LIGHTING INFLUENCE ON CARNATION DEVELOPMENT AND FLOWERING, BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

TRICHODERMA, TRUBAN[®], PYTHIUM AND LIGHTING
INFLUENCE ON CARNATION DEVELOPMENT AND FLOWERING

Investigations were carried out on the merit of utilizing Trichoderma in the production of carnations. Two different strains of Trichoderma harzianum, T-12 and T-95 were used. The effectiveness of Trichoderma as a control agent against Pythium root rot was found to be small. Some alterations in carnation development were observed, but were insufficient to warrant recommending its use. Growth response as a consequence of Trichoderma were insignificant. If a soil-borne disease problem, such as Pythium root rot, does exist, Truban[®] is recommended for control, as it was very effective in these experiments.

Two experiments were conducted on photoperiodism and timing in the carnation. These were designed to determine if current carnation culture should be modified. Potential benefit was observed, for the grower, in the use of a long day treatment for an older crop of carnations, increasing production prior to a holiday demand period or a crop's removal from production. The use of a period of short days,

prior to the long day period, was also investigated. Results were ambiguous, and no recommendations could be made.

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ACKNOWLEDGEMENTS

The preparation of this thesis would never have been possible without the help and guidance of many individuals. Foremost among these are the members of my graduate committee; Dr. Joe Hanan, Dr. Ralph Baker, Dr. Kenneth Goldsberry and Dr. William Healy. Special recognition must go to the American Florist Endowment, Colorado Greenhouse Grower's Association Inc. and the Colorado Agricultural Experiment Station for supplying the funding necessary for the research done in preparation for this thesis. It is also important that other members of the faculty and staff, at CSU be recognized for their help and support; Dr. Charles Basham, Charles Christensen, Judy Croissant, Ann McSay and others.

Last, but certainly not least, I wish to express my sincere thanks to my family; Hugo and Beverly Graper and Randy, and also my freinds, who are very important to me and without which, I would have not been able to complete this thesis and thus continue my education.

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CHAPTER 1

INTRODUCTION

Carnations have long been, a very important floricultural crop in Colorado, as well as in many other areas of the US and world. Most grower's goal is to produce a high quality product, however, many obstacles exist, as it is pursued. One problem that is omnipresent, plant diseases, can prevent a grower from realizing success.

Much research has been done, to combat the ever-present threat of plant pathogens. Most recommendations have been through the use of chemicals, however, biological control can be a viable alternative, which has recently, begun to show its true potential for many horticultural crops.

Another realm of possibility exists, for the use of chemicals and biological that of inducing a plant to further approach production at its genetic potential. It is of course, extremely difficult to determine when that point is reached. Organisms such as Trichoderma may be an answer in finding a solution in both biological disease control and increasing production.

Carnation production, requires synchronization to the highest demand periods, to provide maximum return. If one is to obtain maximum return, the grower must devise a method of programming crop production. Programming, or timing of the crop, can be accomplished through such

procedures as time of planting, pinching and altering the photoperiod under which the crop is grown.

Much of the photoperiod and timing research was done during the late 1950's and early 1960's. Many cultural changes have been implemented in carnation production that have an effect on plant development and its subsequent timing. In light of current production techniques, research was needed to determine if the old timing practices should be updated to further enhance carnation crop programming.

The research conducted for this thesis was carried out under the auspices of the American Florists Endowment, the Colorado Greenhouse Growers Association Inc. and the Colorado Agricultural Experiment Station. The funding supplied, was used to conduct research into the merit of using Trichoderma sp. in carnation production, and if so, what effect would result in timing of the carnation crop. Therefore, due to this nature of the research, each of the major sections of this thesis are divided to correspond to these two major related topic areas of research. The first of these deals with soil borne disease pathogens: their control and predators. The second deals with photoperiodism and timing in carnations.

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CHAPTER 2

LITERATURE REVIEW

A. Soil borne disease pathogens: their control and predators.

1. Soil borne disease pathogens of carnation and their control.

Disease control is an integral part of carnation production. A wide variety of disease pathogens may effect carnationplants by decreasing yield and vigor. Pathogens which are common in carnation production are: Fusarium, Pectobacterium, Phialophora, Pythium, and Rhizoctonia (9, 34, 47, 50, 54). The severity of each of these pathognes in carnation production has been greatly reduced since the advent of soil fumigation and steam pasteurization. The utilization of raised benches for production, clean stock, a clean water supply and good sanitation practices (9, 24) greatly reduce the chances of disease reinfestation after pasturization or fumigating. If no severe problems were encountered with the previous carnation crop, pasturization may not be needed if a soil fungicide is used at planting time (9).

Despite using the best cultural methods, disease outbreaks will sometimes occur. Many soil fungicides are available including: Dexon[@], Subdue[@], Terrachlor[@] and Truban[@] which will provide good control of soil

borne diseases (8, 9, 24, 50). Certain pathogens may be controlled or suppressed by biological or other means as will be discussed later.

2. Pythium root rot.

One of the most innocuous soil fungal organisms that is pathogenic to many different plant species is Pythium. Many Pythium species have rapid growth rates, as much as $15 \text{ mm (24 hr)}^{-1}$ (21), making them a good initial invader of pasturized or fumigated soil, where it may quickly take over the soil substrate and which could act to deter colonization by other organisms (7).

The pathogen, Pythium, is commonly associated with preemergent and post emergent damping off of seedlings (54). However, it can also reduce plant growth and flower production of well developed plants including carnation (49). Carnation growth may be inhibited even with the absence of foliar symptoms, such as chlorosis, a common symptom of plants suffering root damage. Carnations planted in newly steam pasturized beds, which were reinfected, may have reduced vigor due to the pathogen causing damage to root tips (24).

Pythium development is favored by high moisture in the growing medium which tends to create a more favorable condition for infection. Many plant species' roots develop poorly in a low oxygen atmosphere, but Pythium can develop quite well. High moisture may also lead to an increased shoot-to-root ratio which can put the plant under additional stress, thus further predisposing the plant to infection. High nitrate levels in the soil may also lead to an increase in shoot-to-root ratio (19, 21, 42, 54).

Pythium can generally be controlled using several of the fungicides listed earlier. Of these, Truban[®] was found to give good control and increased flower production (54). Rigorous use of sanitation procedures also helped to maintain a low Pythium population (23).

Pythium ultimum, originally described by Trow in 1901, like other members of the Pythiaceae family, belongs to the phycomycete group of fungi. Its development is favored by soils which have a fairly neutral pH, high water holding capacity and rich in organic matter (54). It is considered to be the most pathogenic species of Pythium causing damping off. P. ultimum has rapid propagule germination and high growth rate. Natural populations are commonly found to have 64 to 3800 propagules g⁻¹ of soil (56).

3. Biological control of disease pathogens; Trichoderma.

Biological control is the suppression of pests with biotic agents using such techniques as: sterility, modifying pest behavior through the use of chemicals or the use of antagonism which involves processes such as: antibiosis, competition, predation, and cross protection (8).

A wide range of biotic agents have been developed and utilized, often giving excellent results, especially in the control of soil borne diseases as well. One of the most prominent examples is the control of Fusarium wilts employing Bacillus subtilis, Chaetomium glabosum and Pseudomonas putida as biocontrol agents (3, 12, 17, 45). Another example can be found in controlling Pythium ultimum- induced damping off by utilizing an antibiotic, pyoluteorin, excreted by the fungus Pseudomonas fluorescens (37). Recently, a new species of Pythium has

been discovered which is not pathogenic to most plants and also acts as a mycoparasite to several pathogenic Pythium and Phytophthora species as well as Rhizoctonia solani. P. nunn, as it has been named for the city in Colorado where it was first isolated, has even worked to reduce Pythium species that were not suppressed by Trichoderma (42, 44, 45).

Other types of biological control of soil borne diseases have been developed. Wilt disease in peas, is prevented by inhibiting the action of the enzyme cutinase, secreted by Fusarium solani during the development of the disease to allow fungal penetration of the host. This inhibition is created by using rabbit antibodies in conjunction with dusoprylfluorophosphate (DEP). The treatment did not kill the fungus, but was very effective in preventing infection. This type of product could lead to the development of enzyme targeted fungicides (44).

One of the most well known soil mycoparasites is Trichoderma. This fungus, belongs to the group fungi imperfecti, and is found in many habitats in nature, especially in moist non-alkaline areas (15, 26, 41). Of the nine species described by Riffai in 1965 (6), several have been utilized for their mycoparasitic abilities against such pathogens as Pythium, Rhizoctonia and Sclerotium (5, 18, 53).

The main action of Trichoderma, in reducing disease inoculum, is the hyphae coiling around the thalli of the fungi, obtaining nourishment through the process of lysis. Certain strains of Trichoderma may be integrated into a control program involving fungicides with the development of mutations which are tolerant to the use of fungicides. An example is the T-95 strain of T. harzianum which is tolerant to benomyl (8).

Disease development can also be suppressed by Trichoderma through the release of certain metabolites which have an antibiotic effect.

T. viride is reported to produce viridin and glyotoxin, two compounds which have some antibiotic effect on the disease organism Pythium (16). Certain other species also produce compounds which may inhibit mycelial growth or possibly promote development of the host. Rhizoctonia has been suppressed by T. hamatum and T. harzianum which produce gluconase and chitinase enzymes, degrading glucose and chitin in the cell walls of Rhizoctonia. It is conjectured that these Trichoderma species should also control Pythium since cellulose is a constituent of its cell walls (13, 14, 16, 26).

Agnihorti (2) found that Trichoderma caused a growth response in yet another way, through the solubilization of insoluble phosphates in the soil. Pythium and Rhizoctonia develop poorly in low phosphate soils, therefore inoculum of the antagonist, Trichoderma, could build up to provide increased disease control. Some of the newly available phosphorous could be used by the host plant if it was deficient. Another way disease could be suppressed would simply be by the physical occupation of the substrate, thus excluding the other organisms (26).

4. Summary.

Thus far, much of this discussion has dealt with various means of controlling a variety of soil borne disease pathogens. When one encounters, or typically experiences disease problems, control measures should be implemented to maintain quality and production. However, the conscientious grower, who is careful to follow stringent sanitation and cultural procedures, may have little use for either chemical or biological control measures, since positive responses may not be observed. Perhaps

the elimination of the need for disease control measures should be the ultimate goal of all growers in their carnation production program as well as the production of all floricultural crops.

B. Photoperiodism and timing in the carnation.

Radiant energy is the most influential of environmental factor which affects the growth, production and quality of the carnation plant. Solar radiation is often deficient during the winter months and may limit production unless supplemental irradiation is supplied. In fact, an energy deficiency may delay the interval from pinching to peak production as much as eight months during the late fall to winter period compared to a three month requirement during the summer (10, 33, 48).

Based on total irradiation available, one could deduce that a carnation plant can support different numbers of good quality shoots and flowers during different seasons of the year. Flower size can be reduced significantly as radiation levels decrease (29). Therefore, to maintain production of high quality flowers, one should optimize the number of shoots allowed to develop on the plant during a particular season (31). Other than modifying cutting practices to control vegetative breaks, one can modify the photoperiod which the carnation receives (35, 36).

Since the carnation is a quantitative long day plant, altering the photoperiod can hasten or delay flower initiation and thus subsequent flowering. Side break development, unlike flower initiation, is inhibited by an increased photoperiod (1, 10, 27, 28, 35, 36, 40, 48, 51).

Research has shown that critical day lengths do not exist for cut carnations, but, flowering is best promoted as daylength equals 24 hours. Flower initiation has been found to be hastened by using dusk-to-dawn lighting to lengthen the photoperiod (28). Other researchers (10, 30) concur with the use of dusk-to-dawn lighting, however, there were some who felt that the response using night interruption lighting was adequate and would also conserve energy (11, 32, 52).

Side break development can be a major problem when using artificial lighting to lengthen photoperiod for the promotion of flower initiation (26, 32, 39, 51). One researcher (36) found little use for lighting young plants in Colorado to achieve hastened flower initiation. It was specifically advised, that the young plants should not be lighted before Christmas as production from the return crops would be limited for the important spring holiday demand periods. Perhaps one of the best uses for lighting might be in lighting a one to three year old crop to inflate production just prior to its removal (35).

Long photoperiods tend to enhance flower initiation and short days tend to encourage side break development. Side break development and flower initiation can be enhanced if short days are used in conjunction with long days (39). Rather than four weeks of long days, as had been the practice, a six week cycle of long days and short days was proposed using three weeks of long days followed by three weeks of short days to provide for good lateral shoot development, thus providing for the return crop (39).

Koon's research (39), was later confirmed, emphasizing the need for a period of short days for side break development. Another six week cycle was proposed, however it differed in that long days were given on

alternate weeks during the period, it was felt that the alternating treatment would be a more beneficial treatment. This latest approach did give better results because it was more likely to stimulate shoots in the proper stage of development and also conserve electricity (11). Researchers in Minnesota (31) recommended a short day treatment for five to seven weeks after pinching and then three weeks of long days to obtain an optimum response of flowering and side break development.

The last proposal was taken a bit further and was developed into yet another photoperiod scheme which was quite effective in producing an increased number of flowers. In this scheme, cuttings planted in June were pinched to four nodes, given 30 natural days, followed by 75 short days to promote vegetative lateral development and then finally a period of 30 long days was given to promote rapid floral initiation of the developing laterals (31).

Once a shoot's meristem has made the transition from vegetative to reproductive, photoperiod no longer plays a role in its development, therefore, the timing of the long day treatment is crucial for an optimum response to be obtained. The transition of the meristem has been determined to occur at different stages in the development of the shoot by different authors. Healy, who has done the latest research in this area, found transition occurred at the five-leaf-pair stage. Consequently, the shoot must be in a long day photoperiod prior to the shoot's development to this stage or else transition will be delayed (31). Earlier researchers in Minnesota determined that this transition occurs during the seventh to ninth week after pinching (30), although this may not be a very accurate method in determining the proper stage since development can be greatly slowed by low radiation levels or

conversely increased if radiation levels are unusually high (10, 33, 48). Another researcher (51) felt that the shoot was in this transition stage when it had three to five leaf pairs visible and was one to two inches long. Still another view was that it occurred at the four to six leaf pair stage (39, 48). The consensus of opinion seems to be that when the majority of the shoots are approaching the five leaf stage, the long day photoperiod treatment should begin.

Flower quality is always of extreme importance. Generally it was discovered that the use of artificial irradiation to modify photoperiod did not significantly reduce flower quality if it was done at the proper time, keeping in mind the total available radiation of the period during which the flowers will be developing (11, 39, 40, 48, 51).

CHAPTER THREE

MATERIALS AND METHODS

A. General Methods

All plants used in these experiments were standard varieties of Dianthus caryophyllus, either 'White Sim' or 'CSU Red' as noted for each experiment. All were grown as single pinch plants in raised benches, except where noted, planted at a 18 by 18 cm spacing. Greenhouses were controlled at 11 C night and 17 C day setting. All benched plants were irrigated using a Chapin double wall trickle system with automatic nutrient injection. The growing media consisted of a 1:1:1 soil:peat:perlite mix except where noted. Each bench was planted with a 50 cm buffer at either end. Benches were steamed prior to planting.

As plants were developing, their flowering shoots were disbudded by taking all reproductive and one vegetative break. When cut, one to two vegetative breaks were removed with the flower stem. The cut flower stems were then graded into the four standard grades of fancy, standard, short, and design. Grade of each individual cut flower was recorded. Flowers were harvested three times weekly except where noted.

B. Trichoderma, Truban[@] and Pythium and their effect on carnation development: Methods.

The following experiments were basically designed to determine what effect Trichoderma had on the development of carnation. Since this was a soil treatment, benches were built so that each treatment plot, in a bench, would be isolated from either plot adjacent to it. This was either accomplished by using a polyethelene and board divider, a double board divider (Fig. 1), or through the use of individual pots.

Two strains of Trichoderma harzianum were used, T-95 and T-12. The T-95 inoculum was acquired from the MYCOGEN Corporation of California while the T-12 inoculum was produced at CSU in the Gnotobiotics laboratory. The T-12 inoculum was grown by a simple procedure. Cultured T-12 inoculum was placed on a peat bran medium (56) consisting of one part milled peat bran, one part sphagnum peat moss and one part water. Before this medium was inoculated, the medium was placed in two liter jars plugged with cotton. The jars are then autoclaved for 30 min., allowed to sit for two days and then autoclaved again for another 30 min. and allowed to cool before inoculation.

Approximately seven days after inoculation, the Trichoderma usually filled the medium with mycelium and spores, and was ready to be processed (Fig. 2). First, the material was removed from the jars and allowed to air-dry for two to three days, at which point it was collected and ground using a Wiley mill with a 2 mm sieve. Contamination in the jars was a serious problem and was very undesirable since the Trichoderma population decreased markedly. Only non-contaminated inoculum was used to insure better results.



Fig. 1: Bench used in Experiment II, showing the use of a double board divider to separate treatments, lessening chances of cross contamination.

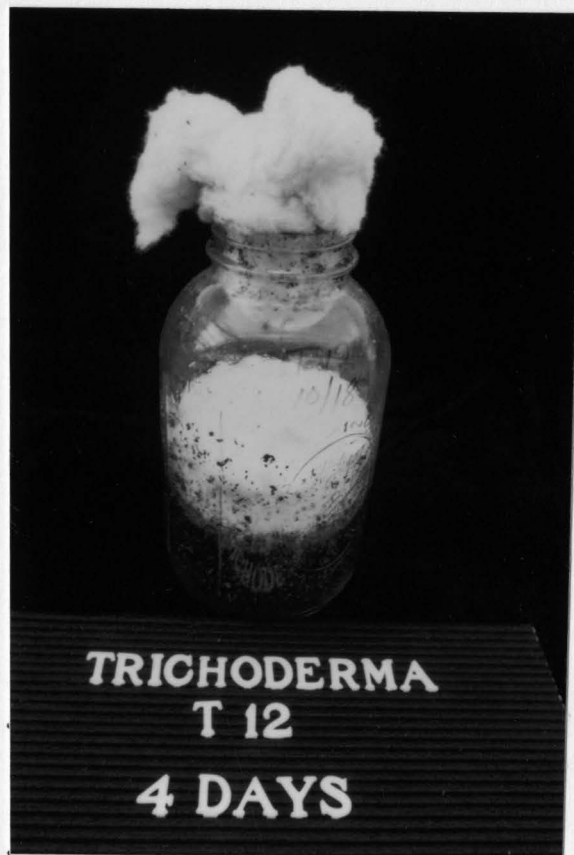


Fig. 2: Two liter jars showing T-12 mycelium development four days after being inoculated. Generally after seven days, the jar was filled with mycelium and ready to be processed.

Once the inoculum was processed, it was mixed and sampled for its level of Trichoderma using standard dilution procedures on a selective medium (18). Once the population density was determined, it was possible to calculate the amount of inoculum to be added to the plant growing medium to give the desired Trichoderma populations.

Two experiments used Pythium ultimum in one or more treatments. This fungus was also supplied through inoculum produced in the Gnotobiotics lab at CSU using procedures described by Lumsden (47) and Stanghellini (58). Unlike the T-12 and T-95, this inoculum was based in a soil medium. Population levels were taken of the P. ultimum in a similar manner as the Trichoderma, using a Pythium selective medium. The desired population level was then obtained by mixing the proper amount of Pythium inoculum with the growing medium.

Experiment I.

Experiment I was begun June 16, 1983 when 1200 'White Sim' #1 and 300 'Dusty' cuttings were propagated. The rooting medium consisted of a 1:1 peat:perlite mixture. The rooting bench was supplied with an intermittent mist system and bottom heat. One third of the main treatment cuttings, 'White Sim', were rooted in an isolated portion of the bench. To this isolated area, a sufficient amount of T-95 inoculum was added to bring the population level to 1×10^7 cfu g⁻¹.

Rooted cuttings were then planted into the four benches which were divided into six plots and two buffer zones at each end with measurements of 105 X 170 cm and 105 X 50 cm respectively. The buffer zones were planted with 18 cuttings each, in three rows, while the treatment

plots were planted with 54 cuttings in a 18 X 18 cm spacing giving nine rows of six plants.

Six treatments were used in this experiment, each bench representing a block. Treatments were assigned to each of the plots within a block at random. The six treatments used included:

1. T-95 in propagation bench.
2. T-95 in growing bench.
3. Truban[®] in growing bench.
4. T-95 in growing bench plus Truban[®].
5. T-95 in propagation bench plus Truban[®].
6. Control.

Two treatments had T-95 added to the growing bench. This was done in much the same manner as described in the addition of T-95 to the propagation bench resulting in a similar population level. Truban[®] was added to the proper treatments at the time of planting at a rate of 5.5 g m⁻². In treatments where Truban[®] and T-95 were used in conjunction, the individual rates of each remained the same.

Experiment II.

The cuttings utilized in Experiment II were handled in much the same manner as Experiment I except 'Elliot White' was used as the main treatment cultivar and 'Quinn's Yellow' was used as the buffer plants. T-95 was used in the rooting of one half of the treatment cuttings at a rate sufficient to yield a 1×10^6 cfu g⁻¹ population level. Cuttings were stuck May 18, 1984. Only one bench was used in this experiment, divided into eight treatment plots and two buffer zones measuring 105 X 125 cm

and 105 X 50 cm respectively. Treatment plots contained seven rows of six plants in a 18 X 18 cm spacing. Buffer zones were planted with 18 plants, as in Experiment I.

Four treatments, replicated twice, were used in this experiment in a randomized complete block design. Treatments included:

1. T-95 in propagation bench.
2. Truban[®].
3. T-95 in propagation bench plus Truban[®].
4. Control.

Rooted cuttings were planted June 6, 1984 and pinched two weeks later. At this time the Truban[®] was applied to the plots which required it as well as approximately 6×10^4 cfu plot⁻¹ Pythium ultimum inoculum to all treatments and thoroughly watered in.

Soil samples were taken September 24, 1984 and assayed for both Trichoderma and Pythium inoculum levels utilizing procedures described earlier. The soil analyses showed extremely low populations of both organisms. It was felt that at this point there was little benefit to be gained in continuing the experiment beyond the harvest of the first crop of flowers due to the low population levels and lack of any visual growth response.

Experiment III.

This experiment was begun on October 22, 1984 when the cuttings of cultivar 'CSU Red' were stuck into the propagation bench. As described previously, Trichoderma was used in the propagation medium for four of the treatments. A different strain of Trichoderma harzianum, T-12 was

used. A propagule level of 5×10^5 cfu g⁻¹ was used for the rooting treatment of those cuttings.

Six-inch azalea pots were used in this experiment, as well as a different growing medium consisting of a 1:1 peat:perlite mixture. It was hoped that the shallower pots would give better isolation and control of each treatment. Also the medium, used in conjunction with the pots would have a higher water holding capacity providing a more conducive environment for development of both Trichoderma and Pythium (7, 14, 15, 16, 21, 22, 42, 45, 49, 50, 54).

Irrigation was accomplished through the use of spaghetti tubes with two emitters placed in each pot. This was to better insure proper wetting of the entire root ball and guard against cross contamination between pots and treatments (Fig. 3).

Twelve different treatments were used in this experiment consisting of T-12, Pythium ultimum and Truban[®] used alone and in combinations.

The resulting treatments were:

1. Control.
2. Truban[®].
3. T-12 in propagation bench.
4. T-12 in pot.
5. Pythium.
6. Truban[®] plus T-12 in propagation bench.
7. Truban[®] plus T-12 in pot.
8. Truban[®] plus Pythium.
9. T-12 in propagation bench plus Pythium.

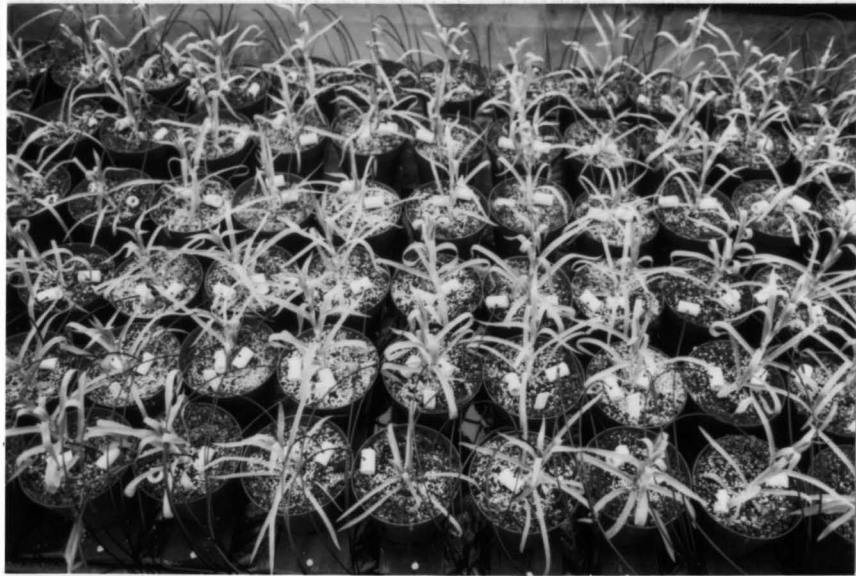


Fig. 3: Six inch azalea pot setup for Experiment III showing the use of spaghetti tube irrigation system. Note the use of two emitters in each pot.

10. T-12 in pot plus Pythium.
11. T-12 in propagation bench plus Pythium plus Truban[®].
12. T-12 in pot plus Pythium plus Truban[®].

Treatments received Truban[®] at the rate of $0.8 \text{ g (1 H}_2\text{O)}^{-1} \text{ (4 pots)}^{-1}$ directly after planting. T-12 and Pythium inoculum were thoroughly mixed with the growing medium, using a cement mixer, at inoculum levels of 1×10^6 and $2 \times 10^2 \text{ cfu g}^{-1}$ respectively. The mixer was cleaned with an Amphyl[®] solution between treatments to avoid contamination.

Four pots were used for each treatment with four replications. The experiment was set up in a randomized complete block design with six pots placed across the bench, with the outer two being buffer plants. Buffer zones were also set up between replicates and at each end of the bench.

Three weeks after planting, the plants had begun to elongate. At this point all plants were pinched to four nodes and dry weights were taken on the portion removed from each treatment. Starting six weeks after pinching, measurements, including height, number of nodes and number and length of breaks were taken and recorded from representative plants from each treatment. Photographs were also taken of plants from each treatment. This process was continued at two week intervals until the development of the plants was such that damage to the plants was likely occur as a result of the measurement process. Samples of the medium were also taken periodically to check for Trichoderma and Pythium population levels.

As the plants developed, they were disbudded, removing all elongated reproductive shoots when the terminal bud had reached a diameter of approximately 1 cm. The fully developed flowers were harvested on a

daily basis, removing them at the origin of the main stem. Days to harvest from pinching, overall length, number of nodes and breaks, mean length of two uppermost breaks and grade were recorded. The uppermost breaks generally develop more quickly and uniformly than breaks developing more basipetally (53), thus more concern was focused on these. Each flower was then oven dried at approximately 185 C for 48 h, and its dry weight recorded.

Experiment IV.

The main objective in conducting this experiment was to determine the effect of Trichoderma on rooting of carnations. Enhanced rooting could lead to faster plant development and ultimately earlier flowering. Therefore, it was deemed appropriate to check for this response. Once again T-12 was used for this experiment. However, this time it was used at two levels of inoculum in the propagation bench; 1×10^6 and 5×10^7 g^{-1} , and also a control of no T-12. Ten cuttings were used for each treatment which was contained in a six-inch azalea pot. Each treatment was replicated five times and set up in a randomized complete block design. Pots were placed on top of the medium already in the mist propagation bench but later sunk approximately 5 cm into the medium to obtain better bottom heat. This resulted in an average rooting medium temperature from 60 F to 76 F.

When the cuttings had been in the rooting medium for seven days, one pot from each treatment was selected at random and the cuttings checked for callous and root development. Criteria for development was rated according to the following scale:

0. No callous development.
1. slight callous development.
2. Well developed callous.
3. Root initials present.
4. Roots 5 mm in length.
5. Roots 1 cm in length.
6. Roots 2 cm in length.
7. Roots 3 cm in length.
8. Roots 4 cm in length.
9. Roots 5 cm or more in length.

The harvested cuttings were then oven dried for 48 h at 85 C and dry weights measured. Four more additional harvests were made at five day intervals. Rooting medium samples were also taken at each harvest and a final composite sample of all treatments within a replicate were assayed for its T-12 inoculum level. The rooting medium was retained from the last three harvests and used in the growing of some representative cuttings from the last harvest. Three cuttings from each treatment, of the final harvest, were grown in pots, replicated five times, in a greenhouse kept at 17 C night and 23 C day settings, to determine if the higher temperature would better elucidate a growth response.

These plants were kept unpinched and harvested, cutting at the base of the plant, when the plant came into bloom. As before, each plant was disbudded, removing all elongated reproductive breaks when the terminal bud was approximately 1 cm in diameter. Days to flower, overall length, number of nodes and breaks, average length of two uppermost breaks and dry weight was measured and recorded at harvest.

C. Photoperiodism and timing in the carnation: Methods.

Experiment V.

Additional cultural techniques have been recommended since the majority of the timing research was done on carnations in Colorado in the 1950's and 1960's. Therefore, this experiment was devised to ascertain the response of the carnation, to current cultural methods used in conjunction with some of the recent timing practices developed in Minnesota (31).

Rooted cuttings, of the variety 'CSU Red' were planted into each of 16 plots measuring 125 X 105 cm, in two benches. Each bench consisted of two replicates of four treatments assigned at random. Each treatment plot included a buffer row at each end and all plants were in an 18 X 18 cm spacing, as in previous experiments. Buffer plants were of either 'Quinn's Yellow' or 'Safari' cultivars.

On July 2, 1984, three weeks of natural days after planting, all plants were pinched to four nodes. By July 19, 1984, plants had developed sufficiently to be approaching the period of growth when the meristematic regions of the shoots would become responsive to photoperiod (31). At this time, the four short day photoperiod treatments were commenced. Treatments consisted of:

1. Control; natural photoperiod.
2. Thirty short days.
3. Forty-five short days.
4. Sixty short days.

Each treatment, which was to receive a short day photoperiod, was prepared in the following manner. A conduit framework was constructed and fastened to the bench at each corner of each plot. The framework was built so that it would extend approximately 125 cm above the side of the bench. Each day, a black sateen shade cloth was placed over the framework from 18:00 to 08:00 h at which time it was rolled up and placed over the edge of the framework of each treatment. When adjacent plots required shading at the same time, a single cloth was used over both of the plots.

At the conclusion of each of the 30, 45 or 60 short day period, the long day treatment was started. This was produced with a single 60-watt incandescent light bulb, equipped with a reflector, placed approximately 25 cm above the center of each treatment plot. These lights were in operation from 22:00 to 02:00 h each night for a 45 day period following the short day treatment. Each plot, receiving a long day treatment, was isolated from adjacent plots, not receiving the long day treatment, by the placement of a 1/4 inch wafer board between the plots. Slots were cut into the board so that it would fit down into the caging to eliminate as much of the light leakage as possible. Pieces of cardboard were also placed between lower layers of caging to further block light leakage into adjacent plots. A black polyethelene shade cloth was also pulled between each bench.

As each of the treatment was completed, the long day photoperiod was discontinued. Flowers were harvested three times weekly, as described in Experiments I and II, recording grade and total from each treatment.

Experiment VI.

This experiment was an attempt to determine the response of an older crop of carnations to a long day photoperiod. Sixty-watt reflectorized incandescent light bulbs spaced 150 cm apart were suspended approximately 40 cm above the center of two of the benches used for Experiment I. Lighting was done from dusk-to-dawn during the period from September 20 to October 20, 1984. Black polyethelene was suspended between benches to avoid light contamination of non-lighted benches. Flowers were harvested as they had been for Experiment I.

Despite the fact that these benches already had been used for another experiment, it was felt that this would be of little consequence, since no differences had been observed between treatments in Experiment I.

CHAPTER FOUR

RESULTS AND DISCUSSION

A. Trichoderma, Truban[@] and Pythium and their effect on Carnation development.

Experiment I.

General observation of plant development throughout this experiment provided little evidence of differences between treatments. All plants appeared to develop at the same rate and had similar stem lengths, vigor, break development and flowering characteristics. Preliminary data showed no significant differences between treatments in flower production. The final data was analyzed at the conclusion of the experiment (Table 1 and Fig. 4 - 6), and also indicated no differences.

Reasons for the lack of significant differences between treatments, were somewhat unclear. Perhaps the T-95 was not at a high enough concentration in the treatments where it was used or the original inoculum was of poor quality. It is possible the Trichoderma did not yield a response with carnations under the cultural conditions in which the plants were grown. This hypothesis will be discussed in greater detail at a later point when all experimental results have been discussed.

Table 1: Mean yield ft^{-2} of 'Improved White Sim' carnation flowers, planted July 11, 1983, in each of the six treatments in Experiment I in four grade categories during the 70 wk period beginning October 9, 1983

TREATMENT	FLOWER PRODUCTION BY GRADE				
	FANCY	STANDARD	SHORT	DESIGN	TOTAL
Control	8.8 ^z	30.8	31.2	21.2	89.6
Truban [@]	8.7	30.0	30.8	20.4	90.4
T-95 in bench	8.4	31.2	30.4	19.2	89.2
T-95 in propagation	8.4	30.8	30.8	23.2	93.2
Truban [@] + T-95 in bench	9.6	31.2	30.9	21.6	92.8
Truban [@] + T-95 in prop.	8.5	30.4	29.6	21.2	89.6

^z Values for each of the flower grades and total were not significantly different from each other. $\underline{P} = 0.05$. Four replicates were used in a randomized complete block design.

Fig. 4: Weekly production patterns of the control and Truban[®] treatments during Experiment I. Data was transformed using a three-week moving mean to remove some of the variation in production from week to week that often results from changing weather conditions and other factors, unrelated to the treatments used in this experiment. The high demand periods of Christmas, Valentines Day and Mother's Day are shown.

EXPERIMENT I

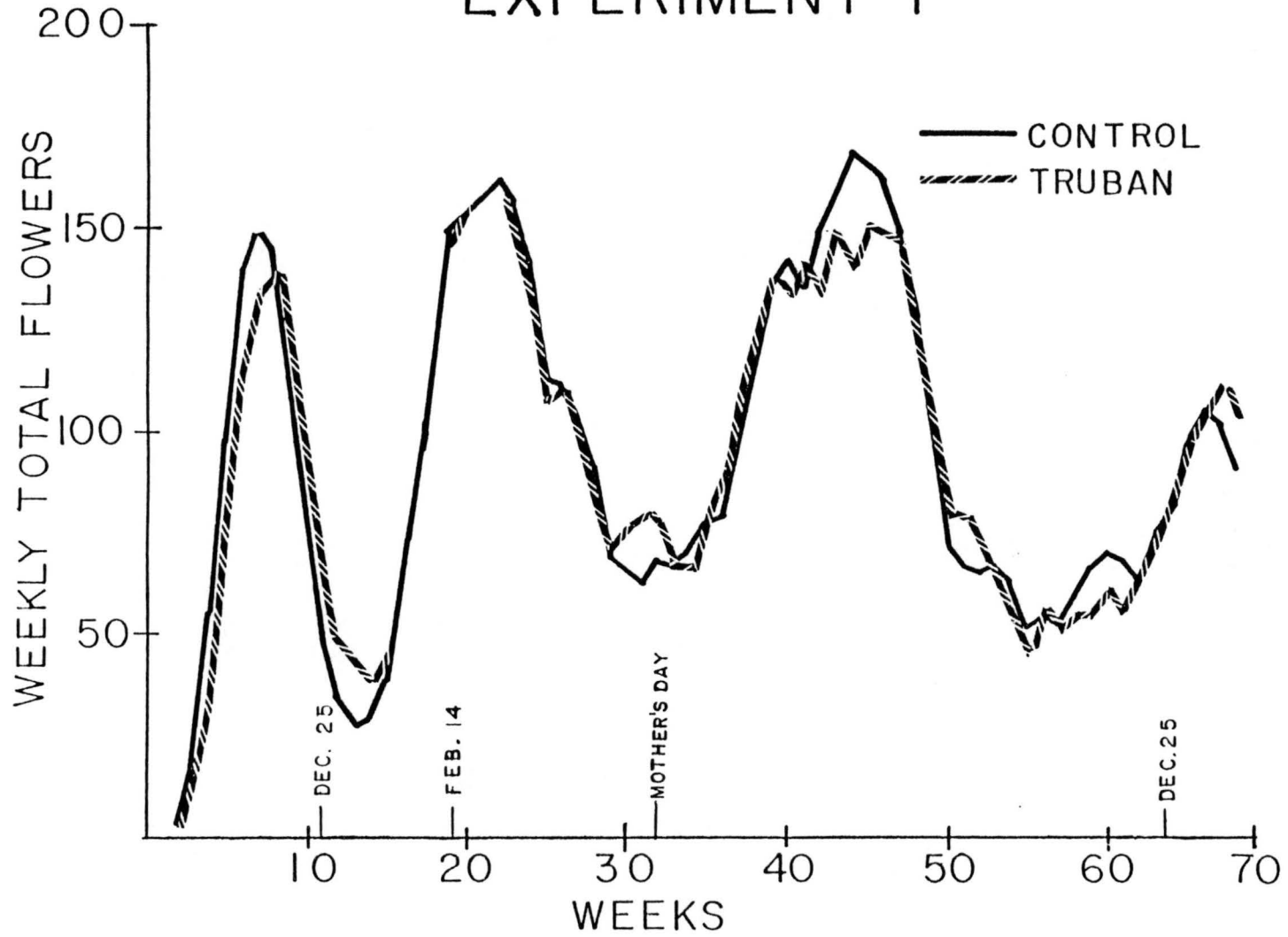


Fig. 5: Weekly production patterns of the treatments in which T-95 was used in propagation and in the bench, of Experiment I. Data was transformed using a three-week floating mean to remove some of the variation in production from week to week that often results from changing weather conditions and other factors, unrelated to the treatments used in this experiment. The high demand periods of Christmas, Valentines Day and Mother's Day are shown.

EXPERIMENT I

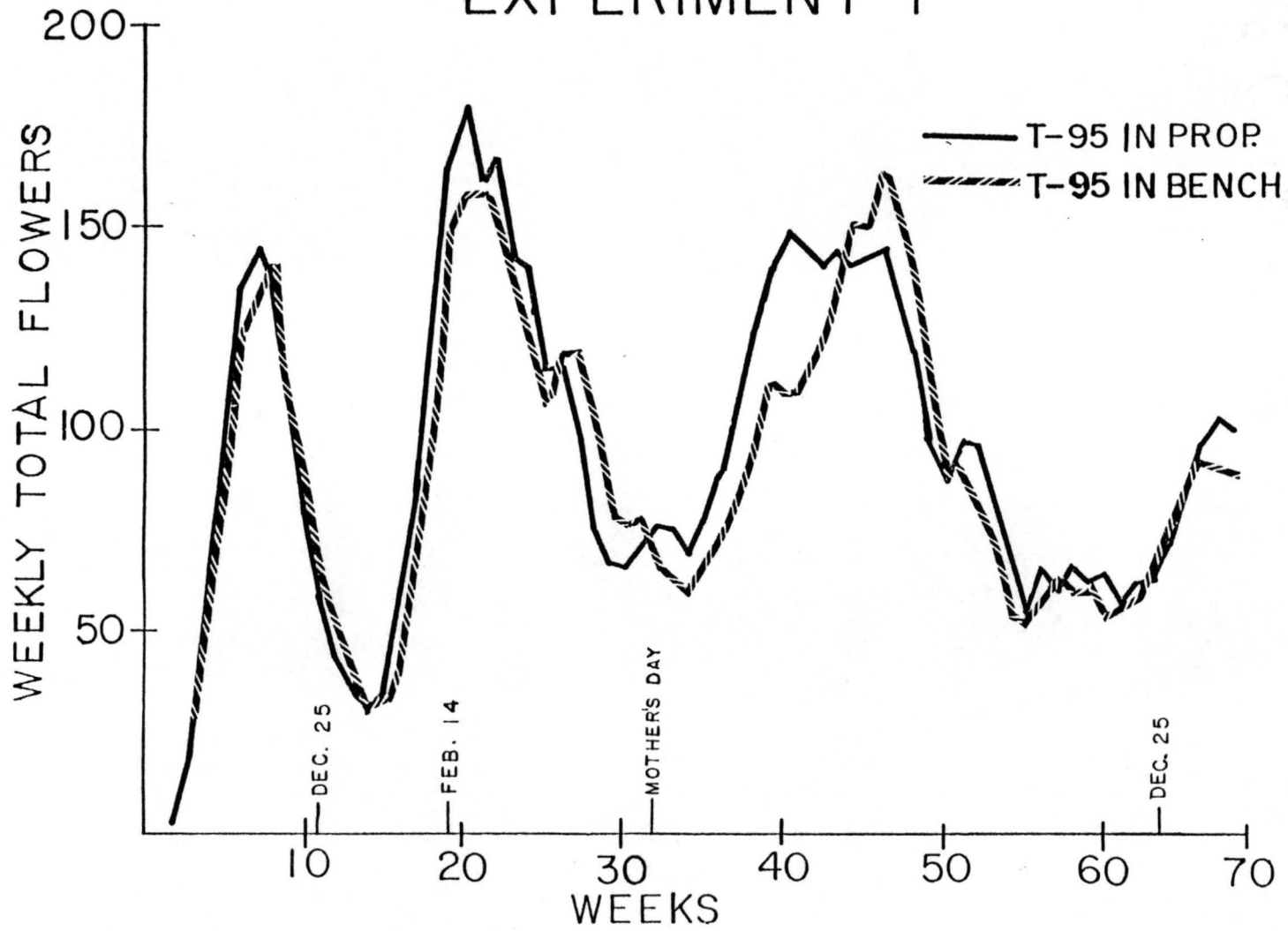
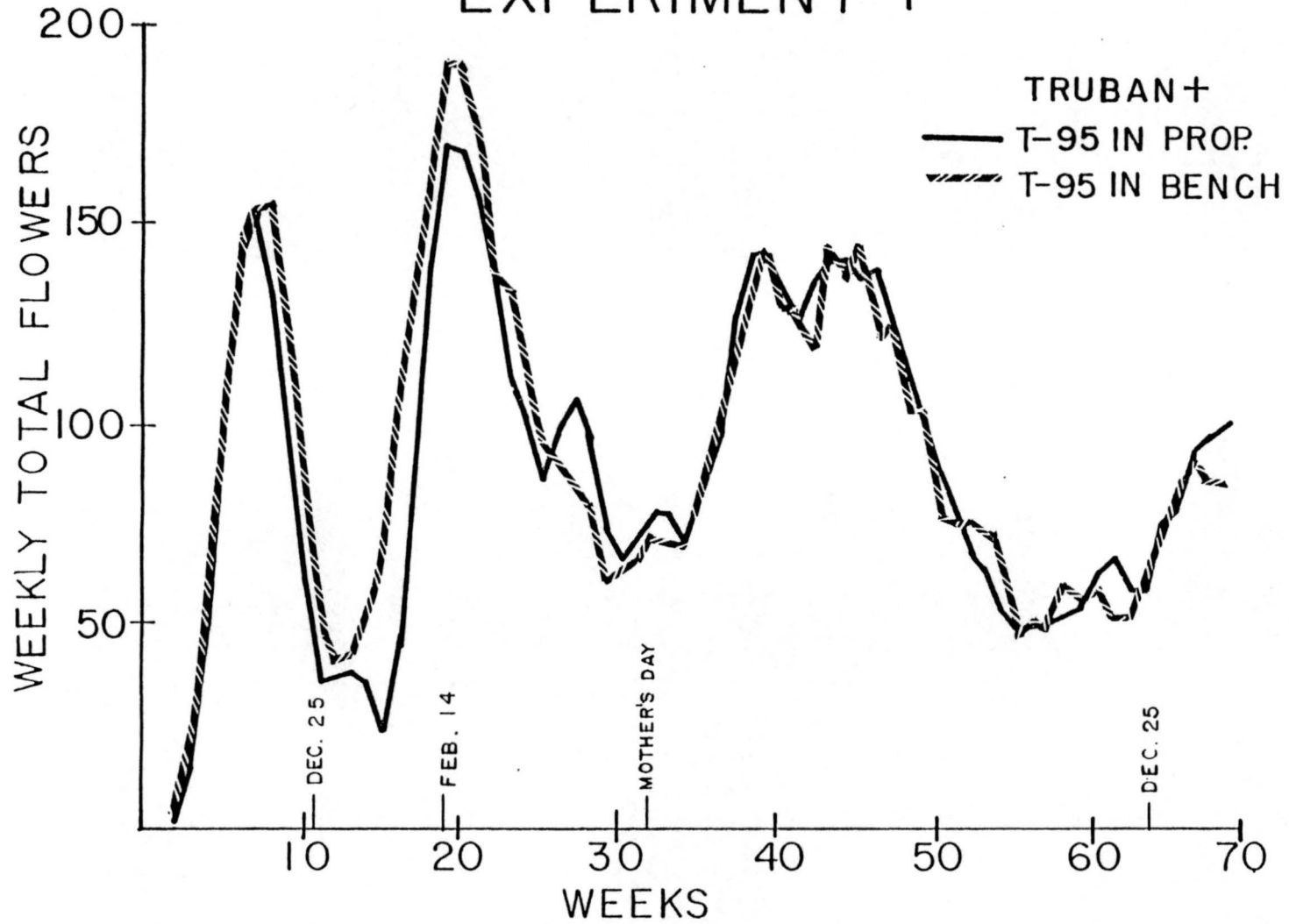


Fig. 6: Weekly Production patterns of the treatments in which T-95 was used in propagation and in the bench with the application of Truban[®] for Experiment I. Data was transformed using a three-week floating mean to remove some of the variation in production from week to week that often results from changing weather conditions and other factors, unrelated to the treatments used in this experiment. The high demand periods of Christmas, Valentines Day and Mother's Day are shown.

EXPERIMENT I



Experiment II.

The plants in this experiment progressed in much the same manner as described in Experiment I. Analysis of data, after the harvest of the first crop showed no significant differences between treatments in timing, quality or production (Table 2 and Figs. 7 - 8).

Soil samples were analyzed for T-95 and Pythium inoculum levels, and they were found to be very low, less than 100 and 1×10^3 cfu g⁻¹ respectively. At these levels, which were felt to be too low to achieve a response, it was decided that the experiment would be terminated. No response was seen under these growing conditions.

Experiment III.

Early in this experiment, differences between treatments could be observed. However, plant development, in general, was quite slow for all treatments due to the lower radiation levels commonly associated with late fall. Plant measurements were taken for the first time, six weeks after the plants had been pinched. Plant height, number of breaks and length of breaks was measured at this time. Each of these measurements were taken again at eight and ten weeks after pinching. At that point, the development of the plants in the caging made it too difficult to take further break development measurements, however, they were taken at the time of each flower's harvest.

Significant differences were observed, in each of these measurements. Although, other differences were observed, the main

Table 2: Mean yield ft^{-2} of 'Elliot White' carnation flowers, planted June 6, 1984, in each of four treatments in Experiment II in four grade categories during the nine week period beginning September 9, 1984

TREATMENT	FLOWER PRODUCTION BY GRADE				
	FANCY	STANDARD	SHORT	DESIGN	TOTAL
Control	0.1 ^z	2.8	6.6	3.3	13.0
Truban [@]	0.1	2.2	5.9	4.6	12.7
T-95 in propagation	0.1	3.3	6.6	3.1	13.0
T-95 in prop. + Truban [@]	0.1	3.2	8.2	3.1	12.6

^z Values for each of the flower grades and total were not significantly different from each other. $\underline{P} = 0.05$. Two replicates were used in a randomized complete block design.

Fig. 7: Total weekly flower production of each of the four treatments in Experiment II. Although there appear to be differences between the flowers produced at the peak of production, at about week 5, they were not significantly different. Only one crop was harvested from this experiment.

TRICHODERMA EXPERIMENT 2

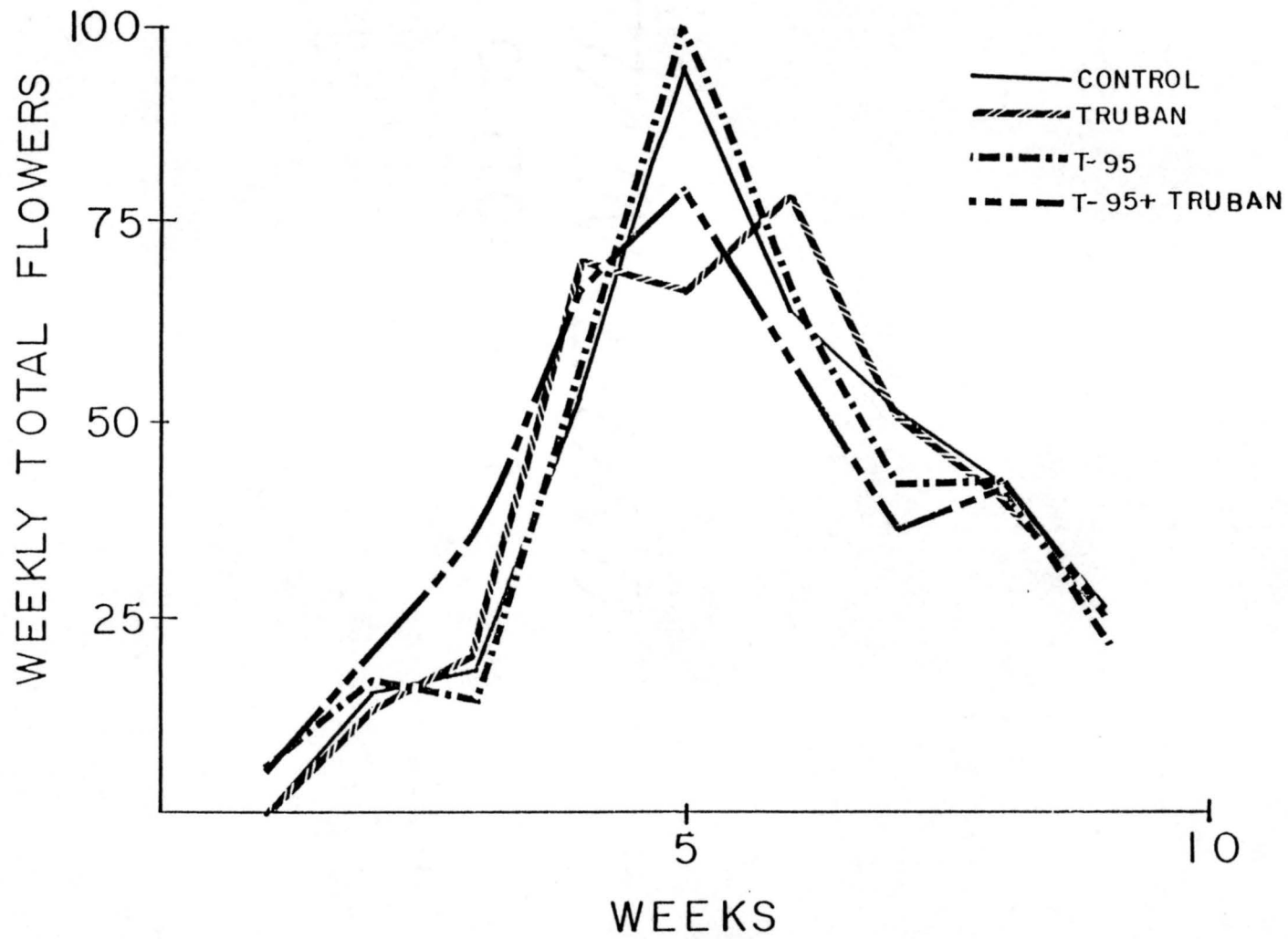




Fig. 8: Developing plants used in Experiment II, just prior to first flower harvest, showing a comparison between the control plots, Truban[®] and T-95

consistent significant differences were among the treatments which utilized either Pythium alone, or in combination with T-12 in the pot or in propagation. These three treatments were consistently shorter and had fewer, less developed breaks than the other treatments in the experiment (Tables 3 - 5 and Fig. 9).

As plant development proceeded, differences between treatments remained apparent, especially plant height. Radiation levels were gradually increasing, as spring approached and had several effects on the plants. First, the majority of the plants were quite well developed, except for those which were delayed due to the effects of Pythium, water use was increasing which led to more frequent irrigations. Since the treatments having Pythium were less developed, they received more water than necessary. This resulted in a growing medium which was near saturation most of the time, thus becoming a more favorable environment for Pythium root rot development putting further stress on these plants. Secondly, the Pythium treated plants were shorter and often shaded by adjacent treatment rows which led to slower growth (Fig. 10).

As these two conditions continued, their interaction progressively slowed development. This situation slowly changed, however, as the more developed plants began to reach maturity and began to flower. As plants continued to grow and radiation levels increased, the differences in plant height diminished. Height measurements for weeks 12, 14 and 16 show this trend (Table 6).

Approximately 19 weeks after the plants had been pinched, some of the first flowers were harvested. As was expected, the treatments which had been developing at a faster rate, throughout the experiment, were the first to come into production.

Table 3: Height measurements for developing 'CSU Red' carnation plants, planted November 21, 1984, in each of the treatments in Experiment III, taken six, eight and ten weeks after the plants were pinched on December 14, 1984. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

<u>HEIGHT MEASUREMENT</u>					
6 WEEKS		8 WEEKS		10 WEEKS	
TRT.	HEIGHT (cm)	TRT.	HEIGHT (cm)	TRT.	HEIGHT (cm)
1	21.6 ^Z	4	26.8	4	34.8
4	21.1	1	26.3	1	33.9
3	20.9	12	26.0	8	32.9
6	20.1	3	25.6	2	32.8
8	19.5	6	24.8	6	32.5
12	19.4	2	24.5	11	32.5
7	19.1	11	24.5	7	32.4
2	18.9	7	24.3	3	32.3
11	18.8	8	24.3	12	32.3
5	17.8	5	20.4	9	24.3
9	14.0	9	19.3	5	24.0
10	13.5	10	15.6	10	21.9

^Z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

Table 4: Break counts for developing 'CSU Red' carnation plants, planted November 21, 1984, in each of the treatment in Experiment III, taken six, eight and ten weeks after the plants were pinched on December 14, 1984. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [@] + T-12 in prop. |
| 2. Truban [@] | 7. Truban [@] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [@] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [@] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [@] |

<u>NUMBER OF BREAKS</u>					
6 WEEKS		8 WEEKS		10 WEEKS	
TRT.	NUMBER	TRT.	NUMBER	TRT.	NUMBER
4	11.5 ^z	12	16.9	7	26.3
12	11.3	7	16.2	12	26.3
7	11.2	4	14.8	4	25.0
2	10.5	6	14.8	8	22.8
6	10.3	8	14.0	1	22.0
8	10.0	11	13.3	3	22.0
1	9.3	2	13.0	2	21.5
3	9.1	3	12.9	6	21.5
11	8.8	1	12.4	11	18.0
5	4.5	5	6.3	5	10.5
10	2.9	9	3.6	10	7.3
9	2.6	10	3.3	9	5.6

^z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

Table 5: Mean length of the two top-most breaks for developing 'CSU Red' carnation plants, planted November 21, 1984, in each of the treatments in Experiment III, taken six, eight and ten weeks after the plants were pinched on December 14, 1984. The numbered treatments, 1-12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

MEAN LENGTH OF DEVELOPING BREAKS					
6 WEEKS		8 WEEKS		10 WEEKS	
TRT.	LENGTH (cm.)	TRT.	LENGTH (cm.)	TRT.	LENGTH (cm.)
8	10.1 ^z	6	14.7	4	21.2
12	10.1	3	14.2	2	20.0
11	9.4	8	13.3	3	19.1
4	9.2	11	13.3	11	18.8
2	8.9	7	12.8	7	18.8
6	8.9	4	12.7	6	18.7
3	8.2	2	12.6	8	18.1
1	8.0	12	12.4	1	17.8
7	7.7	1	11.7	12	17.3
10	5.7	5	7.4	10	14.0
5	5.5	10	7.2	5	11.6
9	3.9	9	5.3	9	11.2

^z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.



Fig. 9: Comparison of plant development of representative plants chosen from four of the twelve treatments in Experiment III. Photo was taken ten weeks after pinching.



Fig. 10: Development of plants, stressed by Pythium, were stunted and received decreased radiation due to shading by adjacent treatment plants. Treatment row in center of photo, taken 12 weeks after pinching, is treated with Pythium

Table 6: Height measurements for developing 'CSU Red' carnation plants, planted November 21, 1984, in each of the treatments in Experiment III, taken 12, 14 and 16 weeks after the plants were pinched on December 14, 1984. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

<u>HEIGHT MEASUREMENT</u>					
12 WEEKS		14 WEEKS		16 WEEKS	
TRT.	HEIGHT (cm.)	TRT.	HEIGHT (cm.)	TRT.	HEIGHT (cm.)
4	48.2 ^Z	6	68.8	4	89.6
3	46.0	4	68.5	3	88.3
1	45.8	3	67.0	1	87.0
6	45.6	8	66.2	2	86.8
8	45.3	12	65.1	8	86.3
12	45.1	1	64.5	6	85.7
11	44.7	7	62.8	7	83.8
7	44.3	11	62.6	12	83.7
2	43.8	2	62.5	11	82.7
5	35.9	5	46.9	10	65.5
10	35.0	10	44.8	5	63.9
9	31.4	9	41.2	9	56.1

^Z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

After approximately six weeks of harvesting flowers, 200 days after pinching, the experiment was terminated. Similar trends appeared in the final analysis of the data as had been evident throughout the experiment. As had been expected, the three treatments, which allowed the development of Pythium, were shown to be significantly lower quality, producing fewer flowers and were delayed in flowering (Table 7).

Other characteristics, of the harvested flowers, were not as clear-cut as those just described, however, some interesting interactions were observed. In the case of stem length, Pythium, significantly shortened the stems. Truban[®], used alone or in combination with another treatment, resulted in significantly shorter stem length than the control, which had the longest overall stem length. The addition of T-12 in the propagation bench, to the treatments containing Pythium, did not significantly increase stem length over Pythium alone. However, when T-12 was used in the pot, it effectively increased stem length as compared to the propagation bench treatment, when Pythium was a part of the treatment. The failure of T-12 to control Pythium was evident in treatments where Pythium was used with T-12, in either the pot or in the propagation bench; the addition of Truban[®] gave a significant increase in stem length in both cases (Table 8).

Pythium caused a reduction in stem length apparently resulting from decreased internode length. Generally, it was found that Pythium treated plants had an increased total number of nodes but decreased internode length. As an example, Pythium significantly increased number of stem nodes when it was added to a pot in which T-12 was applied.

Table 7: Flower production data of 'CSU Red' carnation plants planted November 21, 1984, for the 200 day period following pinching on December 14, 1984, for each of the treatments in Experiment III. Mean grade values, 1 through 4, are based on the standard grading system used for carnations. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

FINAL PRODUCTION RESULTS

TOTAL FLOWERS CUT		NUMBER OF DAYS FROM PINCH		MEAN GRADE	
TRT.	NUMBER	TRT.	DAYS	TRT.	GRADE
7	14.5 ^z	12	156.4	12	3.8
12	14.5	7	158.4	1	3.7
1	14.3	4	158.5	3	3.7
3	14.3	8	159.1	7	3.6
2	14.0	11	159.4	4	3.6
4	14.0	2	159.6	8	3.6
11	14.0	1	159.9	2	3.5
8	13.8	3	160.4	11	3.5
6	13.5	6	160.4	6	3.4
5	9.5	5	169.8	10	3.4
10	9.0	9	169.9	5	2.9
9	8.5	10	172.7	9	2.6

^z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

Table 8: Mean cut flower stem length from 'CSU Red' carnation plants, planted, November 21, 1985, in each of the treatments in Experiment III, during the 200 day period after the plants were pinched on December 14, 1985.

<u>MEAN STEM LENGTH</u>	
TREATMENT	LENGTH (cm.)
Control	98.9 ^z
T-12 in pot	96.9
T-12 in propagation bench	96.2
Truban [@]	94.5
Truban [@] + <u>Pythium</u>	93.8
Truban [@] + T-12 in propagation bench	93.7
Truban [@] + T-12 in pot	93.0
T-12 in pot + <u>Pythium</u> + Truban [@]	91.7
T-12 in prop. bench + <u>Pythium</u> + Truban [@]	89.8
T-12 in pot + <u>Pythium</u>	87.9
<u>Pythium</u>	84.9
T-12 in propagation bench + <u>Pythium</u>	82.5

^z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

Internode length was significantly decreased when Pythium was added to a treatment containing either T-12 in the pot or in propagation. Conversely, Truban[®], had somewhat the opposite effect of Pythium. When it was added, in conjunction with Pythium alone or with T-12, the number of nodes was reduced. In the latter, it also acted to increase internode length. In one instance, T-12 showed a significant effect. When a plant, which was propagated using T-12, had Pythium applied to it, internodes were longer than when Pythium was applied alone (Table 9).

Break development, another important component of carnation production, was significantly altered by the treatments when looking at harvest data. Mean break length followed the common pattern of being reduced by the addition of Pythium alone or in combination with T-12. The number of breaks developed, was also affected, as was seen earlier. Truban[®] showed the most impact in this case, by decreasing the number of breaks when it was added to plants which had either Pythium alone or in combination with T-12 in the pot. The treatment using both Truban[®] and T-12 in the pot also produced more breaks than the control. This showed some interaction, or synergism, between Truban[®] and T-12, since neither used alone caused a significant increase over the control (Table 10).

Dry weights of flower stems were lowest in treatments containing either Pythium alone or in combination with T-12 in the propagation bench. The addition of either Truban[®] or T-12 in the pot increased dry weight. Neither Truban[®] or T-12 used alone, or in combination with each other, resulted in an increased dry weight over the control (Table 11).

In previous experiments, there was a question as to whether the Trichoderma and Pythium populations were at a high enough level to elicit a response. Inoculum levels were much higher in this experiment

Table 9: Mean internode length and number of nodes for cut flower stems of 'CSU Red' carnations planted November 21, 1985, harvested in each of the treatments in Experiment III, taken during the 200 day period after the plants were pinched on December 14, 1984. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

INTERNODE LENGTH AND NUMBER OF NODES

MEAN INTERNODE LENGTH		MEAN NUMBER OF NODES	
TRT.	LENGTH (cm.)	TRT.	NUMBER
2	7.3 ^z	6	13.3
4	7.2	7	13.4
1	7.1	12	13.4
3	7.0	2	13.5
6	7.0	11	13.5
12	7.0	8	13.5
7	7.0	4	13.6
8	7.0	3	13.9
11	6.6	1	14.1
10	6.0	9	14.3
5	5.8	5	14.4
9	5.5	10	14.8

^z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

Table 10: Mean break length and number of breaks for the 'CSU Red' flower stems harvested from plants planted November 21, 1984, in each of the treatments in Experiment III, taken during the 200 day period after the plants were pinched on December 14, 1984. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

BREAK DEVELOPMENT

BREAK LENGTH		NUMBER OF BREAKS	
TRT.	LENGTH (cm.)	TRT.	NUMBER
7	44.0 ^z	7	5.2
2	43.9	12	5.2
6	43.8	8	5.0
4	43.0	2	4.7
8	41.7	6	4.6
1	40.0	11	4.6
12	39.9	4	4.6
3	39.8	3	4.2
11	39.3	5	4.2
5	27.0	1	4.2
10	26.8	9	4.2
9	22.7	10	4.0

^z Means not followed by the same letter differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

Table 11: Mean dry weight of 'CSU Red' carnation flower stems cut from the plants planted November 21, 1984, in each of the treatments in Experiment III, during the 200 day period after the plants were pinched on December 14, 1984.

<u>FINAL MEAN DRY WEIGHT</u>	
TREATMENT	WEIGHT (g)
T-12 in pot	17.6 ^z
T-12 in propagation bench	17.2
Truban [@] + T-12 in pot	17.1
Control	17.0
Truban [@] + T-12 in propagation bench	16.2
Truban [@]	16.0
T-12 in pot + <u>Pythium</u> + Truban [@]	15.6
Truban [@] + <u>Pythium</u>	15.5
T-12 in prop. bench + <u>Pythium</u> + Truban [@]	14.4
T-12 in pot + <u>Pythium</u>	12.4
<u>Pythium</u>	9.9
T-12 in propagation bench + <u>Pythium</u>	8.3

^z Means not followed by the same vertical line differed significantly. $P = 0.05$. Four replicates were used in a randomized complete block design.

and were apparently high enough to give a response. Soil samples were taken at the beginning of the experiment, near the middle of the experiment and at its conclusion.

Trichoderma levels in the medium showed a general decrease throughout the course of the experiment. This was possibly due to a natural mortality of the fungus over time, in this environment. It was also very interesting to detect the rise in the Pythium inoculum level in those treatments in which Truban[®] had been applied. Truban[®] seemed to persist approximately six months before it became ineffective. If another application had been made at six months, Pythium build up probably would not have occurred (Tables 12 and 13).

Experiment IV.

As described earlier, the main objective of this experiment was to determine if Trichoderma, used in the propagation bed, at different levels, would increase or hasten rooting. Using the root development scale, which was described in Chapter 3, treatments generally made no difference in carnation root development. Some significant differences were seen early in the experiment, at seven days and again at 17 days, however, root development was still insufficient for cuttings to be transplanted with any degree of success. These differences were of little importance (Table 14 and Fig. 11).

Dry weights were taken of cuttings at each of the harvests and compared as well, showing that the control had greater dry weights at 17, 22 and 27 days, after the cuttings were stuck, than did the cuttings which were rooted in the low concentration of T-12 (Table 15).

Table 12: Soil Trichoderma, T-12, inoculum cfu g⁻¹ levels found in each of the 12 treatments used in Experiment III. Samples were taken near the beginning, middle and conclusion of the experiment on January 8, March 10 and June 28, 1985 respectively. A composite sample was taken from each of the replicates for each treatment for analysis. Values in the table of -100 were used when no development was detected in a 1 in 100 dilution. The fungus may be present in very low levels, not detected at that dilution level. The numbered treatments, 1 through 12, refer to:

1. Control
2. Truban[®]
3. T-12 in propagation
4. T-12 in pot
5. Pythium
6. Truban[®] + T-12 in prop.
7. Truban[®] + T-12 in pot
8. Truban[®] + Pythium
9. T-12 in prop. + Pythium
10. T-12 in pot + Pythium
11. T-12 in propagation + Pythium + Truban[®]
12. T-12 in pot + Pythium + Truban[®]

TRICHODERMA INOCULUM LEVELS

TRT.	JANUARY 8 INOCULUM LEVEL	MARCH 10 INOCULUM LEVEL	JUNE 28 INOCULUM LEVEL
1	-100	-100	-100
2	-100	-100	-100
3	3.0 X 10 ⁵	8.5 X 10 ⁴	7.9 X 10 ³
4	4.8 X 10 ⁶	1.5 X 10 ⁶	4.4 X 10 ⁵
5	-100	-100	-100
6	9.0 X 10 ⁴	1.8 X 10 ⁵	7.0 X 10 ³
7	2.2 X 10 ⁶	5.0 X 10 ⁵	1.1 X 10 ⁵
8	-100	-100	-100
9	3.4 X 10 ⁶	2.8 X 10 ⁴	1.7 X 10 ³
10	1.9 X 10 ⁶	1.0 X 10 ⁶	1.7 X 10 ⁵
11	7.5 X 10 ⁴	3.4 X 10 ⁴	1.3 X 10 ⁵
12	2.5 X 10 ⁶	2.9 X 10 ⁶	3.5 X 10 ⁵

Table 13: Soil Pythium inoculum cfu g⁻¹ levels found in each of the 12 treatments used in Experiment III. Samples were taken near the beginning, middle and conclusion of the experiment on January 8, March 10 and June 28, 1985 respectively. A composite sample was taken from each of the replicates for each treatment for analysis. Values in the table of -100 were used when no development was detected in a 1 in 100 dilution. The fungus may be present in very low levels, not detected at that dilution level. The numbered treatments, 1 through 12, refer to:

- | | |
|------------------------|--|
| 1. Control | 6. Truban [®] + T-12 in prop. |
| 2. Truban [®] | 7. Truban [®] + T-12 in pot |
| 3. T-12 in propagation | 8. Truban [®] + <u>Pythium</u> |
| 4. T-12 in pot | 9. T-12 in prop. + <u>Pythium</u> |
| 5. <u>Pythium</u> | 10. T-12 in pot + <u>Pythium</u> |
| | 11. T-12 in propagation + <u>Pythium</u> + Truban [®] |
| | 12. T-12 in pot + <u>Pythium</u> + Truban [®] |

PYTHIUM INOCULUM LEVELS

TRT.	JANUARY 8 INOCULUM LEVEL	MARCH 10 INOCULUM LEVEL	JUNE 28 INOCULUM LEVEL
1	-100	-100	-100
2	-100	-100	-100
3	-100	-100	-100
4	-100	-100	-100
5	3.0 X 10 ²	6.0 X 10 ²	8.3 X 10 ²
6	1.5 X 10 ²	-100	-100
7	-100	-100	-100
8	-100	-100	8.0 X 10 ²
9	5.0 X 10 ²	3.3 X 10 ³	7.5 X 10 ²
10	2.0 X 10 ²	3.0 X 10 ²	7.3 X 10 ²
11	1.5 X 10 ²	-100	4.8 X 10 ²
12	-100	-100	6.0 X 10 ²

Table 14: Mean root development ratings for 'CSU Red' carnation cuttings in each of the treatments used in Experiment IV, at each of the five harvests, 7, 12, 17, 22 and 27 days after being stuck in the mist bed on February 14, 1985. Development was rated on a scale of 0 to 9:

- | | |
|--------------------------------|----------------------------|
| 0. No callous development. | 5. Roots 1 cm in length. |
| 1. Slight callous development. | 6. Roots 2 cm in length. |
| 2. Well developed callous. | 7. Roots 3 cm in length. |
| 3. Root initials present. | 8. Roots 4 cm in length. |
| 4. Roots 5mm in length. | 9. Roots 5 cm + in length. |

ROOTING DEVELOPMENT

ROOT DEVELOPMENT AT EACH T-12 LEVEL

DAYS	CONTROL	1.0×10^6 cfu g ⁻¹	5.0×10^7 cfu g ⁻¹
7	1.0 ^z b	1.1 ab	1.2 a
12	2.5 a	2.4 a	2.3 a
17	4.0 ab	4.4 a	3.5 b
22	5.0 a	5.4 a	4.7 a
27	6.4 a	7.3 a	5.7 a

^z Means not followed by the same letter differed significantly. $P = 0.05$. Five replicates were used in a randomized complete block design.



Fig. 11: Root development of cuttings rooted in each of the three treatments used in Experiment IV. Photos taken at 22 and 27 days after being stuck in the propagation bench

Table 15: Mean dry weights of the 'CSU Red' carnation cuttings from each of the treatments used in Experiment IV for each of the five harvests made at 7, 12, 17, 22, and 27 days after the cuttings had been stuck in the rooting bench on February 14, 1985.

ROOTING DEVELOPMENT

DRY WEIGHTS AT EACH T-12 LEVEL

DAYS	CONTROL	1.0×10^6 cfu g ⁻¹	5.0×10^7 cfu g ⁻¹
7	6.7 ^z a	5.2 b	5.4 b
12	7.2 a	5.6 a	7.2 a
17	7.7 a	6.0 b	7.0 ab
22	9.0 a	6.2 b	7.7 ab
27	8.6 a	7.1 b	8.2 ab

^z Means not followed by the same letter differed significantly. $P = 0.05$. Five replicates were used in a randomized complete block design.

Observations of the cuttings in harvest one, seven days after the cuttings had been stuck, showed that the control cuttings appeared to have a more blueish green color than the treatments which had T-12 applied to them. It was also observed that certain pots were not getting sufficient mist in the propagation bed, this could account for decreased rooting in some of the cuttings. Despite the lack of mist on some of the cuttings, if the T-12 was going to yield a significant response, it should have been evident. T-12 was at high enough levels to elicit a response, if one was to be seen, as evidenced by the T-12 inoculum levels found in analysis of the rooting media. Although the T-12 levels found were lower than the original prepared levels, a low and high level of inoculum was maintained (Table 16).

Rooting media temperatures were fairly low, especially during the early part of the experiment before pots were sunk in the media. This low rooting temperature may have been too low for the T-12 to give an optimum response in this experiment or in the previous experiments. To check this hypothesis, representative rooted cuttings, from the final harvest of cuttings, were potted in the treatment media and grown in a greenhouse with higher temperature settings. These plants were harvested when the terminal flower was fully open. Data was taken in a similar manner to that of Experiment III, including: days to flower, overall length, number of nodes, number of breaks, length of breaks and dry weight. No significant differences were found between the plants which developed from the three treatments used in Experiment IV. Thus the lower temperatures were apparently not a limiting factor for a positive T-12 response.

Table 16: T-12 rooting media cfu g⁻¹ levels for each of the treatments and harvests, 7, 12, 17, 22 and 27 days after the cuttings were stuck in the mist bed on February 14, 1985, used in Experiment IV. Inoculum levels are for composite samples taken from each of the five replicates at each harvest. Values of -100, signify that no T-12 was detected at that dilution level, but may be present in small numbers. The mean inoculum level over the entire experiment is also given.

ROOTING MEDIA T-12 INOCULUM LEVELS

INOCULUM LEVEL AT EACH INITIAL T-12 LEVEL

DAYS	CONTROL	1.0 X 10 ⁶	5.0 X 10 ⁶
7	-100	9.0 X 10 ⁵	2.7 X 10 ⁷
12	-100	4.5 X 10 ⁵	1.5 X 10 ⁷
17	-100	8.0 X 10 ⁵	1.3 X 10 ⁷
22	-100	4.0 X 10 ⁵	5.0 X 10 ⁶
27	-100	1.0 X 10 ⁶	1.2 X 10 ⁷
mean	-100	7.1 X 10 ⁵	1.4 X 10 ⁷

B. Photoperiodism and timing in the carnation.

Experiment V.

The effect of short days, used in conjunction with long days, on carnations, was investigated in this experiment. Analysis of 34 weeks of data yielded no significant differences between mean total weekly flower production or production in any of the four quality grades of flowers (Table 17). It had been hoped that treatments receiving 45 and 60 short days would produce more flowers and in a shorter length of time, however, this was not evident in analysis of the data.

The reason for this lack of significance probably can be seen when production of each of the four replicates, used in the experiment, are compared (Fig. 8). In total production and in production of flowers in each of the four quality grades, significant differences were found. Generally replicates one and two produced fewer flowers than replicates three and four. This seemed a viable conclusion since replicates one and two were in a bench located just north of the bench containing replicates three and four. The latter bench was located along the south wall of the greenhouse, which provided a location with warmer temperatures and more radiation than the bench containing replicates one and two. These warmer temperatures, supplied by a peripheral heating pipe located on the wall, in conjunction with the greater radiation, produced a growing environment which hastened growth and flower development. This variation between replicates was so great that it effectively

Table 17: Mean number of 'CSU Red' carnation flowers harvested ft² over the 35 week period of production beginning September 2, 1984, for Experiment IV for each of the four treatments, and each of the four replicates showing total production in each of the quality grades. Plants were planted July 11, 1983.

TREATMENT	FANCY	STANDARD	SHORT	DESIGN	TOTAL
CONTROL	0.8 ^y	9.3	14.8	6.5	31.0
30 SHORT DAYS	0.8	7.8	16.3	8.0	32.3
45 SHORT DAYS	0.5	7.5	16.0	8.0	32.0
60 SHORT DAYS	0.5	7.0	16.0	7.8	32.8

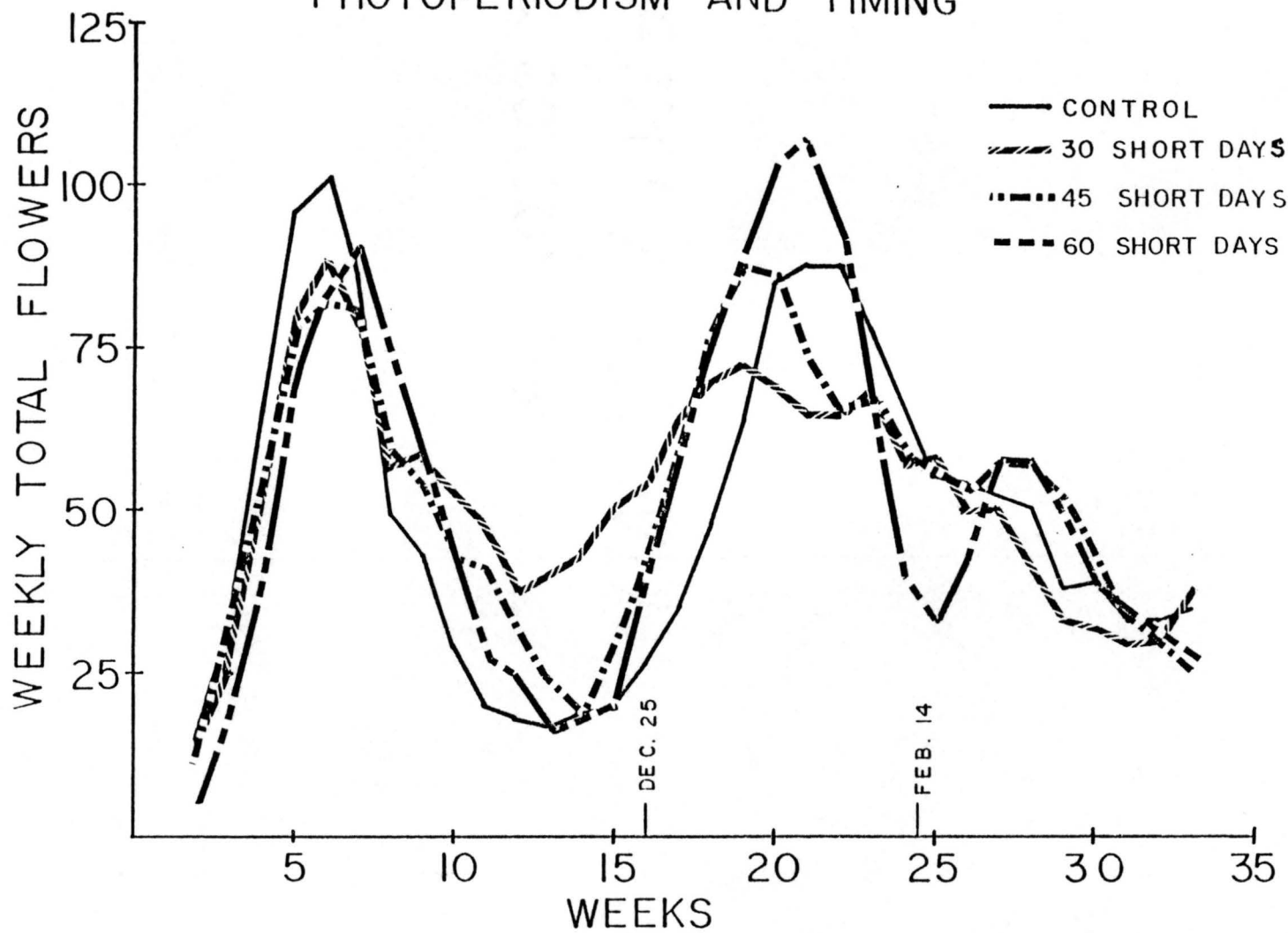
REPLICATE	FANCY	STANDARD	SHORT	DESIGN	TOTAL
1	0.3 ^z	6.5	14.3	7.3	28.3
2	0.5	6.5	13.8	5.8	26.5
3	0.8	9.3	18.3	9.3	37.3
4	1.0	9.3	16.8	8.0	34.8
HSD _{.05}	0.5	2.3	3.4	2.3	6.3

^y Values for each of the flower grades and total were not significantly different from each other. $\bar{P} = 0.05$.

^z Replicate differences differed significantly. The HSD value is given for each of these. $P = 0.05$

Fig. 12: Mean weekly production of each of the treatments used in Experiment V. Weekly values plotted were totals of four replicates which have been transformed using a three-week floating mean. This procedure helps to smooth the data removing some of the variation in production which often results due to changing weather conditions and other factors unrelated to the treatments. The high demand periods of Christmas and Valentines Day are shown.

PHOTOPERIODISM AND TIMING



"diluted" the production results from each of the treatments, yielding nonsignificant differences (Table 17).

In general, the timing of the crops was quite similar during the first crop and somewhat different during the second crop peak, however, these differences were not large enough to be significant. As mentioned earlier, the hastened flowering of replicates three and four tended to decrease the overall differences between the treatments.

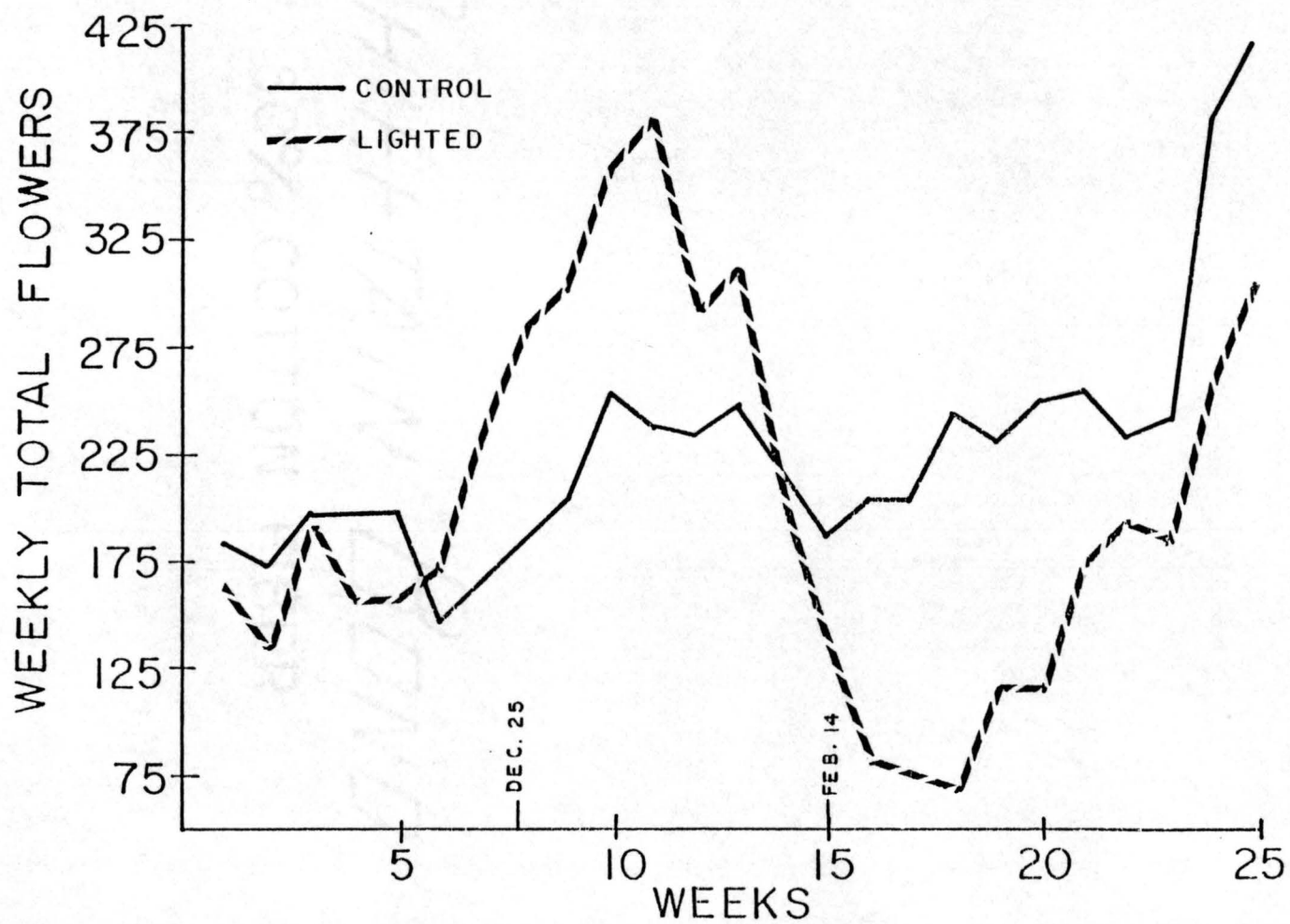
Experiment VI.

In this experiment, a lighting treatment of 30 long days was applied to two of four benches of carnations which had been in production for approximately one year. The response of the lighting treatment was quite dramatic. Approximately seven weeks after the conclusion of the 30 long days, the lighted benches showed a rapid increase in production, followed by a very drastic decline in production, after the crop had peaked. The nonlighted benches, in contrast, showed a gradual increase in production followed by a gradual decrease in production, after the crop had peaked. (Fig. 9).

Analysis of data over the entire 25 week period of the experiment yielded no significant differences between total flower production, but did show that the lighted treatment produced fewer short flowers than did the control. When the 25 week period was broken into two smaller periods of weeks 5 to 15 and weeks 15 to 25, significant differences were then evident both between total flower production and production within the quality grades.

Fig. 13: Mean weekly production for each of the two treatments, control and lighted for 30 days of Experiment V. Weekly values plotted were total production from two replicates. The lighting treatment was applied for the 30 day period prior to the origin of this graph. The high demand periods of Christmas and Valentines Day are shown.

PHOTOPERIODISM AND TIMING



During weeks five to 15, the lighted plants produced more total flowers and more flowers in the design grade than did the control. The long day treatment may have caused some of the lower breaks, on the plants to come into production, which probably produced flowers of inferior quality, accounting for the increase in this grade.

Long day plants also produced more flowers in each of the other grades, but these were not significant except in contributing to the total production of each of the benches. Weeks 15 to 25 were significantly lower production, of the lighted benches, in total production as well as in the production of standard, short and design grade flowers (Table 18).

Only two replicates were used in this experiment. Had more replicates been used, perhaps the differences in production of flowers in the grades of fancy and standard would have been evident. If this were the case, it would provide a good reason for the use of lighting to hasten or increase production, especially if one wanted high production before an upcoming holiday demand period or before a bench was to be removed from production. If one was timing for a holiday, it might be advisable not to give the long day treatment to all the benches of a particular variety since production will drop to a very low level after the crop peaks.

Table 18: Mean weekly number of 'Improved White Sim' carnation flowers ft² produced over each period for the two treatments in each of the quality grades and total in Experiment VI for the overall period of the experiment beginning November 4, 1984, weeks 1 to 25, and two smaller periods during the experiment, weeks 1 - 15 and weeks 15 to 25. Plants were planted October 9, 1983.

TREATMENT	<u>WEEKS 1 - 25</u>				
	FANCY	STANDARD	SHORT	DESIGN	TOTAL
LIGHTED	0.8 ^z	6.2	8.3	6.4	21.7
CONTROL	0.5	6.2	10.8	7.1	24.6
HSD _{.05}	NS	NS	1.5	NS	NS
TREATMENT	<u>WEEKS 5 - 15</u>				
	FANCY	STANDARD	SHORT	DESIGN	TOTAL
LIGHTED	0.1	2.4	4.6	3.9	11.0
CONTROL	0.1	1.8	4.2	2.8	9.0
HSD _{.05}	NS	NS	NS	0.6	NS
TREATMENT	<u>WEEKS 15 - 25</u>				
	FANCY	STANDARD	SHORT	DESIGN	TOTAL
LIGHTED	0.6	2.4	2.2	1.4	6.6
CONTROL	0.3	3.2	4.6	3.1	11.2
HSD _{.05}	NS	0.8	0.8	0.6	2.0

^z When values listed were significantly different from each other, the HSD value is given, or "NS" if not significant. $\underline{P} = 0.05$.

CHAPTER FIVE

CONCLUSIONS

- A. Trichoderma, Truban[@] and Pythium and their effect on carnation development.

Overall results from each of the four experiments, led to several conclusions. First, one must be assured of having a viable population of the organism that is being examined. This was a problem in Experiments I and II. It was difficult to ascertain, whether the negative results, from these experiments, were due to the poor fungal development or some other factor. Failure to find a high Trichoderma and Pythium population in soil samples, from these experiments, lead one to believe that this was the problem. Little evidence as to the possible benefit of Trichoderma was gained from these two experiments.

Experiment III was done in a more precise manner to hopefully overcome some of the difficulties encountered in Experiments I and II. Good quality inoculum was used in conjunction with more plant observations and measurements. One of the first conclusions to be drawn, was that Pythium definitely decreased carnation growth and development. This was seen in overall length, and development of internodes, breaks and flowers as well as dry weight of harvested flower stems. It

was also found that Truban[®] effectively controlled Pythium and generally resulted in plant development similar to that of the control plants.

Trichoderma, though it did, in some instances, significantly alter carnation development, was generally ineffective in the control of Pythium, or in protecting against the effects of the fungus. A synergistic effect was seen in one case; increasing break development, when Trichoderma was used in conjunction with Truban[®]. T-12 in the pot with Pythium also gave an increase in dry weight over Pythium alone, however, these two factors, did not constitute a legitimate reason to highly recommend its use.

Rooting, like other carnation development characteristics, was not significantly altered to the point of strong visual improvement. Rooting temperature was somewhat low, at least in the early part of this experiment, which could have caused decreased Trichoderma activity, resulting in a poor rooting response. Under optimum conditions, an increased rooting response may have been observed.

In summary, a carnation crop, in good condition, relatively free of disease, would not benefit from the addition of disease control measures. This was seen in both the use of Trichoderma and Truban[®]. In neither case, was development improved by its addition, over the development of the control plants. When Pythium was present and reducing plant growth, Truban[®], by far, gave the most consistent control of the fungus and contributed to better development than Trichoderma treatments. The response gotten from Trichoderma, was generally too subtle to be of benefit to the grower. Perhaps, under less favorable growing conditions, Trichoderma would prove beneficial.

B. Photoperiodism and timing in the carnation.

Experiment V.

Due to negative results, few conclusions can be drawn from this experiment. The biggest problem with this experiment, was poor design and implementation of the long and short day treatments. First of all, it was somewhat difficult to assure the absence of light contamination between treatments. Caging and other factors limited the use of simple shading and lighting techniques. Secondly, due to some confusion, short day photoperiods were somewhat inconsistent in their length. These two factors, coupled with the location of the replicates, within the two benches which were used, were most likely responsible for the lack of good visible plant responses to the different photoperiods.

Based on Minnesota studies, the treatments receiving 45 and 60 short days should have had significantly higher production, due to increased break development enhanced by the shortened photoperiod. The return crop should have been larger. Unfortunately this was not evident.

If this experiment were repeated, one should devise a different method of isolating each of the treatments, to avoid possible light contamination between treatments. Bench location should also be considered carefully as well, to avoid extreme differences in temperature and radiation levels between replicates.

Experiment VI.

This experiment was much more successful in determining effects of long day photoperiods on carnations than the previous experiment. The one drawback, that could have led to more expressed results, was that only two replicates were used in the experiment. If more had been used, differences in production would have been clearer. Despite this, however, a significant response was seen.

Production was increased in all quality grades of flowers, as well as total flowers produced. As discussed in Chapter four, this could be quite usefull in preparation for a holiday demand period or to increase production just prior to a crops removal from production. In looking at the data, peak production occurred approximately 11 weeks after the termination of the long day treatment. More experimentation is needed, to give more precision in determining the length of time between treatment and peak response. Radiation levels, during production, also need to be taken into consideration in determining when a crop will peak after a photoperiod treatment has been implemented.

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