AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF PLANT REGULATORS ON GROWTH, DEVELOPMENT, AND SEED PRODUCTION OF ALFALFA

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Abstract approved: Dr. D. O. Chilcote

Experiments were conducted to determine the effects of certain plant regulatory chemicals on seed production of alfalfa (Medicago sativa L.). Two plant regulators, SADH (succinic acid 2,2-dimethylhydrazide) and TIBA (2,3,5-triiodobenzoic acid), received particular attention.

Replicated field tests of the plant regulators were carried out on the cultivar 'Talent' in southern Oregon and on 'DuPuits' in the Willamette Valley. Other experiments were conducted in the greenhouse, laboratory, and in controlled environment chambers to explore possible modes of action.

In 1972 foliar sprays of SADH and an experimental formulation of succinic acid derivatives, TD-6266-R, resulted in seed yields of 'Talent' of approximately 400 kg ha^{-1} compared to 168 kg ha^{-1} in untreated plots. These

responses were statistically significant, while lesser seed yield increases due to 2,4-DB [4-(2,4-dichlorophenoxy) butyric acid] and chlormequat [(2-chloroethyl)trimethyl-ammonium chloride] were not significant.

Similar field testing in 1973 resulted in no seed yield enhancement by any plant regulator. However, precipitation at the experimental site during the 1973 growing season was only 0.4 cm in contrast to the 30-year average of 4.3 cm. A plant regulator-environment interaction is suggested.

Yield component analysis showed that the number of seeds per pod was increased significantly by the same treatments which promoted yield. Number of pods per raceme was also a strong determinant of seed yield. The relative importance of one component of yield, the number of seed-bearing racemes per unit land area, is yet to be established.

After treatment with SADH and TIBA in 1973, field-grown 'DuPuits' alfalfa accumulated more total dry matter and up to twice as much dry matter in pods as did untreated plots. Shoots treated with TIBA exhibited a mean net carbon dioxide exchange (NCE) rate of 21 mg CO_2 dm^{-2} leaf area hr^{-1} compared to 16 mg CO_2 dm^{-2} hr^{-1} for SADH-treated and control shoots. However, dry matter accumulation and NCE responses were statistically nonsignificant at the .05 probability level. Specific leaf weight exhibited an

increased diurnal maximum when treated with SADH and TIBA, while the diurnal minimum remained equal to that of control plants.

The principal effect of TIBA on reproductive development was via its promotion of the growth and raceme initiation of axillary branches. In a dosage response study, 10 ppm TIBA was most effective, resulting in a fourfold increase in total branch length per primary stem and a highly significant increase in total raceme production. The finding that two axillary structures generally arise from an alfalfa leaf axil may be useful in understanding the phenomena of branching and floral initiation in this crop.

Alfalfa genotypes differed in their growth response to environmental conditions. It appeared possible that genotypes also differed in response to applied plant regulators. This research has shown that applied plant regulators can promote flowering in alfalfa and has suggested that the carbon budget of alfalfa may also be affected. These changes may or may not be translated into increased seed production. The net effects are a result of plant regulator interaction with environmental conditions and plant genotype. Thus, variability in the response to plant regulators can be expected.

Effects of Plant Regulators on Growth, Development, and Seed Production of Alfalfa

by

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EFFECTS OF PLANT REGULATORS ON GROWTH, DEVELOPMENT, AND SEED PRODUCTION OF ALFALFA

GENERAL INTRODUCTION

Alfalfa (Medicago sativa L.) is the most widely grown forage legume, and perhaps the most extensively investigated. Yet, as with agronomic crops in general, relatively few studies on the effect of chemical plant regulators have been conducted with alfalfa. Some reports suggest that plant regulators have potential for enhancing alfalfa seed production (Feltner and Sackett, 1964; Yeh and Bingham, 1969; Hale, 1971; Miller, Huffaker, and Jones, 1972). The influence of synthetic plant regulators on alfalfa seed production is the concern of this report.

The objective of the research is to describe the effects of certain plant regulators on the growth and development of alfalfa. The perspective is an agronomic one, in which plant regulator effects on growth and development are viewed as they relate to the production of seed by the alfalfa crop. Consequently, the influence of plant regulators is evaluated via three approaches: 1) direct observations of economic seed yield and its components, 2) analyses of the carbon budget of alfalfa, and 3) developmental studies. Each of these approaches is treated separately in the following sections of the thesis.

(1964) reported an average of 19 percent more seed produced

ALFALFA SEED YIELD AND YIELD COMPONENTS AS INFLUENCED BY PLANT REGULATORS

INTRODUCTION

The seed yield of a crop is defined as the weight or mass of seed harvested per unit area of land under production. Components of seed yield in alfalfa are the number of seed-bearing inflorescences (racemes) per unit land area, the number of pods per raceme, the number of seeds per pod, and weight per seed.

Certain plant regulators applied at low concentrations to alfalfa foliage have shown promise for significant increases in seed yield. TIBA (2,3,5-triiodobenzoic acid), ethephon [(2-chloroethyl)phosphonic acid], 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid], and TD-6266-R (undisclosed derivatives of succinic acid) have been reported to provide seed yield increases of 12 to 51 percent (Miller et al., 1972). Hale (1971) earlier had suggested that TIBA is capable of enhancing alfalfa seed production, and evidence was presented that TIBA increased the mean number of seed-bearing stems per plant. There appeared to be no effect on the number of pods per raceme, number of seeds per pod, or seed weight. Cowett and Sprague (1962) also found that TIBA increased the number of stems per plant. However, they did not evaluate seed yield. Feltner and Sackett (1964) reported an average of 19 percent more seed produced

on alfalfa treated with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) than on control plants. But, Massengale et al. (1968) observed no increase in seed yield in a test of different rates of 2,4,5-T. Yeh and Bingham (1969) reported that proper concentrations of either IAA or GA increased the selfed seed yield of a multifoliate alfalfa clone in a greenhouse experiment. In the same study 50 ppm TIBA decreased selfed seed yield.

The use of these plant regulators to improve seed yield has been investigated for other crop species. TIBA has been reported to enhance seed yield of Ladino clover (Miller, Huffaker, and Jones, 1973), soybeans (Greer and Anderson, 1965; Wax and Pendleton, 1968; Bauer, Sherbeck, and Ohlrogge, 1969; Hume, Tanner, and Criswell, 1972; Clapp, 1973), southern peas (Hipp and Cowley, 1969a, 1969b), bengal gram, a legume important in Africa and Asia (Sinha and Ghildiyal, 1973), and cotton (Freytag and Coleman, 1973). However, positive yield responses to TIBA were not obtained with lentils (Muehlbauer and Miller, 1971) and flax (Vetter, Holden, and Albrechtsen, 1970) and in other studies on soybeans (Burton and Curley, 1966; Hicks, Pendleton, and Scott, 1967).

Increases in seed yield have also been obtained by the use of the growth retardant, chlormequat, but such responses have been confirmed only in crop species belonging to the grass family. Yield increases have often

been attributed to the prevention of lodging. However, there are a number of reports of chlormequat causing higher yields of wheat in the absence of a lodging problem (El-Damaty, Kuhn, and Linser, 1965; Humphries, Welbank, and Witts, 1965; Appleby, Kronstad, and Rhode, 1966; Humphries, 1968a; Humphries, 1968b; Humphries and Bond, 1969; Lowe and Carter, 1970; Bokhari and Youngner, 1971b; Philpotts, 1972). Bokhari and Youngner (1971a) increased grain yield per plant in a greenhouse study of chlormequat on a uniculm mutant of barley. Chlormequat has also been reported to increase the number of heads per plant in timothy (Stoddart, 1964).

Another plant growth retardant, succinic acid 2,2-dimethylhydrazide (SADH), has shown promise for raising seed yield of red clover (Holm, 1972) and arrowleaf clover (Ball, Hoveland, and Buchanan, 1973). SADH is also reported to increase yields of peanut (U. S. Rubber Company, technical information on Kylar) and potato tubers (Bodlaender and Algra, 1966).

Ethephon, a compound which releases ethylene, is reported to enhance yields of peas (Oplinger and Gritton, 1973), Ladino clover (Miller et al., 1973), and tomatoes (Dostal and Wilcox, 1970). However, ethephon decreased yield of soybean (Slife and Earley, 1970).

It should be noted that products containing as active ingredients either SADH or ethephon have been commercially

available for several years, but have been recommended principally for modifying flowering and fruit maturation in ornamentals and tree-fruit crops. The references cited here represent the comparatively few studies of SADH and ethephon on agronomic crops.

2,4-DB, which has been used as a selective herbicide to control broadleaf weeds in legumes, is also considered to be a seed yield promoter (Miller et al., 1972, 1973). Klingman (1961) suggests that the conversion of 2,4-DB to 2,4-D (2,4-dichlorophenoxyacetic acid) is very slow in legumes. If this is so, the lower concentrations of 2,4-D in alfalfa tissue may result in promotion of growth or development rather than herbicidal effects.

Miller et al. (1972) have demonstrated that application of plant regulators can enhance alfalfa seed production in California. However, data are lacking on the value of such chemicals under environmental conditions in Oregon. Experiments were designed to evaluate the effects of selected plant regulators on alfalfa seed production under field conditions in Oregon. Specific objectives were 1) to identify treatments causing seed yield increases, and 2) to discover which of the components of seed yield were influenced by those treatments.

MATERIALS AND METHODS

Experiments were conducted in 1972 and 1973 at the Southern Oregon Experiment Station near Medford, Oregon. Plant material was 'Talent' alfalfa (Medicago sativa L.) sown in 30-cm rows on Central Point sandy loam soil in 1971. The plant regulators used in 1972 were 2,4-DB, chlormequat, ethephon, SADH, TIBA, and TD-6266-R, an experimental formulation. Three ingredients of TD-6266-R were offered separately by the manufacturer in 1973. These new formulations, designated TD-6732, TD-6733, and TD-6817, replaced TD-6266-R, ethephon, and chlormequat in the 1973 experiment. Table 1 further identifies these chemicals. All plant regulators were in aqueous solutions containing 0.4 percent non-ionic spray adjuvant. Rates of application are included in Tables 2 and 6.

1972 Experiment

Foliar sprays of each plant regulator were applied to plots with dimensions of 4 meters by 12.2 meters. The spray volume was equivalent to 468 liters per hectare. Each plot received a single application.

Stage of alfalfa development at the time of treatment was introduced as another variable according to the following plan: half of the total number of plots were sprayed on 13 June before any racemes were visible (pre-bud stage), and the remainder of the plots were sprayed on

Table 1. Identities and sources of plant regulators used in field trials on alfalfa for seed production.

Plant Regulator	Chemical Nomenclature	Trade Name	Source
2,4-DB*	4-(2,4-dichlorophenoxy-butyric acid	Butoxone amine	Rhodia, Inc. Portland, Oregon
chlormequat	(2-chlorethyl) trimethyl-ammonium chloride	Cycocel	American Cyanamid Co. Wayne, New Jersey
ethephon	(2-chlorethyl) phosphonic acid	Ethrel	Amchem Products, Inc. Ambler, Pennsylvania
SADH	succinic acid 2,2-dimethyl-hydrazide	Alar	Uniroyal, Inc. Naugatuck, Conn.
TIBA*	2,3,5-triiodo-benzoic acid	Regim-8	Chemagro Corporation Kansas City, Mo.
TD-6266-R TD-6732 TD-6733 TD-6817	derivatives of succinic acid; structures undisclosed		Pennwalt Corporation Tacoma, Washington

*The formulation used contained as active ingredient the dimethylamine salt of the acid.

23 June when the alfalfa racemes were in an early bud stage. Control plots were not sprayed. All treatments were replicated three times using a randomized block design.

Cultural practices included harvest of the first growth for hay on 28 May, sprinkler application of approximately 7.5 cm of water on 6 June and again on 27 July, and aerial application of Dylox insecticide (1 kg active ingredient per hectare) on 26 July. Honeybees and leafcutter bees were present as pollinators.

On 18 August samples consisting of 20 stems cut at the soil surface were taken from each plot. These were used to determine the number of racemes per stem. On 13 September ten racemes bearing mature pods were collected from each plot. The number of pods per raceme, number of seeds per pod, and seed weight were determined from these samples. The alfalfa was swathed on 15 September and allowed to cure in windrows for five days prior to combine harvesting of the seed. Harvested area per plot was 20.5 m^2. Germination tests on alfalfa seed harvested from these plots were performed by the laboratory staff of Dr. D. F. Grabe at Oregon State University.

1973 Experiment

Plot dimensions were 3 m by 12.2 m. A single foliar application of each plant regulator was made on 9 July with an estimated 25 to 40 percent of the alfalfa stems bearing

racemes in bloom. Spray volume per hectare was greater for some treatments than for others (Table 6) in an effort to improve spray effectiveness. Fifteen treatments were replicated four times in a randomized block design.

Cultural practices included harvest of the first growth of the season for hay on 22 May, sprinkler irrigation with 12 cm water on 1 June, and application of the insecticides Cygon at a rate of 1.17 l ha^{-1} on 15 June and Dylox at 1.4 kg ha^{-1} on 20 June. Honeybees were used as pollinators.

On 31 August a sample of 20 alfalfa stems cut at the soil surface was taken from each plot to provide data on seed yield components. Plots were swathed on 4 September and seed was harvested by combine on 13 September. Harvest area was again 20.5 m^2.

Statistical Analysis of the Data

Analyses of variance were performed on seed yield and yield component data, followed by pairwise comparison of appropriate means via the Least Significant Difference (LSD). In order to obtain an estimate of the independent contributions of each component of yield in 1972, the path coefficient analysis was employed. This method was introduced by Wright (1921), reviewed by Li (1956), and recently applied to seed yield component analysis (Vetter et al., 1970; Duarte and Adams, 1972).

Figure 1 depicts the logical relationships between

alfalfa seed yield and its components, and defines the symbols used. The path coefficient (P) is the standardized partial regression coefficient given by:

$$P = b(\frac{s_x}{s_y})$$

where:

b = partial regression coefficient

s_x = standard deviation of X

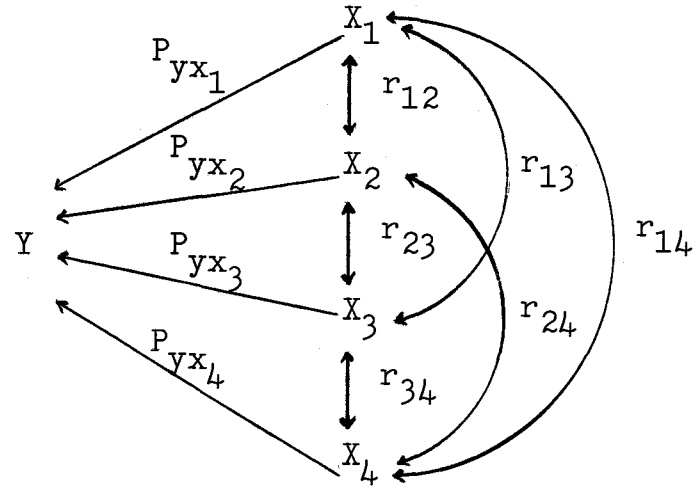
s_y = standard deviation of Y

The object is to learn the relative strength of the relation between Y and each of the X variables. However, direct comparison of partial regression coefficients, for example b_{yx_1} with b_{yx_2}, is not adequate if there is unequal variation in X_1 and X_2. The standardized partial regression coefficients (P) may be compared as suggested by Snedecor (1956, p. 416) since the sample standard deviations of the several X are taken into account.

The correlation coefficients between Y and each X were partitioned into direct and indirect effects as shown in the example equation below:

$$r_{yx_1} = P_{yx_1} + P_{yx_2}(r_{12}) + P_{yx_3}(r_{13}) + P_{yx_4}(r_{14})$$

where r is the correlation coefficient between two variables, P_{yx_1} is the path coefficient measuring the direct effect of X_1 on Y, and the products P(r) are the indirect effects of X_1 on Y through each of the other X variables.



P_{yx} = path coefficient measuring direct effect of X on Y; also termed standardized partial regression coefficient

r = correlation coefficient

Y = seed yield

X_1 = number of racemes per stem

X_2 = number of pods per raceme

X_3 = number of seeds per pod

X_4 = weight per seed

Figure 1. Association of alfalfa seed yield and yield components.

The multiple coefficient of determination, R^2, is the sum of its components as shown in the equation

$$R^2 = P_{yx_1}(r_{yx_1}) + P_{yx_2}(r_{yx_2}) + P_{yx_3}(r_{yx_3}) + P_{yx_4}(r_{yx_4})$$

The numerical value of each component is interpreted as the fraction of the total variation in Y accounted for by variation in a given X. The residual, $1-R^2$, is the fraction of variation in Y which is not accounted for.

The multiple regressions for the path coefficient analysis, as well as nearly all other calculations reported here, were performed with the aid of the Oregon State University OS-3 computer subsystems.

RESULTS AND DISCUSSION

Table 2 lists the treatments imposed and the resultant yields in the 1972 experiment. Application of SADH and TD-6266-R resulted in statistically significant increases in seed yield. No other treatments differed significantly from the control, although there appeared to be a substantial positive yield response to 2,4-DB. The rates of TIBA applied in 1972 were herbicidal, so no yield response occurred. Yields were highly variable as evidenced by the coefficient of variation equal to 67 percent.

Yield component analysis showed that the number of seeds per pod was increased significantly by the same treatments which promoted yield (Table 2). None of the other treatments resulted in statistically significant changes in any of the observed components of seed yield.

There was only one significant response attributable to stage of development at the time of treatment. Plots treated on 13 June averaged 7.0 pods per raceme compared to 7.8 pods per raceme for plots sprayed ten days later.

Numbers of seeds per pod are low, due at least in part to the method of evaluation. While all stages of pod development, from barely visible pods to those obviously filled with seeds, were included in pod numbers, only fully developed seeds were counted. Large numbers of small seeds which had not completed development were obtained upon threshing of pod samples. These were not recorded since

Table 2. Seed yield and yield components of alfalfa as affected by plant regulators. Medford, Oregon, 1972.

Plant Regulator	Rate of Application (g a.i. ha^{-1})	Yield Components				Seed Yield (kg ha^{-1})
		Racemes $Stem^{-1}$	Pods $Raceme^{-1}$	Seeds Pod^{-1}	1000-Seed Wt. (g)	
Control	0	20.5	7.8	0.50	2.138	168
TD-6266-R	530	28.5	7.5	1.33*	2.145	412*
SADH	1120	26.5	8.0	1.17*	2.147	410*
TD-6266-R	795	29.0	7.2	1.33*	2.162	370*
2,4-DB	560	25.7	7.8	1.00	2.145	354
chlormequat	560	25.0	7.2	0.33	2.173	270
chlormequat	1680	28.5	8.3	0.83	2.137	230
TIBA	1120	17.0	7.3	0.50	2.112	123
TIBA	560	26.8	6.7	0.67	2.108	119
ethephon	140	17.0	7.7	0.67	2.187	115
ethephon	280	12.8	6.3	0.33	2.150	95
c.v., %				51		67
$LSD_{.05}$				0.64		191

* Differs from Control in same column at .05 probability level.

such seeds are lost during commercial harvesting operations and thus are not a part of the harvestable seed yield. The numerical estimates of all other yield components in Table 2 are of the same order of magnitude as those reported by other workers (Grandfield, 1945; Pederson and Nye, 1962).

Seed size, indicated by 1000-seed weight in Table 2, was least from alfalfa sprayed with TIBA. Decreased seed size has been frequently noted when TIBA was applied to soybeans (Anderson, Greer, and Tanner, 1965; Greer and Anderson, 1965; Burton and Curley, 1966; Hicks et al., 1967; Wax and Pendleton, 1968; Bauer et al., 1969).

The one component of alfalfa seed yield for which data were not obtained was the number of seed-bearing racemes per unit land area. Numbers of seed-bearing racemes per stem were evaluated, but no data were collected on the number of such stems which occupy a unit of land area. At the time of sample collection it was felt that this component could not be increased by plant regulators. The rationale was that even if additional stems were initiated in response to plant regulators, these would have insufficient time to bear mature seed. From subsequent observation of alfalfa development it appears that, on the contrary, the interval from plant regulator application to seed harvest (50 to 75 days) is of sufficient length to allow stems to develop and bear pods with mature seed.

Plant regulators that decrease apical dominance, such

as TIBA, would be expected to induce development of axillary and crown buds into branches and primary stems, respectively. This would provide additional leaf axils, potential sites for the formation of racemes.

It now appears that the most efficient method of completing seed yield component analysis is to evaluate directly the numbers of seed-bearing racemes per unit of land area. This obviates the determination of both raceme number per stem and stem number per unit land area.

An estimate of the number of seed-bearing stems per square meter may be calculated from the data on yield and yield components by the equation

$$\text{stems } m^{-2} = \frac{\text{kg seed } ha^{-1} \ (1000)}{(\frac{\text{racemes}}{\text{stem}})(\frac{\text{pods}}{\text{raceme}})(\frac{\text{seeds}}{\text{pod}})(\frac{\text{gms}}{1000 \text{ seeds}})}$$

The mean number of seed-bearing stems per square meter, calculated in this manner for all treatments in 1972, is 92. Considering the 30-cm inter-row spacing, this means an average of one seed-bearing stem approximately every 4 cm within a row. This is a credible description of a one-year-old drill sown stand of alfalfa.

Table 3 summarizes the direct and indirect effects on seed yield of those yield components for which data were obtained. The analysis demonstrates the relatively strong direct effect of seed number per pod and pod number per raceme as contrasted with number of racemes per stem and seed weight. Indirect effects were consistently slight.

Table 3. Path coefficient analysis of direct and indirect influences of yield components on seed yield for treatments which increased yield and for those which resulted in no yield response.

Type of Effect	Coefficients calculated for treatments that resulted in	
	Yield Increase	No Yield Response
Effect of racemes stem^{-1}		
Direct effect	-0.0170	0.1667
Indirect effect via pods raceme^{-1}	0.0416	-0.0023
Indirect effect via seeds pod^{-1}	0.0503	0.0192
Indirect effect via seed weight	0.0158	0.0111
Total correlation (r_{yx_1})	0.0907	0.1940
Effect of pods raceme^{-1}		
Direct effect	0.2948	0.2363
Indirect effect via racemes stem^{-1}	-0.0024	-0.0016
Indirect effect via seeds pod^{-1}	0.1781	0.1571
Indirect effect via seed weight	0.0010	0.0138
Total correlation (r_{yx_2})	0.4717	0.4058**
Effect of seeds pod^{-1}		
Direct effect	0.4839	0.4371
Indirect effect via racemes stem^{-1}	-0.0018	0.0073
Indirect effect via pods raceme^{-1}	0.1085	0.0849
Indirect effect via seed weight	0.0011	0.0038
Total correlation (r_{yx_3})	0.5918*	0.5393**
Effect of seed weight		
Direct effect	0.0586	0.0841
Indirect effect via racemes stem^{-1}	-0.0046	0.0219
Indirect effect via pods raceme^{-1}	0.0052	0.0389
Indirect effect via seeds pod^{-1}	0.0091	0.0191
Total correlation (r_{yx_4})	0.0684	0.1581

* Differs from zero at .05 probability level.

** Differs from zero at .01 probability level.

Both seed number per pod and pod number per raceme were highly correlated with seed yield based on tests of significance for partial coefficients of correlation (Steel and Torrie, 1960, p. 287). Seed number per pod has consistently been found to be among the best predictors of seed yield, according to a recent review (Pederson et al., 1972).

Table 4 lists the coefficients of determination for the 1972 alfalfa seed yield study. In plots where seed yield responded to plant regulators, the determining influence of pod number per raceme and seed number per pod on yield was increased relative to other plots. This suggests again that increases in seed yield resulting from application of TD-6266-R or SADH were expressed through increases in these two yield components. Such an effect on pod number per raceme is consistent with observations that SADH increases fruit set in apples and grapes (U. S. Rubber Co., technical information for Alar-85). It may be significant that Feltner and Sackett (1964) likewise concluded that number of seed pods per raceme was the yield component primarily responsible for increased seed production of alfalfa treated with 2,4,5-T.

Ovule fertilization and development of the alfalfa ovary into a pod may occur after either self- or cross-pollination. So increases in pod number per raceme might be the result of increases in either type of pollination,

or both. However, reports that the attraction of pollinating bees to alfalfa flowers is related to the volume, sugar concentration, or aroma of the nectar (Pedersen, 1953; Loper and Waller, 1970) and that these factors could be influenced by the plant regulator 2,4,5-T (Feltner and Sackett, 1964) suggest that cross-pollination is the mode of pollination affected. Further, if self-pollinated flowers had given rise to the additional pods per raceme observed, fewer seeds per pod could be expected (Bradner and Frakes, 1964; Pankiw and Bolton, 1965; Barnes and Stephenson, 1971). However, cross-pollination was apparently benefited in this experiment since yield responses observed here are due in part to a greater number of seeds per pod.

Seed number per pod, which responded to TD-6266-R and SADH application, is determined by frequency of ovule fertilization and ovule abortion. Interactions between pollen and maternal tissue of the stigma, style, and ovary apparently control the entry and depth of penetration of pollen tubes into the ovary. There is little basis even for speculation on whether the applied plant regulators may affect these factors.

It is important to note the magnitude of the residual terms in Table 4. Approximately 60 percent of the variation in seed yield was not accounted for in this model. The residual term in this study includes one important

component for which data were not obtained, namely number of seed-bearing stems per unit land area. If this is interpreted as meaning that potentially as much as 60 percent of variation in seed yield is due to variation in number of seed-bearing stems per unit area, it supports Hale's (1971) contention that increased seed yield from treatment with TIBA was likely due to an increase in the average number of seed-bearing stems per plant.

When seed yield was increased by plant regulators, germinability and the proportion of hard seed were not affected (Table 5).

A second field experiment was performed in 1973. The treatments imposed and the resultant alfalfa seed yields are given in Table 6. None of the plant regulators caused a statistically significant seed yield response, including those which had done so in 1972. Yields appeared to have been decreased by 2,4-DB. There was much variation in seed yield which could not be accounted for by plant regulators or replication. This is apparent from the high coefficient of variation, 34 percent, and is implied by the unexpected large difference in yield (over 30 kg ha^{-1}) between plots sprayed only with water and those receiving no treatment whatsoever. Under these circumstances yield responses have to be large, as were those observed in the 1972 experiment, in order to become statistically significant. Yield component analysis was not performed in view of the lack of

Table 5. Characteristics of alfalfa seed harvested in 1972 from plots treated with plant regulators.

Plant Regulator	Date Applied	Seed Parameters				
		Germination %		Hard Seed %	Dead Seed %	1000-Seed Wt. (g)
		4 days	7 days			
Control	13 June	37	38	36	26	2.140
	23 June	38	40	38	23	2.137
SADH	13 June	42	43	29	28	2.143
	23 June	40	43	33	27	2.150
TD-6266-R ($530\ g\ ha^{-1}$)	13 June	37	39	37	24	2.120
	23 June	45	47	23	30	2.170

Table 6. Seed yield of alfalfa as affected by plant regulators. Medford, Oregon, 1973.

Plant Regulator	Rate of Application (g a.i. ha^{-1})	Seed Yield (kg ha^{-1})
Controls		
Untreated	0	302
Water	0	269
Water + adjuvant	0	258
TIBA	70	343
TD-6817	185	321
SADH	1120	318
TIBA	17.5	311
TD-6733	31	307
TD-6817	370	298
TD-6732	295	298
TIBA	35	291
2,4-DB	35	278
TD-6732	147	267
TD-6733	62	250
2,4-DB	560	136
c.v., %		34
$LSD_{.05}$		124

No mean differs from Control (Water + adjuvant) at .05 probability level.

a yield response to plant regulators in 1973.

It is speculated that the failure of alfalfa to respond to any of the plant regulators in 1973 may have been due to the dry growing season. For the period 1 June to 31 August, the experimental site received 6.1 cm of precipitation in 1972 in contrast to only 0.4 cm in 1973. The 30-year average is 4.3 cm of rainfall during these months.

Stutte, Cothran, and Rudolph (1974) have observed that adequate water must be available during flowering and pod-filling stages of soybean development in order to permit evaluation of plant regulator effects on seed yield. Likewise, Hume, Tanner, and Criswell (1972) have reported positive soybean yield responses to TIBA only in years when precipitation was above normal. Ohki and McBride (1972) generally obtained increases in soybean pod number using TIBA in greenhouse and growth chamber studies. However, when they imposed low soil moisture conditions the response to TIBA was sometimes negative. Vetter et al. (1970) suggested that a moisture deficiency may have restricted the favorable response of flax to their applications of TIBA. The potential importance of soil moisture to seed production was also demonstrated in a greenhouse study of potted alfalfa plants (Grandfield, 1945). Mean numbers of racemes per plant were 32 and 66 for plants grown in soil maintained at 12 and 22 percent water, respectively. No

significant changes in pod number per raceme or seed number per pod resulted from different soil moisture contents.

Results of the present research corroborate the findings of other workers that seed yield responses to applied plant regulators can be obtained on field-grown alfalfa. The fact that these responses remain highly unpredictable is indicative of the limited understanding of the specific effects plant regulators have on crop plants. Such specific effects have been the subject of investigations reported in the next sections.

THE EFFECTS OF SADH AND TIBA ON THE CARBON BUDGET OF ALFALFA

INTRODUCTION

Dry matter accumulation in seeds is ultimately determined by the photosynthetic assimilation of carbon and its subsequent distribution, storage, and utilization. Thus, the rates of photosynthesis, respiration, and assimilate translocation are important elements of the plant carbon budget. The specific leaf weight (dry weight per unit leaf area) may be a physical manifestation of the leaf carbon budget if it accurately reflects the relative rates of net CO_2 uptake and assimilate export.

Maximum rates of photosynthetic CO_2 uptake by young leaves reported in the literature surveyed by Brown et al. (1972) vary from 20 to almost 70 mg dm^{-2} hr^{-1}. Maximum photosynthetic rates of alfalfa canopies are reported at 7 to 9 g CO_2 m^{-2} hr^{-1} (Thomas and Hill, 1949; King and Evans, 1967; Wilfong, Brown, and Blaser, 1967). Dark respiration rates determined by the same workers range from 0.3 to 2 g CO_2 m^{-2} hr^{-1}. Calculations based on these and other measurements have yielded maximum dry matter accumulation, or crop growth rates, on the order of 20 to 25 g m^{-2} day^{-1} (Thomas and Hill, 1937a; Thomas and Hill, 1937b; Nelson and Smith, 1968; Hunt, Moore, and Winch, 1970).

Net carbon dioxide exchange (NCE) rate of alfalfa has

been studied in relation to light intensity, leaf age and position in the canopy, temperature, soil moisture, and potassium nutrition. Uptake of CO_2 by young individual leaves is light-saturated at about 3000 foot-candles (32.4 klux), while entire canopies become light-saturated at about 8000 foot-candles (86.4 klux) (Brown, Cooper, and Blaser, 1966; King and Evans, 1967; Wilfong et al., 1967; Pearce et al., 1969). Light saturation of CO_2 assimilation by alfalfa has also been expressed in units of radiant flux density, namely 0.12 cal cm^{-2} min^{-1} for single leaves (Gaastra, 1962) and 0.45 to 0.80 cal cm^{-2} min^{-1} for canopies (Thomas and Hill, 1937a; Gaastra, 1962). Unfortunately, precise comparison of these findings is not possible because foot-candles and cal cm^{-2} min^{-1} are not readily inter-converted.

Photosynthetic capacity declines with leaf age and depth within the canopy (Brown et al., 1966; Fuess and Tesar, 1968; Pearce, Brown, and Blaser, 1968; Wolf and Blaser, 1971; Wolf and Blaser, 1972). Other evidence indicates that photosynthetic CO_2 assimilation has a broad temperature optimum (Thomas and Hill, 1949; Murata, Iyama, and Honma, 1965), is affected relatively little by soil moisture shortage (Murata, Iyama, and Honma, 1966), and generally decreases as leaf potassium content decreases from 5 to 0.5 percent of dry weight (Cooper, Blaser, and Brown, 1967).

Though specific investigations of translocation in alfalfa stems or petioles have not been reported, there is no reason to doubt that most photosynthate is exported from fully expanded leaves for storage and utilization elsewhere in the plant. Brown et al. (1972) cite unpublished data of D. D. Wolf which show a large increase in starch concentration of alfalfa leaves during the day, followed by a decline at night. Holt and Hilst (1969) and Lechtenberg, Holt, and Youngberg (1971) reported similar diurnal changes in starch content of alfalfa leaves. Since there was no increase in other carbohydrates corresponding to the decline of starch at night, this decline was attributed to export of carbohydrate from the leaves.

Chatterton, Lee, and Hungerford (1972) observed diurnal variation in specific leaf weight (SLW) of alfalfa, with SLW increasing from a morning low to an afternoon high. They interpreted changes in SLW as an indication of the photosynthate production-translocation balance. Increasing SLW during the day is evidence that photosynthate production exceeds export from the leaves. Chatterton (1973) has further reported that NCE rate and SLW vary inversely during the course of a day implying that end-product inhibition of photosynthesis occurs in alfalfa. Brown et al. (1972) cited unpublished data of Wolf supporting the hypothesis that photosynthesis can be inhibited in the early afternoon by starch accumulation

during the morning.

Studies of partitioning of assimilates in alfalfa have emphasized the seasonal demands for growth by the shoot and the root. In a review of this topic, Brown et al. (1972) state that shoots apparently have priority for photosynthate early in the growing season, while root growth appears to increase later in the season. When season or cutting schedule dictate rapid shoot growth, translocation of assimilates to the roots is decreased (Wolf, 1967; Pearce, Fissel, and Carlson, 1969) and total available carbohydrates in the roots decline (Smith, 1962). However, when assimilation overtakes requirements for shoot growth, carbohydrates again begin to build up in the roots.

Data are limited on the partitioning of assimilates among organs of the shoot. Wolf and Blaser (1971) provided evidence that $^{14}CO_2$ assimilated by the upper portion of alfalfa shoots either remains there or moves to the crown and roots. In their review Brown et al. (1972) point out that during seed development, crown buds as well as seeds appear to be strong sinks for assimilates. They suggest that photosynthates are the primary source for developing seeds, and stored carbohydrates in roots are the primary source for new basal shoots.

It is not difficult to speculate that chemical plant regulators could affect the carbon budget. They might do so, for example, by controlling the amount of assimilatory

material or the development of sinks. Little information is available on the effects of plant regulators on photosynthetic CO_2 assimilation. Hew, Nelson, and Krotkov (1967) applied either IAA or GA to decapitated soybean shoots, then allowed the leaves to assimilate $^{14}CO_2$. There was no effect on the rate of photosynthesis. However, it was reported that both IAA and GA increased the rate, total amount, and pattern of ^{14}C-sucrose translocation.

The pattern of translocation of assimilates has been altered in several species by auxin-harbicides. Translocation of labeled photosynthate to bean roots was enhanced by picloram (Leonard, Glenn, and Bayer, 1972). Abnormal accumulation of ^{14}C-assimilates in mature leaves resulted from herbicidal dosages of amitrole and 2,4,5-T on red maple and white ash (Leonard, Bayer, and Glenn, 1966) and 2,4-D on beans (Leonard, Donaldson, and Bayer, 1968). Transport of ^{14}C-assimilates from leaves to grape clusters continued under treatment with picloram or 2,4-D, whereas translocation toward the roots was disrupted (Leonard, Weaver, and Glenn, 1967). Blomquist and Kust (1971) reported that neither ethephon nor SADH affected the translocation pattern of labeled assimilates in soybean. There was some indication that plants treated with ethephon transported more ^{14}C-assimilates to pods at the axil of the fed leaf.

As already mentioned, there is evidence for product

inhibition of photosynthesis in alfalfa (Chatterton, 1973). Limiting translocation rate is one factor which could cause accumulation of the products which may inhibit photosynthesis. Effects of plant regulators on translocation therefore merit continued attention.

The objectives of this research were to determine what influence, if any, two synthetic plant regulators exert on 1) dry matter accumulation and partitioning in an alfalfa canopy, 2) NCE rate, 3) export of labeled carbohydrate from primary leaves, and 4) specific leaf weight. Evaluation of genotypic differences in growth response to the plant regulators was a fifth objective.

MATERIALS AND METHODS

Dry Matter Accumulation Studies

Plant material was a one-year-old stand of 'DuPuits' alfalfa located on Woodburn silty clay loam soil near Corvallis, Oregon. Inter-row spacing was 20 cm.

Plant regulators were applied in aqueous foliar sprays on 13 June 1973 when the first growth of the season was estimated to be at the 50 percent bloom stage of development. Experimental plots were 10 meters in length and 3 meters wide. SADH was applied at a rate of 1120 g ha^{-1} in a spray volume of 636 l ha^{-1}. TIBA rates were 17.5, 35, and 70 g ha^{-1} applied in a volume equivalent to 1272 l ha^{-1}. Control plots were sprayed with water. All spray liquids, including the control, contained 0.4 percent non-ionic adjuvant.

During the flowering period a honeybee colony was placed adjacent to the plots. Endemic pollinators were also present.

At intervals during the period from plant regulator application to crop maturity, samples comprising 20 shoots cut at the soil surface were taken at random from each plot. Samples were separated into 1) stems, 2) leaves, 3) racemes, and 4) pods, if any existed. More closely defined, these fractions were: 1) principal stem and its branches, 2) all leaves including petioles, 3) racemes of any stage of development from buds to bare peduncles from

which all flowers had abscised, and 4) visible pods of any stage of development including the seed within and vestigial floral parts still attached to pods. Peduncles supporting pods were placed in the raceme fraction. Plant fractions were dried to constant weight at 90 C in a forced-air oven, then weighed. On 11 September 1973 a forage harvester was used to remove all aboveground alfalfa from a 9.2 m^2 area within each plot. Total plant mass and grams of clean seed were determined from this harvest, following drying for ten days at approximately 35 C.

Net Carbon Dioxide Exchange (NCE) Study

Four genotypes of alfalfa served as the plant material. Genotype 72-12 is a selection from 'Vernal' made by R. V. Frakes and co-workers in Umatilla County, Oregon, in 1972. Genotypes 466, 529, and 759 are selections obtained from a regional alfalfa nursery at Reno, Nevada. The four genotypes were grown in a controlled environment, treated with plant regulators, and subsequently monitored to determine their NCE rates under illumination.

Specifically, plants were potted in soil with nutrient solution supplied daily by an automatic subirrigation system (Stanwood, Phillips, and Chilcote, in press). Growth conditions were a 14-hour photoperiod at a radiation flux density of 12,000 microwatts cm^{-2} (400-700 nm) and a temperature of 20 $\pm$ 2 C. Temperature during the 10-hour dark period was 16 $\pm$ 2 C. Concentration of CO_2 in ambient

air varied from 320 to 380 volumes per million (vpm) during the course of these experiments. Plants were maintained through several cycles of shoot removal and regrowth prior to the study. Plant regulators were sprayed on the foliage of selected plants when shoots were about 40 cm in length and initial raceme buds were appearing. Three treatments consisting of SADH (2000 ppm), TIBA (50 ppm), and a control (water) were applied as mists with an atomizer. Each treatment was administered to each genotype. Between 3 and 20 days after treatment NCE rate under illumination was monitored on three individual shoots from each combination of plant regulator and alfalfa genotype. Each shoot represented one observation. An observation of NCE rate was obtained as follows. The shoot to be monitored was positioned at a standard distance from the overhead light source in the controlled environment chamber by supporting its pot on a ringstand. The uppermost 22 cm of the shoot was enclosed in a sealed acrylic cuvette. Ambient air was conditioned to a near-constant temperature of about 22 C and relative humidity of 55 to 60 percent, then passed through the cuvette containing the alfalfa shoot. Two simultaneous and continuous samples of this airstream were taken, one each immediately upstream and downstream from the cuvette. These paired sample airstreams flowed through a low temperature condensor to remove water vapor and then an infrared gas analyzer for determination of $\Delta[CO_2]$, i.e.,

the difference in CO_2 concentration between the two sample airstreams. As soon as a steady state $\Delta[CO_2]$ was obtained, the main airstream was diverted through rotameter to measure air flow rate. The shoot was removed from the cuvette, the terminal 22-cm portion detached, and fresh weight determined. All leaflets on this portion of the shoot were detached and then photo-copied. Leaflets and the remainder of the shoot were dried separately to constant weight in a forced-air oven at 90 C, then weighed. Leaf area was determined by planimetry of the leaflet photo-copies.

This procedure provided data on $\Delta[CO_2]$, the rate of flow of air past the shoot, and the amount of assimilatory plant material involved. From these data NCE rates were calculated. Appendix I explains the necessary calculations. Notes on light measurement are provided in Appendix II for readers interested in light units other than those reported in this text.

Flap-Feeding of Labeled Glucose

Potted plants of the genotype 72-12 were cultured in the same manner as described in the preceding section. When shoots had reached a length of about 50 cm and flowers of the most advanced racemes were beginning to open, the plant regulators TIBA (50 ppm) and SADH (2000 ppm) were each applied as aqueous foliar sprays. Similar plants sprayed with water served as controls. Seven days after

application of plant regulators, one primary leaf from each treatment was chosen as a site for administering a solution of uniformly labeled glucose. Such leaves were selected for uniformity in both apparent physiological age and physical location on the shoot in relation to potential assimilate sinks such as young elongating branches and developing inflorescences. The median leaflet of selected leaves was immersed in water while a three-sided incision was made in the lamina across and along each side of a first order lateral vein. The reverse flap (Biddulph, 1941) thus created permitted the distal portion of the severed lateral vein to be inserted in a capillary containing 15 microliter of 0.01 M D-glucose-^{14}C with total activity of 1.5 microcurie. Flap-feeding of the leaflet took place for two hours. At the end of this time period the capillary was removed and the shoot bearing the fed leaf was severed, immersed in liquid nitrogen for 30 minutes, and stored at -22 C while awaiting subsequent extraction. Frozen shoots were dissected, and pieces separately extracted for 90 minutes in 80 percent ethanol at 90 to 100 C. Resulting extracts were built up to a volume of 2.5 ml each. One-ml aliquots of this were added to 10 ml of liquid scintillation solvent made up of toluene and 100 percent ethanol (2:1), 0.6 percent 2,5-diphenyloxazole (PPO), and 0.01 percent 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP). Radioactivity was

determined using a Beta Mate II liquid scintillation counter. Extracts containing ^{14}C-activity were compared with sucrose, glucose, and fructose by descending paper chromatography. A solvent system of n-butanol:ethanol: water (4:2:1) was employed and sugars subsequently detected with aniline phthalate reagent. Activity of ^{14}C on chromatograms was located using a Packard model 7200 radio-chromatogram scanner with recording ratemeter.

Specific Leaf Weight Study

Plant material for this greenhouse study of specific leaf weight change in response to plant regulators was a group of several hundred potted alfalfa plants vegetatively propagated from a single plant designated 72-7. This genotype was selected from 'Apex' in Umatilla County, Oregon, in 1972. Fluorescent lamps supplemented incident sunlight and extended photoperiod to a length of 14 hours. Temperatures approximated 24 C during the day and 13 C at night.

One third of the plants were treated with a foliar spray of 50 ppm TIBA and 0.4 percent X-77 adjuvant. Another third received 2000 ppm SADH plus adjuvant. The remaining control plants were sprayed only with water containing adjuvant.

Determinations of specific leaf weight were begun three days after foliar sprays were applied. Leaves were sampled at four-hour intervals. The third fully-expanded

primary leaf, counting basipetally from the shoot apex, was detached from five randomly selected shoots in each of the three treatments. Leaves were photo-copied, dried to constant weight at 100 C in a forced-air oven, and weighed. Leaf areas were determined by planimetry of the photo-copies.

Comparative Study of Alfalfa Genotypes

Potted plants of three genotypes, C-84, 466, and 759 from the regional alfalfa nursery at Reno, Nevada, served as the plant material. Controlled environment cabinets provided two distinct environments for growth. One environment was characterized by light/dark air temperatures of 21 $\pm$ 1 C/10 $\pm$ 1 C, the other by light/dark temperatures of 28 $\pm$ 1 C/12 $\pm$ 1 C. Radiation flux density in the 400 to 700 nm wavelength range was near 6,400 microwatts cm^{-2} in both environments. Photoperiod was 14 hours in both cases. Relative humidity of the air, although not measured, was less in the higher temperature cabinet.

After two weeks of preconditioning in these environments the foliage of the potted plants was clipped to a uniform height of about 8 cm. When regrowth was approximately 12 cm high, sprays of SADH at concentrations of 100, 500, 1000, or 2000 ppm and TIBA at 10, 50, 100, or 200 ppm were applied until foliage was thoroughly wet. After 54 days the aboveground plant material was removed from each pot, dried to constant weight, and weighed.

RESULTS AND DISCUSSION

Dry Matter Accumulation

Figure 2 shows the course of dry matter accumulation during the period from mid-bloom to pod maturity. The dry weight of 20 shoots, averaged over all treatments, increased from about 75 g immediately after regulators were applied (15 June) to about 110 g at harvest on 7 September. Note that apparent decreases in 20-shoot dry weight during the first 40 days after plant regulator application (Figure 2) are considered to be due to sampling variation. Real decreases are ruled out since dry weight of the stem fraction of the same 20-shoot samples showed corresponding decreases (data not shown). In theory, dry weight of the stem fraction increases continually throughout the growing season.

In general, the rank of plant regulator treatments according to amount of dry matter in a 20-shoot sample was the same at 40 and 86 days after treatment application. The two treatments with the most dry matter per sample on day 40 accumulated the most additional dry matter after that time. The average dry weight increment per sample after day 40 was 46 g for plots treated with TIBA at 35 g ha^{-1}, 40 g for SADH, and 30 to 35 g for other treatments, including the control.

Figure 3 shows the general pattern of dry matter partitioning among four shoot fractions: 1) stems,

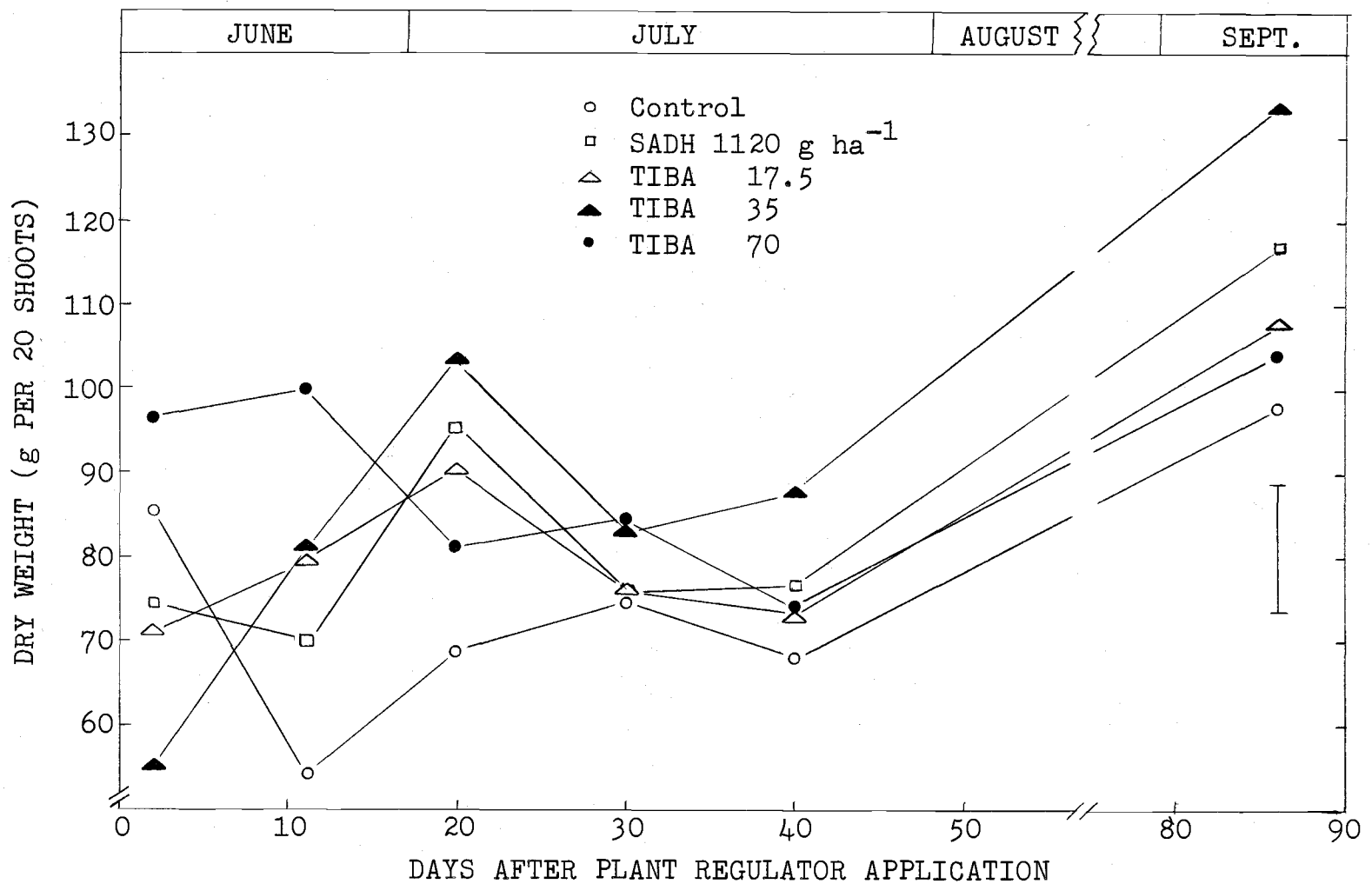


Figure 2. Seasonal course of total 20-shoot weight as affected by plant regulator application. Vertical bracket indicates standard error of the mean at date of harvest.

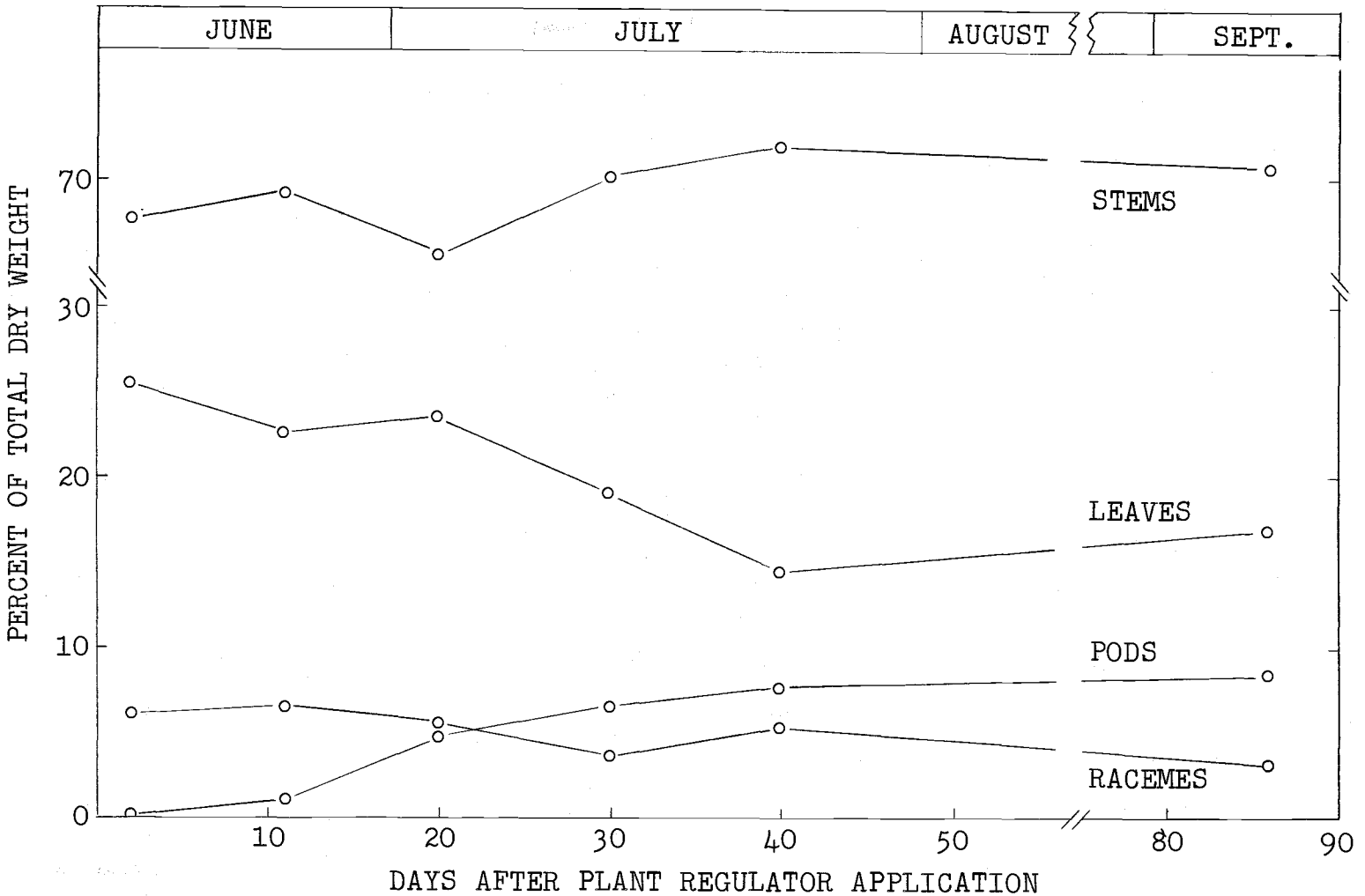


Figure 3. Partitioning of dry weight of 20 shoots into stem, leaf, raceme, and pod fractions.

2) leaves, 3) racemes, and 4) pods. The data plotted are means of all plant regulator treatments. This time interval, largely occupied by seed development, was characterized by a marked decrease in the leaf fraction and a marked increase in the pod fraction. The decrease in the leaf fraction represents abscission of senescent leaves, not a transfer of dry matter to other plant fractions. The fraction of dry weight in stems and in racemes underwent only minor changes.

The effect of plant regulator application on the mass of dry matter accumulated in pods is illustrated in Figure 4. Dry matter in pods was first notable on 24 June, 11 days after plant regulators were applied. Accumulation was most rapid between 20 and 30 days after treatment but apparently continued until harvest. Alfalfa treated with TIBA at either 17.5 or 35 g ha^{-1} accumulated more than twice as much dry matter in pods as did the control. SADH application also resulted in increased pod weight in a 20-shoot sample. In a dry matter accumulation study in soybeans, the fraction of the total dry matter in the form of reproductive structures was increased by treatment with TIBA (Greer and Anderson, 1965).

Figure 4 shows absolute pod weights, while Figure 5 expresses these data as a fraction of the total dry weight in a sample. This clearly reveals the period of most rapid increase in pod weight lasting for approximately 30 days

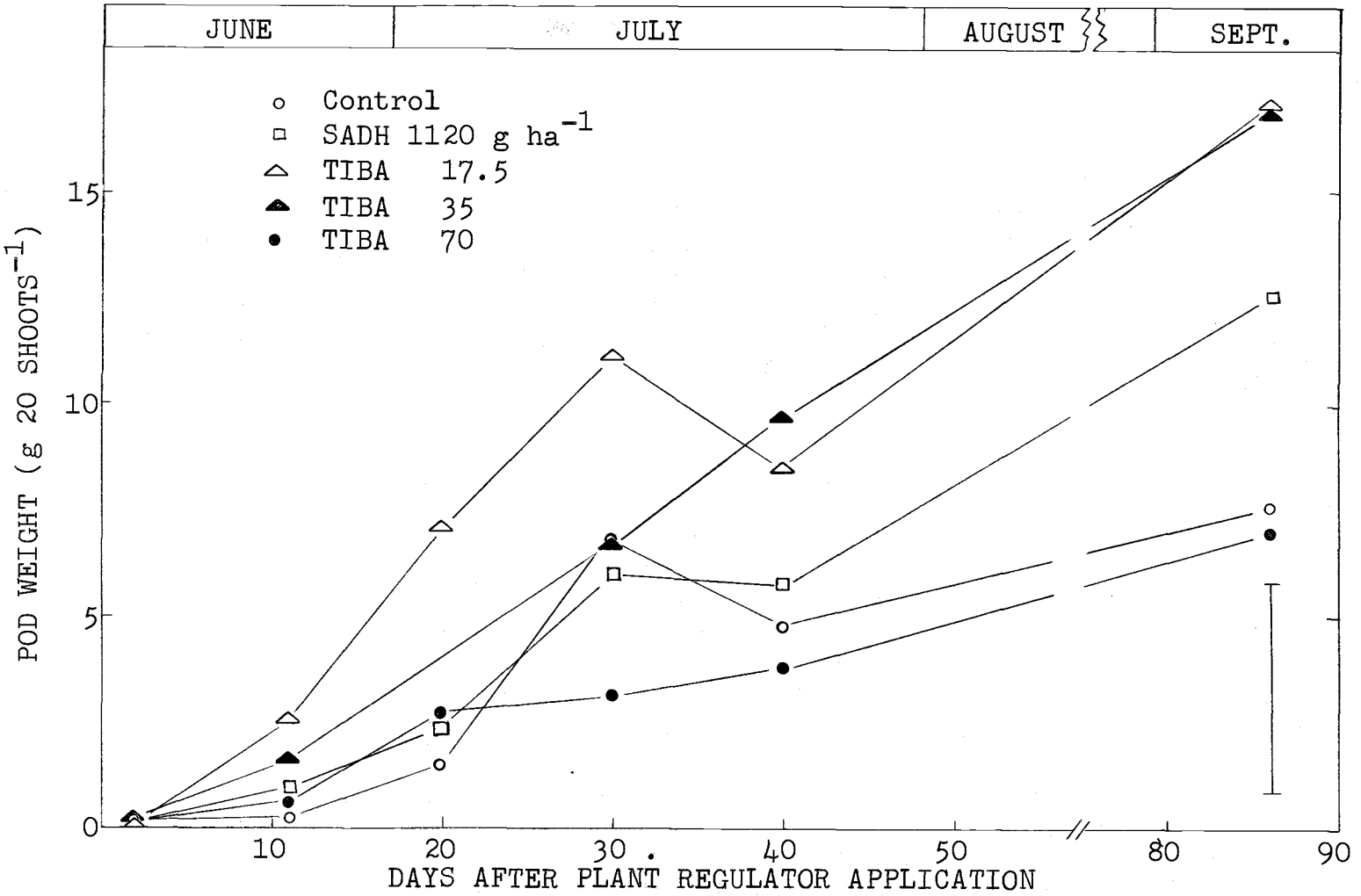


Figure 4. Seasonal course of pod weight per 20 shoots as affected by plant regulator application. Vertical bracket indicates standard error of the mean at date of harvest.

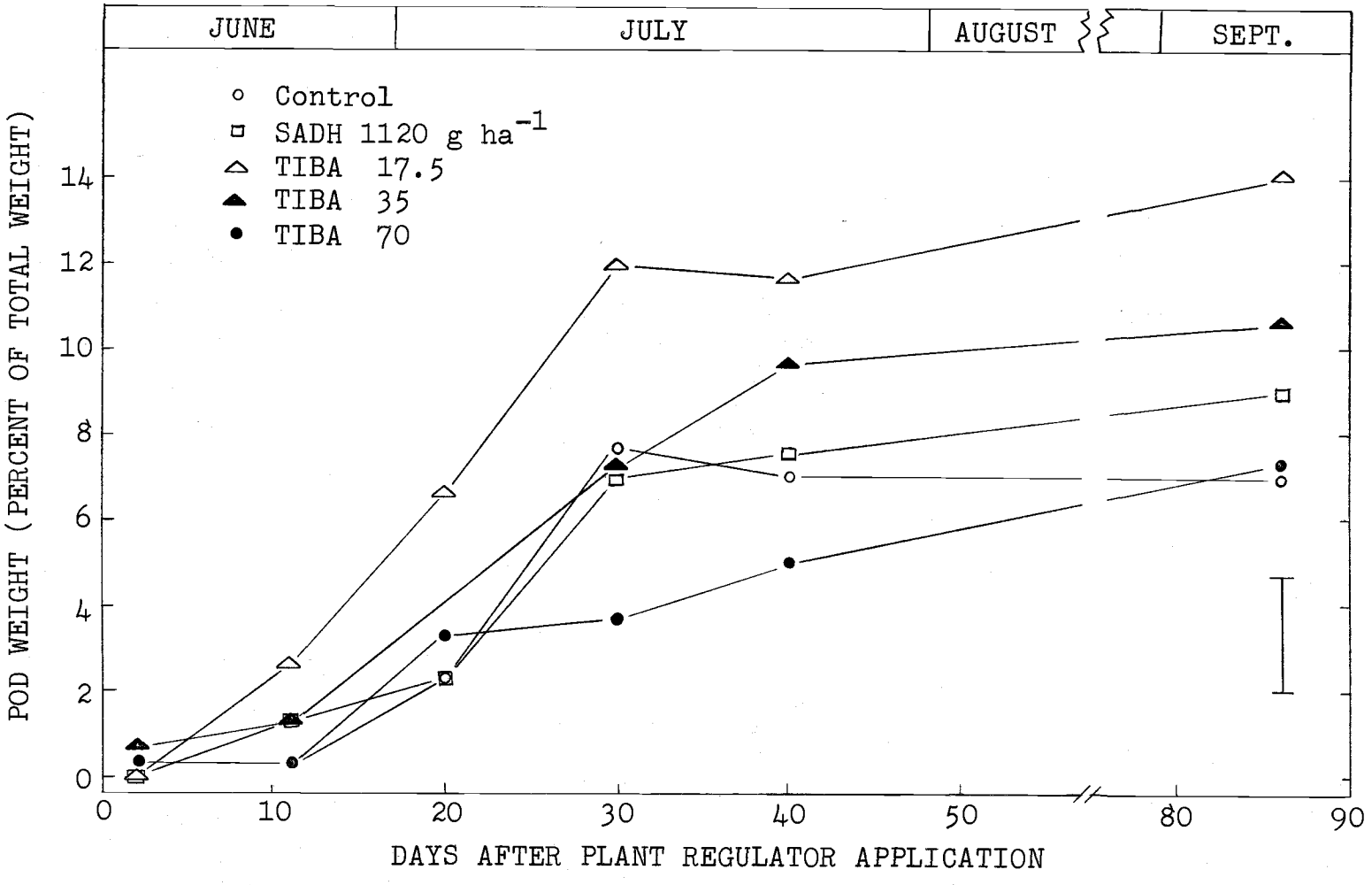


Figure 5. Seasonal course of pod weight expressed as a percent of the total dry weight of 20 shoots. Vertical bracket indicates standard error of the mean at date of harvest.

after mid-bloom. During this period the weight of pods increased much more rapidly than weight of other above-ground organs. After about 30 days the rate of pod weight increase was distinctly less. Thus, dry weight increase of alfalfa pods and the seeds within them conforms to the typical sigmoid growth curve. Turner and Turner (1957) observed a similar pattern for seeds of pea, another legume.

The rank of plant regulator treatments according to the fraction of dry matter found in pods on the final sample data (Figure 5) is similar to their rank according to the ratio of seed mass:total mass (Table 7) as determined from the harvest on 11 September. This indicates that the 20-stem samples were representative of the canopy, at least in the latter part of the season.

The data also provide insight into the relative mass of pods versus the seed alone. For example, samples from control plots indicate that pods plus seeds represented about seven percent of the total dry matter in a mature canopy (Figure 5), whereas clean seed represented only one percent of total harvested biomass (Table 7).

The yields of seed in Table 7 range from approximately 50 to 150 kg ha^{-1}, much less than typical commercial yields in seed production areas. The location of the plots in the Willamette Valley, not an alfalfa seed producing area, probably is responsible in large part for the low seed

Table 7. Production of seed and total biomass by alfalfa as influenced by plant regulators. Corvallis, Oregon, 1973.

Plant Regulator	Rate of Application (g a.i. ha^{-1})	Dry Matter (kg ha^{-1})		Ratio of Seed Per Total Biomass (%)
		Seed	Total Biomass	
Control	0	57	5852	1.0
TIBA	17.5	144	6187	2.3
TIBA	35	124	6382	1.9
SADH	1120	113	6902	1.6
TIBA	70	52	6619	0.8
c.v., %		89	22	
$LSD_{.05}$		125	2248	

yields. Management practices as well as climate were dissimilar to those in areas, such as extreme eastern Oregon, where alfalfa seed yields exceed 1000 kg ha^{-1}.

The data summarized in Table 7 indicate that the best rates of application of TIBA for promoting seed production were in the range of 17.5 to 35 g ha^{-1}. However, the results obtained at Medford (Table 6) suggest that the response to these rates was not different from the response to 70 g ha^{-1}. This points to the problem of variable amounts of exogenous regulators reaching sites of action even when rates of application are similar. Based on Table 7 and the results of Miller et al. (1972) it may be concluded that the optimum rate of application of TIBA to alfalfa seed fields is generally 20 to 40 g a.i. ha^{-1}.

Net Carbon Dioxide Exchange (NCE)

The NCE rates of four alfalfa genotypes treated with SADH and TIBA are given in Table 8. The mean value for NCE rate for all shoots treated with TIBA, 20.8, does not differ statistically from the mean NCE rate for untreated shoots, 16.4. All genotypes had mean NCE rates of about 20 mg CO_2 dm^{-2} leaf area hr^{-1} except for genotype 529. Its NCE rate was consistently only about 12 mg CO_2 dm^{-2} hr^{-1}. However, this difference was also statistically nonsignificant.

Genetic makeup exerts a large measure of control over physiological processes, including NCE rate. Thus it would

Table 8. Net carbon dioxide exchange rates of four alfalfa genotypes treated with plant regulators.

Genotype	NCE Rate (mg CO_2 dm^{-2} hr^{-1}) Plant Regulator Treatment			Mean
	SADH 2000 ppm	TIBA 50 ppm	Control	
72-12	14.3	24.6	21.8	20.2
466	20.7	21.0	15.8	19.2
529	12.5	12.3	12.4	12.4
759	17.4	27.5	15.8	20.2
Mean	16.2	20.8	16.4	

No treatment mean differs from Control in same genotype.

Genotypic means do not differ at the .05 probability level.

not be surprising that genotypes vary in their response when attempts are made to modify plant processes with chemical regulators. This principle also applies to cultivars. For example, Fisher and Looney (1967) found that apple cultivars differed in the degree of flower bud induction which resulted from SADH application. The diverse germplasm utilized in alfalfa breeding increases the potential for such interaction between genotypes and applied plant regulators.

The lack of a significant response of net CO_2 assimilation rate to the plant regulators SADH and TIBA complements the findings of Hew et al. (1967) that photosynthesis of soybean seedlings was not affected by exogenous IAA or GA. Halfacre, Barden, and Rollins (1968) reported that application of SADH resulted in significant decreases in NCE of apples. Cultivar differences in response were also noted. Kirkland (1973) found that net photosynthesis of wheat expressed as mg CO_2 per gram dry weight per unit time was increased by seed soaks with CCC. However, when net photosynthesis was expressed on a per plant basis, CCC had no effect. In general, then, applications of plant regulators have not increased NCE rates.

In this study SADH and TIBA had no significant effect, regardless of whether NCE rate was expressed on a per shoot basis or on the basis of area, fresh weight, or dry weight of leaves. Leaf area ($r = 0.42$) and shoot fresh weight

($r = 0.48$) were more closely correlated with NCE rate than were number of leaflets, leaf dry weight, or shoot dry weight.

The possibility exists that responses of alfalfa NCE rate to plant regulators were prevented by light limitation. However, when a single shoot was isolated within the cuvette for these measurements of NCE rate, mutual shading of leaves was minimal. It is assumed that under these conditions the light response curve for solitary shoots is similar to that for individual leaves reported by Gaastra (1962). If this assumption is correct the radiant flux density of 0.1 to 0.2 cal cm^{-2} min^{-1} should not have limited photosynthetic rate in this experiment.

The NCE rate of the terminal 22 cm of a shoot, as measured in the present research, is an integral of the NCE rates of all included leaves. Photosynthetic rates of any leaves more than about ten days old will have begun to decline from the maximum (Pearce et al., 1968). Hence, NCE rates on a shoot basis should be somewhat less than those for young leaves.

Movement of Labeled Carbon from Leaves

Movement of ^{14}C-labeled substances from alfalfa leaves was not affected by SADH or TIBA in one experiment, yet in a duplicate experiment such movement was apparently enhanced by both plant regulators (Table 9). Blomquist and Kust (1971) reported SADH had no effect on translocation in

Table 9. Effect of plant regulators on distribution of ^{14}C activity in alfalfa following two hours of introduction of D-glucose-^{14}C via a leaf flap.

Plant Regulator	Replicate	^{14}C Activity (cpm)		Maximum Distance of ^{14}C Movement (cm)
		Fed Leaflet	Other Plant Parts	
Control	I	420,000	2,200	1.2
	II	220,000	1,200	0.3
SADH	I	320,000	1,800	0.8
	II	180,000	55,050	≥ 16.5
TIBA	I	320,000	4,600	0.9
	II	380,000	7,700	9.3

soybean. This supports the results of the first replicate of the present data. However, in view of the discrepancy between replicates of the present experiment, results are not considered conclusive.

The limited distance of transport during two hours suggests that the flap-feeding technique as employed here may not have been effective in introducing ^{14}C to the translocation system. Other workers (Biddulph, 1941; Trip and Gorham, 1968; Trip, 1969; Hendrix, 1973) have successfully employed the reverse flap technique to supply labeled substances to leaves of bean, soybean, sugar beet, and squash. Each of these species has leaves that are much larger than those of alfalfa. If the dimensions of leaf veins are proportionate to leaf area, then alfalfa may be able to take up lesser volumes of liquid via leaf flaps

than the other species mentioned. In contrast to the transport distances recorded in Table 9, Hendrix (1973) found label distributed 20 cm or more from the site of introduction after 30 minutes of flap-feeding sucrose-^{14}C to squash. Trip and Gorham (1968) first detected label in soybean petiole after 30 minutes, and after 2 hours label had moved about 25 cm from the fed leaf blade.

Although the amount of radioactivity supplied to a leaf flap in this study, 1.5 microcurie, was much less than other workers (Trip and Gorham, 1968) have supplied, there was no difficulty in locating the front of radioactivity, with one exception. In the second feeding experiment, ^{14}C in the SADH-treated plant moved beyond the point where the main stem was severed after two hours of transport. The extent of movement of the ^{14}C front could not be determined in this case.

In most alfalfa petioles the radioactivity gradient was nearly flat (Figure 6). This was also characteristic of flap-feeding experiments reported by Trip and Gorham (1968). Hew et al. (1967) reasoned that the slope of such a profile reflects the rate of translocation. Figure 6 shows that the radioactivity gradient of greatest slope occurred in plants treated with TIBA. In this case, level of ^{14}C activity and distance of transport were inversely related. Yet even this slope is slight compared to that reported by previous authors, for example Hew et al. (1967)

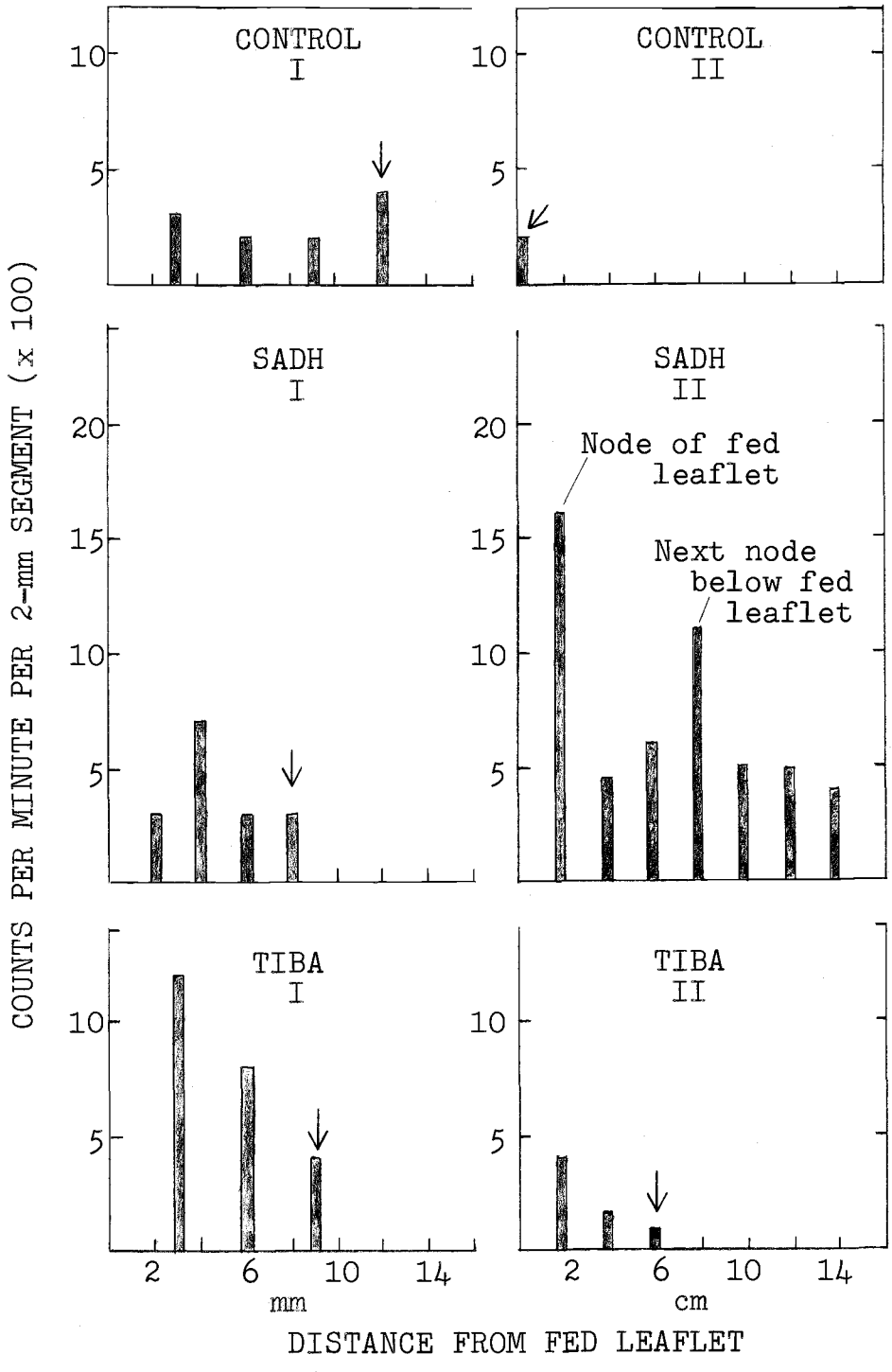


Figure 6. Profile of ^{14}C distribution after two hours of continuous flap-feeding as affected by plant regulators. Arrows indicate positions of ^{14}C front.

who plotted their gradient on a logarithmic scale.

In Figure 6 the profile for an SADH-treated plant (Experiment II) shows peaks at distances of 2 and 8 cm from the fed leaflet. These distances coincided with the positions of nodes. It is not known whether the higher level of activity in these segments was due to an accumulation of labeled substances in the node or due to more tissue being present in node segments. The latter is a possibility since the stem diameter is somewhat greater at the nodes than in the internodes.

When translocated label reached the main stem it always moved basipetally. Even after six hours of continuous feeding no radioactivity was located in the stem internode immediately above the fed leaf. Activity was found in young branches arising from the axil of the fed leaf. In some instances activity was located in both lateral leaflets of the fed leaf, while in others it was found in only one or neither lateral leaflet.

Scanning of chromatograms showed a concentration of ^{14}C activity at a position corresponding to sucrose. Lesser concentrations of ^{14}C were apparently located in fructose and glucose. This is evidence that in alfalfa, as in many other species, sucrose is the principal translocated sugar. There was no evidence that applied regulators influenced the species of sugar translocated.

Specific Leaf Weight

Figure 7 shows that the pattern of diurnal variation in specific leaf weight (SLW) was altered by both SADH and TIBA. Differences were apparent both in mean daily SLW and in the amplitude of the diurnal cycle, i.e., maximum SLW minus minimum SLW. Statistics describing the diurnal variation of SLW during a 48-hour observation period are presented in Table 10. Application of either SADH or TIBA resulted in greater SLW averaged over an entire day, as well as a greater amplitude of variation. A similar result was obtained from TIBA in a preliminary experiment. Figure 7 and Table 10 show that the diurnal maxima were affected more than the minima. This has been interpreted to indicate that all leaves had a common minimum SLW which was reached when export of photosynthates was complete. This point usually occurred between the hours of 2400 and 0800 in the present study. Observed differences in SLW maxima attained during the photoperiod may logically be attributed to differential rates of net synthesis or export of carbon compounds, or both. For example, in leaves treated with TIBA or SADH, net photosynthesis evidently exceeds export to a greater extent than in untreated leaves.

The observed effects of TIBA on SLW and NCE rate are compatible. NCE rate was increased slightly over the control, perhaps leading to the greater SLW maximum during the day. However, it is important to point out that the

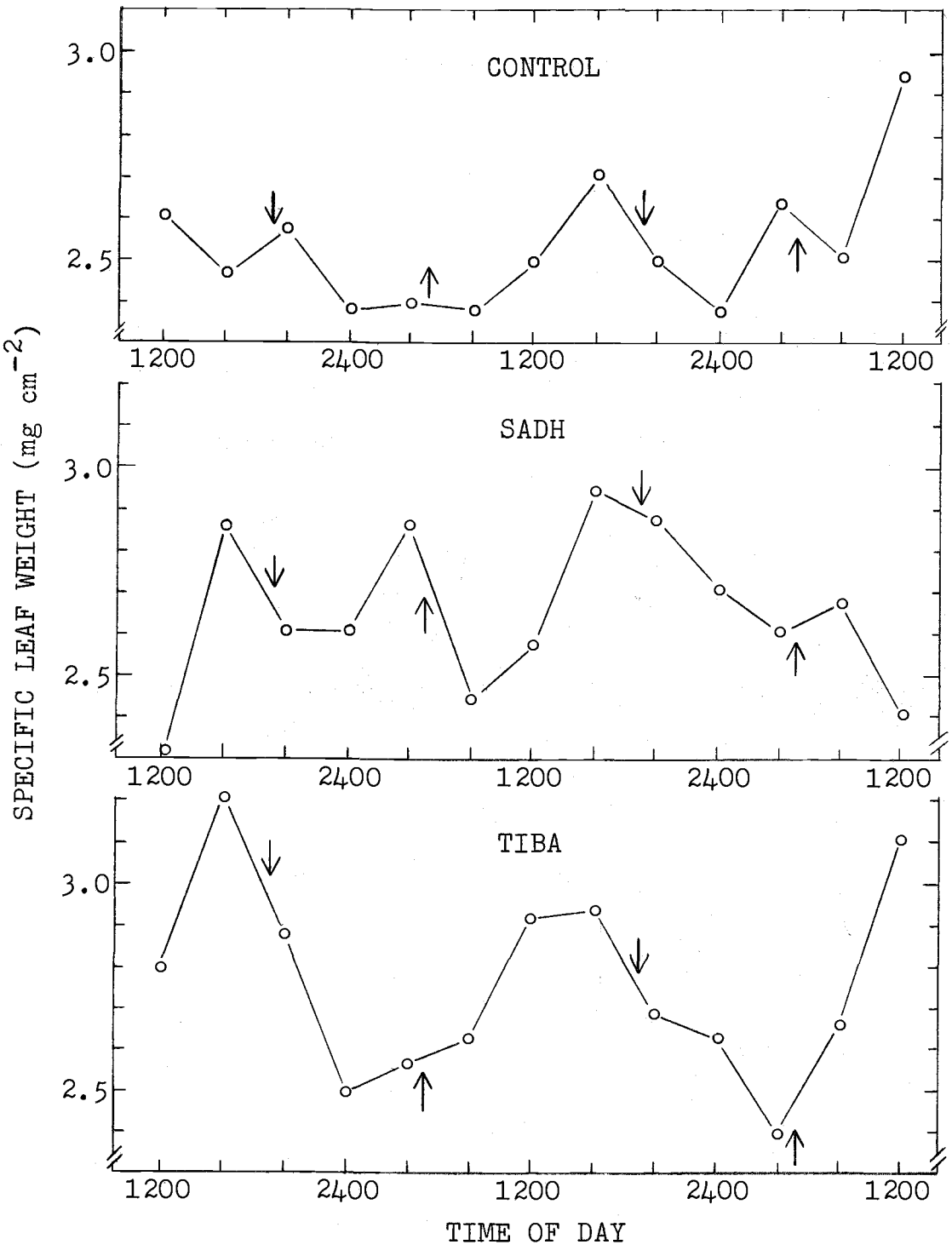


Figure 7. Diurnal variation in SLW of alfalfa treated with plant regulators. Photoperiod begins(↑) at 0500 and ends(↓) at 1900.

Table 10. Diurnal means and extremes of SLW of alfalfa treated with plant regulators.

Plant Regulator	Specific Leaf Weight (mg cm^{-2})			
	Mean	Maximum	Minimum	Amplitude of Variation
Control	2.54	2.76	2.38	0.38
SADH	2.66	2.90	2.35	0.55
TIBA	2.76	3.09	2.45	0.64

genotype of alfalfa in which SLW was studied was different from those in the study of NCE rate.

All treatments in Figure 7 appear to have had maximum SLW at about 1200 to 1600 (after 7 to 11 hours in light). However, the diurnal minima of SLW occurred at different times of day for plants treated with different regulators. Control leaves appear to have remained at a minimum SLW for several hours from about 2400 to 0800. Under treatment with TIBA, SLW was at a minimum from 2400 to 0400 and had increased by 0800. However, it was not determined from these data at what hour between 0400 and 0800 the rise in SLW began. Presumably such a rise would not have been possible until after light was provided at 0500. The minimum SLW of leaves from SADH-treated plants occurred somewhat later in the day, between 0800 and 1200. These plants thus required a longer period of time to decline from the diurnal maximum to the minimum.

Comparative Response of Alfalfa Genotypes

In controlled environment studies there were genotypic differences in dry matter accumulation in response to temperature but not to plant regulators. Genotype C-84 responded very differently to temperature than the other two genotypes, 466 and 759 (Table 11). Accumulation of aboveground dry matter by C-84 was approximately half of that by the other genotypes at 21 C. However, at 28 C, C-84 produced at least as much dry matter as either 466 or 759. Coincidentally, genotype C-84 appeared to differ in water status from 466 and 759. A limited number of plant water potential (ψ_c) measurements with a pressure bomb apparatus indicated that C-84 shoots had a midafternoon ψ_c of about -13 bars compared to -6 to -8 bars for shoots of 466 and 759. These genotypic values of ψ_c were found for shoots at 21 C as well as at 28 C. While ψ_c measurements suggest that C-84 differed from the other genotypes in water status, such measurements do not explain the lesser dry matter accumulation by C-84 in the 21 C regime.

There were no significant differences in aboveground dry matter production due to either SADH or TIBA application (Table 12). Dry matter per plant averaged over both temperature regimes was decreased by all plant regulator treatments except SADH at 1000 ppm. Small decreases in dry weight corresponded with increases in concentration of either SADH or TIBA, again with the exception of 1000 ppm

Table 11. Production of dry matter by three alfalfa genotypes under two temperature regimes.

Genotype	Aboveground Dry Matter Per Plant (grams)		
	21 C	28 C	Mean
C-84	16.9	22.2	19.5
466	30.2	20.6	25.4
759	30.7	17.7	24.2
Mean	25.9	20.2	

Standard error of the mean = 1.5 for interaction means, 1.1 for genotypic means, and 0.9 for temperature means.

SADH. This treatment resulted in a dry weight per plant greater than the control. This was true both at 21 C and 28 C. This concentration of SADH in the foliar spray compares with 1760 ppm applied in the field study of dry matter accumulation. The effect of SADH at 1000 ppm cannot be explained simply on the basis of dosage since 100 ppm SADH also resulted in an apparent increase in dry matter accumulation relative to the intermediate concentration, 500 ppm. Inherent heterogeneity among the plants in this study is indicated.

In summary, foliar application of SADH and TIBA resulted in some interesting effects on the carbon budget of alfalfa. After treatment with these compounds, plots of field-grown 'DuPuits' alfalfa accumulated up to twice as much dry matter in pods as did control plots. Shoots

Table 12. Production of dry matter by alfalfa as influenced by temperature and plant regulators.

Plant Regulator	Aboveground Dry Matter Per Plant (grams)		
	21 C	28 C	Mean
Control	28.8	21.6	25.2
SADH (ppm)			
100	28.2	18.0	23.1
500	23.4	20.4	21.9
1000	31.2	22.2	26.7
2000	26.1	16.8	21.4
TIBA (ppm)			
10	26.1	21.3	23.7
50	24.9	19.5	22.2
100	20.7	23.1	21.9
200	24.0	18.0	21.0

Means do not differ from the Control in the same column at the .05 probability level.

c.v. = 28%

treated with TIBA had a mean NCE rate of 21 mg CO_2 dm^{-2} hr^{-1} compared with 16 mg CO_2 dm^{-2} hr^{-1} for SADH-treated and control shoots. However, these responses were statistically nonsignificant. Specific leaf weight (SLW) exhibited an increased diurnal maximum under treatment with SADH or TIBA, while the diurnal minimum SLW remained equal to that of control plants. All three of these effects are compatible with the hypothesis that TIBA and, to a lesser extent SADH, increased the rate of net photosynthesis in alfalfa. The lack of statistical significance in these results dictates additional research and the need for more homogeneous plant material.

REPRODUCTIVE DEVELOPMENT OF ALFALFA AS INFLUENCED BY PLANT REGULATORS

INTRODUCTION

Two plant regulators which have shown promise for increasing seed production in alfalfa are SADH and TIBA. In order to explain the effect of these chemicals on seed production it seemed logical to study their effect on flowering since: 1) flowers are a prerequisite for seed formation, 2) endogenous regulators almost certainly control flowering (Chailakhyan, 1968; Evans, 1971), and 3) flowering is commonly modified by application of exogenous plant regulators (Weaver, 1972, Chapter 7).

According to Cathey (1964) SADH is a growth retardant to which legumes are particularly responsive. Besides its retarding effect on stem elongation SADH has been reported to promote or induce flowering in various species, for example, apple (Batjer, Williams, and Martin, 1964; Edgerton and Hoffman, 1965; Fisher and Looney, 1967), pear (Griggs and Iwakiri, 1968; Dennis, 1968; Rogers and Thompson, 1968), lemon (Monselise and Halevy, 1964; Monselise, Goren, and Halevy, 1966), blueberry (Shutak, 1968; Hapitan, Shutak, and Kitchin, 1969), Rhododendron spp. (Stuart, 1961; Stuart, 1965; McDowell and Larson, 1966; Ryan, 1972), Bougainvillea (Hackett and Sachs, 1967), and Fuchsia (Ryugo and Sachs, 1969). However, Zeevart

(1966) reported inhibition of flower formation when SADH was applied to Pharbitis nil.

The effect of SADH on flowering in legumes has received less attention. Coyne (1969) found that SADH did not affect earliness of flowering in field beans. Although SADH has been applied to alfalfa and Ladino clover (Calder, Canham, and Fensom, 1973), flowering was not among the responses studied.

The principal observed effect of TIBA on plants is the loss of apical dominance. Increases in branching and promotion of axillary bud development in field crop plants have been attributed to TIBA by a number of workers (Galston, 1947; Cumming, 1959; Anderson, Greer, and Tanner, 1965; Burton and Curley, 1966; Hicks et al., 1967; Bauer et al., 1969; Vetter et al., 1970; Basnet, Paulsen, and Nickell, 1972). The loss of apical dominance in TIBA-treated plants may be due to decreased transport of auxin from the shoot apex. The inhibition of basipetal transport of IAA by TIBA is well documented, including several reports on leguminous systems (Hay, 1956; Zwar and Rijven, 1956; Pilet, 1965; Keitt and Baker, 1966; Winter, 1968).

There are also reports that TIBA influences flowering. Zimmerman and Hitchcock (1942) found that TIBA caused normally vegetative tomato buds to produce flowers. The number of flower buds on photoinduced soybean plants was greatly enhanced by TIBA (Galston, 1947). Gorter (1949)

reported that TIBA caused tomato plants to produce more flowers than untreated plants. Leopold and Guernsey (1954) treated pea seeds with TIBA and found that a very low concentration resulted in occurrence of the first flower at a lower node. Bean plants produced as much as 50 percent more flowers when watered with TIBA solutions (Gorter, 1954). Cumming (1959) stated that under photoperiods of 16 or 24 hours the number of red clover inflorescences formed per mature primary stem was increased by TIBA. Zawawi and Irving (1968) reported nearly doubling the amount of flowering of Kalanchoe by applying TIBA under short day conditions.

Applications of TIBA to the apexes of Impatiens balsamina plants resulted in floral initiation under non-inductive photoperiods (Sawhney, Toky, and Nanda, 1970). The number of pistillate flowers, and consequently, the number of fruits per plant was increased when 25 or 50 ppm TIBA was sprayed on squash melon plants (Saimbhi and Thakur, 1973).

When axillary buds are stimulated, they develop into either branches of the primary stem or inflorescences. Thus an effect of TIBA on flowering might be achieved indirectly via its promotion of axillary bud development. In alfalfa, axillary buds nearest the stem apex nearly always develop into racemes, while more basally located axillary buds generally produce branches. It would be

useful to know if buds in both of these positions are stimulated by TIBA application. If flowers produced in response to applied plant regulators are to contribute to seed production, such flowers must develop in time to allow an effective seed filling period. Hence, the kinetics of axillary bud promotion by TIBA is also of interest.

Experiments were carried out to determine the effect of SADH and TIBA application on raceme initiation in different alfalfa genotypes. Two temperature regimes were selected to create contrasting environments in which to evaluate plant regulator effects. The influence of TIBA on axillary bud development was also evaluated by examining a) the concentration of nucleic acids in alfalfa node tissue, and b) the anatomy of the axillary meristem.

MATERIALS AND METHODS

Studies of Shoot Development and Raceme Initiation

Controlled environment cabinets provided two temperature regimes for alfalfa growth. Light/dark temperatures were 21 ± 1 C/10 ± 1 C in one cabinet and 28 ± 1 C/12 ± 1 C in the other. Radiation flux density in the 400 to 700 nm range was near 6,400 microwatts cm^{-2} throughout a 14-hour photoperiod in both cabinets. Plant pots were filled with water once daily and a mineral nutrient solution once weekly. Relative humidity of the air inside the cabinets was not determined but was obviously less in the higher temperature regime. Evaporation of water from plant pots at 28 C was clearly more rapid than at 21 C. Visible symptoms of plant water stress were never observed, however. At the end of the study, water potential (Ψ_c) of alfalfa shoots was determined with a pressure bomb apparatus. Ψ_c was determined for three shoots per genotype in each temperature regime. The mean Ψ_c for shoots in the 21 and 28 C regimes were 8.9 and 9.7 bars, respectively.

After two weeks of conditioning in these environments the foliage on potted plants of three alfalfa genotypes was clipped to a height of about 8 cm, then allow to regrow to approximately 12 cm in height. At this point regrowth was sufficient to permit selection of three young shoots per plant. Numbered tags were attached to these shoots. SADH at concentrations of 100, 500, 1000, and 2000 ppm and TIBA

at concentrations of 10, 50, 100, and 200 ppm were sprayed on the foliage to thorough wetting. Two potted plants of each genotype were sprayed with a given plant regulator and concentration. This provided 54 plants per temperature regime. These plants were arranged in a completely randomized design within each of the two controlled environment cabinets.

On the day following spray application, the length of each tagged shoot was measured and the numbers of nodes, branches, and racemes recorded. Similar data were collected for the same stems 29 days later, and again after another 25 days. In addition to data from tagged stems, at termination of the experiment the number of raceme-bearing stems per plant was recorded.

The genotypes studied were C-84, 466, and 759, all clonally propagated from plants obtained from a regional alfalfa nursery at Reno, Nevada.

The dosage response of a single genotype, designated 72-12, to a single plant regulator, TIBA, was also examined in a separate study. Genotype 72-12 is a selection from 'Vernal' made by R. V. Frakes and co-workers in 1972. Solutions of TIBA at concentrations of 0, 10, 50, 100, or 200 ppm were applied to two primary leaves at adjacent nodes of selected alfalfa stems. Four random stems in an alfalfa canopy received each level of TIBA. Treated leaves were located at approximately one half the vertical depth

of the canopy.

The alfalfa canopy was grown in a controlled environment chamber having a 14-hour photoperiod with radiant flux density of 12,000 microwatts cm^{-2} (400 to 700 nm) and a temperature of 20 $\pm$ 2 C. Temperature during the 10-hour dark period was 16 $\pm$ 2 C.

The TIBA solutions, each containing 0.2 percent nonionic adjuvant, were spread on the adaxial surface of the leaves with a soft-bristled brush. Application was prior to the appearance of any floral buds. Daily observations of raceme initiation were begun eight days after TIBA application and continued until 34 days after treatment.

Study of Nucleic Acid Content of Nodes

Plants of genotype 72-12 were grown under the conditions stated for the dosage response study. The adaxial surface of the five uppermost fully expanded leaves on selected vegetative stems were coated with either 0 or 50 ppm TIBA solutions using a soft-bristled brush. Stems were harvested seven days after treatment and nucleic acids were extracted and estimated using existing procedures. The cold perchloric acid extraction of ribonucleic acid (RNA) followed by hydrolysis in hot alkali is essentially the procedure of Broughton (1970). Deoxyribonucleic acid (DNA) was then extracted using the method of Ingle (1963).

Specifics of the methods used are as follows. Operations were performed at 0 to 4 C unless otherwise stated.

From several stems within a given treatment, 2-cm stem segments containing the nodes were excised and pooled. Internode segments from the same stems were also pooled. Duplicate samples of nodal or internodal tissue, each about two grams fresh weight, were cut to small pieces, then ground in a mortar with pestle in 0.25 N perchloric acid. Ground material was homogenized (ten minutes in a Sorvall Omni-mixer at 60 volts) in perchloric acid, allowed to stand ten minutes, and then centrifuged at 10,000 g for five minutes. The supernatant, containing nucleotides, sugars, and organic acids, was discarded. The steps from homogenization were repeated twice to complete the extraction. The precipitate, containing nucleic acids, was freed of pigments and lipids by repeated extraction with ethanol: diethyl ether (2:1 by volume) at room temperature. The precipitate was next hydrolyzed in 0.3 N potassium hydroxide at 37 C for one hour. Hydrolysis was stopped by rapid cooling to 0 C (about 10 minutes in an ice bath) followed by addition of 1.0 N perchloric acid. Following repeated centrifugation and washing of the precipitate, RNA in the combined supernatants was estimated by the orcinol reaction catalyzed by cupric ion (Lin and Schjeide, 1969). Finally, DNA was extracted from the precipitate by adding 0.5 N perchloric acid, hydrolyzing at 70 C for 20 minutes, centrifuging, and decanting the supernatant hydrolysate. The extraction was repeated once and DNA estimated in the

combined hydrolysate by the method of Burton (1968).

Anatomical and Histological Studies

Plant materials, growing conditions, and TIBA treatments were identical to those used in the study of nucleic acids in the stem. Two-cm stem segments each including a node were fixed in formalin-acetic acid-alcohol (FAA) and embedded in "Paraplast". Techniques generally were those of Jensen (1962). Longitudinal sections were mounted and stained with safranin-fast green. Additional observations of intact nodes and the structures arising therefrom were made under a dissecting microscope.

RESULTS AND DISCUSSION

Shoot Development and Raceme Initiation

The effect of plant regulators on the development of alfalfa stems, with particular interest in floral initiation, was investigated under controlled environmental conditions. The method gave attention to three factors which may interact with plant regulators to control flowering under field conditions: 1) time, 2) climate, and 3) plant genotype. Since the flowering period of an alfalfa stand may last several weeks under field conditions, floral initiation in a controlled environment potentially occurs on the same time scale. Hence, alfalfa was allowed to develop for approximately ten weeks after clipping. Soil moisture and climatic conditions such as temperature may affect plant response to applied plant regulators. This study therefore included a comparison of two temperature regimes for plant growth. Finally, a cultivar of alfalfa is a composite of an indefinite number of genotypes which conceivably vary in their response to chemical or environmental stimuli. This study allowed a comparison of the responses of three different alfalfa genotypes.

A broad overview of the results of this experiment is given by Table 13. The table indicates which of the three variables were sources of significant variation in observed plant characteristics. Of primary interest is the finding that the plant regulators SADH and TIBA apparently affected

Table 13. Sources of statistically significant variation in stem length, number of nodes, number of branches, and number of racemes determined at 29 and 54 days after plant regulator application. Statistical significance was determined in a three-factor analysis of variance.

Shoot Character	Days After Application	Sources of Variation			
		Plant Regulators	Temperatures	Genotypes	Temp. x Geno. Interaction
Stem length	29		**	**	**
Nodes	29	*		**	**
Branches	29			**	**
Racemes	29		*	**	
Stem length	54			**	**
Nodes	54		**	**	**
Branches	54			**	**
Racemes	54			**	**

* Significant variation at the .05 probability level.

** Significant variation at the .01 probability level.

neither stem elongation nor the numbers of axillary branches and racemes which developed on a stem. This was true 29 days after regulator application and did not change with additional time. Analysis of variance indicated plant regulators to be a significant cause of variation in the number of nodes per primary stem after 29 days. However, treatment means ranged only from 11.0 to 12.5 nodes, and variation due to applied plant regulators was not significant in data collected for the same stems after 54 days. Thus, it seems questionable whether this single instance of statistically significant variation due to plant regulators is biologically meaningful.

Since raceme initiation was the plant response of primary interest, these data were examined further despite the lack of statistically significant variation. Table 14 presents the data on number of racemes per shoot 54 days after plant regulator application. SADH tended to promote raceme initiation in genotype 466 at either 21 C or 28 C. SADH had no consistent effect on raceme initiation in genotype C-84, whereas it apparently inhibited flowering of genotype 759 at 21 C. TIBA also tended to promote flowering in genotype 466 at both temperatures and inhibit 759 at the lower temperature. Fifty parts per million TIBA generally resulted in more racemes per shoot than other concentrations tested. There was no consistent relation between floral initiation and SADH concentration.

Table 14. The number of racemes initiated per alfalfa shoot as affected by plant regulators, temperature regimes, and genotypes. Data were obtained 54 days after plant regulator application.

Plant Regulator	Number Of Racemes Per Shoot For Three Genotypes At 21 C				28 C			
	C-84	466	759	Mean	C-84	466	759	Mean
Control	0.2	2.3	8.2	3.6	2.0	1.0	2.5	1.8
SADH (ppm)								
100	0	15.5	2.7	6.1	0.7	3.0	2.2	1.9
500	0	6.0	2.8	2.9	1.8	4.3	1.7	2.6
1000	0	7.3	2.0	3.1	2.7	7.3	2.8	4.3
2000	0.2	6.7	1.5	2.8	2.2	3.3	3.2	2.9
TIBA (ppm)								
10	0	2.8	4.8	2.6	0	4.5	3.3	2.6
50	0	4.5	5.0	3.2	1.8	2.7	5.5	3.3
100	0	4.2	1.0	1.7	0.2	0.8	0	0.3
200	0	3.7	2.3	2.0	0.8	2.3	3.5	2.2
Mean	0.0	5.9	3.4		1.4	3.3	2.7	

Standard error of the mean for temperature regimes = 0.36

Standard error of the mean for genotypes = 0.44

Figure 8 illustrates the number of racemes initiated versus time for each concentration of SADH and TIBA. Results obtained in different temperature regimes are plotted separately. These data show that: 1) there was not an obvious dosage response to plant regulators, 2) most treatments tended to inhibit raceme initiation at 21 C, while the majority tended to promote raceme initiation at 28 C, and 3) 50 ppm TIBA and 1000 ppm SADH tended to result in greater raceme initiation than that which occurred under most other treatments in either temperature regime.

Although raceme initiation did not always respond quantitatively according to concentration of applied SADH or TIBA, it can be seen that 100 ppm and 200 ppm TIBA were inhibitory compared to 10 ppm and 50 ppm. No pattern was apparent in the dose response to SADH in the 21 C regime. However, 1000 ppm and 2000 ppm tended to be more effective than lesser concentrations in the higher temperature regime. The latter observation concurs with the literature surveyed by Wittwer (1971) which indicates that SADH is generally effective at concentrations of 2000 to 5000 ppm.

Most of the plant regulator treatments elicited a similar response in number of racemes per shoot regardless of temperature regime. Control plants were an exception, initiating fewer racemes under the higher temperature conditions. A possible explanation for this result is that plant water status may have become suboptimal in untreated

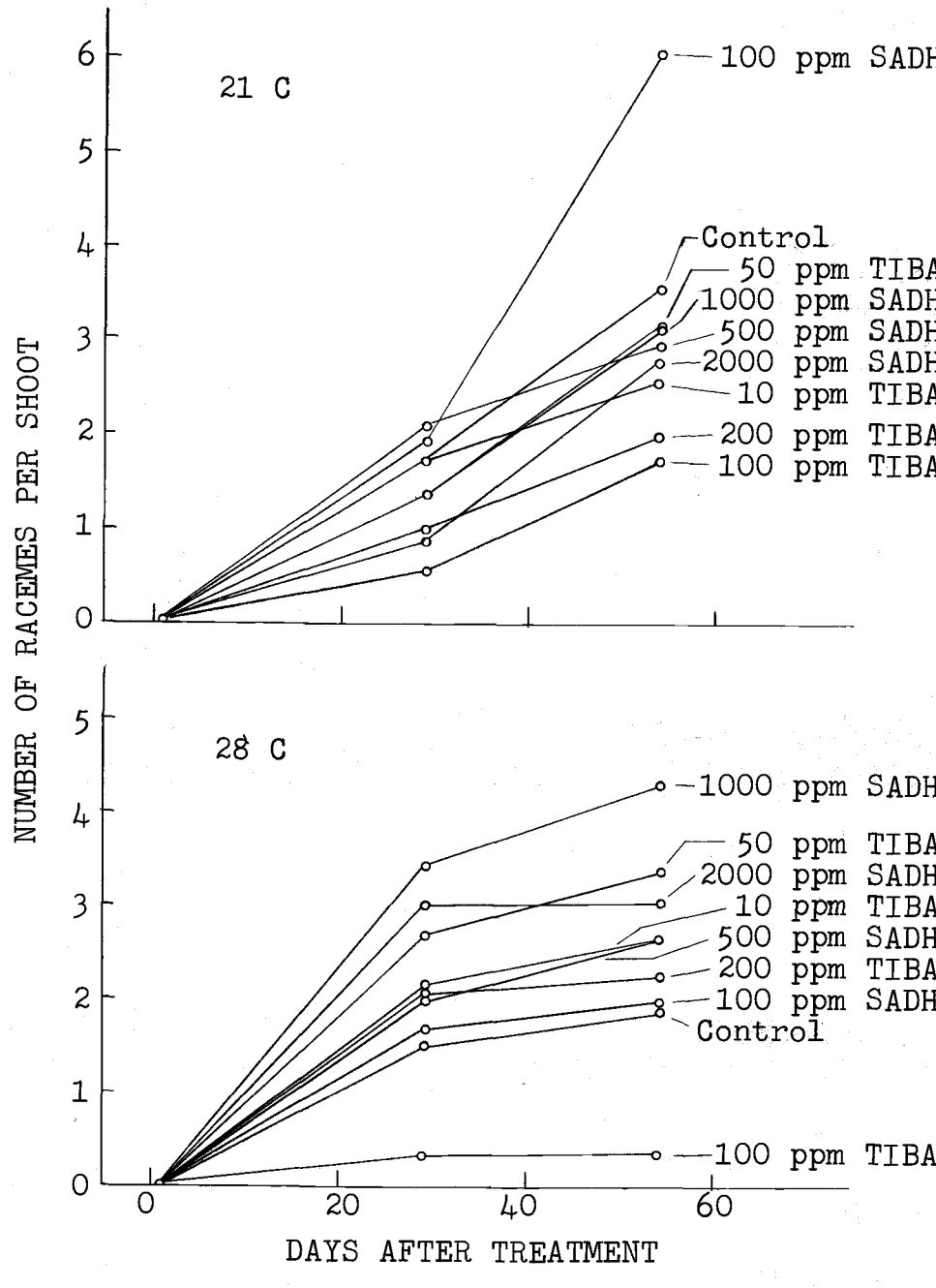


Figure 8. Kinetics of raceme initiation by alfalfa as affected by plant regulator application and temperature regimes.

controls but not in treated plants. SADH and other growth retardants have been found to increase the drought resistance of various plant species (Weaver, 1972).

The analysis of variance compares variation due to treatment, in this case plant regulators, with variation inherent in the plant population. If the ratio of treatment variation to inherent variation is great enough, treatments are considered a significant source of variation. The population of alfalfa shoots on which this experiment was based was very heterogeneous. This explains why even the most noticeable responses in Table 14, namely the apparent promotion of flowering in genotype 466 by both SADH and TIBA, are not statistically significant. The cause of at least part of the heterogeneity in the population of shoots was discovered during the course of this experiment. The shoots that were tagged and from which data were periodically taken were selected about ten days after plants were clipped to a uniform height. Many of these shoots making up the initial regrowth arose from leaf axils at the nodes of old stem bases remaining after clipping. However, it was found that this initial regrowth did not persist and that more vigorous shoots subsequently arose from the crown. Such new shoots arising from crown buds produced numerous racemes and appeared typical of regrowth which occurs under favorable field conditions. The fact that tagged shoots in this experiment included

both shoots from the initial axillary regrowth as well as some originating from the crown undoubtedly contributed to the heterogeneity of the samples.

Variability in plant material was still evident when observations were based upon entire plants rather than individual shoots. Data on the number of raceme-bearing shoots per plant, shown in Table 15, provide an example. These data are not restricted to the tagged shoots only, but represent the performance of whole plants. Although SADH and TIBA tended to decrease the number of raceme-bearing shoots, the effect was statistically nonsignificant.

In view of the recognized action of TIBA in promoting branching, it is of interest to examine the data on production of axillary branches even though plant regulators caused no statistically significant responses. Table 16 summarizes these data for each of the three genotypes. Under most regulator treatments plants produced a number of branches per primary stem equal to or slightly greater than the control plants. Both SADH and TIBA tended to promote branching of genotype 466. There were also instances of apparent inhibition of branching, particularly when SADH was applied to genotype 759. All four concentrations of SADH resulted in fewer branches than untreated controls for this genotype. In general, the concentrations which resulted in the most branches were 1000 ppm of SADH and 50 to 200 ppm of TIBA.

Table 15. The number of shoots per alfalfa plant which bore fully developed racemes as affected by plant regulators, temperature regimes, and genotypes. Data were obtained 54 days after plant regulator application.

Plant Regulator	Number Of Shoots Per Plant Bearing Fully Developed Racemes For Three Genotypes At 21 C				28 C			
	C-84	466	759	Mean	C-84	466	759	Mean
Control	0	3	7	3.3	5	8	6	6.3
SADH (ppm)								
100	0	8	8	5.3	3	3	5	3.7
500	0	4	6	3.3	3	5	6	4.7
1000	0	7	3	3.3	6	5	4	5.0
2000	0	0	3	1.0	4	2	8	4.7
TIBA (ppm)								
10	0	2	8	3.3	2	4	8	4.7
50	0	6	6	4.0	3	2	7	4.0
100	0	4	3	2.3	0	2	5	2.3
200	0	6	2	2.7	0	2	5	2.3
Mean	0	4.4	5.1		2.9	3.7	6.0	

Means do not differ from the Control in the same column at the .05 probability level.

Standard error of the mean for temperature regimes = 0.32

Standard error of the mean for genotypes = 0.39

c.v. = 63%

Table 16. Production of axillary branches of alfalfa as affected by plant regulators and genotypes. Data were obtained 54 days after plant regulator application.

Plant Regulator	Concentration (ppm)	Axillary Branches Per Primary Stem In Genotype			
		C-84	466	759	Mean
Control	0	4.2	5.6	4.2	4.7
SADH	100	4.2	7.8	2.8	4.9
	500	4.9	8.0	3.1	5.3
	1000	4.4	10.2	3.8	6.2
	2000	3.8	5.9	2.2	4.0
TIBA	10	4.4	5.7	4.4	4.8
	50	3.9	8.2	4.9	5.7
	100	4.2	6.0	2.6	4.3
	200	5.0	7.9	4.9	6.0
Mean		4.3	7.3	3.7	

No means differ from the Control in the same column at the .05 probability level.

Standard error of the mean for genotypes = 0.38

c.v. = 78%

Growth and development of alfalfa shoots were influenced by temperature regimes. It is worth noting that plant responses to the two different temperature regimes in this experiment cannot be attributed to temperature per se, but only to the combined effects of temperatures during the photo- and nyctoperiods, water vapor pressure of the air, and soil moisture content. To facilitate discussion, however, the regimes are often referred to simply by their photoperiod temperatures, namely 21 or 28° C.

Table 13 indicates that temperature regimes were a source of significant variation in stem length and raceme initiation at 29 days after plant regulator application, and in number of nodes per primary stem after 54 days. The mean stem length at 29 days in the 21 C environment was 30.4 cm and at 28° C it was 35.9 cm. Thus, stem elongation during the first six weeks following clipping proceeded more rapidly under the warmer conditions. It is interesting to note that this temperature effect of growth was not reflected in the differentiation of nodes or branches during the same time period. The mean number of nodes per primary stem was 11.8 and 11.5, and the mean number of branches per stem was 3.8 and 4.3, in the 21 and 28° C regimes, respectively.

Environment did have an influence on flowering as measured by the extent of raceme initiation within 29 days after regulators were applied. This is evident from

Figure 8 which shows a greater initial rate of raceme initiation at the higher temperature. The mean number of racemes per shoot after 29 days was 1.4 and 2.1 for the 21 and 28 C regimes, respectively.

By the time observations of shoot development were made again after 54 days, there were no longer any significant differences in stem length due to temperature, the mean being 37 cm for both environments. Stems which developed in the 21 C regime tended to have a greater number of nodes, branches, and racemes than those which developed at 28 C. However, only the difference in number of nodes was significant.

The final variable in this experiment to be discussed is plant genotype. As seen in Table 13, genotype was a source of highly significant variation in all shoot characteristics studied, both at 29 and 54 days after treatment. Genotype C-84 was primarily responsible for this variation, as shown by the data in Table 17. Shoot development of C-84 consistently did not keep pace with that of two other genotypes under the lower temperature conditions. However, in the higher temperature regime the development of C-84 was comparable to the other genotypes, except in terms of floral initiation. C-84 still produced fewer racemes. This noticeable effect of temperature regime on only one genotype, C-84, is reflected in the analysis of variance. The temperature-genotype interaction

Table 17. Shoot development of three alfalfa genotypes under two temperature regimes, as measured by stem length and number of nodes, branches, and racemes.

Shoot Character	21 C			28 C		
	C-84	466	759	C-84	466	759
At 29 days						
Length (cm)	22.0 b	35.3 a	33.9 a	37.9 a	35.7 a	34.2 a
Nodes	9.7 c	12.8 a	12.8 a	11.6 b	11.2 b	11.8 b
Branches	2.2 d	5.7 a	3.6 bc	5.7 a	4.8 ab	2.4 cd
Racemes	0.0 c	2.1 ab	2.2 ab	1.3 b	2.5 a	2.4 a
At 54 days						
Length (cm)	24.0 c	49.0 a	38.5 b	37.8 b	37.9 b	35.3 b
Nodes	10.0 d	15.7 a	14.0 b	12.2 c	12.0 c	12.0 c
Branches	2.7 d	8.6 a	4.3 c	6.0 b	6.0 b	3.0 cd
Racemes	0.0 d	5.9 a	3.4 b	1.4 cd	3.2 b	2.7 bc

Means in the same row followed by a common letter are not statistically different at the .05 level according to Duncan's multiple range test.

was found to be a highly significant source of variation in all shoot characteristics except the number of racemes initiated within 29 days of plant regulator application (Table 13).

The kinetics of initiation of racemes and branches in this experiment are shown in Figures 9 and 10, respectively. These are plots of the data on racemes and branches found in Table 17. Figures 9 and 10 both serve to illustrate the differences among genotypes, not only in terms of the ultimate production of racemes or branches but also in terms of the time frame within which initiation occurs. Most notable is the fact that after 29 days genotype 466 continued to initiate racemes and branches at or near its original rate. The rate of initiation by the other two genotypes usually declined more than did that of 466. The prolonged high rate of floral initiation by 466 accounted for the difference in number of racemes produced by 466 and 759. During the first 29 days of the study raceme production by these two genotypes was essentially equal. Perhaps a longer period of active flowering is inherent in certain alfalfa genotypes such as 466. If so, the action of plant regulators or environment might be sustained in such genotypes, and any promotion of flowering thereby enhanced.

The underlying reasons for the differential responses of the three genotypes to the temperature regimes imposed

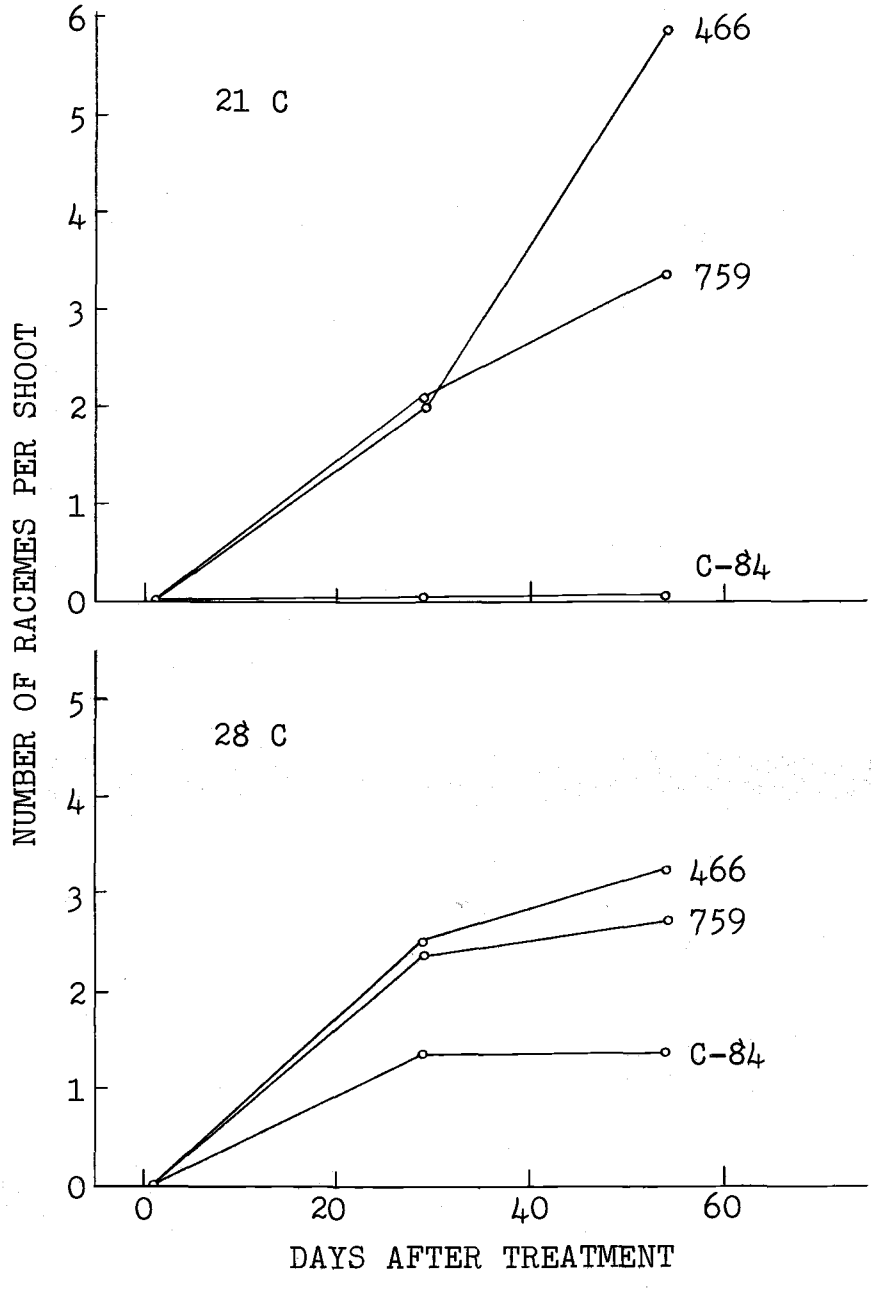


Figure 9. Kinetics of raceme initiation by three alfalfa genotypes under two temperature regimes. Each point is a mean of all plant regulator treatments, which were applied on day zero.

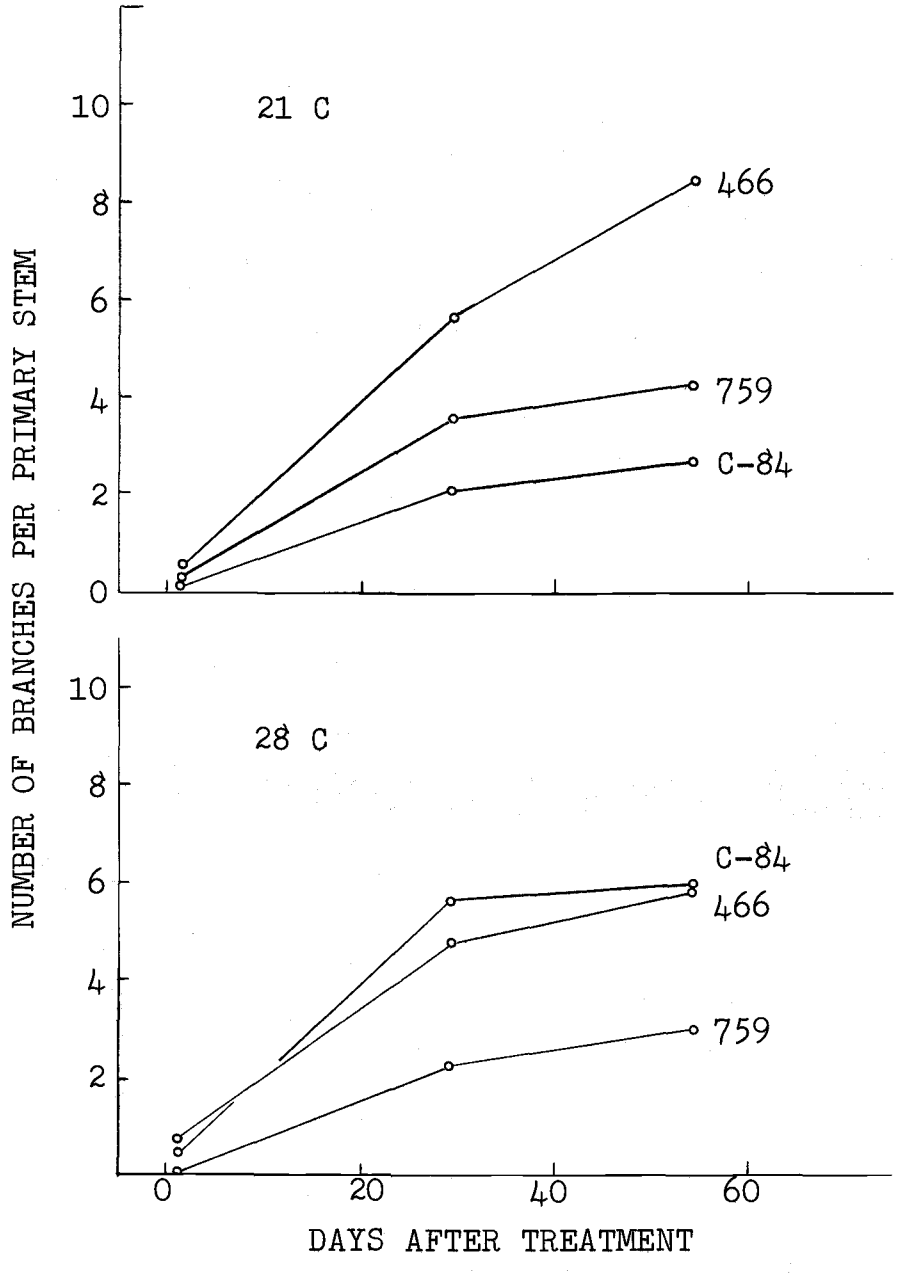


Figure 10. Kinetics of axillary branch initiation by three alfalfa genotypes under two temperature regimes. Each point is a mean of all plant regulator treatments, which were applied on day zero.

are not known. It is possible that genotype C-84 attains its maximum growth rate at a temperature between 21 and 28 C, while 466 and 759 reach maximum growth rate at some temperature below 21 C. Thus, growth and development of the three genotypes were not significantly different so long as temperature conditions were satisfactory for all three as, for example, in the 28 C regime. However, the conditions of the 21 C regime limited the growth rate of C-84 but not those of 466 and 759. Such genotypic variability in response to environment is the basis for the natural selection of plant species and ecotypes to suit specific habitats.

In this experiment there was a tendency for genotypes to also vary in their response to applied plant regulators. The implications of this variability, should it be verified, are that crop cultivars, which are composed of many genotypes, can be expected to respond differently to plant regulators. Responses may vary by degree or by nature. The latter case, (e.g., in Table 14 at 28 C, TIBA tended to inhibit flowering of genotype C-84 but tended to promote flowering of genotype 466), may be explained on the basis of effective concentration of the regulator. It is accepted that endogenous growth regulators can promote or inhibit plant processes depending on their concentration. Physiological or morphological differences among genotypes could result in different concentrations of applied

regulators in the plant even though application is uniform over all genotypes.

The results of the study of dosage response to TIBA are shown in Figure 11. Ten parts TIBA per million resulted in the maximum primary stem length and number of nodes (Figure 11, A and B). Above 10 ppm there was an inverse relationship between concentration and growth. At 200 ppm stem growth was clearly inhibited. The number of axillary branches per primary stem was not promoted or otherwise affected by any concentration of applied TIBA (Figure 11, C). This is in agreement with results obtained with other genotypes (Table 16) but contrasts with reports of increased branching in other crops. Although numbers of branches were not affected, the combined length of all branches on a stem was greatly enhanced by TIBA at 10 ppm and to a lesser extent at 50 and 100 ppm (Figure 11, D). Indeed the growth of axillary branches was more than quadrupled by 10 ppm TIBA as compared to the untreated controls. It appears that under the conditions of this experiment the effect of TIBA is to promote the growth of axillary branches which normally exist but often do not elongate. The significance of this promotion of branch growth depends on the extent to which such branches become reproductive.

In Figure 12 the number of racemes borne on axillary branches and on the primary stem is shown in response to

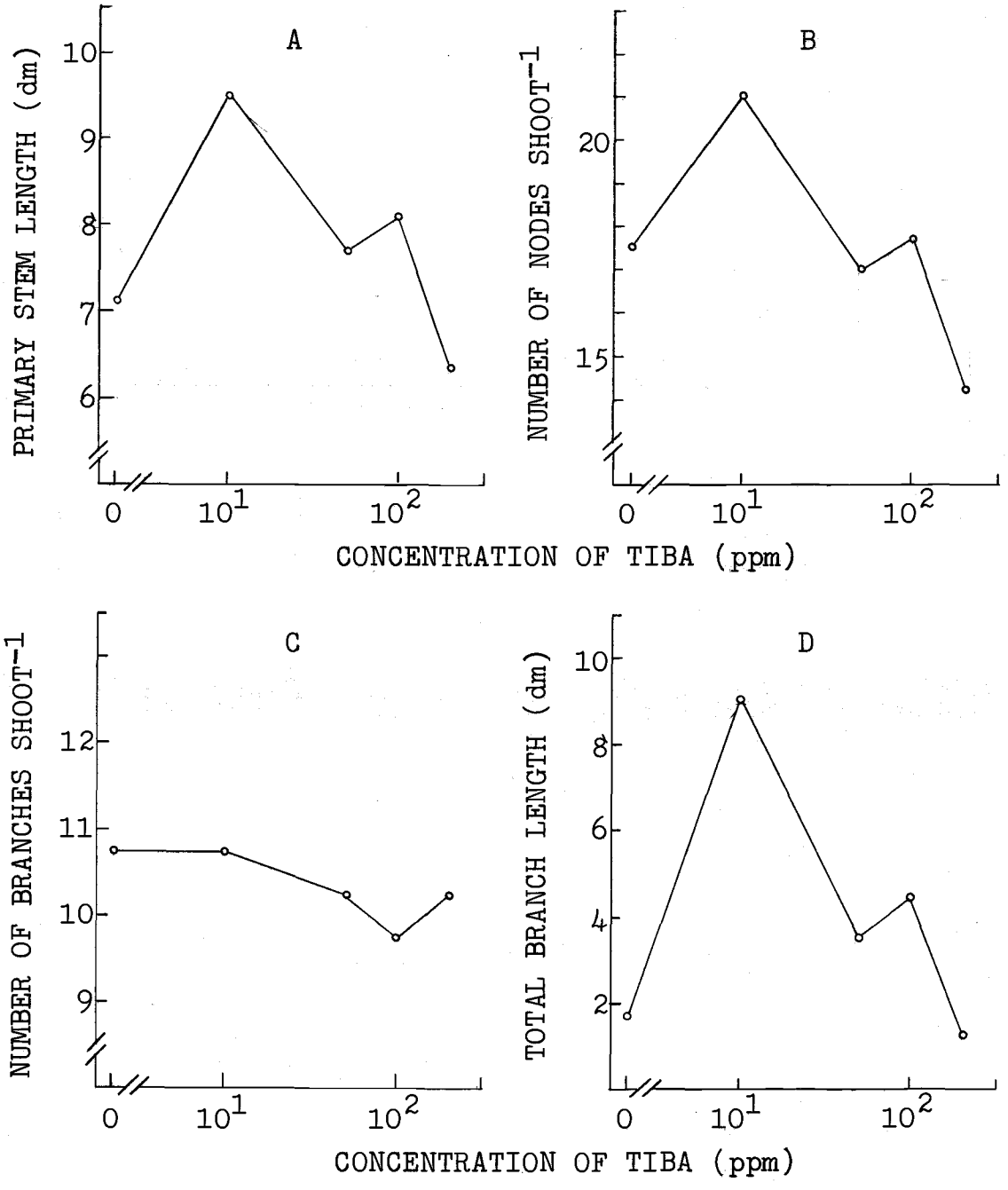


Figure 11. Shoot development of alfalfa in response to concentration of TIBA applied prior to raceme initiation. Observations made 34 days after application of TIBA to two primary leaves per shoot.

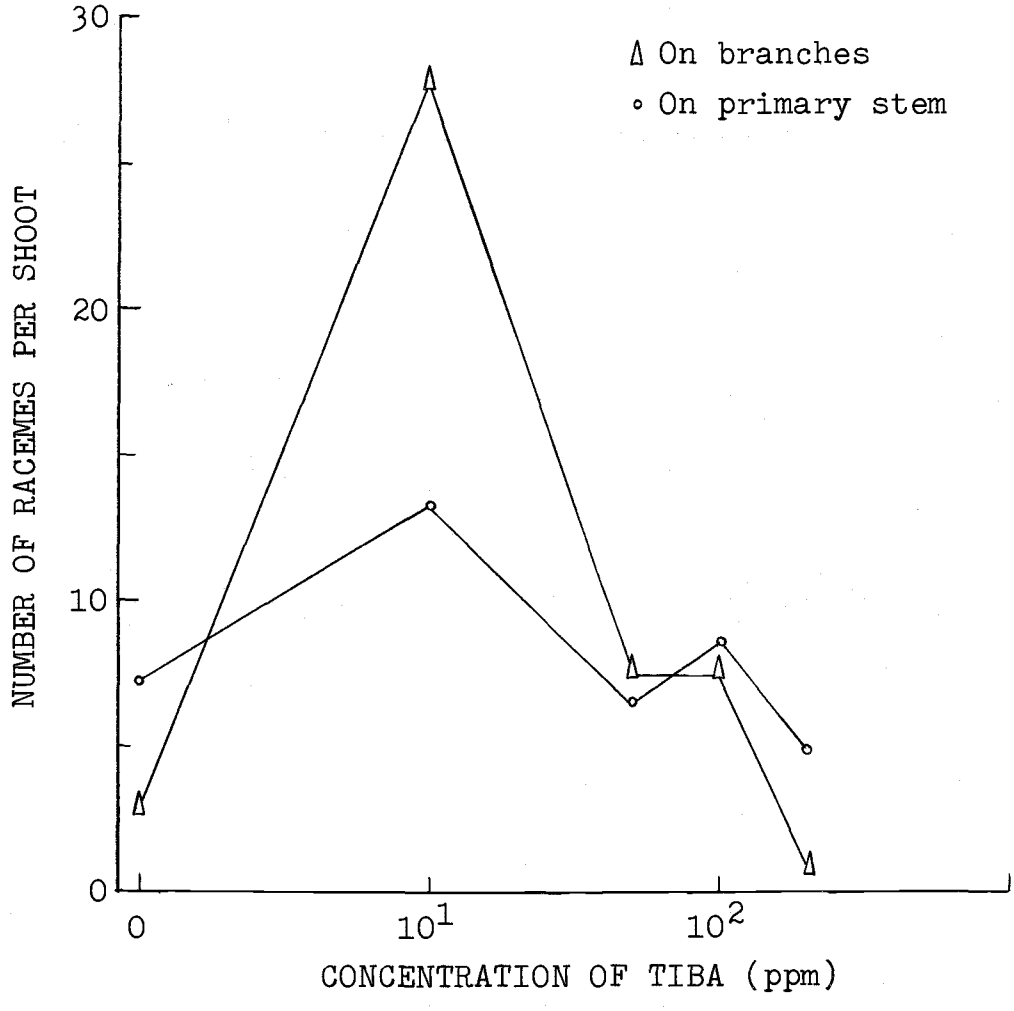


Figure 12. Initiation of racemes on primary stems and on branches of alfalfa in response to concentration of applied TIBA. Observations made 34 days after application of TIBA to two primary leaves per shoot.

concentration of applied TIBA. Like Figure 11, this is based on observations made 34 days after TIBA application. Stems treated with 10 ppm TIBA produced a large number of branch-borne racemes, accounting for nearly 70 percent of the total racemes initiated. On untreated stems less than 30 percent of racemes were located on branches. Figure 12 clearly shows that inflorescence initiation on branches was promoted not at the expense of initiation on the primary stem but in addition to it. Since raceme initiation on the primary stem was enhanced by the same concentration of TIBA which promoted the growth and floral initiation by branches, it appears that once axillary branches are stimulated to elongate they begin to function reproductively much like primary stems. Thus, the stimulation of short, vegetative branches on alfalfa stems by low concentrations of TIBA may make possible substantial increases in total raceme initiation and, consequently, the potential for seed production.

The preceding discussion has established that the effect of TIBA on initiation of racemes is exerted within 34 days after application at the pre-bud stage. The pattern of raceme initiation during that 34 day period is shown by Figure 13 with separate curves for primary stems and branches. Comparison of Figures 13A and 13B reveals that initiation of racemes generally began two to four days earlier on the primary stem than it did on branches. As

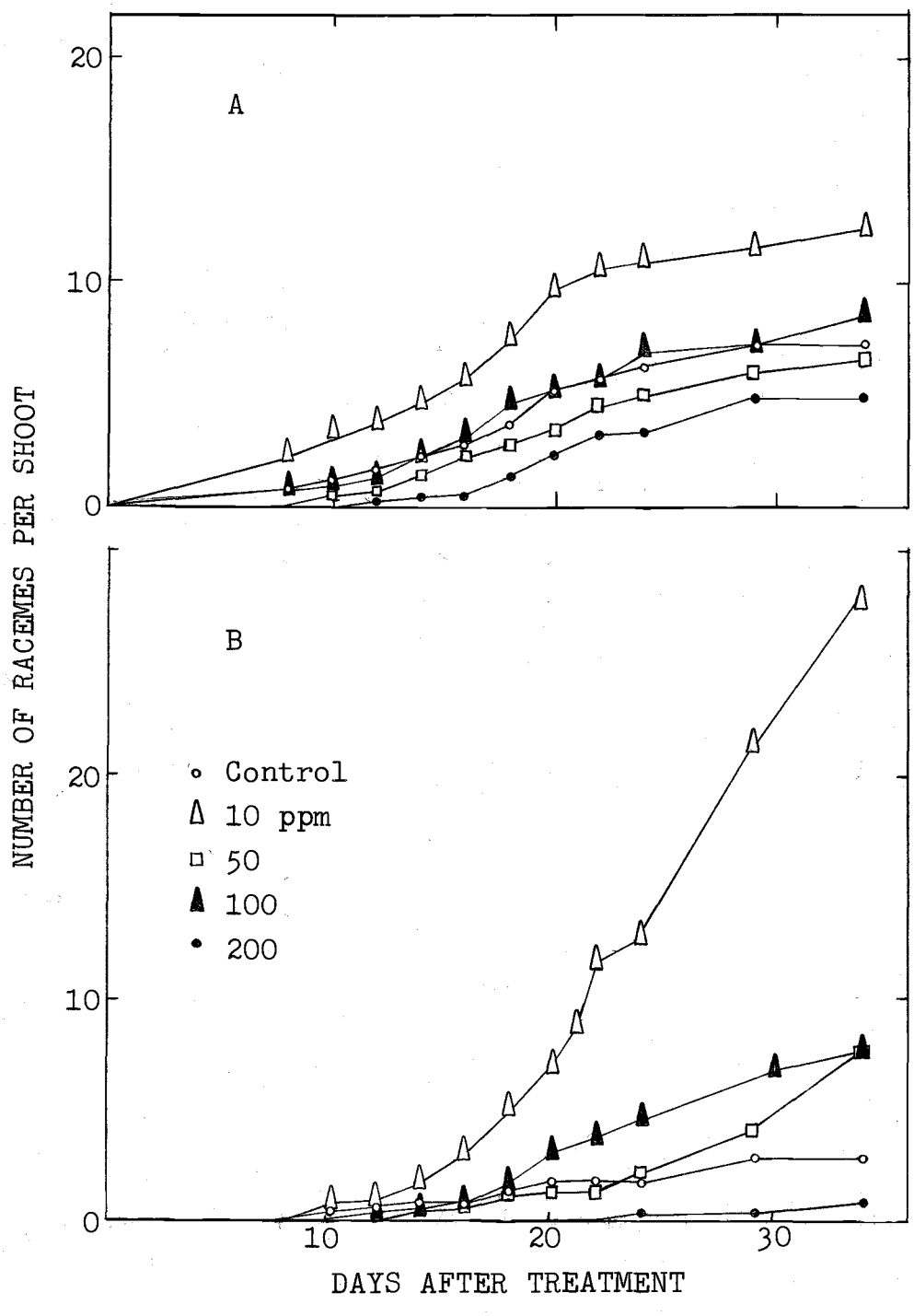


Figure 13. Kinetics of raceme initiation on (A) the primary stem and (B) axillary branches of alfalfa in response to TIBA.

early as the eighth day there were more racemes noted on primary stems treated with 10 ppm TIBA than on untreated stems. Promotion of branch-borne racemes by 10 ppm TIBA was not apparent until about day 16. Floral initiation on both the primary stem and branches was promoted by the lowest concentration of TIBA, yet the kinetics of such initiation differed as shown in Figure 13. While the rate of raceme initiation on the primary stem declined after 20 days, the rate of raceme initiation on branches continued undiminished through the termination of the study on day 34. Thus, the promotion of branch-borne racemes by 10 ppm TIBA was a relatively lasting effect.

Nucleic Acid Content of Nodes

Seven days after TIBA application to leaves of alfalfa the concentration of DNA and RNA in primary stem nodes was greater than in nodes of control plants (Table 18). The nodes sampled exhibited no visible differences in development of axillary buds. Leaves from the axillary bud were visible in the axil of the primary leaf. The increase in nucleic acid content of the node could be interpreted as a sign of activation of the axillary meristem. Goodwin and Mercer (1972, p. 337) state that one of the initial biochemical effects of floral initiation in the shoot apex is the stimulation of RNA synthesis. Evans (1971) mentions in his review of floral induction that increased synthesis of all species of RNA might be an initial event in floral

Table 18. Nucleic acid content of nodal and internodal regions of alfalfa stems in response to TIBA. Each value is the mean of duplicate observations.

Stem Tissue	TIBA Concentration (ppm)	Nucleic Acid Content (percent fresh weight)	
		RNA	DNA
Internode	0	0.098	0.0073
	50	0.116	0.0070
Node	0	0.124	0.0079
	50	0.141	0.0118

evocation. Increased DNA content in buds is an early indication of the transition from vegetative to reproductive growth (Wardell and Skoog, 1973, and references cited). Thus, the data of Table 18 are indirect evidence that a) leaf-applied TIBA evoked some activity in axillary meristems of alfalfa, and b) this activity may have led to the formation of floral primordia.

In the tissue of the stem internodes, RNA content increased while DNA content was unchanged (Table 18). It is possible that TIBA had a general effect on RNA synthesis in all stem tissues, but that this resulted in DNA synthesis only in the axillary meristem, not in internode tissue.

It should be recognized that actual meristematic tissue comprised only a small percentage of the alfalfa node tissue which was analyzed for nucleic acids. The analyses tell nothing about the true concentrations of DNA and RNA in the axillary meristem. Despite the mass of non-meristematic tissue which diluted the samples, the greater RNA content of nodal regions of the stem may be linked to the presence of the axillary meristem. If this assumption is correct, then RNA in the meristem proper must be very concentrated relative to other tissue. If it is further assumed that DNA is likewise very concentrated in the meristem it follows that a substantial change in nucleic acid content of a meristem could be detected as a relatively

modest change in nucleic acid content of the nodal region, such as the changes indicated in Table 18.

Anatomy of Nodes and Axillary Buds

The branches and inflorescences of alfalfa originate from apical meristems situated in the axils of the leaves. In the nascent state these meristems are referred to as axillary buds. Observation of the gross morphology of alfalfa indicates that an axillary bud apparently gives rise to either one or two branches or racemes. When there are two structures both may be branches, both may be racemes, or there may be one of each. Usually one of these structures is more advanced in development than the other. For example, the folded, immature leaves of a very young branch may be visible at the base of a more mature branch which is several centimeters in length (Figure 14). Low-power microscopy of leaf axils bearing a single raceme or branch revealed that there were in fact two structures present. In such cases the difference in stage of development was extreme. Figure 15 depicts such a case, in which a bud less than 2 mm long is situated at the base of a fully developed branch. Dissection of the minute structures verified that these were buds enclosing an apical meristem.

A question arises as to whether the two axillary structures derive from a single, or separate, axillary bud. The author is aware of no reports in the literature on

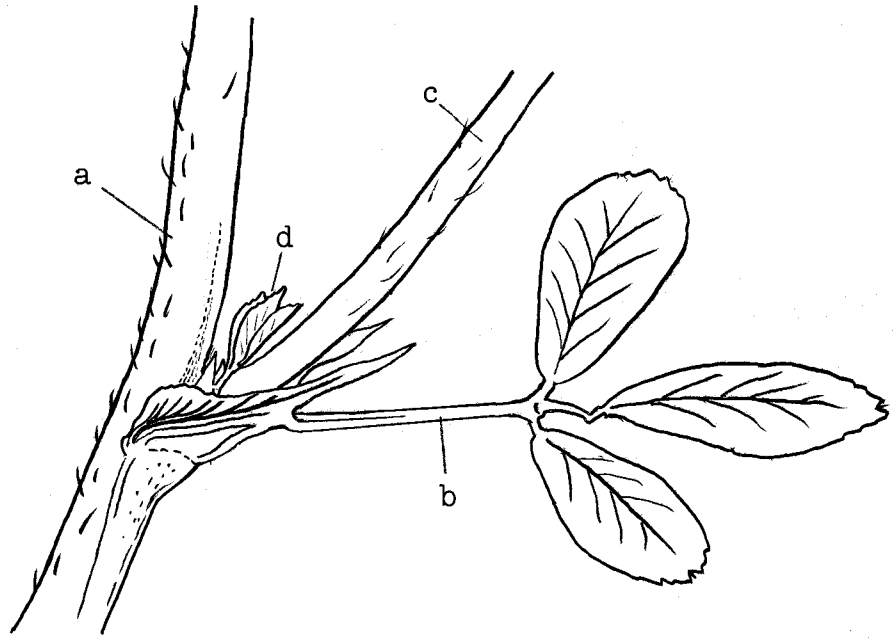


Figure 14. Diagram of a node of alfalfa showing (a) primary stem, (b) primary leaf, (c) well developed axillary branch, and (d) a second emergent axillary branch. Approximately three times natural size.

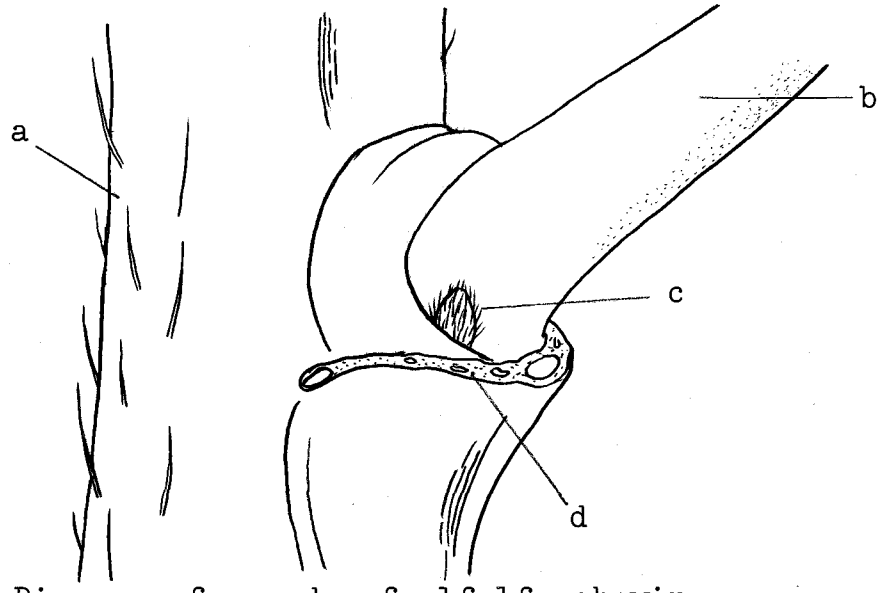


Figure 15. Diagram of a node of alfalfa showing (a) primary stem, (b) well developed axillary branch, and (c) an axillary bud. The primary leaf has been removed, leaving the cut surface (d) at the base of the stipules.

alfalfa that deal with this point. In all specimens examined it was noted that the lesser developed member of a pair of axillary structures was either a branch or simply a bud. In either case an entire apical meristem is included. The more advanced member of a pair of axillary structures was either a branch or a raceme. Presumably such a raceme originates as a primordium on an axillary apical meristem. If it is assumed that the axillary buds are solitary, perhaps the more advanced member of a pair originates as a raceme or branch primordium of the axillary bud and for unexplained reasons develops more rapidly than the remainder of the bud. The remainder of the bud, including the apical meristem proper, would be the lesser developed member of a pair. An alternative hypothesis might be that the axillary apical meristem is dichotomous. This is unlikely since the paired structures at a node usually develop unequally. Further, dichotomy generally occurs in lower vascular plants rather than the angiosperms.

Figure 16 shows a longitudinal section of an axillary bud of alfalfa. This meristem was the apex of a young axillary branch 4 mm in length. The meristem is considered vegetative if an axillary bud primordium is not present in the axil of the youngest leaf primordium. This criterion for the onset of the reproductive state has been established in alfalfa by Dobrenz, Massengale, and Phillips (1965) and previously in white clover by Thomas (1962). It cannot be

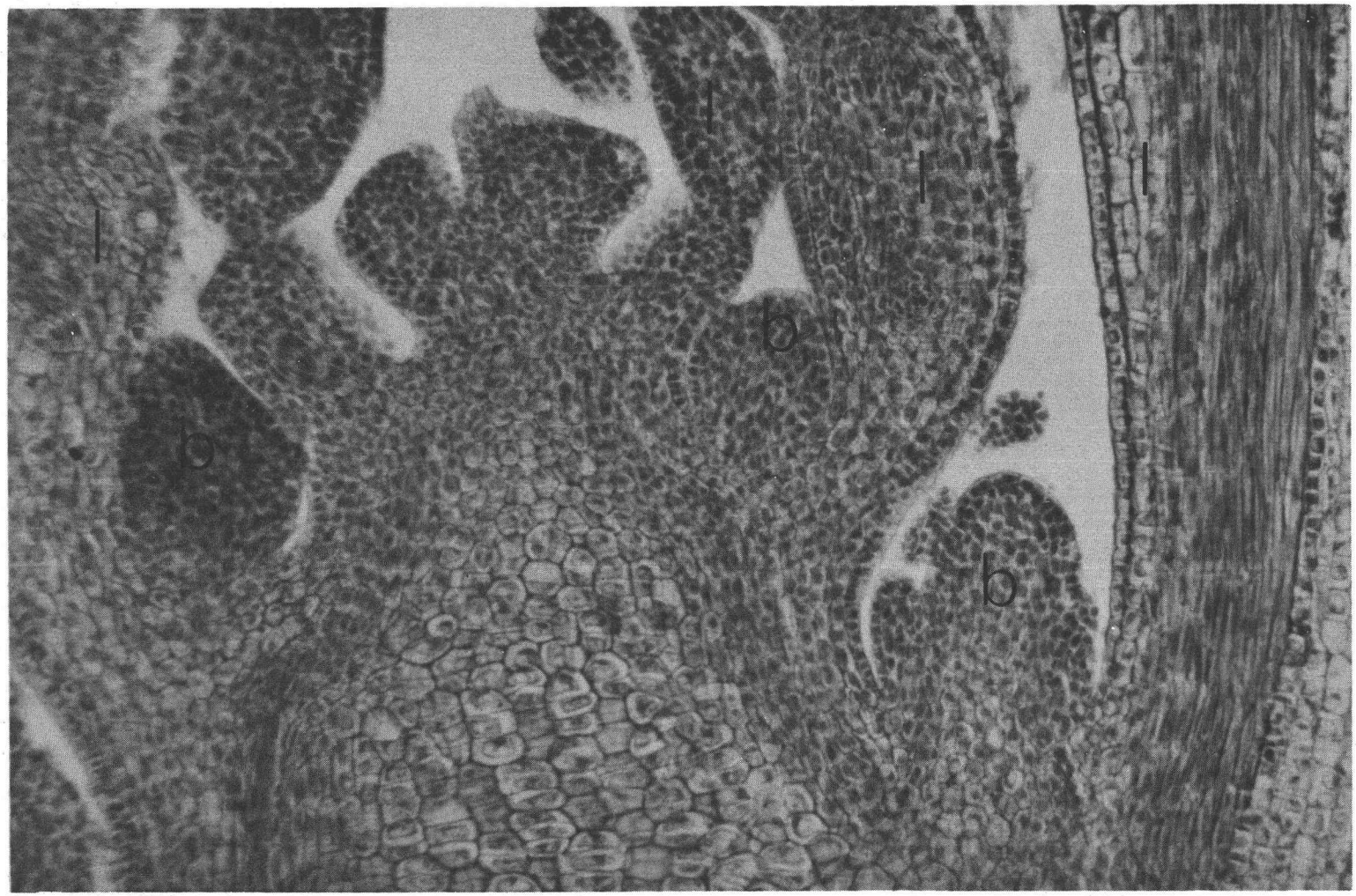


Figure 16. A longitudinal section of the apical meristem of an axillary bud of alfalfa, showing bud primordia (b) in axils of leaf primordia (l). x1100.

determined from sections in a single plane whether this criterion is met by the meristem in Figure 16. However, several axillary bud primordia are visible in the axils of older leaf primordia. Such axillary bud primordia apparently would not have given rise to branches since second-order branches have not been observed on alfalfa grown in controlled environments. Only a few second-order branches have been observed on field-grown alfalfa. It is concluded that axillary bud primordia such as those in Figure 16 develop into raceme primordia. In this context axillary apical meristems may be classified as reproductive as soon as these initiate axillary bud primordia of their own.

The meristem pictured in Figure 16 arose at the node of an untreated control plant. Longisections of such nodes were compared with analagous nodes from plants treated with 50 ppm TIBA. No histological differences were noted. Dimensions of the axillary branch and development of vascular tissue supplying the branch were comparable. Comparison of the apical meristems was not possible, as those from TIBA-treated stems were destroyed during preparation.

In summary, anatomical and histological studies provided no evidence that TIBA, at 50 ppm, affected the transition of axillary buds to the reproductive state. It was determined, however, that primordia which probably become floral structures had been initiated even in control

plants at the time of sampling. Other results show that at this same sampling time the nodal regions of TIBA-treated plants contained more RNA and DNA than those of control plants. This suggests that floral initiation may have been accelerated slightly by TIBA. However, the results in this section of the thesis show that the primary effect of TIBA on flowering was via its promotion of the growth and raceme initiation of axillary branches. Finally it was found that two axillary structures are generally found in an alfalfa leaf axil. This may be important in understanding the phenomena of branching and floral initiation in this crop.

GENERAL CONCLUSIONS

Field experiments in southern Oregon show that the harvestable seed yield of alfalfa can be increased by the application of chemicals which regulate plant growth and development. Succinic acid 2,2-dimethylhydrazide (SADH), TD-6266-R (structure undisclosed, but also a derivative, or derivatives, of succinic acid), and 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB) each provided substantial increases in seed yield of 'Talent' alfalfa. SADH and 2,3,5-triiodobenzoic acid (TIBA) also demonstrated activity when applied to 'DuPuits'. These and other compounds deserve further field testing, particularly in production areas where high seed yields are typical, such as eastern Oregon.

Yield responses to plant regulators were variable from one year to the next. Such variability remains unexplained but may be due, at least in part, to different environmental conditions. Precipitation during the growing season differed markedly from year to year in this study.

Seed yield responses to SADH and TD-6266-R were most closely associated with increased numbers of seeds per pod and pods per raceme. Substantial but statistically nonsignificant yield increases following application of 2,4-DB and TIBA also appeared to be via these two seed yield components. However, the number of seed-bearing racemes per unit land area is a yield component which has not yet been adequately described. Raceme production needs to be

related to area in order to eliminate problems in evaluating the numbers of stems or plants per unit area. Further, controlled environment studies indicated that raceme production was promoted by TIBA applied in low concentration.

Plant regulators were equally effective in promoting seed yield when applied either prior to or at the onset of visible floral development. Other workers (Miller et al., 1972) reported yield responses when regulators were applied still later, at the 50 percent bloom stage. The modes of action of these chemicals are evidently such that alfalfa is capable of responding throughout a range of developmental stages.

Foliar application of SADH and TIBA tended to result in increased accumulation of dry matter in pods and seeds, increased NCE rate, and an increased diurnal maximum for specific leaf weight (SLW). All of these results are accomodated by an hypothesis that the net photosynthetic rate was increased by application of the plant regulators. Although responses were in many instances substantial, units of plant material were sufficiently variable to prevent demonstration of statistical significance. For further studies of NCE rate, the use of leaves selected for uniformity in age and position on a shoot as well as genetic composition might eliminate much of the variability encountered in these studies involving entire shoots. By

thus minimizing variability in plant material it may be possible to either demonstrate the reality of plant regulator responses suggested by these data, or to confirm the conclusion that must be made at this time, namely that neither SADH nor TIBA have a significant effect on the carbon budget of alfalfa as investigated here.

Some of the experiments on plant regulator effects on reproductive development were also complicated by the heterogeneous nature of alfalfa shoots. One source of heterogeneity was the fact that two distinct types of shoots make up the regrowth following top removal. Initial regrowth arose from axillary buds of the stubble, while more vigorous shoots subsequently arose from crown buds.

In other studies confined to the latter type of shoot, it was found that TIBA promoted the growth and development of axillary branches. However, TIBA did not affect the number of branches per shoot. Existing branches which developed in apparent response to TIBA initiated large numbers of racemes, resulting in a highly significant increase in total raceme production. Thus, studies relating to floral initiation are perhaps the most promising approach for future research. Further research should include an investigation of the development of axillary buds of alfalfa. The discovery that two axillary structures, either branches or racemes or one of each, generally occur in a single axil may be significant, particularly if it can

be demonstrated that both such structures can be promoted by plant regulators.

Effective concentrations of SADH and TIBA for use on alfalfa were more closely defined. When applied as a foliar spray to alfalfa, SADH was generally most effective at concentrations of 1000 to 2000 ppm. TIBA was generally most effective at 50 to 100 ppm as a spray, while 10 ppm was most effective when applied directly to leaf surfaces with a brush.

It was also demonstrated that genotypes of alfalfa differed markedly in their growth response to two temperature regimes. Alfalfa genotypes tended to differ in their response to plant regulators. At this point, it is not possible to say to what extent environment conditions interacted with plant regulators in determining genotypic responses. In any event, such variability due to genetic makeup may mean that alfalfa cultivars will differ markedly in their response to applied plant regulators.

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APPENDICES

APPENDIX I

CALCULATION OF NET CARBON DIOXIDE EXCHANGE RATE

Net carbon dioxide exchange (NCE) rates reported in the text were calculated as follows. Measurements of each alfalfa shoot provided data on the net removal of CO_2 from air. The units of these data are volumes CO_2 per million (vpm), or microliters CO_2 per liter of air. Conversion to units of mg CO_2 per liter is achieved via equation (1) which accounts for the volume occupied by a mole of gas, corrected for existing air temperature and pressure, as well as the molecular weight of CO_2.

$$\frac{\text{mg } CO_2}{\text{liter}} = \left(\frac{\text{ul } CO_2}{\text{liter}}\right)\left(\frac{273 \text{ K}}{t}\right)\left(\frac{P}{1013 \text{ mb}}\right)\left(\frac{44{,}000 \text{ mg}}{\text{mole } CO_2}\right)\left(\frac{\text{mole } CO_2}{22.4 \text{ l } CO_2}\right)\left(10^{-6}\right) \quad (1)$$

The flow of air over the shoot and a measure of the assimilatory material, commonly leaf area, are next used to calculate net photosynthesis, or NCE rate, as in equation (2).

$$\text{NCE rate} = \left(\frac{\text{mg } CO_2}{\text{liter}}\right)\left(\frac{\text{liters}}{\text{hour}}\right)\left(\frac{1}{\text{dm}^2 \text{ leaf area}}\right) \quad (2)$$

The units reduce to mg CO_2 dm^{-2} hr^{-1}, which are perhaps the most widely employed units for expressing rates of photosynthetic CO_2 assimilation.

APPENDIX II

LIGHT MEASUREMENTS

Many plant scientists are accustomed to description of light in terms of intensity or illumination, the units of which are foot-candles in the English system and lux in the metric system. However, in order to relate to physiological processes of plants, especially photosynthesis, it is useful to describe light environments in terms of energy. Energy units currently in use include langleys, watts, and joules. Each of these is used to describe the light energy flowing through a unit area of medium (i.e., air) per unit time. These units are related as follows:

$$1 \text{ langley min}^{-1} = 1 \text{ cal cm}^{-2} \text{ min}^{-1}$$

$$1 \text{ watt m}^{-2} = 0.239 \text{ cal m}^{-2} \text{ sec}^{-1}$$

$$1 \text{ watt m}^{-2} = 1 \text{ joule m}^{-2} \text{ sec}^{-1}$$

$$1 \text{ watt m}^{-2} = 100 \text{ } \mu\text{watts cm}^{-2}$$

Still another unit of light measurement in use is the einstein, which describes light in terms of incident photons or quanta. A microeinstein equals 6.023×10^{17} photons. Photometers are available which measure light in units of μeinsteins cm^{-2} sec^{-1}. If desired, these may be converted to light energy by use of published tables which relate kg-cal to einsteins for each 10- or 20-nm wavelength band of the spectrum.

To convert foot-candles to μwatts cm^{-2}, or vice versa, the spectral energy distribution of the light source must be known. In the present study of NCE rate, this spectral distribution was determined with a spectroradiometer. A light intensity of 3,600 to 4,000 foot-candles (38.9 to 43.2 klux) was then calculated by the method outlined in the manual which accompanies the ISCO Model SR Spectroradiometer. This light intensity was in agreement with direct measurements of light intensity with a Weston light meter.

APPENDIX III

CONCENTRATIONS OF SADH AND TIBA USED IN THIS REPORT

SADH		TIBA	
ppm	Molarity	ppm	Molarity
100	6.29×10^{-4}	10	2.0×10^{-5}
500	3.14×10^{-3}	50	1.0×10^{-4}
1000	6.29×10^{-3}	100	2.0×10^{-4}
2000	1.26×10^{-2}	200	4.0×10^{-4}

Calculated molar equivalents are based on molecular weights of 160.17 for SADH and 499.81 for TIBA.