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Effect of plant age on the form and amount of nitrogen uptake by greenhouse plants /

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EFFECT OF PLANT AGE ON THE FORM AND AMOUNT OF NITROGEN UPTAKE BY GREENHOUSE PLANTS

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DEDICATION

To my mother: Lalla Fettouma El Idrissi

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ABSTRACT

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NITROGEN UPTAKE BY GREENHOUSE PLANTS

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Nitrogen fertilizer is intensively used in greenhouses and nurseries. Any N not used by a crop is subject to leaching, which may pollute groundwater. A close correlation between N supply and N uptake by plants would increase the efficiency of N fertilization and minimize the possibility of N pollution. The objectives of this study were to measure N uptake by American marigold (*Tagetes erecta* L. 'First Lady') and (*Impatiens sp.* hybrids 'Selenia') (NGI) as they grow, determine the effect of plant age on N uptake, the total N required for 70 days of growth, and if the two species have a preference for N form, NO_3-N or NH_4-N.

The plants were grown in solution culture using solutions supplying 120 mg NO_3-N and 120 mg NH_4-N. At ten day intervals, six cultures were chosen at random for nutrient solution analysis and plant sampling for dry weight and tissue analysis. NO_3-N and NH_4-N uptake were determined by potentiometric analysis of the nutrient solution. The

Kjeldahl method of plant analysis was used to determine the amount of N recovered in shoots and roots.

NO_3-N uptake was greater than NH_4-N uptake throughout the experiment for both marigold and NGI. Total N uptake by marigold was greater during the first 50 days after transplanting with maximum N uptake during the period 30 to 50 days. In contrast, N uptake by NGI was greater during the period 40 to 70 days after transplanting. Maximum N uptake for NGI occurred during the period 60 to 70 days. Results of this study suggest that early N fertilization of marigold could be more important for their growth and quality than N applied later on. For NGI, N fertilization later in the crop's development appears to be more important than early on. The total N required by marigold was ≈1.2 gm N/plant or 38 mg N/gm dry weight (DW), for NGI the total N required was ≈0.5 gm N/plant or 52 mg N/gm DW.

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INTRODUCTION

1. 1 Problem statement

As a promotor of plant growth, N fertilizer is used in large quantities in greenhouses and nurseries. It has been estimated that commercial greenhouses and nurseries may use up to 5,000 kg N/ha every year (Morey, 1987 and Green, 1989).

Growing greenhouse crops by conventional irrigation and fertilization may give conditions subject to considerable N leaching. Much of the N available to crops is vulnerable to leaching or runoff because most greenhouses are built with earthen floors where drainage can occur freely. Any N not used by a crop or retained in the growth medium may be carried into groundwater causing N pollution. In 1985, for example, three major nurseries in Southern California (Orange County) were accused of dumping more than 6123.6 kg of N per month into groundwater (Whitesides, 1989).

Fertilizers are still applied in greenhouses according to traditional experience. Some of the common expressions used in fertilization practices are "100 to 200 part per million (ppm) N in the growth medium allows maximum plant growth" and "best growth occurs when nitrate represents 50% or more of the total N supplied." The frequency of

application, volume of fertilizer solution applied per pot, the amount of N per pot, and relationship to stage of growth are rarely specified.

Thus it would be desirable to know how much N the plant needs during its growth and how N absorption and use change during development. This information could lead to the development of more rational and less wasteful fertilizer practices.

1. 2 Objectives

The objectives of this study are to:

1. Measure N uptake by marigold and New Guinea impatiens during growth and determine its relationship to plant growth and stage of development.

2. Determine the total N requirement of the two species during a growth period of typical length.

3. Determine if the two species have a preference for NO_3-N or NH_4-N during growth.

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CHAPTER 2

LITERATURE REVIEW

Probably no other type of agricultural production uses N fertilizer more intensively than do commercial greenhouses and nurseries. Morey (1987) estimates that the industry uses up to 4,400 kg N/ha annually. In the greenhouse, as much as 450 to 600 kg N/ha are applied annually in contrast to about 225 kg N/ha annually for many field crops (Green, 1989; Morey, 1987). Fertilization is seasonal in the field, but is carried on year-round in the greenhouse and is often combined with large volumes of irrigation water (Biernbaum, 1992; Biernbaum et al., 1988).

It is common for growers to use more water and N than plants need for growth (Morey, 1987; Kuack, 1989; Biernbaum, 1992; Biernbaum et al., 1988). Low to moderate N rates (100 -200 mg N/liter) in the root medium generally result in optimum plant growth and quality (Green, 1989), but often higher rates are used (Biernbaum, 1992; Kuack, 1989; Morey, 1987). This high level of application allows even untrained growers to produce greenhouse crops without worrying about N deficiency (Biernbaum, 1990).

Growers have been taught to water containers in excess to cause leaching. Leaching reduces the level of soluble salts in the root medium. Most greenhouse media are porous

and well-drained and leaching occurs very readily. Potentially, runoff from containers carrying N can flow into groundwater, especially when the greenhouse is located on a sandy, well-drained soil (Biernbaum, 1992; Kuack, 1989). Growing greenhouse crops by conventional methods to maximize growth and quality can result in N leaching rates of 100 to 200 mg N/liter of leachate (Green, 1989). Greenhouse operations are thus potential sources of significant surface and groundwater pollution.

The importance of greenhouses as sources of NO_3-N pollution of drinking water has not been completely assessed, but monitoring programs are underway in a number of states. As early as 1983, the Monterey County (California) Water Quality Control Board assessed and reported the impact of commercial greenhouses in the central coastal region of California (Biernbaum et al., 1988). There is potential groundwater pollution in Connecticut where greenhouses are concentrated (Rathier and Frink, 1989). Analyses of groundwater in many agricultural areas show NO_3-N concentrations exceeding the Public Health Service and U.S. Environmental Protection Agency (EPA) drinking water standard of 10 mg/liter (Rathier and Frink, 1989).

Since it appears that greenhouses may be a source of pollution, protecting surface and groundwater from runoff

and percolation of nutrients, especially NO_3-N, has become a concern of researchers and greenhouse operators. Controlling and reducing NO_3-N fertilization is considered the first priority (Biernbaum, 1992). Better fertilization and irrigation techniques are needed for the greenhouse industry as well as a better understanding of the N requirements of greenhouse crops.

2. 1 Irrigation practices

Methods of irrigation need more exacting attention in the greenhouse than in the field. The quantity and quality of water supply is very important for optimum growth and quality.

2. 1. 1 Water quantity

The amount of water applied depends on the type of soil or soil mix and the size and type of container. It is recommended a daily application of 0.3 gallons of water/ft^2 per feet square (Aldrich, 1990). Often, larger quantities of irrigation water are used, which causes excess runoff.

In a study of the effect of water application level on water percolation and nitrate leaching, Timmons (1984) found that an increase in weekly water supply from 1.3 to 3.4 cm resulted in an increase in the annual percolation of runoff water from 36 to 65 mm. McAvoy et al. (1993) found that

increasing the leaching fraction from poinsettia stock plants resulted in NO_3-N moving to greater depths in soil profile.

2. 1. 2 Water quality

Water quality is very important for plant growth and appearance. Water is considered adequate for irrigation if it has a neutral pH, low levels of iron and soluble salt, and no suspended solids (Smeal and Coartney, 1987).

When pH is outside the 6.5 - 7.5 range, root growth and nutrient uptake may be affected, resulting in unbalanced nutrition and poor growth (Smeal and Coarney, 1987). The electrical conductivity of water used for irrigation should not exceed 450 ppm because high salt levels in water can be detrimental to plants (Smeal and Coartney, 1987).

2. 1. 3 Irrigation systems

Traditionally, greenhouse operators fertilize while irrigating with overhead systems. Overhead irrigation may cause a considerable amount of nutrient runoff. Indeed, a significant quantity of fertilizer solution is lost in the space between containers. By some estimates, at a 10-cm container spacing, about 30% of fertilizer solution lands in the container, and the rest ends up on the greenhouse floor as waste (Morey, 1987; Biernbaum et al., 1988).

Nitrate pollution of surface and groundwater, then, is attributed as much to the volume of leached water as to the concentration of NO_3-N in the leachate. Using an annual rate of 1800 kg N per acre in nursery production, Morey (1987) estimated that over 1400 kg N are wasted annually even though the liquid feed contains only 200 mg N/liter. Green (1989) stated that overhead irrigation systems may result in excess application of water and considerable loss of water as runoff. He reported that the excess volume of water combined with nontarget application can result in a daily use of 26,000 litersof water/ha with as much as 10,000 to 23,000 liters/ha lost to evaporation and runoff.

The problem of runoff caused by overhead irrigation can be partially or completely solved in several ways:

2. 1. 3. 1 Leachate can be collected and recirculated

Excess water applied by overhead irrigation systems and leachate from containers can be collected and reused thus preventing nutrients from reaching water supplies. Collection and reuse may reduce the amount of N required for optimum growth and quality by as much as 50% (Green, 1989). However, various contaminants in runoff can affect plant growth and quality. Skimina (1986) pointed out that overhead irrigation with dirty water can result in dirty plants. In addition, the growth of some plants was reduced

when irrigated with recycled water. Growth inhibition may be due to the presence of phytotoxic levels of pesticides or unbalanced nutrients in the leachate. Runoff water can contain levels of sand, silt, clay, organic matter particles, humic acid, fulvic acid, tannins, herbicides, pesticides, fertilizers, fungi, weed seeds, nematodes, bacteria, algae, and salts which may adversely affect plants (Smeal and Coartney, 1987; Skimina, 1986). All these compounds can be harmful, necessitating filtering and purification of runoff water before it is reused.

The reuse of leachate not only recycles water but also nutrients. However, the concentration will vary with nutrient uptake, water uptake, dilution effect of added water, and addition of nutrients. So, if leached water is recycled, the concentration of nutrients must be monitored regularly and adjusted if necessary (Biernbaum et al., 1988).

Recycling leached water from overhead irrigation systems is a good way to decrease runoff and cut fertilizer use, but it can be very expensive. Recycling leachate is costly in terms of equipment and energy, but may save labor and chemicals. In 1979, a plant designed for recycling runoff at a large Los Angeles nursery cost $1.3 million (Skimina, 1986).

Subirrigation is another type of collecting and recycling system. It consists of flooding floors or benches or flowing water in troughs (Biernbaum et al., 1988). Plants absorb the water and nutrients they need, and the unused portion is collected and used again in subsequent irrigation. This system has the advantage of 'zero runoff', but it requires a large capital investment in benching and other equipment.

Another method to reduce leaching is to use trays or saucers under individual pots to subirrigate pots or to collect the leachate and excess solution from overhead irrigation. Depending on their shape and spacing on the bench, water trays and saucers can hold the water drained from the growth medium, and they catch the water missing the pots during overhead irrigation. This water either evaporates or is reabsorbed by the containers. Hasek et al. (1986) reported that the use of plastic saucers during the last six weeks of chrysanthemum production cycle, combined with monitored irrigation and adjusted nutrition, resulted in 'zero runoff' without salt injury. The use of this technique also cut water and fertilizer use by about 25%. This method is similar to the subirrigation system, but leaching-loss is not completely prevented but is reduced considerably at a lower cost. However, it should not be used unless good quality water is available for irrigation

or excess soluble salts could be a problem (Hasek et al., 1986).

2. 1. 3. 2 Excess water can be avoided by adopting drip irrigation techniques

Drip irrigation systems can eliminate runoff of water missing the pot during overhead irrigation and can also be used to control the volume of water applied to the pot (Cox, 1991). Some Israeli drip systems have low water flow rates allowing a complete wetting of the pot with little or no leaching (Biernbaum et al., 1988).

Advanced drip systems consist of a tensiometer and a small computer. The tensiometer senses the moisture level in the medium, and the computer is programmed to turn the system on or off once preset moisture levels are reached. Lieth et al. (1990) described such a system and found that its use reduced runoff from potted chrysanthemums and poinsettias to near zero while maintaining adequate soil moisture and nutrition.

2. 2 Greenhouse design

Traditionally, most greenhouses are built with earthen floors. Sometimes the surface soil on a greenhouse site is removed or otherwise modified to improve drainage, which allows soluble fertilizer to flow to groundwater causing

NO_3-N pollution (Green, 1989; Rathier and Frink, 1989). Often a drainage system is installed beneath a greenhouse to carry off excess water (Green, 1989). The drainage system carries wastewater and its contents away from the greenhouse and may release it where it can cause pollution.

Collecting leached water from a drainage system and reusing it is a solution for limiting fertilizer losses from the greenhouse (Biernbaum, 1992). A solid, non-porous floor combined with a non-polluting drain system is another solution. However, it is probably less expensive to reduce or stop runoff before it reaches the soil than to collect excess water and reuse it after drainage (Biernbaum, 1992, Biernbaum et al., 1988).

2. 3 Growth media

Modern greenhouse media are generally soilless with peat moss, pine bark, vermiculite, and perlite being common substitutes for field soil. Soilless media are generally very well-drained and have limited nutrient-holding capacity in relation to nutrient levels applied and applied by plants. Biernbaum (1992) suggests that media containing large proportions of peat moss may help reduce runoff because peat holds more water than other materials. The growth medium affects N leaching, interacting with irrigation frequency and container type. Stewart et al.,

(1981) found that the lowest rate of N leaching was obtained with a peat mix in a clay container irrigated every other day. However, no other research of this type is available studying the effects of greenhouse growth media on leaching.

2. 4 Fertilization practices

Plants take up nutrients continuously throughout their growth cycle. A fairly constant supply of N is thus necessary for optimum growth. Even though the nutrient requirement could be higher at one point in the growing period than another, a constant supply has traditionally been satisfactory. The small reserve of water and N in greenhouse growth media necessitates frequent irrigation and application of fertilizer. Under some conditions growers irrigate even twice a day (Furuta, 1976). Frequent irrigation with water alone can result in the loss of the limited reserve of soluble N existing in the pot. Thus, frequent irrigation combined with the leachability of N from the growth medium and the limited volume of the container are factors which make it difficult for growers to maintain an optimum level of N in the pot.

One solution is the adoption of a N fertilizer program involving injection of N into the irrigation water (Furuta, 1976). Under this system, the plants are fertilized each time they are irrigated unless leaching of excess salts is required. Until the problem of N runoff was recognized,

irrigation with fertilizer solutions was acceptable and is still the industry standard.

An alternative to water-soluble fertilizers is controlled-release fertilizers (CRF). CRFs are encapsulated materials containing one or more essential elements that become available to the plant over an extended period of time. Resin-coated fertilizers, such as Osmocote and Nutricote, are complete NPK fertilizers coated with a resin. The nutrient release rate depends on the thickness of the coat, soil temperature, and to some extent soil moisture. Holcomb (1980) reported that the fertilizer becomes more available to plants as the thickness of the coat decreases and the soil temperature increases. When the resin-coated fertilizers come in contact with water, the particles swell and their pores increase in size releasing the soluble fertilizer. Resin-coated fertilizers release the materials faster when mixed with the growth medium than when they are applied to the surface (Holcomb, 1980).

CRFs can help reduce nutrient runoff. The use of CRFs can partially reduce N runoff even though the quantity of N lost by leaching can attributed more to the volume of leached water than to the N concentration of the leachate (Holcomb, 1980, Jarrell et al. 1983). Holcomb (1980) reported that, at the same N rate, leachate NO_3-N concentration was 204 ppm with liquid fertilization system

in comparison to 2.2 ppm with Osmocote. Furuta (1976) reported that the use of CRF at low rates of application and low rates of leaching (leaching fraction ≤ 0.25) resulted in low concentrations of NO_3-N in the leachate. Leachate NO_3-N concentration was 4.9 ppm with Osmocote versus 277 ppm with liquid fertilization. Hershey and Paul (1981) found that N leaching with Osmocote fertilizer was about one-half that with liquid feeding at the same rate of N.

Plants can recover a higher percentage of N when it is supplied from CRFs. Holcomb (1980) found that 89% of the N supplied by CRFs was recovered in chrysanthemum compared to 46% when N was applied by irrigation. The amount of N prone to leaching is generally smaller with CRFs compared to water-soluble fertilizers. Ideally the rate of nutrient release by CRFs will correlate with the plant needs and thus N loss is limited (Holcomb, 1980; Hershey and Paul, 1982).

The effectiveness of CRFs in controlling N leaching depends on how they are applied. Nitrogen is used more efficiently when CRFs are split into two small applications, spaced several weeks apart, instead of one large application at planting (Cox, 1993; Jarrell et al. 1983; Rathier and Frink 1989). At planting, the plants are small and do not benefit from the large quantities of N released at this time. Hershey and Paul (1982) found that the efficiency of N recovery by chrysanthemums with Osmocote 14-14-14 was

very low during the first half of the growth cycle. Nitrogen release by the fertilizer was much greater than N uptake by plants, which resulted in substantial leaching-loss. Nitrogen recovery was much higher during the final four weeks of growth. The increase in N recovery was attributed to the increased rate of N uptake by plants in the second half of their growth cycle.

The method of application affects the efficiency of CRFs. Less N leaching occurs with CRFs when they are applied to the surface of the growth medium after planting versus mixing the same amount with the growth medium prior to planting (Cox, 1993). Preplant incorporation may not match N release with N uptake, which results in N loss (Green, 1989).

2. 5 Quantity and form of nitrogen

The quantity of N applied is crucial for greenhouse crop production. If the quantity of a supplied is lower than the quantity needed, plants will be N deficient and growth and quality will suffer. On the other hand, if N level is greater than the plant needs, ammonium toxicity, excess salinity, unbalanced nutrition, and, of course, excessive N leaching may result. For many greenhouse crops, plant growth occurs when 100 to 200 ppm N is present in the growth medium (Jarrell et al., 1983; Green, 1989). McElhannon and Mills (1978) reported that N was used

efficiently by lima beans in solution culture when its concentration was 150 ppm. Jarrell et al. (1983) found that the yields of *Ligustrum texanum* were lower than optimum when the rate of N was less than 100 ppm or greater than 200 ppm.

Nitrogen requirement may depend on the plant's stage of development. Thermon and Noogle (1973) reported that N uptake by corn was greatest up to tasseling then declined significantly after silking. Lathwell and Evans (1951) grew soybeans in solution culture and found that the plants absorbed 79% of their N requirement within the period of full bloom to the pod-filling stages. Boatwright and Haas (1961) found that wheat absorbed most its N prior to heading. With lima beans the most N absorption was during inflorescence initiation, pod initiation, and pod-filling (McElhannon and Mills, 1978).

Application of the appropriate form of N increases the efficiency of fertilizer use. Since the nitrate ion is subject to loss by leaching and denitrification, NH_4-N would be the ideal form for N fertilization. However, many plants do not tolerate continuous ammonium nutrition (Barker, 1980). For example, tomato and eggplant develop stem lesions when fertilized by NH_4-N only (Herman et al. 1970). Cox and Seeley (1984) reported that poinsettia developed ammonium injury symptoms in response to exclusive ammonium nutrition. The growth was depressed, the leaves were

chlorotic indicating N deficiency and the roots were brown with short, thick and poorly developed laterals. Nitrogen absorption was also significantly less with ammonium versus nitrate nutrition or a combination of both.

Other studies demonstrate that some plants have a N form preference at particular stages in their development. McElhannon and Mills (1978) grew lima bean in solution culture and found that more NO_3-N was absorbed during initiation of inflorescence and pod filling than at other times and NH_4-N was absorbed to the greatest extent during initiation of inflorescence, pod development, and pod filling. Wheat prefers NH_4-N to NO_3-N when a seedling, but later in its development absorbs more NO_3-N than NH_4-N (Spratt, 1974).

Coordinating the rate of N uptake with the amount of N supplied, and the application with the peak absorption periods, may offer growers a cultural practice that maximizes N utilization to optimize growth and quality and minimize N runoff.

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CHAPTER 3

MATERIALS AND METHODS

3. 1 Plant material

The two species of plants chosen for this study were American Marigold (*Tagetes erecta* L. 'First Lady') and New Guinea Impatiens (*Impatiens* sp. hybrid 'Selenia') (NGI). Both plants are commercially important spring season crops in Massachusetts and most other regions of the United States. For this reason, and their ease of year-round propagation and general uniformity of growth and development, these plants were chosen for this research.

Marigold propagates by seed and is a quantitative short-day plant, flowering more rapidly at photoperiods less than 12.5 hours (Mastalerz, 1977). 'First Lady' flowers about 8 to 11 weeks after seeding.

NGI normally are propagated by terminal stem cuttings. 'Selenia' displays strong basal branching and compact growth habit. NGI flower continuously with no known response to photoperiod and are known to be sensitive to soluble salts accumulation in the growth medium (Judd and Cox, 1992).

3. 2 Procedure

3. 2. 1 Propagation

Experiment 1. Seeds of marigold were sown in #3 vermiculite on 1 April 1992 and transplanted to solution culture when the first set of true leaves was well developed, 23 April. The experiment ended 2 July.

Experiment 2. Terminal stem cuttings of NGI 5 to 7 cm long were taken 15 January 1993 and rooted in #3 vermiculite under intermittent mist. On 2 February rooted cuttings were transplanted to solution culture. The experiment ended 13 April.

3. 2. 2 Solution culture and conditions of growth

Plants in both experiments were grown in 1.6-liter opaque plastic containers. Solutions were aerated constantly and deionized water was added as needed to maintain volume. Nutrient solutions had a NH_4-N:NO_3-N ratio of 1:1 and concentration of each N form was 75 ppm N (Table 1). Thus, 120 mg N/culture container of each N form was available to the plants from the solution. The plants were grown in a greenhouse under natural photoperiod, and temperature was maintained as closely as possible to 21/17 °C (day/night).

Table 1. The nutrient solution used for growing 'First Lady' marigold and 'Selenia' New Guinea impatiens.

Material	mg/liter	Element	Concentration (ppm)
	Macronutrient		
$Ca(NO_3)_2.4H_2O$	632.5	Ca	108
		NO_3-N	75
$(NH_4)_2SO_4$	353.1	NH_4-N	75
		S	86
$MgSO_4.7H_2O$	484.8	Mg	48
		S	64
KH_2PO_4	276.8	K	79
		P	62
	Micronutrient		
H_3BO_3	28.6	B	0.5
$MnCl_2.4H_2O$	18.1	Mn	0.5
$ZnSO_4.7H_2O$	2.2	Zn	0.05
$CuSO_4.5H_2O$	0.8	Cu	0.02
$(NH_4)_6Mo_7O_{24}.4H_2O$	9.2	Mo	0.5
Sequestrene 330 Iron (Technical sodium ferric diethylene-thiamine penta-acetate)	0.05	Fe	2.3

3. 3 Solution and plant sampling methods

Experiment duration was for 70 days after transplanting. During the growth period, plants and solutions were sampled at 10-day intervals for dry weight determination and N analysis, respectively. There were six single plants replicates for each sampling date or 42 plants total (seven sample dates x six replicates = 42). On each sampling date, six containers were chosen randomly for plant and solution sampling. Nutrient solutions in the remaining containers were replaced with freshly-prepared solutions (Table 1).

3. 3. 1 Plant samples

Plants were harvested and divided into root and shoot samples. The roots were rinsed with deionized water prior to drying in order to remove nutrient solution residue. The shoots and roots were dried to a constant weight in a forced-draft oven at 75 °C for dry weight determination and tissue was saved for total N analysis.

3. 3. 2 Nutrient solutions

The pH of the depleted nutrient solutions from which plants were harvested was measured on each sample date. The nutrient solutions were then brought up to their original volume by adding deionized water, mixed well, and two 125 ml

samples were taken, one for pH and NO_3-N analysis and the other for NH_4-N analysis.

3. 4 Nutrient solution and plant tissue analysis

3. 4. 1 Determination of NH_4-N and NO_3-N in depleted solutions

The Orion ammonia gas-sensing electrode (Model 95-10, Orion Research Inc., Cambridge, Mass.) was used to determine NH_4-N concentration in the depleted solutions following the addition of 10M NaOH.

NO_3-N was determined using the Orion nitrate specific ion electrode (Model 92-07) in combination with an Orion double-junction reference electrode (Model 900002) with 0.02 M $(NH_4)_2SO_4$ as the outer chamber filling solution. Ionic strength adjustor was 2M $(NH_4)_2SO_4$. In general the methods reviewed by Mills (1980) were followed for solution analysis.

3. 4. 2 Determination of total N in shoots and root systems

The total N content of oven-dry tissues was determined by the Kjeldahl method described by Nelson and Sommer (1980). Once the tissue samples were dried they were ground to pass a 40-mesh screen. The sample size used for analysis was about 500 mg (actual weight was recorded for calculations). The tissue sample was placed in a 350-ml

digestion tube and 15 ml of acid digestion mixture and one Kjeltab ST tablet (Thompson and Copper, Ltd., Runcorn, Cheshire, England) was added. The contents of the tube were mixed by swirling. The tubes were allowed to stand at room temperature for about one hour. The acid digestion mixture consisted of sulfuric acid and salicylic acid (concentrated H_2SO_4 + 33% salicylic acid). Salicylic acid was used to ensure recovery of NO_3-N in the sample. Salicylic acid converts the NO_3-N present in the sample to reduced N compounds which are detectable by NH_3 gas-sensing electrode used later in the analysis (Nelson and Sommer, 1980).

Kjeltabs were used as catalysts and raise the boiling point of H_2SO_4. The catalyst increases the boiling temperature of sulfuric acid and reduces the time required for complete digestion of samples. Kjeltabs used in this research contained 99.90% potassium sulfate and 0.10% selenium.

After one hour, the digestion tubes were placed in an aluminum digestion block (Tecator Digestion System 20, Hoganas, Sweden). It was programmed for a ramp time of 30 minutes and a final digestion temperature of 420 $^{\circ}C$ for about 3 hours. After digestion was complete the contents were allowed to cool. Total N was determined using the NH_3-gas sensing electrode following the procedure of Eastin (1976). Details of the analysis are discussed in Appendix.

3. 5 Data analysis

The statistical analysis of data was carried by using SAS software program (SAS, Institute, 1985) based on regression analysis, General linear models procedure (GLM) and analysis of variance following the procedure of Damon and Harvey (1987).

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CHAPTER 4

RESULTS AND DISCUSSION

4. 1 Experiment 1

4. 1. 1 Marigold stages of development

In this study marigolds had a period of vegetative growth during the first 30 days after transplanting. No flower bud was visible during this time. Between day 30 and day 40 flower buds become visible. By day 40, the terminal bud was about 1.0 cm in diameter, but was tight and did not show color. Fifty days after transplanting, the terminal bud showed some yellow color of the ray flowers, and its diameter was about 4.0 cm. The terminal bud continued to develop becoming a fully-expanded inflorescence, 8 cm in diameter, by 60 days after transplanting. Between day 40 and 60 other flower buds began to develop on the lateral shoots and subtending the terminal inflorescence.

4. 1. 2 Nitrogen uptake by marigold

Nitrogen uptake was affected by N form and plant age (Fig. 1). In general, NO_3-N absorption exceeded NH_4-N absorption throughout the experiment. These findings were similar to those of Sasseville and Mills (1979) who found that southernpeas absorbed more NO_3-N than NH_4-N during their growth; but different from those reported by Edwards and

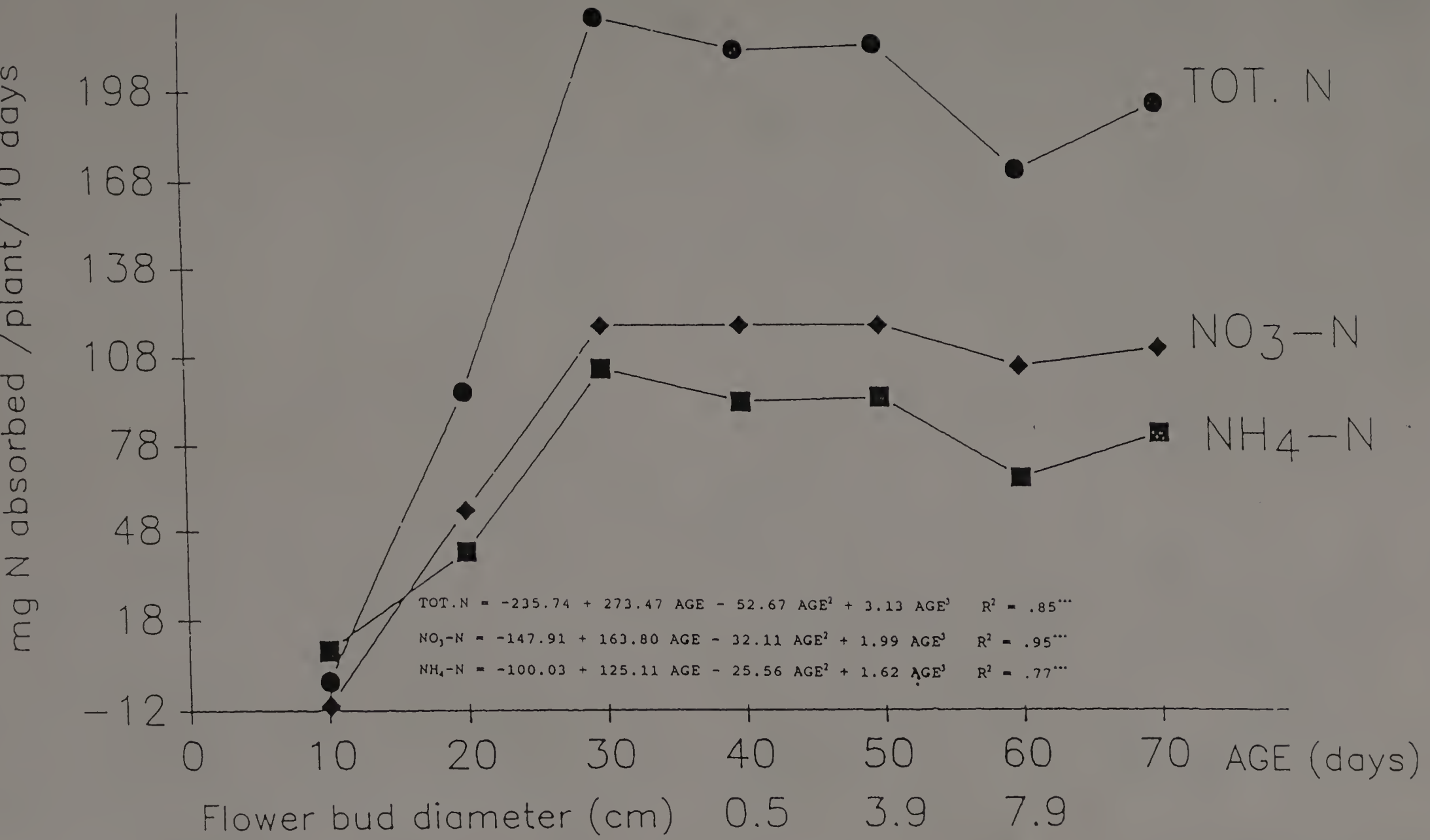

Fig.1 Nitrogen uptake determined by solution depletion during the development of marigold 'First Lady'

Barber (1976) who found that NO_3-N and NH_4-N were absorbed at similar rates by corn roots. On the other hand Schrader et al. (1972) and Warncke and Barber (1973) found that N form had no effect on N uptake by corn.

This preference for NO_3-N shown by marigold might also be related to the capacity of the plant to absorb NH_4-N as influenced by the ratio of NO_3-N to NH_4-N. For example, Sasseville and Mills (1979) found that NO_3-N absorption by southernpea predominated when NO_3-N constituted 50% or less of the total N supplied.

The quantity of N absorbed by marigold plants was significantly influenced by plant age (Fig. 1). Total N, NO_3-N, and NH_4-N absorption paralleled each other through the experiment. Rapid absorption was shown during the first month after transplanting. Maximum uptake occurred between 30 and 50 days and declined between 50 and 60 days. Nitrogen uptake increased slightly between 60 and 70 days.

Total N uptake showed a linear increase during the first month of growth, followed by a plateau between the ages 30 and 50 days, and a gradual decrease between 50 and 70 days. Similar trends have been observed with other plants. Edwards and Barber (1976) found that N uptake by corn reached a maximum when plants were 18 to 21 days old and then decreased as the plants grew older. In an earlier

experiment with corn Warncke and Barber (1973) measured a decrease in N uptake as plant age increased from 18 days to 81 days.

In this study, some of the changes in N absorption by marigold may have been related to stage of plant development. Nitrogen absorption increased rapidly during the apparent vegetative phase of growth (transplanting to day 30), reached a maximum while the terminal flower bud was developing (day 30 to day 60), and declined somewhat as the first inflorescence matured (Fig. 1).

During initiation and early development of the first inflorescence, marigold absorbed N in large amounts. Then the plants absorbed less N as the first inflorescence matured and other flower buds began to initiate and develop. Results indicate that during the later stages of growth, the N requirement of marigold decreases. Perhaps, marigold does not depend entirely on newly absorbed N to meet its needs, but rather on previously absorbed N. Lunt and Kofranek (1958) reported considerable translocation of N from vegetative parts of the chrysanthemum to the flowers during their development. They found that high N fertilization during the early stages of growth was essential for the production of quality blooms. Apparently N is stored during vegetative growth for translocation during flower development.

Marigolds seem to have a high requirement for fertilizer N during their early growth. The N absorbed during this period of growth may be crucial for the later stages of growth. Indeed, the plant's ability to absorb N seemed to decrease as the first inflorescence matures (Fig. 1). Deficiencies suffered during the early stages of growth might not be overcome by later N fertilization, however further study is needed to provide direct evidence.

4. 1. 3 Solution pH during the growth of marigold

The pH of the depleted nutrient solution (Fig. 2) fluctuated within one pH unit during the experiment. pH gradually decreased from about 4.5, at the beginning of the experiment to about 3.3, 50 days later. The acidity of the solution can probably be attributed to NH_4-N absorption and the release of H^+ for cation balance in the plant tissues. After 50 days of growth, the pH of the medium started to increase. This reflect the fact that less NH_4-N was absorbed toward the end of the experiment. It should be pointed out that no attempt was made to control pH and that deionized water was added between samples to maintain volume.

Whereas the absorption of NH_4-N lowers the growth medium pH, NO_3-N absorption increases pH (Street and Sheat, 1958). The changes in pH recorded in this experiment may

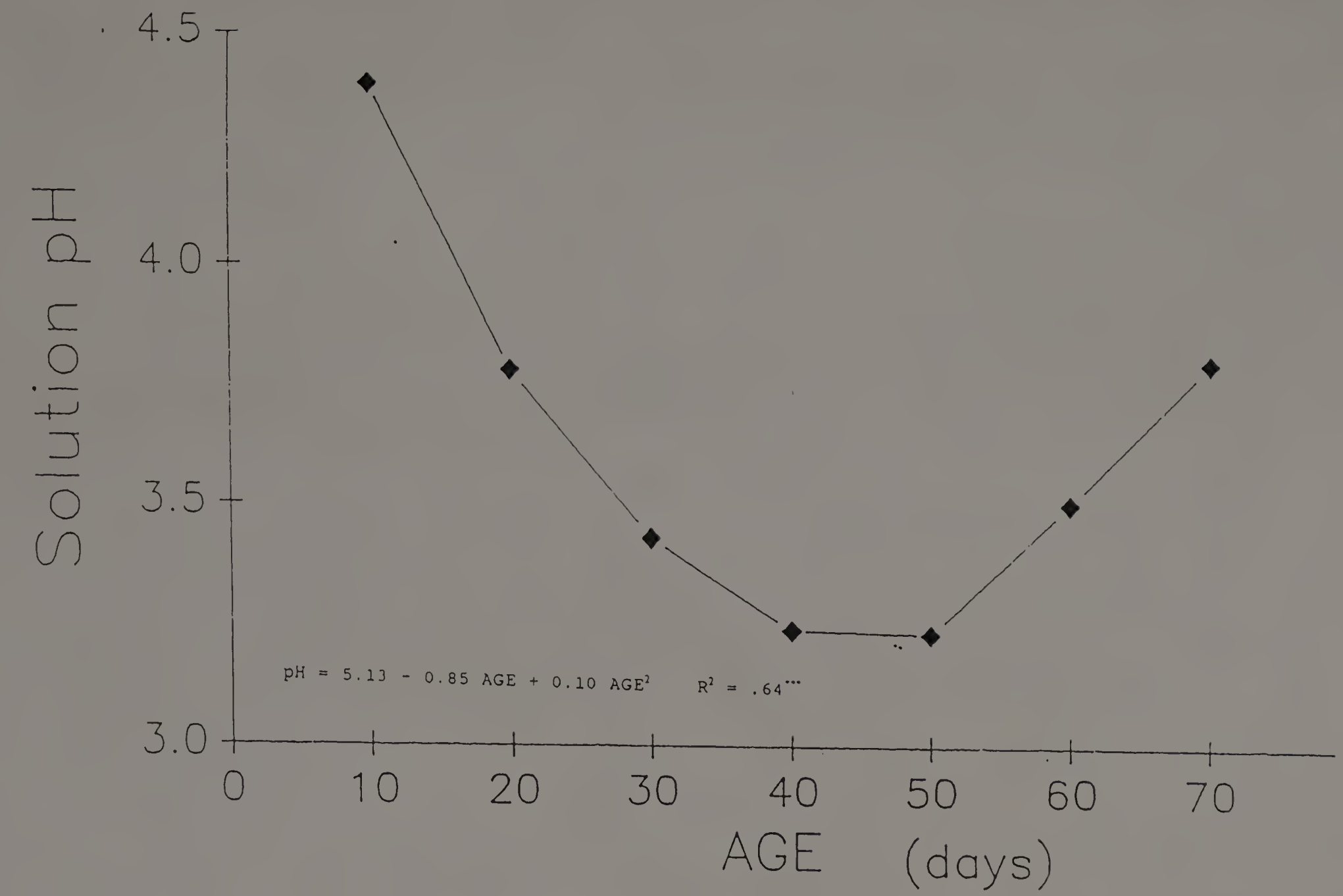

Fig.2 Solution pH during the growth of marigold.

have had an influence on NO_3-N and NH_4-N absorption during plant growth.

Maximum NO_3-N uptake occurs in the pH range of 4.5 to 5.0 and for NH_4-N, pH 6.0 to 6.5 (Wallace and Muller, 1956). This effect of solution pH on N uptake might explain the predominance of NO_3-N absorption throughout the experiment since solution pH was below 6.0.

4. 1. 4 Dry matter accumulation and N recovery

4. 1. 4. 1 Dry weight accumulation

Total dry weight of marigold increased steadily over the course of the experiment (Fig. 3). However, gains in dry weight were largest early in the experiment. Dry weight gains were largest between 10 and 40 days and reached a maximum between 40 and 50 days after transplanting. Dry weight gains were smaller during the period 50-70 days, corresponding to the period of first inflorescence opening and maturation.

Cockshull (1968) reported a cessation of dry weight accumulation by chrysanthemum during flower development and Woodson and Boodley (1983) found that if the flowers of chrysanthemum were removed early in their development dry weight continued to increase. For marigolds, dry weight accumulation did not stop completely, but gains decreased as

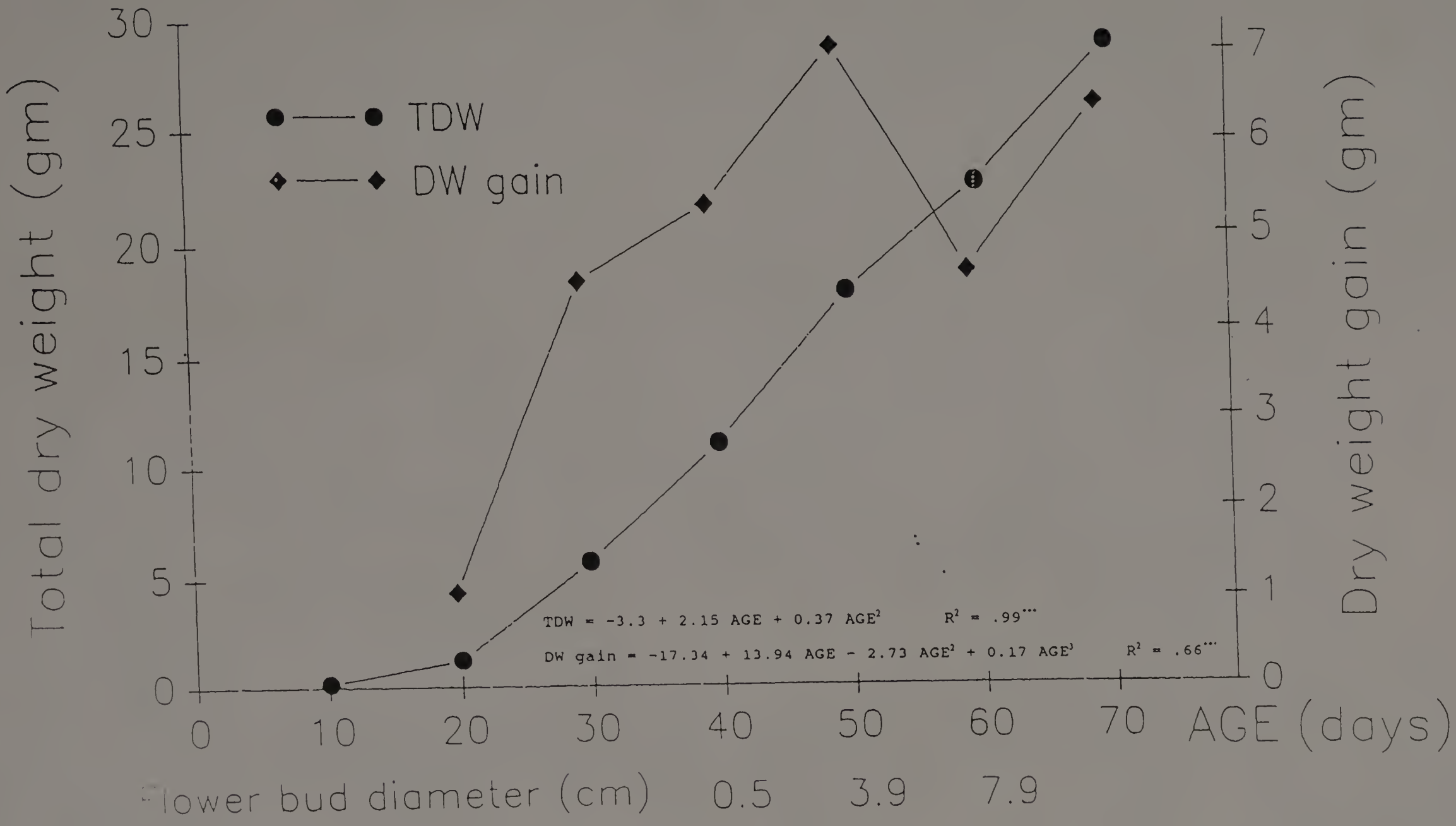

Fig.3 Dry weight of marigold 'First Lady'.

the first inflorescence matured (Fig. 3). This trend might indicate that during inflorescence development the photosynthetic capacity of the plant decreased, but still exceeded the demands of flowers (Woodson and Boodley, 1983).

The decrease in the levels of dry weight gain during the period 50 to 70 days might also be correlated with the decrease in N uptake during this period (Fig. 1).

4. 1. 4. 2 Nitrogen recovery

The amount of N recovered in the whole plant between sample dates paralleled the dry weight gains (Fig. 4). Thus, N recovery was greatest between 10 and 50 days after transplanting and recovery declined between 50 and 70 days. Results show that marigolds accumulate large amounts of N during their early development, but less during the period of first inflorescence development and maturation. A decrease in the rate of N accumulation after 50 days of growth may indicate a decrease in the N requirement of marigold. Nitrogen fertilizer applied during this period might be less effective in promoting plant growth and could be subject to loss by leaching.

Our results are similar to those of Woodson and Boodley (1983) who found that chrysanthemum also accumulated large amounts of N during the early stages of development and much less during the reproductive stage. They found that during

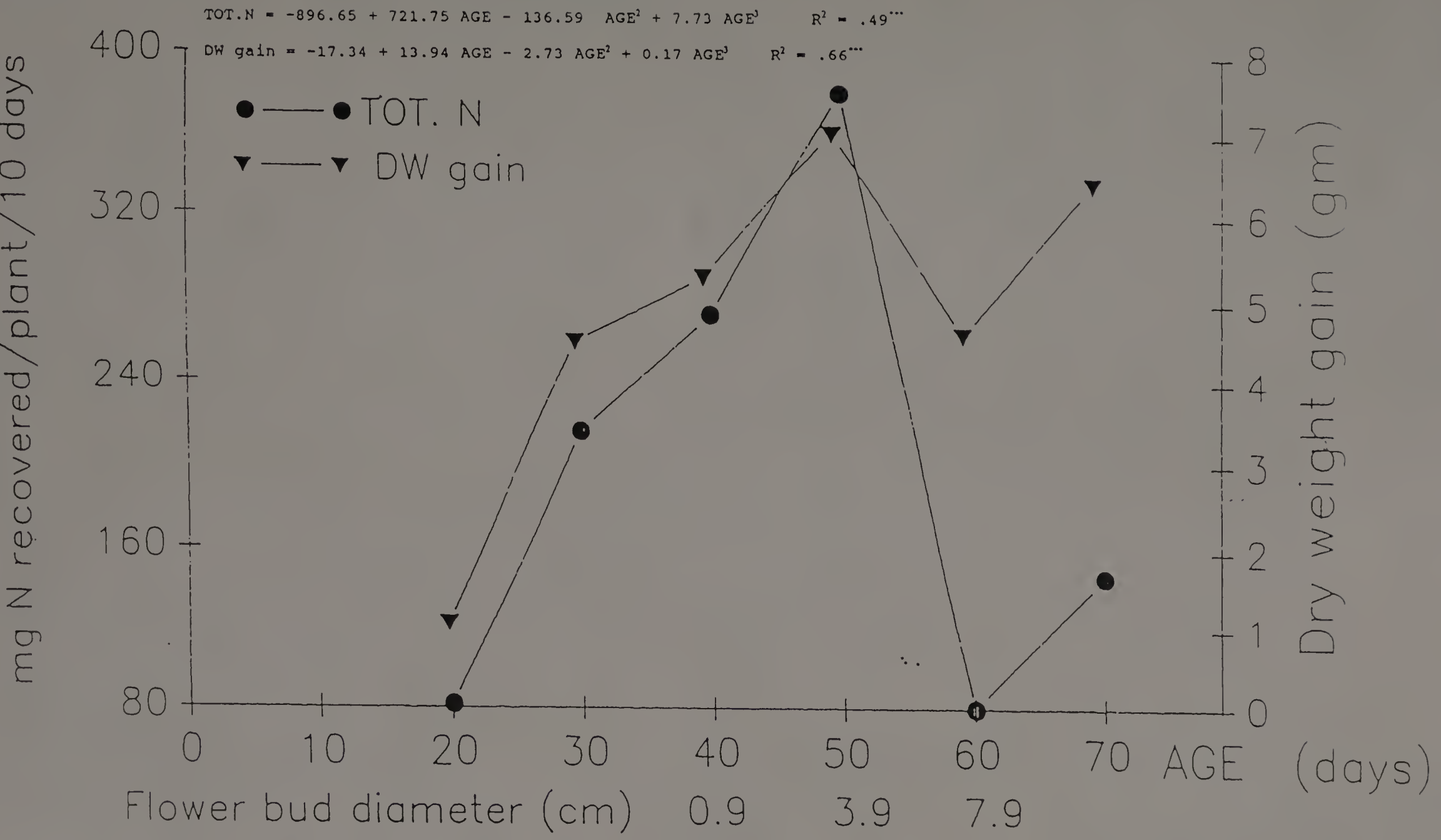

Fig.4 Total dry weight and nitrogen recovery.

the early juvenile growth about one-third of the N accumulated in the tissues was NO_3-N and they suggested that this accumulated NO_3-N might compensate for the progressive decline in N uptake during the reproductive phase. In later work, Woodson et al. (1984) found that the NO_3-N content of the leaves and stems declined during inflorescence maturation suggesting movement to flowers. Lunt and Kofranek (1958) found that about 12% of the total shoot weight of the plant was accounted for by the flower.

4. 1. 5 Dry weight of shoots and roots and N accumulation

Dry weight accumulation and N recovered in the shoots and roots parallel one another throughout the experiment (Fig. 5,6). The ratio of SDW to TDW (shoot dry weight/total dry weight) increased from about 70% at the beginning of the cycle to about 90% at the end of the cycle and the ratio of SN to TN (N recovered in shoots/total N recovered in plant) followed a similar path (Fig. 5).

The ratio of RDW to TDW (root dry weight/total dry weight) decreased over the course of the experiment (Fig. 6). Nitrogen recovered in the roots as a percentage of total in the plant followed a similar course. Thus no changes in N partitioning were evident due to effects other than the relationship between dry weight accumulation in shoots and roots.

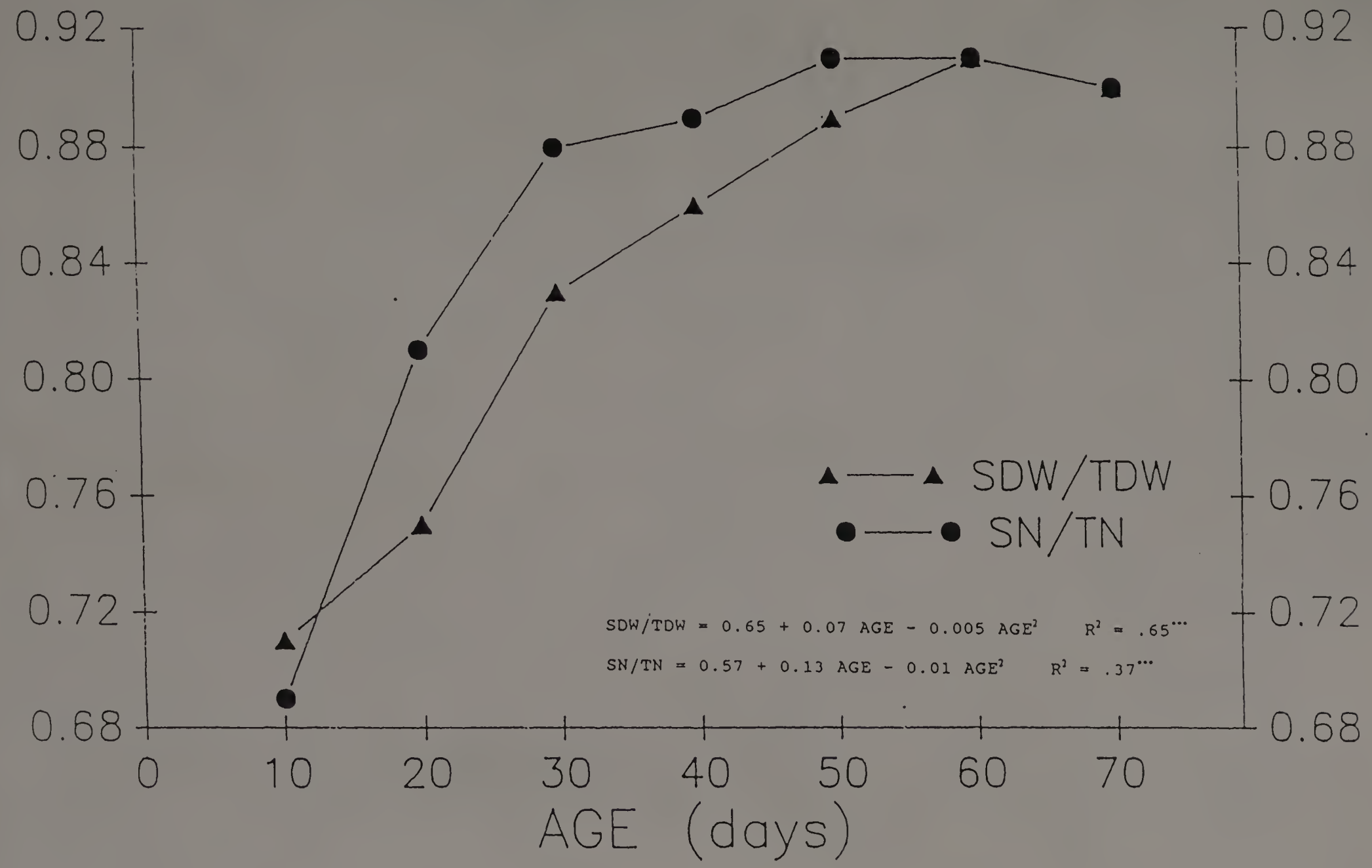

Fig.5 Relationship between shoot dry weight and N recovered in shoots.

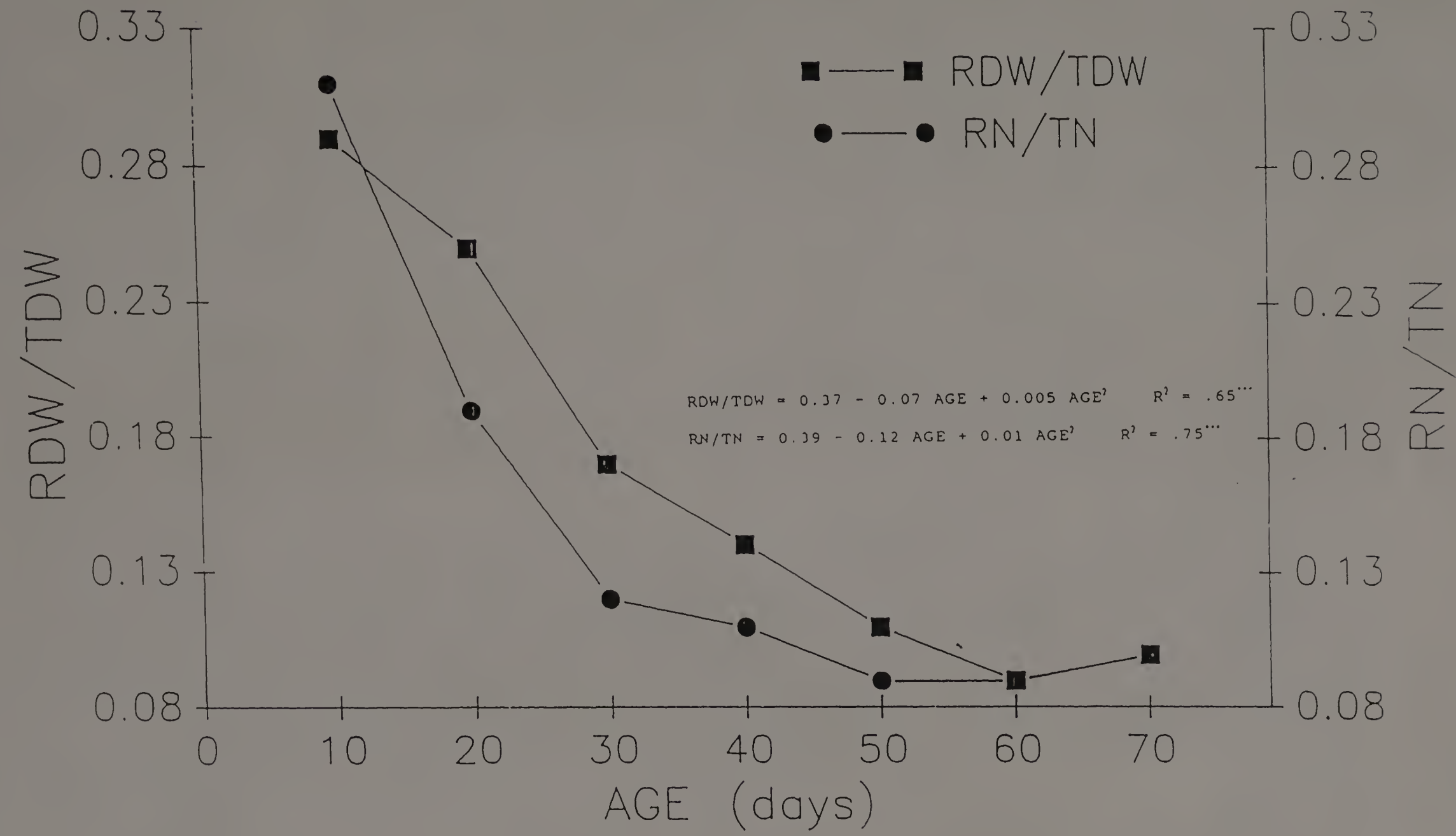

Fig.6 Relationship between root dry weight and N recovered in roots.

4. 1. 6 Nitrogen requirement of marigold

The total amount of N required by marigold, under the conditions of this experiment, was ≈1.2 gm N/plant or 38 mg N/gm DW (Table 2). About one-half of the total requirement was met by 40 days after transplanting. Clearly any number of factors might affect the apparent N requirement of plants that exhibit indeterminate growth. For example, N absorption by 'Inca Gold' marigold grown in peat-perlite medium increased with plant age and solution N concentration (Tolman et al., 1990).

4. 1. 7 Conclusion

In sum, decreases in N absorption, dry matter accumulation, and N recovery that occurred during inflorescence maturity seem to indicate that the capacity of the plant to absorb and accumulate N lessens during this period. It seems that, during this stage, marigold may depend partially on the N already accumulated in the tissues to meet its needs for N. Nitrogen fertilization during inflorescence maturation may not be as important to the plant as fertilization earlier.

This study suggests that the time of application of N is important in the production of marigolds. High N fertilization during the vegetative period of growth is probably to be very important. A Nitrogen deficiency

Table 2. Nitrogen absorbed by 'First Lady ' marigold during 70 day experiment (Expt. 1).

Method of determination	mg N/plant	mg N/gm DW/plant
Solution analysis	1116	--
Plant analysis	1191 ± 22	38

developing in the early ages of growth may irrevocably affect plant quality and corrective N fertilization might not be effective in promoting normal growth and flower development later because the capacity of the plant to absorb N decreases during inflorescence development. Nitrogen applications coming after this period might not compensate for the inhibition of plant growth. On the other hand, sustained high N levels in the root zone late in development might lead to N loss by leaching and potentially N pollution of water. Further research is needed on the effects of N deficiency at different stages of development.

4. 2 Experiment 2

4. 2. 1 General observations on New Guinea impatiens development

During their development, New Guinea impatiens (NGI) grew in size but did not show developmental changes like the marigold (Expt. 1). The plants of NGI were flowering at transplanting and continued to do so for the duration of the experiment.

4. 2. 2 Nitrogen uptake by New Guinea impatiens

The analysis of variance shows that there was a significant interaction between plant age and N form absorbed by the plants (Fig. 7). The plants absorbed more NO_3-N than NH_4-N during their development. These results were similar to those obtained with marigolds (Expt. 1) and with southernpeas (Sasseville and Mills, 1979). Both marigolds and southernpeas seem to prefer NO_3-N over NH_4-N. Our findings, however, contrast with those obtained by Edwards and Barber (1976) and Schrader et al. (1972). They found that NO_3-N and NH_4-N were absorbed at similar rates by corn. Results were also different from those reported by Wallace and Muller (1956) who found that rough lemon cuttings absorbed more NH_4-N than NO_3-N during their growth. Greidanus et al. (1972) reported that NH_4-N was the only N form absorbed by cranberries, apparently because they

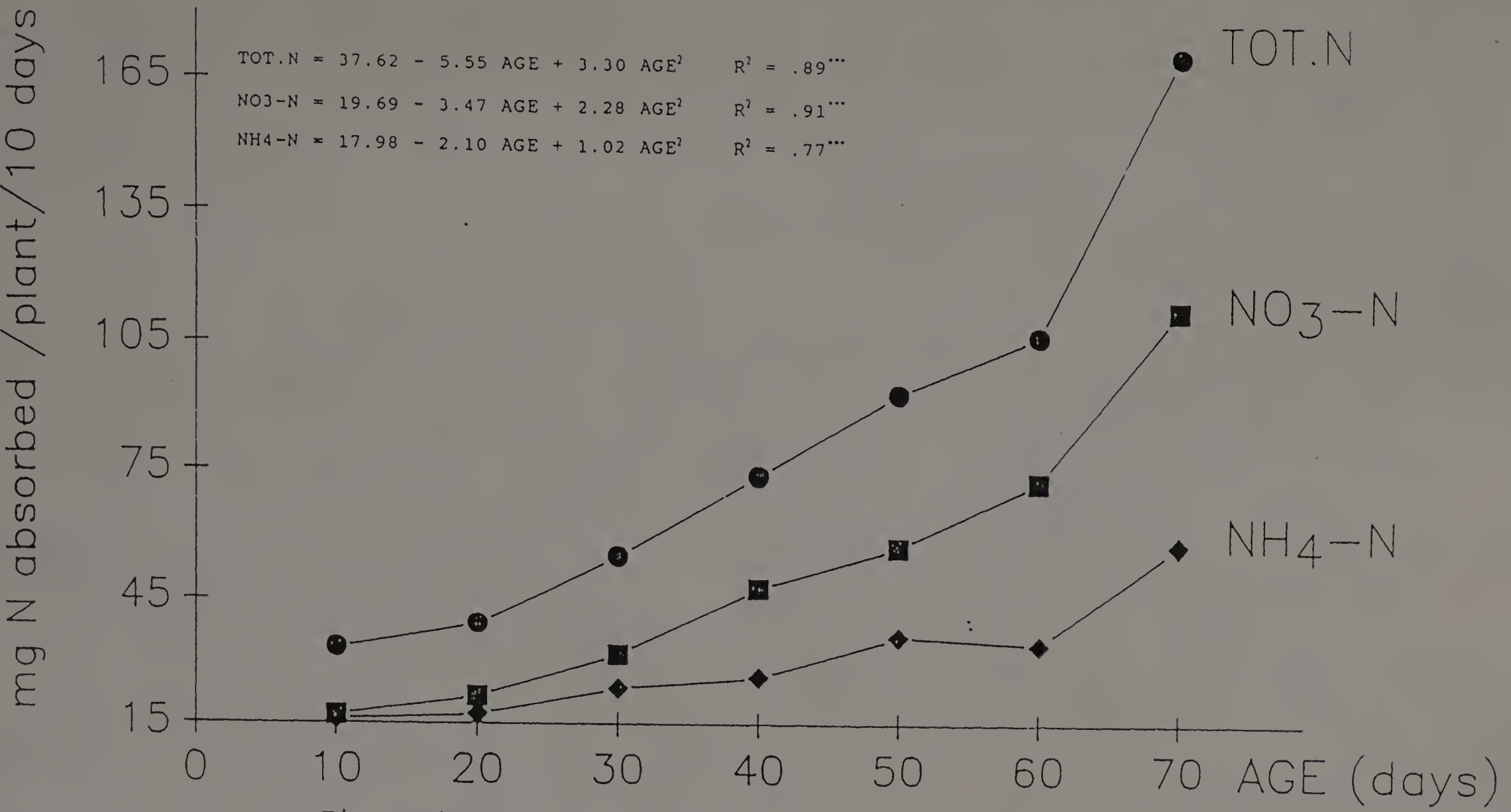

Fig.7 Nitrogen uptake determined by solution depletion during the development of impatiens.

lack the ability to absorb and reduce NO_3-N. Thus, it seems that N form preference depends on the plant species under study.

The preference of NGI for NO_3-N over NH_4-N might also be attributed to the ratio NO_3-N:NH_4-N in solution. It has been found that the ratio NO_3-N:NH_4-N influence the capacity of plants to absorb NH_4-N (Cox and Seeley, 1984). In this case NH_4-N absorption by poinsettia decreased as the NH_4-N content of the medium increased. Sasseville and Mills (1979) reported that southernpeas absorb more NO_3-N than NH_4-N when the ratio of NH_4-N to NO_3-N was 1:1 or greater.

Plant age had a highly significant effect on N uptake during growth (Fig. 7). Total N, NO_3-N, and NH_4-N uptake all increased with time. The curve of total N uptake showed a cubic increase of N absorption as the plants grew older.

Results with NGI were different from those obtained with marigolds in that N uptake increased steadily during the experiment whereas, with marigolds, N uptake was greatest during the period 30-50 days after transplanting and began to decline over the final 20 days of the experiment. In this way NGI were also different from corn and chrysanthemum. Nitrogen uptake by corn increased in the beginning of the cycle, reached a plateau then decreased at the end of the cycle (Edward and Barber,1976 and Warncke and

Barber, 1973). Woodson and Boodley (1983) reported similar changes in N uptake with chrysanthemum.

Marigolds, chrysanthemum, and corn are all similar in that they pass through different stages of vegetative and reproductive development during their growth. These developmental changes may account for their similarities in regard to N uptake. In contrast, NGI do not show distinct stages of vegetative and reproductive development. Thus, N uptake increased continuously during the course of the experiment.

4. 2. 3 Changes in solution pH during the growth of NGI

The pH of the depleted nutrient solution fluctuated within 0.4 pH units (Fig. 8). It must be pointed out that deionized water was being added periodically to containers to maintain volume and that there was no attempt to control pH. Solution pH decreased from about 3.8 at 10 days after transplanting to about 3.4 40 days later. This decrease in pH probably was the result of the acidifying effect of NH_4-N absorption. The pH of the depleted nutrient solution increased slightly during the period 50 to 60 days. This increase may have resulted from the small decrease in NH_4-N uptake during this period and the increasing difference between NO_3-N and NH_4-N uptake. The pH decreased during the

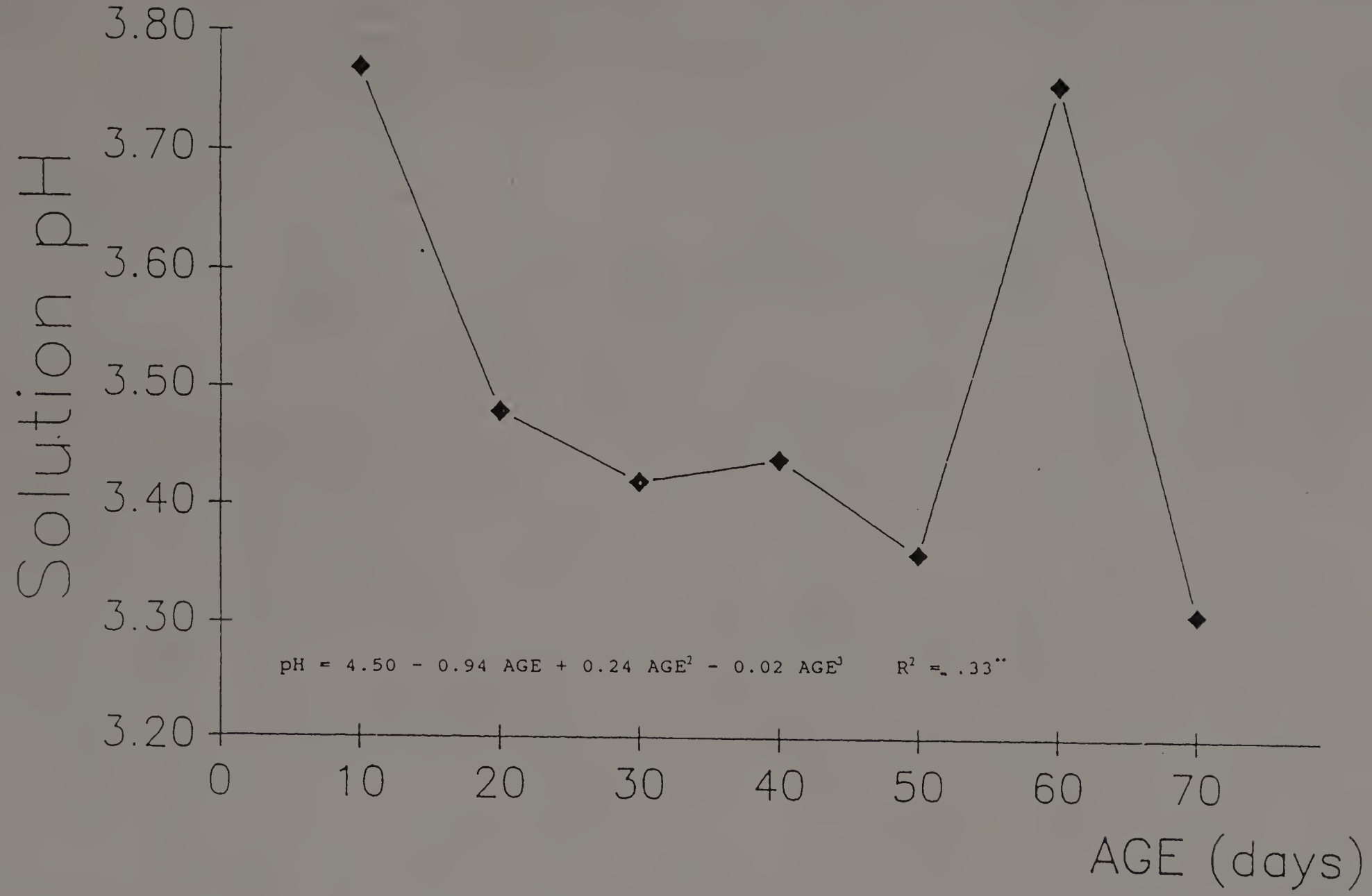

Fig.8 Solution pH during the growth of impatiens.

following 10 days, which might be attributed to the small increase in NH_4-N absorption during this period.

The low pH of the nutrient solution probably influenced NO_3-N and NH_4-N absorption during the experiment. In general the literature (Street and Sheat, 1958 and Wallace and Muller (1956) indicates that NH_4-N absorption increases at high pH (6.0-6.5) while NO_3-N absorption increases at low pH (4.5-5.0). Solution culture pH (Fig. 8) was generally below 6.0, which favors NO_3-N absorption.

4. 2. 4 Dry matter accumulation and N recovery

4. 2. 4. 1 Dry weight accumulation

Dry weight of NGI increased during the experiment with the largest DW gains occurring toward the end of the experiment (Fig.9). Dry weight gains were especially large during the period 50-70 days after transplanting. Gains in dry weight early in the experiment (20 - 50) days were relatively small.

The results obtained with NGI were different from those obtained with chrysanthemum in earlier studies (Elliot and Nelson, 1982 and Woodson and Boodley, 1983) and marigold in Expt. 1. Marigold and chrysanthemum showed a decrease in the rate of dry weight accumulation during flower development. This difference between NGI and the two other species might

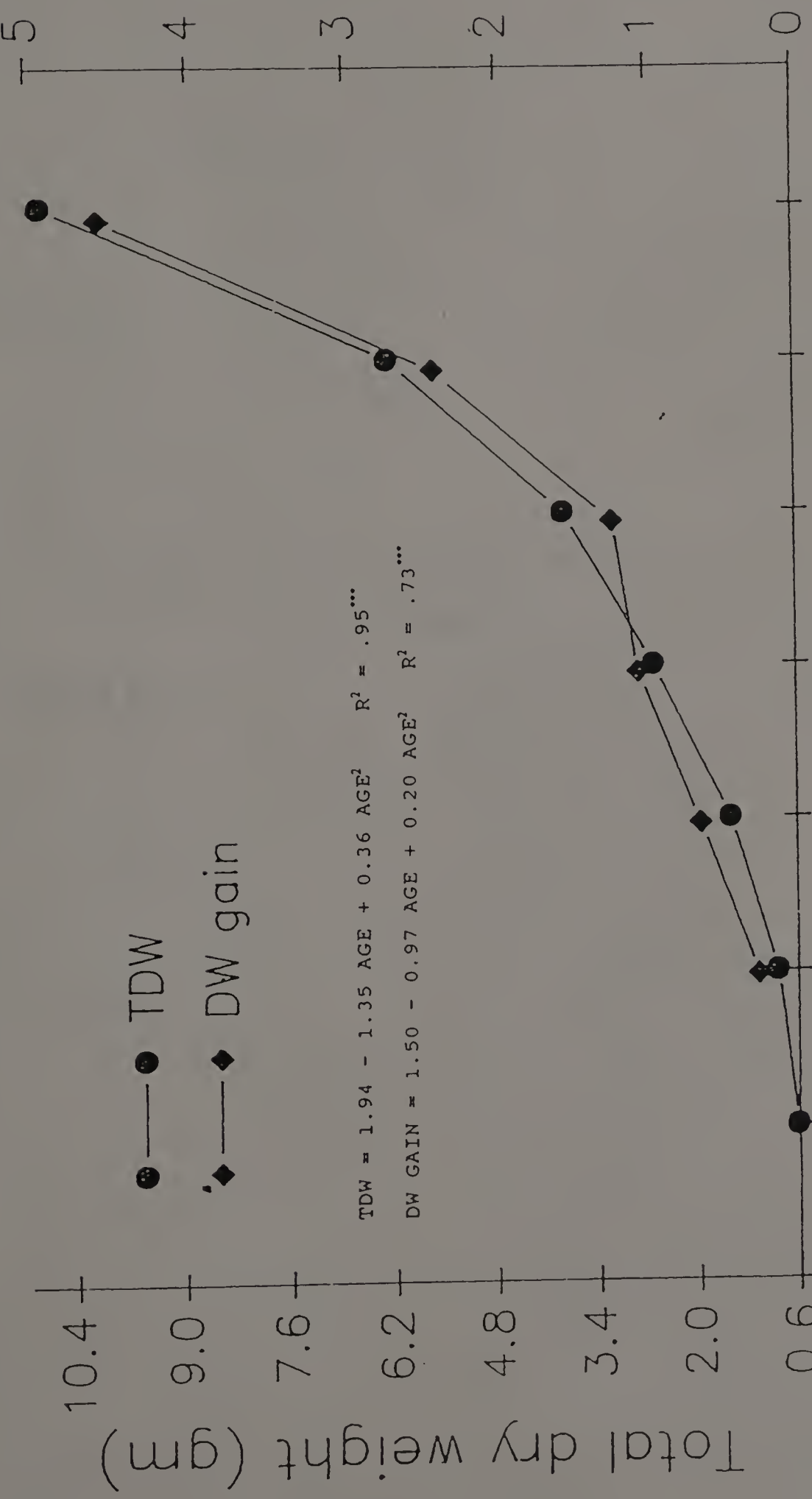

Fig.9 Dry weight of impatiens.

be attributed to the fact that NGI flower continuously rather than after a distinct vegetative growth phase.

4. 2. 4. 2 Nitrogen recovery

Nitrogen recovery and dry weight accumulation closely paralleled one another during the experiment increasing with time (Fig.10). More N accumulated in the plants during the second half of the experiment than during the first half.

Results with NGI contrast with those of marigold. Marigold showed greater N accumulation during the early stages of development than in the later stages of growth. Our findings with NGI were also different from those reported with other plants. Nitrogen recovery by lily showed an increase, a plateau, and then a decrease as the plants matured (Boodley, 1962). Chrysanthemums accumulate most of their N during the first four weeks after potting (Boodley and Meyer, 1965). The work of Woodson et al. (1984) and Lunt and Kofranek (1958) also showed that chrysanthemum have a higher requirement for fertilizer N during the early than later stages of development.

4. 2. 5 Partitioning of dry weight and accumulated N

Dry weight gains and accumulated N were greater in the shoots than in the roots throughout the experiment (Fig. 11, 12). Nitrogen partitioning was a reflection of dry weight partitioning (Fig. 11, 12). The ratio of SDW to TDW (shoot

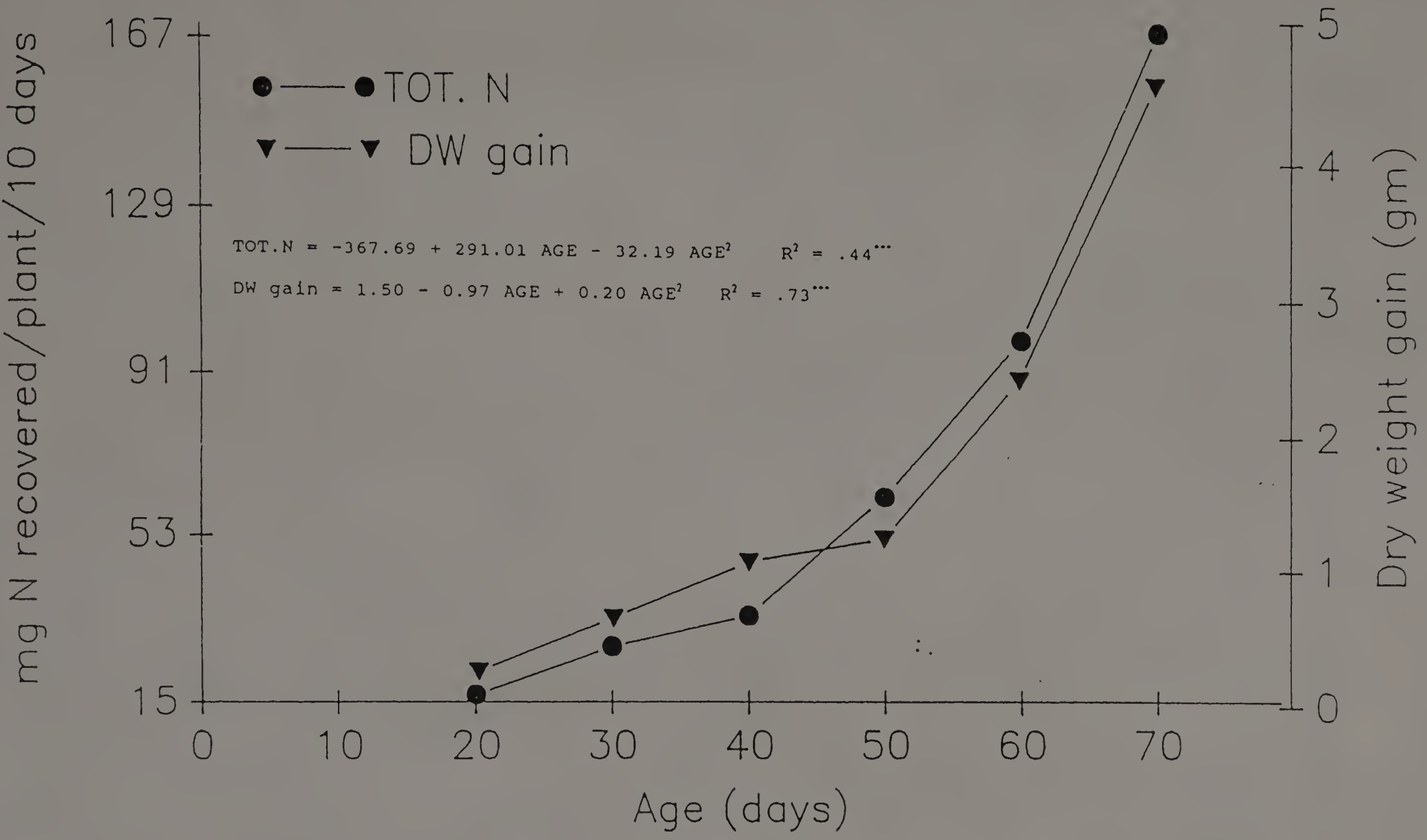

Fig.10 Total dry weight and nitrogen recovery.

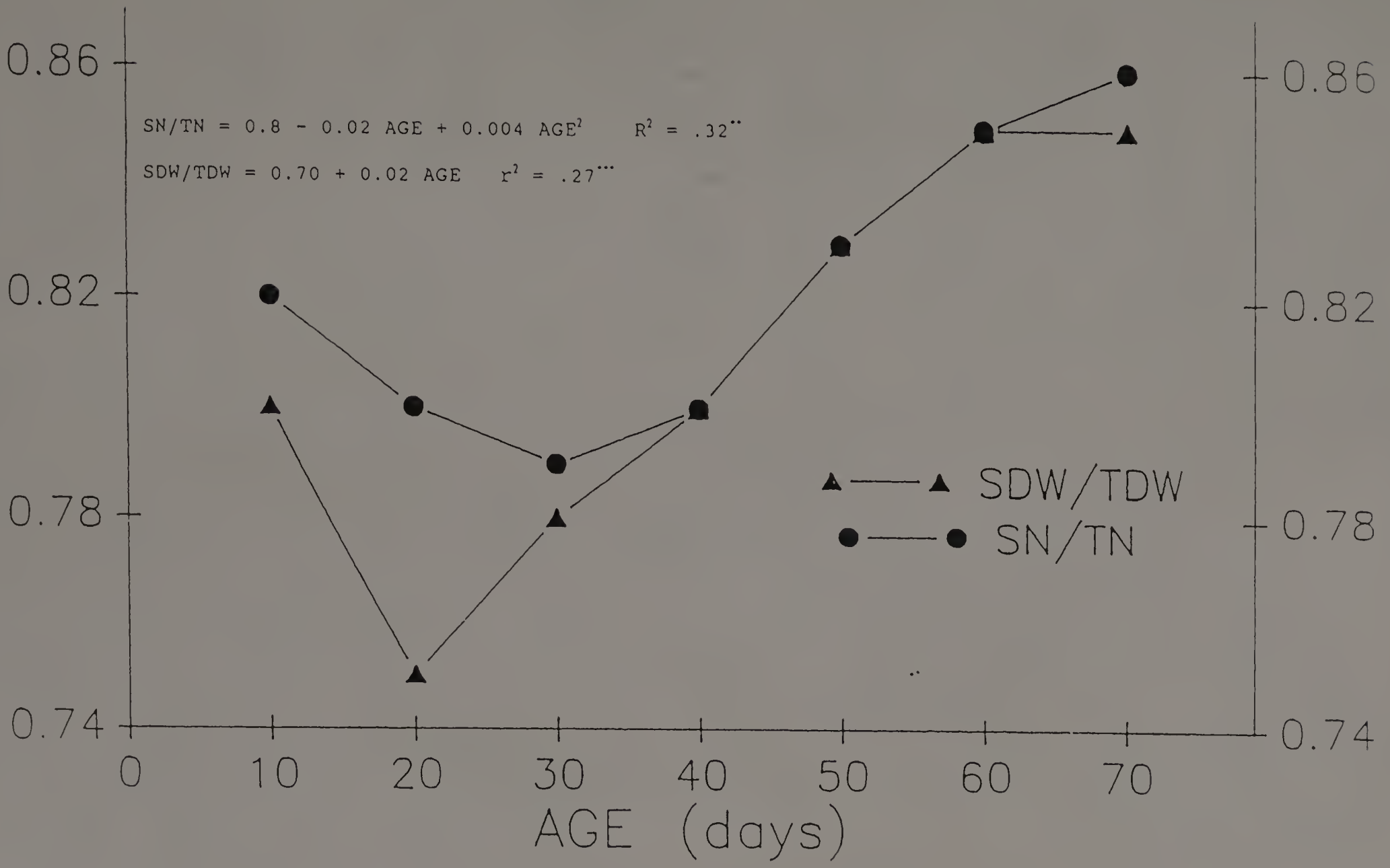

Fig.11 Relationship between shoot dry weight and N recovered in shoots.

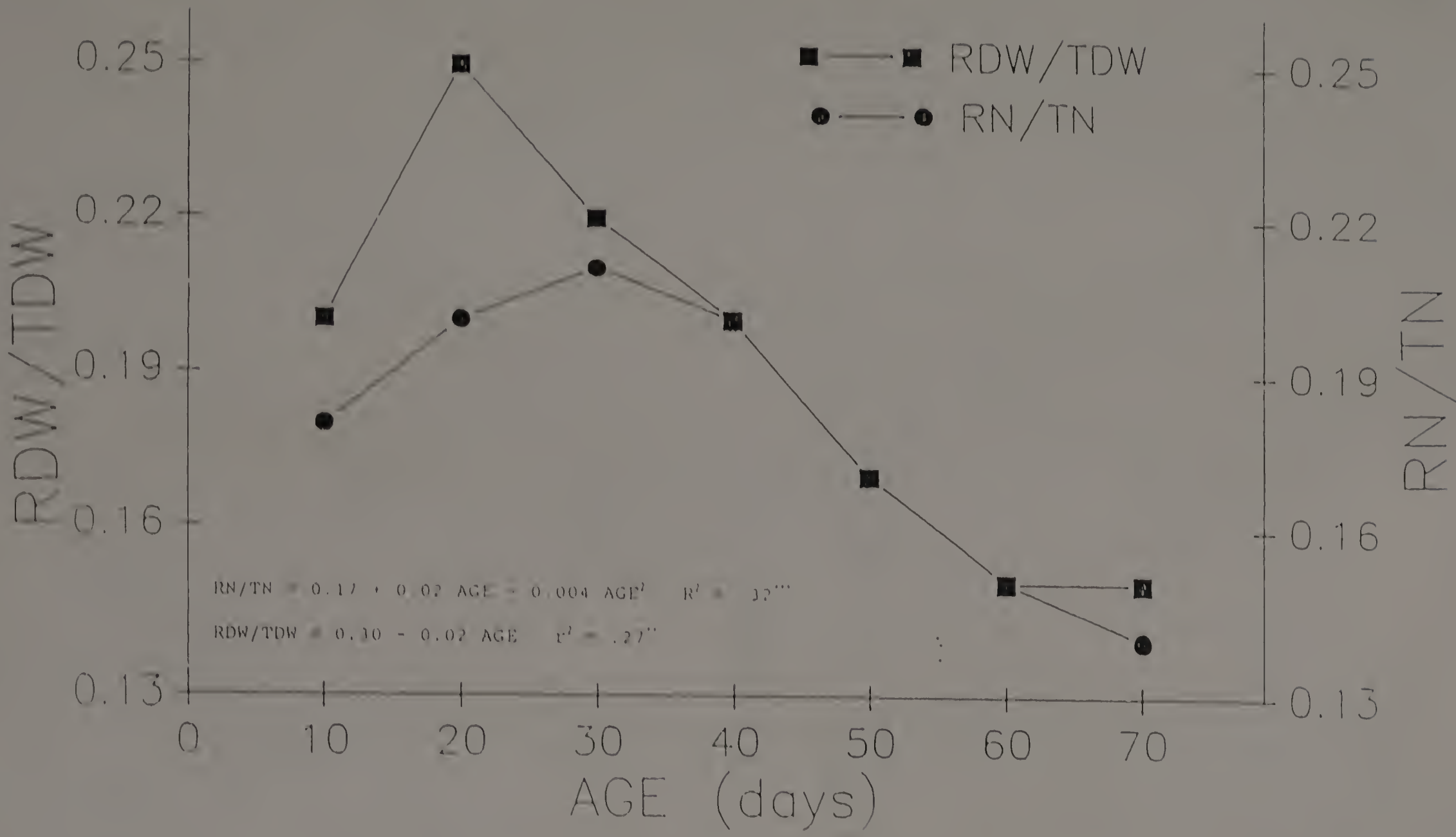

Fig.12 Relationship between root dry weight and N recovered in roots.

dry weight:total dry weight) decreased during the first month of growth from about 0.82 to about 0.79 (Fig. 11) and then increased with time to reach 0.87 at the end of the experiment, the ratio SN to TN (shoot N:total N) followed the same pattern. The SN to TN ratio decreased during the first 20 days of growth, from 0.81 to about 0.75, and then increased gradually to 0.85 when the plants were 70 days old.

Similar results were observed in the roots (Fig. 12). The RDW:TDW ratio increased from about 0.20 10 days after transplanting to about 0.26 during the following 10 days of growth. Then the ratio decreased gradually to 0.16 when the plants were 70 days old. Simultaneously, the RN:TN ratio increased during the first month of growth from about 0.17 10 days after transplanting to about 0.20 30 days after transplanting, then the ratio decreased to about 0.14 at the end of the experiment.

It seems that at the beginning of the experiment, the plant was building its rooting system. When this was completed, the focus appeared to shift to shoot growth. This hypothesis is supported by the general decline in the ratio of root dry weight to total dry weight with time and the low N uptake during the beginning of growth (Fig. 7). The root system was not large enough to absorb high levels

of N. The rate of N uptake was higher when the rooting system was well-developed.

The results obtained here with NGI were similar to those obtained by Mertens and Wright (1978) with Japanese Holly. They found that a chronological relationship existed between root and shoot growth and that N absorbed by the plant was first used to increase the root system. Once the roots were developed enough to absorb large amounts of N nutrients in excess of what was needed for root growth were translocated to the shoots to support growth of the tops.

Perhaps the similarities between NGI and Japanese Holly can be attributed to the fact that both plants are propagated by cuttings. Newly-formed adventitious roots may require more N than a mature root system in order continue developing. Once a root system is established, the plant may then direct more N to shoot growth.

4. 2. 6 Total N required by NGI for 70 days of growth

The total amount of N required by NGI, under the conditions of this experiment, was ≈0.5 gm N/plant or 52 mg N/gm DW (Table 3). In contrast, marigold (Expt. 1) required ≈1.2 gm N/plant for the same length of growth period. Any factor affecting growth might affect the apparent N requirement of NGI.

Table 3. Nitrogen absorbed by New Guinea impatiens during 70 day experiment (Expt. 2).

Method of determination	mg N/plant	mg N/gm DW/plant
Solution analysis	569	--
Plant analysis	428 ± 18	52

4. 2. 7 Conclusion

This experiment shows that N uptake by NGI increases with the plant age. Seventy-day old plants absorbed all of the NO_3-N and about one-half of the NH_4-N being supplied. Impatiens thus have a high requirement for N during later stages of growth than at the beginning. Nitrogen deficiency during the period when NGI are making the largest DW gains might be more detrimental than low N earlier on, however this remains to be studied. Supplying NGI with high levels of fertilizer N during the first half of growth might be less beneficial for the plants. The capacity of plants to absorb N appears to very low in the stages of growth. The unabsorbed N might be subject to loss by leaching or excess soluble salts could result from too much fertilizer may lead to growth inhibition of NGI (Judd and Cox, 1993).

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CHAPTER 5

CONCLUSION

High N fertilization early in the growth period of marigolds appears to be very important. Data from this experiment suggest that maintenance of high levels of N during the first 50 days after transplanting may be crucial for the production of good quality plants because N uptake is greatest during this period. If N deficiency developed during this period, it may not be possible to overcome the deficiency by subsequent fertilization because the capacity of plants to absorb and accumulate N seems to decrease when marigolds are older. On the other hand, supplying marigolds with high levels of N late in the growth cycle, during inflorescence development and maturation, might lead to N leaching and eventually N pollution. Further studies are, however, needed to provide direct evidence.

In contrast, high N fertilization of NGI would probably be more beneficial later in growth. The capacity of NGI to absorb N increased with the time following transplanting. Consequently, maintaining high N fertilization during the period 40 to 70 days after transplanting, during the time of greatest DW gains, is probably crucial for the production of good quality plants because demand appears to be greatest at this time. On the other hand, supplying the plants with

high levels of N during the first month of growth might lead to N loss by leaching and injury from soluble salts because of the low rates of N uptake and accumulation.

Under the conditions of this study results indicate that marigolds need about 1.2 gm N/plant for 70 days of growth or on a dry weight basis, about 38 mg N/gram of dry weight/plant. On the other hand NGI needed only 0.5 gm N/plant for 70 days of growth or about 52 mg of N/gram of dry weight/plant. Clearly the N requirement of both plants could change with any factor affecting rate of growth.

In sum, our data suggest the importance of an early N fertilization for marigolds and a late N fertilization for NGI. Coordination of N supply with plant N requirement would lead to improved fertilization practices allowing the production of good quality plants while minimizing N loss by leaching.

This kind of study should be done on most important commercial plants to reach a better match between the plant's needs in N and the N supply by fertilization. This would decrease the possibility of N pollution while producing good quality plants.

APPENDIX

TOTAL N IN SHOOTS AND ROOTS

The Kjeldahl method was used to determine the total nitrogen recovered in the plant tissue (Nelsson and Sommer, 1980). The reagents used in the Kjeldahl procedure were:

1. Concentrated H_2SO_4 was used as the digesting acid.
2. Salicylic acid (33 gm of salicylic acid was dissolved in one liter of H_2SO_4) was used to capture any NO_3-N in the tissue.
3. Kjeltab ST pellets (potassium sulfate and selenium (Thompson Capper, Ltd., Runcorn, Cheshire, England) used as a catalyst to speed digestion.

The Kjeldahl procedure consisted in two steps:

Step 1: When the tissues were dried and ground, 500 mg of each sample were mixed with one Kjeltab and 15 ml acid digestion mixture (H_2SO_4 + 33 gm/liter salicylic acid). The contents of the tube were mixed by swirling and allowed to stand at room temperature for about one hour and then were subjected to high-temperature digestion at 420 °C. The concentrated H_2SO_4 and catalysts converted organic and inorganic forms of N to NH_4-N. After approximately three hours of digestion, the contents of the tubes were clear, and digestion was complete.

The contents of each tube was then diluted as follows:

Dilution 1: The contents of each tube was transferred to a 100 ml volumetric flask and deionized water was added to bring to volume.

Dilution 2: Two ml of the solution resulting from Dilution 1 was transferred to a 50 ml flask and deionized water was added to bring to volume.

Step 2: Following Dilution 2 NH_4-N in the diluted digest was determined using the Orion 95-10 NH_3 gas-sensing electrode.

To determine the quantity of nitrogen recovered in the tissue, the following calculations were necessary:

Calculations:

$$\frac{\text{Initial volume (100 ml)}}{\text{gm of tissue (~ 0.5)}} = \text{Dilution factor 1 (DF1)}$$

$$\frac{\text{Second volume (50 ml)}}{\text{ml from first dilution (2 ml)}} = \text{Dilution factor 2 (DF2)}$$

$$\frac{(\text{DF1} \times \text{DF2})\ (\text{ppm N by electrode})}{10{,}000} = \%\text{N on a dry weight basis}$$

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