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BARD

FINAL REPORT

PROJECT NO. US-1587-88

Involvement of Different Gibberellins in Flowering of Biennial Bearing Olive Trees
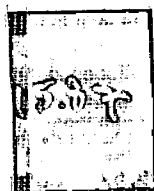
Y. Ben-Tal

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Title:

Involvement of Different Gibberellins in Flowering of Biennial Bearing

Olive Trees.

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Investigators' Names (Principal listed first)	Investigators' Institutions
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Project's starting date: ___October 1988___

Type of Report: 1st Annual _____ 2nd Annual _____ Final __✓__

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Signature
Principal Investigator

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Signature
Institution's Authorizing Official

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Involvement of different gibberellins in flowering of biennial bearing olive trees

Abstract

Biennial bearing is a severe obstacle in olive production for both the producer and the consumer. It causes large fluctuations in growers income and in market prices from one year to the next. As in most species, biennial bearing in olive is characterized by poor blossming during "off" years followed by enormous flowering the next year ("on" years). In many plants, plant hormones, especially gibberellins (GAs), have been shown to be involved in the flowering process.

In this project olive buds taken from "on" and "off" trees, during their differentiation season, were tested for their endogenous GAs. Using a combination of biological and current biochemical and physico-chemical instrumental techniques, enabled us to identify 20 different GAs in these buds. It was found that the buds taken from trees after "off" years, contained more GAs and larger quantities of these GAs than did buds after "on" years. Furthermore, the buds after "off" years, that were suppose to flower during the following spring, contained large amounts of GAs from the GA_4 route, which are known to be associated with flowering, during the early stages of the differentiation. However, during January and February, toward inflorescences evocation, the majority of GAs in these buds ("off") were from the GA_1 route, which are known to be associated with growth and elongation. The extracts from "on" buds contained small GA-like activity in January and February which coincided with their delayed development on the tree as compared with flowering buds.

Objectives of the project:

1. To quantitatively determine the endogenous GAs of apple and olive buds in the course of their development during both "on" and "off" years.

2. To determine if shoots of apple and olive, that are either in the "on" or "off" condition, synthesize GAs that differentially accumulate in their respective buds (axillary and spur buds of apple, and axillary buds of olive).

3. To determine if there is a difference in the biological activity of GA_4 compared to GA_3 on return bloom of apple and olive trees when treated either in the "on" or "off" condition.

4. To determine if there is a difference in the metabolism of GA_4 compared to GA_3 in spur buds of apple and axillary buds of olive in "on" and "off" years.

All 4 objectives presented in the original proposal have been investigated in the olive. Resonable understanding was achieved regarding biennial productivity of the olive tree. It is not clear whether these objectives have been achieved for apple too.

Introduction

Biennial bearing in olive is a severe drawback of the entire olive industry. Both products, olive oil and pickled fruits are stored up to two years in order to meet market demands during "OFF" years. Fruit prices are low and labour costs are high during plentiful seasons, and the opposite occurs during "OFF" years.

The phenomenon of biennial bearing in fruit trees is quite common, and is well known in apple, citrus, avocado, nuts and many other crops (Hennerty and Forshey 1971, Williams and Edgerton 1974, Hoad 1978, Jonkers 1979, Monselise and Goldschmidt 1982). But the extent of alternate bearing in these crops is never as severe as in olive (Lavee 1985, Lavee et al 1986, Stutte and Martin 1986). Many investigations were performed and reviews were published trying to explain the reasons for biennial bearing, but no single prime cause was ever found (Hennerty and Forshey 1971, Williams and Edgerton 1974, Hoad 1978, Monselise and Goldschmidt 1982). Therefore, a thorough investigation is required to study all the factors which are known to be involved in biennial bearing, and establish their relative influence, and their exact role in the sequence of events leading to biennial bearing.

Olive trees bear their fruits on one year old branches. These twigs begin their development in the spring, and grow during summer and fall. By winter they reach length of ca. 30 - 40 cm. Reproductive differentiation in the buds of these twigs occurs probably during winter months (Hartmann 1951, Lavee 1985, Ben-Tal unpublished data). It was established mainly by circumstantial evidence, but its validity is quite strong. The facts that support this conclusion are that warm winters result in poor flowering even after "OFF" years; that girdlings performed during December were most effective in increasing flowering of olive trees (Lavee et al 1983, Ben-Tal and Lavee 1984,1985); that a number of chilling hours requirements for flower differentiation of several olive cultivars were determined (Hartmann and Prolingis 1958, Hackett and Hartmann 1963, Badr and Hartmann 1971, Hartmann and Wisler 1975); and that olive trees grown under tropical conditions never flower.

In the following spring most of the axillary buds develop into inflorescences and set fruits if the winter requirements were fulfilled. During the summer and fall the fruits develop, and in late fall or early winter the crop is harvested. During "OFF" years a large number of twigs develop on the trees due to lack of fruits and therefore intensive vegetative growth. After "ON" years the opposite occurs. Many fruits were developed, the vegetative growth was limited to only a small number of twigs due to severe competition with the fruit load. Yet, comparisons between the percentage of inflorescences developed on similar twigs that had been grown during the two types of years revealed that after an "OFF" year 70 - 80% of the buds had developed inflorescences while only less than 15% of the buds did so after "ON" years (Hartmann and Wisler 1975, Ben-Tal unpublished data). This means that although some twigs did grow and develop during "ON" years, their buds were not capable of producing inflorescences. A few of these buds, that could not flower, did grow vegetatively while the rest of the buds remained dormant. Furthermore, reproductively differentiated buds begin to develop earlier than the vegetative ones. Toward the end of February the reproductive buds begin to swell and change their shape while vegetative buds still look dormant. These facts ruled out the possibility that competition for assimilation products or being stronger sinks advantage vegetative growth over reproductive development and becomes the primary reason for biennial bearing.

Our assumption was that some other factors may control the ability of the buds to flower. We thought that the presence or absence of an endogenous growth regulator might be the major control mechanism. Gibberellins (GAs) were chosen because they are known to be involved in flowering processes in many plants (Hoad 1978, Dunberg 1980, Marino and Greene 1981, Goldschmidt 1984, Pharis and king 1985, Pharis and Ross 1986, Bonnet-Masimbert and Zaerr 1987, Metzger 1988, Takahashi et al 1991). Badr and Hartmann (1972) Pinney and Polito (1986) and Lavee (1989) suggested that endogenous growth regulators were involved in alternate bearing in olive.

In order to study the role of endogenous gibberellins' involvement in flowering ability of olive trees, buds were taken from "OFF" and "ON" trees during fall and winter months. The aim of

this study was to determine and identify differences among endogenous GAs during the differentiation period in buds taken from olive trees after "ON" and "OFF" years.

Materials and methods

Twelve year old "Manzanillo" olive trees (*Olea europeae* L.) located in the experimental olive orchard at the Volcani Research Center in Bet Dagan, were the source of plant material for the entire project. The yield of these trees has been recorded since their first production, and their biennial production pattern was well established (Table 1).

Eight twigs of 30 - 40 cm long were collected from each of 6 olive trees that were after their "ON" year and from 6 trees that were after their "OFF" year, a total of 96 twigs. The twigs were taken from these trees around the middle of the month from October through February in both years 1988/9 and 1989/90. The number of inflorescences was counted on another 8 twigs on each of these trees during the following flowering season (late April - early May of each of these years), and the ratio between the number of inflorescences and the total bud number on these twigs was calculated (Table 2). Upon harvesting the yield of these trees was determined (Table 3).

Table 3. The actual average yields of the trees that were expected to be either "ON" or "OFF".
After recording the yields of 1988, these trees were marked and used for buds analysis throughout the entire project. The actual yields were recorded in both years, 1989 and 1990, in order to validate whether our predictions were correct.

Type of year predicted for 1989 and 1990	1988 Yield (kg) ±SE		1989 Yield (kg) ±SE		1990 Yield (kg) ±SE	
" ON "	14.75	2.62	85.17	3.87	12.5	1.04
" OFF "	69.83	4.89	20.33	2.19	63.5	3.43

Table 1. Yields (kg) of the selected trees in the olive grove of which buds were taken

Tree No.	1985	1986	1987	1988	1989	1990	1991
1	42	18	65	15	74	15	62
2	48	21	60	14.5	86	10	88
3	74	12	63	6	82	9	72
4	64	23	93	25	96	14	62
5	63	14	57	18	86	15	75
6	56	18	78	10	87	12	90
7	28	44	10	81	23	72	28
8	21	51	12	58	16	69	24
9	15	54	2	69	22	48	15
10	28	78	21	86	18	66	11
11	14	59	0	55	29	60	36
12	16	85	12	70	14	66	16

Table 2. Percent of flowering on twigs that grew on the olive trees after "ON" or

Previous yield	Buds/twig 1989	Inflo/twig 1989	Percent Flowering	Buds/twig 1990	Inflo/twig 1990	Percent Flowering
"ON"	41.35 ±0.96	6.69 ±0.68	16.3 ±1.85	29.6 ±1.72	3.37 ±0.57	11.39 ±1.91
"OFF"	32.35 ±1.17	23.3 ±0.59	72.3 ±1.91	22.87 ±1.03	15.29 ±0.85	66.86 ±2.04

These counts, measurements and calculations were made to validate that our predictions concerning the biennial pattern of these olive trees was maintained throughout the duration of this project.

The twigs that were collected for buds' analysis were frozen in the orchard by liquid nitrogen and freeze dried by lyophilization. After drying, approximately 1000 buds were removed from the entire group of twigs of the same kind ("ON" or "OFF") of trees every month. The buds were counted and weighed. The dry weight of each group of buds was ca. 800 - 900 mg. Then, the buds were ground in 80% MeOH for GAs extraction. Two tritiated marker GAs, usually ^{3}H-GA_1 and ^{3}H-GA_4, at known quantities, were added into each extract and were monitored after each and every separation and purification step in order to account for possible loses of endogenous GAs during the purification and separation process. Separation between conjugated and free GAs, and purification of the extracted GAs was made according Koshioka *et al* (1983a) with minor changes (Fig. 1). The resulted dry extract was collected in 100% MeOH and was injected into a C18 reverse phase column on a 'Varian' 5000 high performance liquid chromatography (HPLC) equipped with a Merck Hibar LiChrosorb RP-18 (250 x 10 mm), 7μm column, for analytical separation of the GAs. The range of retention times (Rt) where GAs were suppose to appear was established by injecting a group of standard GAs, including GA_8, 1, 4, 7, and GA_9 which account for a wide range of retention times of GAs on this column with a running phase of increased MeOH percentage program (Fig. 2). This procedure was addopted from Koshioka *et al* (1983b) with our minor changes. Twenty five (46 - 91% program, Fig. 2a) or thirty five (10 - 73% program, Fig. 2b) 2 min fractions at 2 ml/min were collected from every injected sample after HPLC separation. These 4 ml fractions were dried completely by vaccum and N_2 stream.

For a 'Tan ginbozu' dwarf rice bioassay (Koshioka *et al* 1983a). The dried fractions were dissolved in 100 μl of 100% ethanol. Either 1 μl or 0.5 μl from each dissolved fraction was applied on the dwarf rice seedlings to test the various fractions for their ability to promote gibberellin like activity (GA-like activity). Groups of fractions that caused significant seedlings'
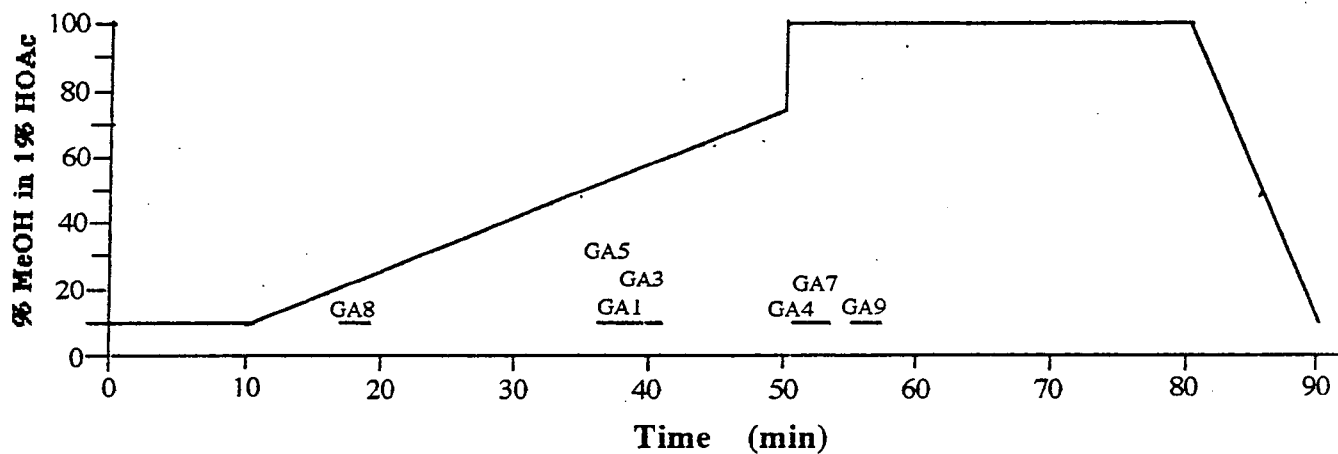
Separation and purification flow chart

Dry buds extracted in 80% MeOH

Separation by Beuchner funnel

pulp discarded

solution, marker GAs added
pH adjusted to 6.7, radioactivity count

C18 bondapak column | rinse with 80% MeOH, 2nd rinse 60 ml of 100% MeOH

100% MeOH rinse radioactivity count (pigments)

80% MeOH soluble fraction, radioactivity count (GAs, precursors, conjugated GAs)
H₂O added, dilution to 40% MeOH

2nd run through C18 bondapak column | rinse with 40% MeOH, 2nd rinse with 30ml of 100% MeOH

100% MeOH rinse radioactivity count (precursors)

40% MeOH soluble fraction, radioactivity count (GAs, conjugated GAs)

dry completely, transfer to celite and run through SiO₂ column in 100 ml of 95:5 | of EtOAc:Hexane. 2nd rinse with 100 ml of 100% MeOH

100% MeOH rinse radioactivity count (conjugated GAs)

95:5 EtOAc:Hexane soluble fraction radioactivity count (GAs)

dry, HPLC C18 column | 46 - 91% MeOH gradient

dwarf rice bioassay

fractions containing GA-like activity | 2nd run in HPLC C18 column 10 - 73% MeOH

2nd dwarf rice bioassay

sharp peaks with GA-like activity derivatized, GC-MS

wide peaks with GA-like activity, 3rd run in HPLC, nucleosil N(CH₃)₂ column, isocratic 99.9% MeOH:0.1% acetic acid

3rd dwarf rice bioassay

sharp peaks with GA-like activity derivatized, GC-MS

Fig. 2b. Running phase program for the 2nd HPLC run including several standards.

Fig. 2a. Running phase program for the 1st HPLC run including several standards.

elongation were combined together, dried by N_2 and re-injected into the HPLC using a different MeOH running phase program (Fig. 2b). Then, all fractions of the 2nd HPLC run were subjected to a 'Tan ginbozu' dwarf rice bioassay. Some of the fractions that caused significant GA-like activity were methylated and trimethylsililated (Steffens *et al* 1991) and injected into a 'Hewlett Packard' 5890 gas-chromatograph combined with a 5791A mass-spectroscopy detector (GC-MSD) equiped with DB1 ($0,25\mu m$, 30m x 0,25mm) column for GAs analysis (Gaskin and MacMillan 1991). Other fractions that promoted GA-like activity in 2-4 adjacent tubes were combined together and were injected again for further separation and purification into HPLC, equipped, this time, with a HiChrom NC100-10, Nucleosil [$N(CH_3)_2$] column (250 x 4.6mm) with isocratic running phase of 99.9% MeOH: 0.1% Acet. Ac. 1ml/min (Yamaguchi *et al* 1982) This 3rd HPLC separation had produced 25 fractions, of 2 minutes each, which were subjected to another 'Tan ginbozu' dwarf rice bioassay. The fractions that promoted GA-like act. were derivatized as described above for GC-MSD analysis. GAs identification was based on comparisons with authentic GA standards (purchased from L.N. Mander, Research School of Chemistry, The Australian University, G.P.O. Box 4, Canberra A.C.T. 2601, Australia). These standards included $GA_{1,3,4,5,7,8}$ and 9. For other GAs comparisons were made with literature published identifications (Koshioka *et al* 1988, Nakayama *et al* 1989, 1990, Talon *et al* 1990, Beale and Willis 1991, Gaskin and MacMillan 1991, Steffens *et al* 1992) that contained lists of Kovatt retention index (KRI) values and ion abundance.

Results

The data in table 1 indicate that the olive trees used for the experimental work were actually representing trees in their "ON" and "OFF" years. The recorded yields of these trees during the two years in which buds were collected, indicated that the predicted production of these trees was mentained (Table 3), and that their "ON" and "OFF" years occured in opposite years.

The low flowering rate after "ON" years (Table 2) is another indication that although twigs and buds were present they did not develop inflorescences as did buds on trees after their "OFF" years.

Bioassays of buds' extracts were made from the buds taken from both kinds of twigs during the 5 consecutive months in both years (1989, 1990). GA-like activity was determined by measuring elongation of the dwarf rice seedlings. October results revealed relatively small quantitative differences between "ON" and "OFF" buds (Fig. 3), and that the major peaks of activity had appeared at the same retention times in both types of buds. It means that they were probably the same GAs. The few qualitative differences resulted probably from the fruit load that was not harvested yet on the "ON" trees. In November, a large quantitative difference was observed in the bioassay at fractions 19 and 27 in "OFF" buds and possible qualitative difference at fractions 25 and 30 in "OFF" buds (Fig. 4). A similar picture was observed in December (Fig. 5). But in January the quantitative diffence in fractions 25-28 almost disappeared while a new peak at fractions 17-19 appeared in the "OFF" extracts (Fig. 6). This new peak in fractions 17-19 grew even larger in February in the "OFF" buds, while the peak in fractions 25-28 almost disappeared in both kinds of extracts (Fig.7).

These results (described in Figs. 3-7) exposed fractions that had promoted rice seedlings elongation, therefore containing GA-like activity. In figure 8, the sequence of HPLC and bioassay steps are presented.

These following HPLC runs were required to achieve satisfactory separation of the final fraction that was derivatized for GC-MSD analysis.

In table 4 the list of GAs identified in both kinds of buds is given.

Table 4. A list of all GAs identified in the olive buds during this project.
In brackets the KRI values.

GA_1 (2667), GA_3 (2695), GA_4 (2500), 3-epi-GA_4 (2621), GA_5 (2477), GA_7 (2522), GA_8 (2825), GA_9 (2386), GA_{15} (2604), GA_{17} (2575), GA_{19} (2598), GA_{24} (2445), GA_{30} (2760), GA_{35} (2648), GA_{37} (2765), GA_{44} (2787), GA_{51} (2522), GA_{53} (2495), GA_{61} (2490) and GA_{63} (2709).

October "ON" 1st HPLC

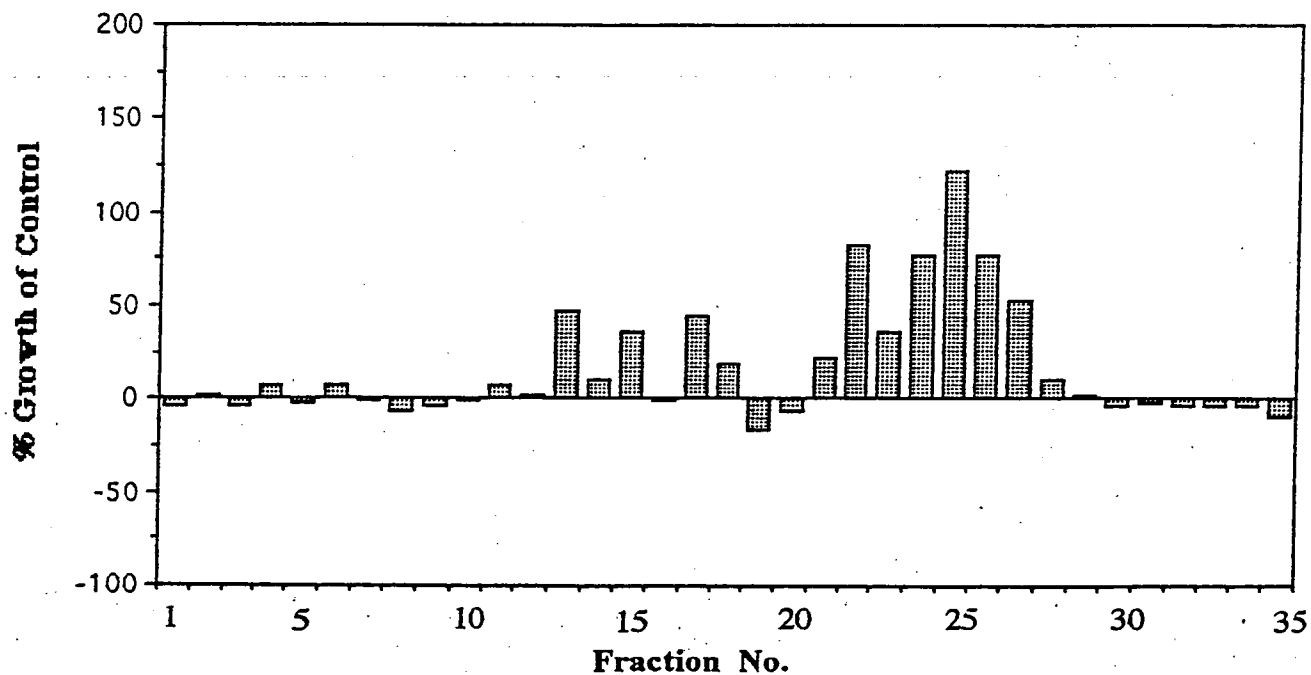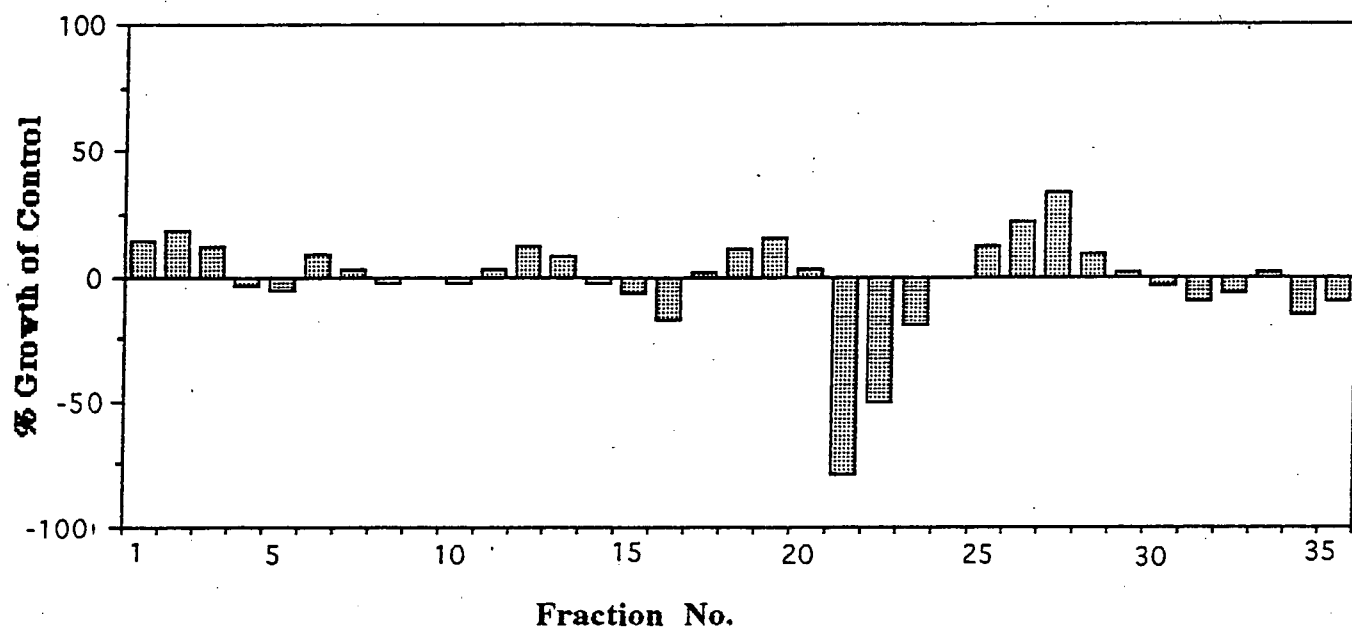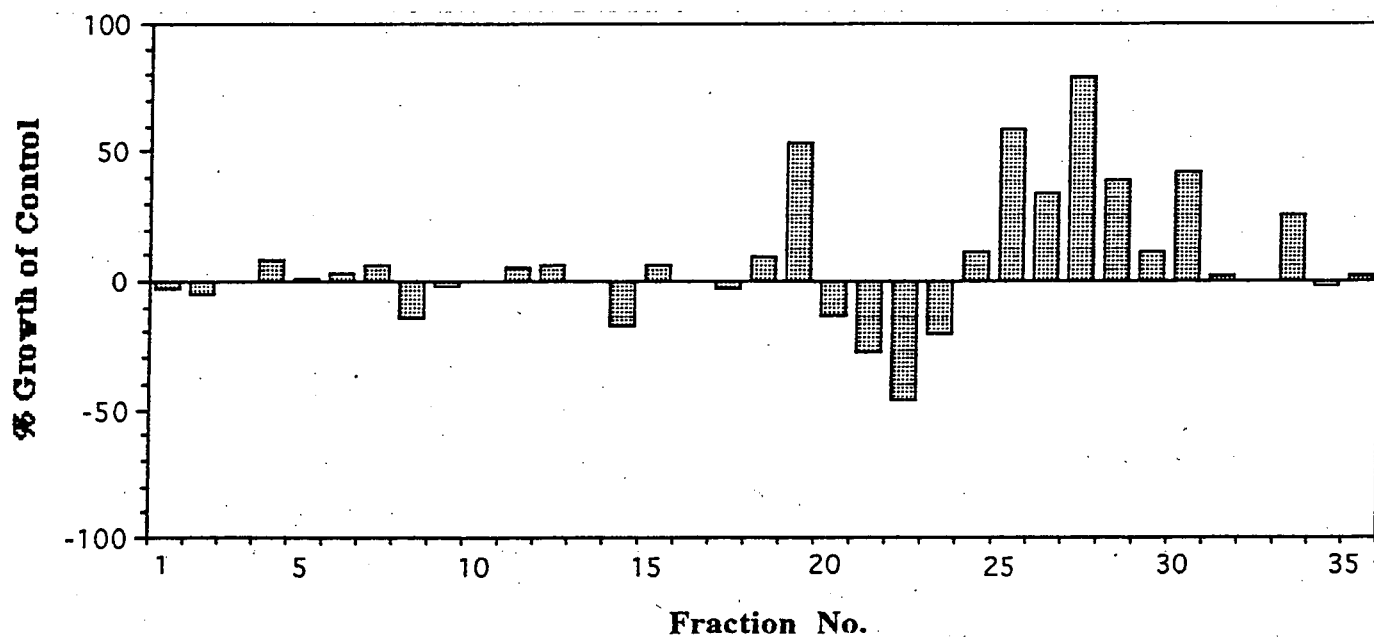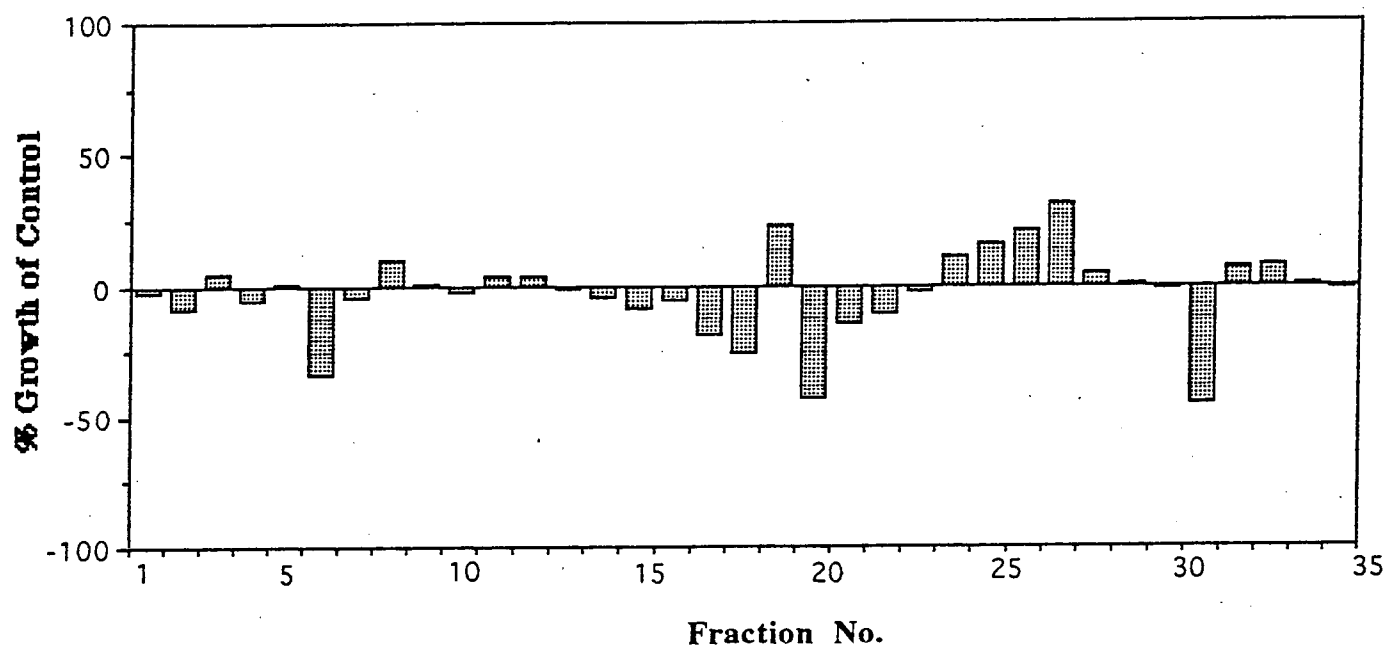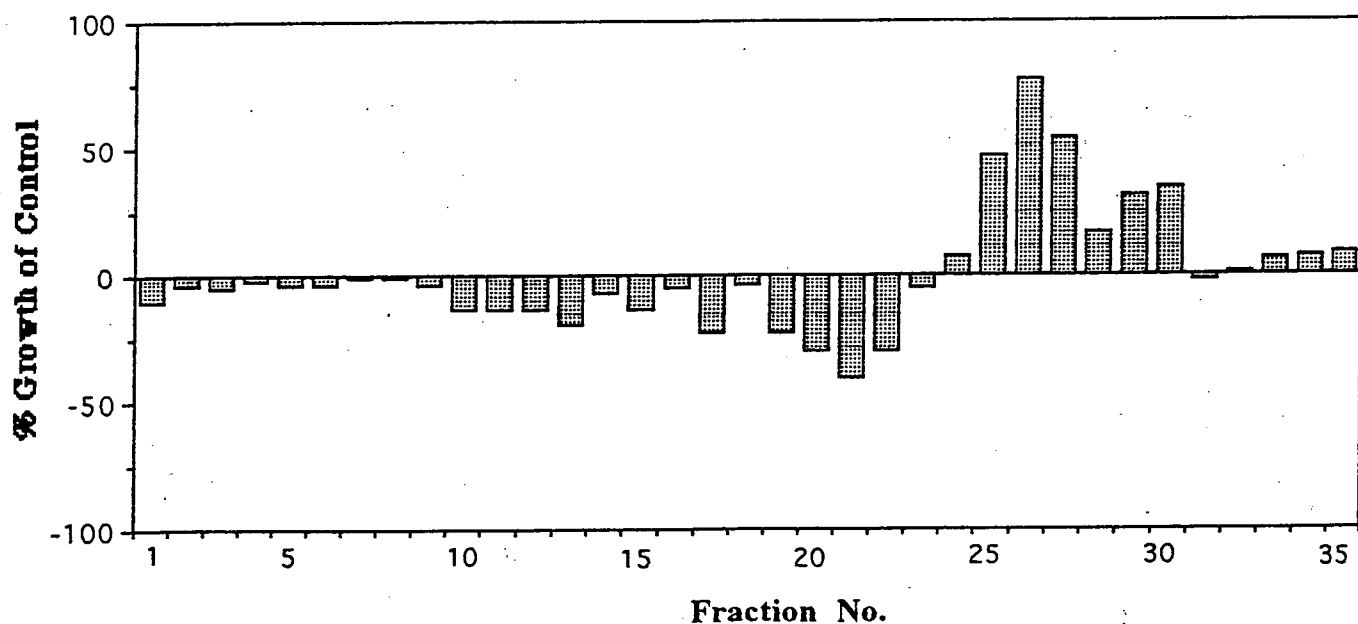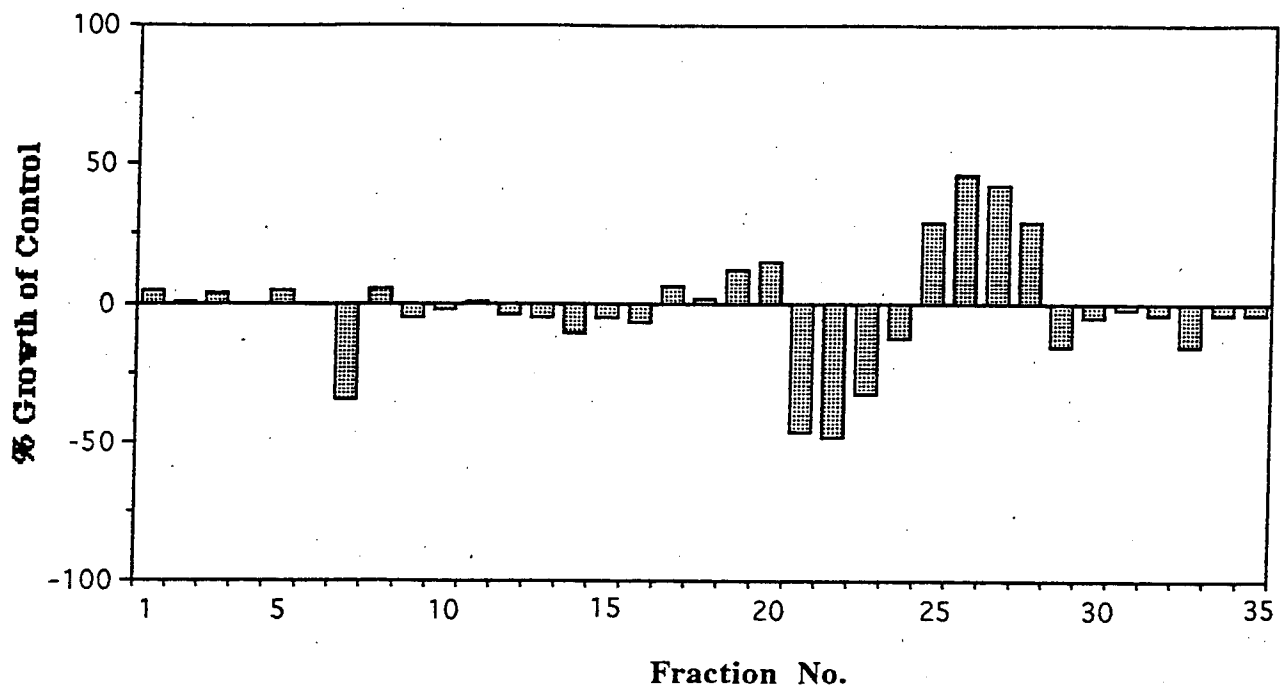

October "OFF" 1st HPLC

Fig. 3. GA-like activity in rice seedlings as a result of buds' extracts.
After HPLC separation samples from every fraction were applied on 'Tan-ginbozu' rice
seedlings. Elongated seedlings are an indication of endogenous GAs. Peaks at different
fractions are an indication of different GAs. The height of the peak is an indication for the
amount of this endogenous GA.

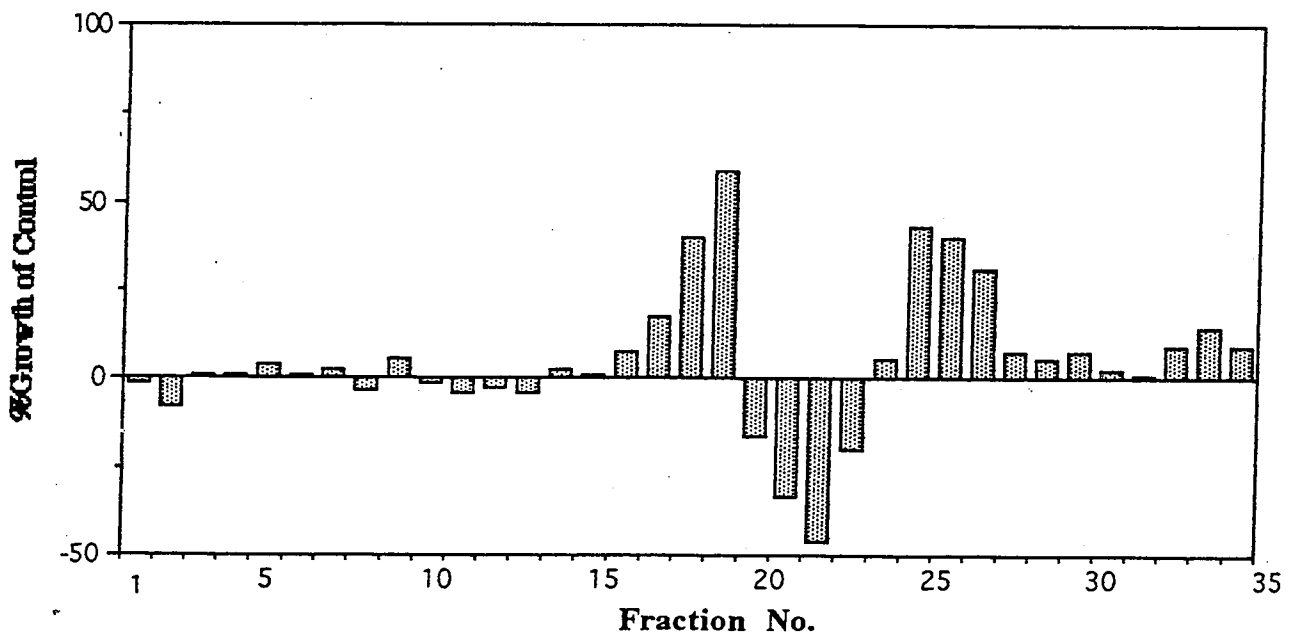## November "ON" 1st HPLC
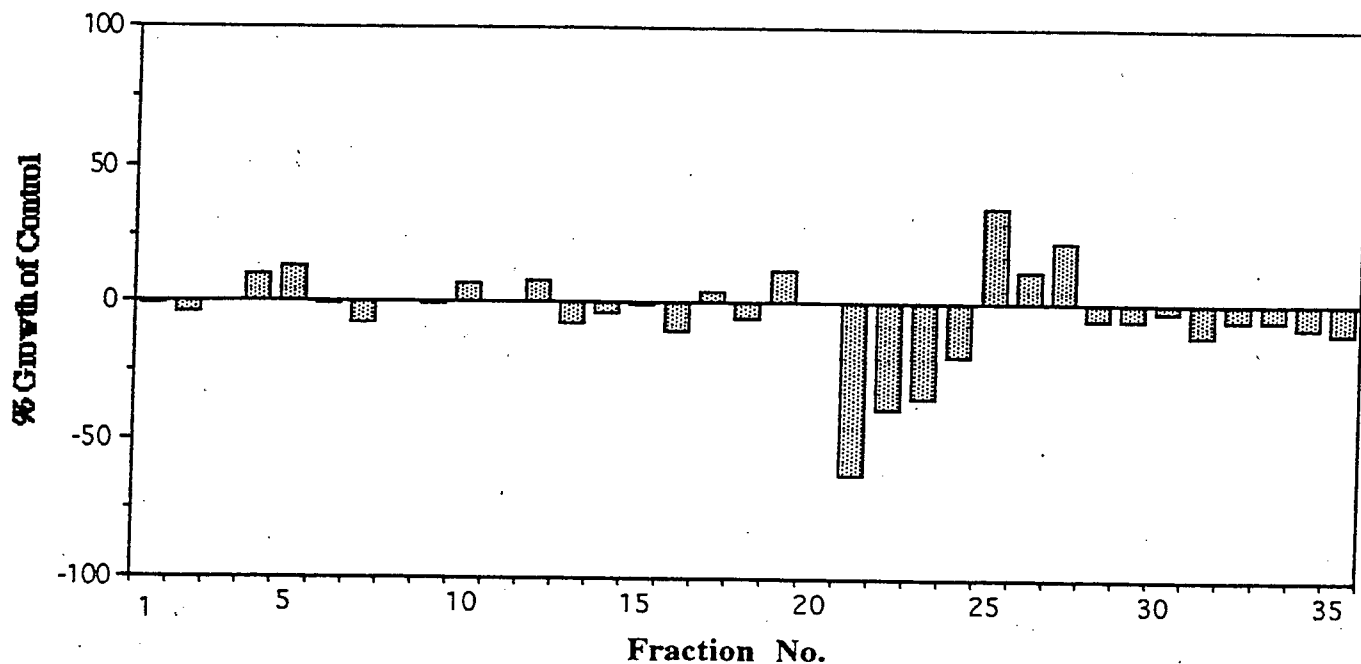

November "OFF" 1st HPLC

Fig. 4. GA-like activity in rice seedlings as a result of buds' extracts.
After HPLC separation samples from every fraction were applied on 'Tan-ginbozu' rice
seedlings. Elongated seedlings are an indication of endogenous GAs. Peaks at different
fractions are an indication of different GAs. The height of the peak is an indication for the
amount of this endogenous GA.

December "ON" 1st HPLC
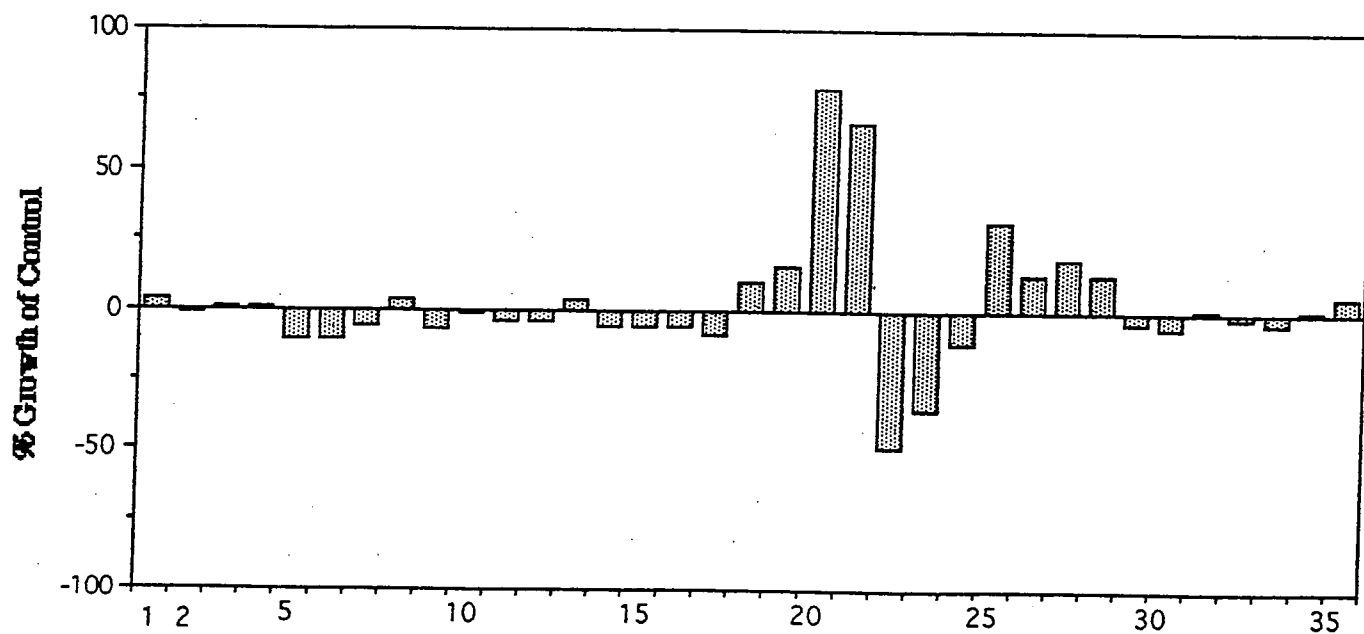

December "OFF" 1st HPLC

Fig. 5. GA-like activity in rice seedlings as a result of buds' extracts.
After HPLC separation samples from every fraction were applied on 'Tan-ginbozu' rice
seedlings. Elongated seedlings are an indication of endogenous GAs. Peaks at different
fractions are an indication of different GAs. The height of the peak is an indication for the
amount of this endogenous GA.

January "ON" 1st HPLC

January "OFF" 1st HPLC

Fig. 6. GA-like activity in rice seedlings as a result of buds' extracts.
After HPLC separation samples from every fraction were applied on 'Tan-ginbozu' rice seedlings. Elongated seedlings are an indication of endogenous GAs. Peaks at different fractions are an indication of different GAs. The height of the peak is an indication for the amount of this endogenous GA.
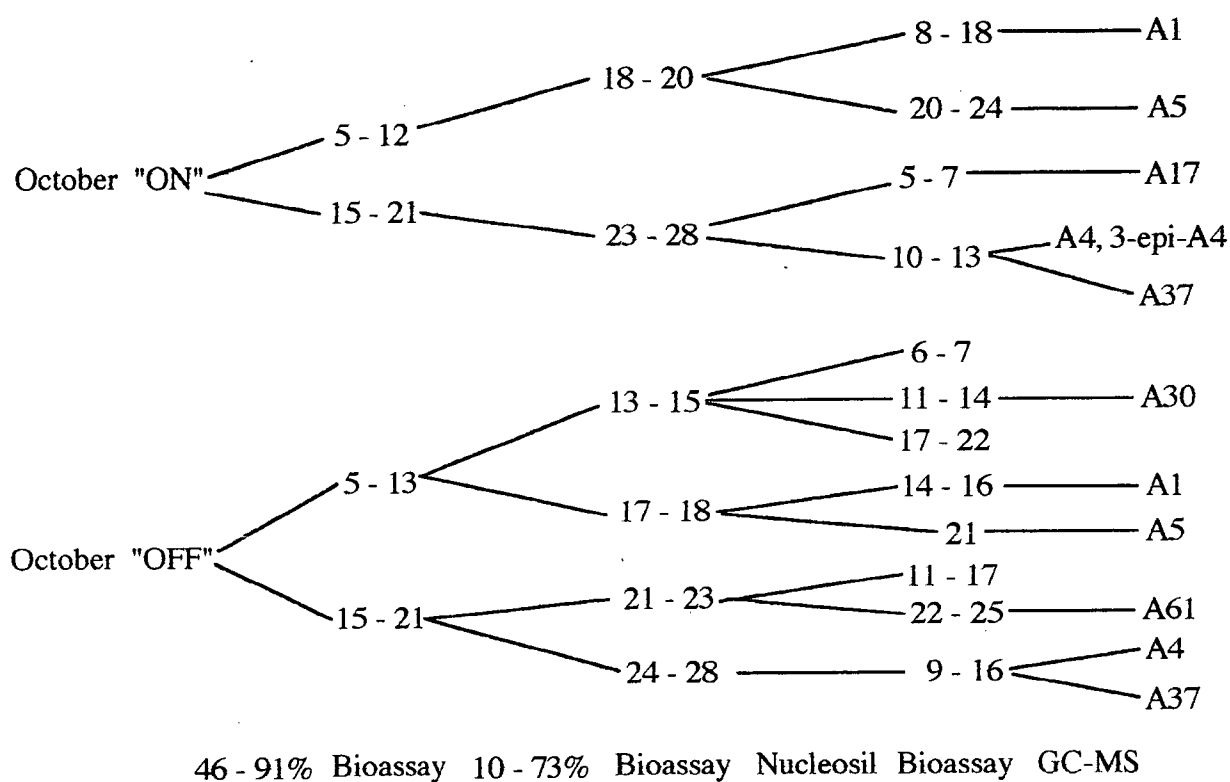
February "ON" 1st HPLC

February "OFF" 1st HPLC

Fig. 7. GA-like activity in rice seedlings as a result of buds' extracts.
After HPLC separation samples from every fraction were applied on 'Tan-ginbozu' rice
seedlings. Elongated seedlings are an indication of endogenous GAs. Peaks at different
fractions are an indication of different GAs. The height of the peak is an indication for the
amount of this endogenous GA.

Fig. 8. Sequence of HPLC and bioassay steps leading to GC-MS identification.
A demonstration of steps required for identifications of GAs in extracts made of buds colected
during October.

The identification procedure is shown for one of the GAs. It appeared as a peak at Rt of 26.14 after a full scan analysis and was expected to be GA_4 (Fig. 9). In the next test the same compound was tested for selected ions monitored (SIM) with hydrocarbons (ion 85) to determine the exact KRI and the fact that all the relevant ions appeared at exactly the same Rt (Fig. 10). The results indicated that it was GA_4 based on the ions ratio and the KRI calculated. The next step compared it with a scan of a standard GA_4 which is stored in the library of the GC-MSD. This comparison established that it actually was GA_4 at 86% validity (Fig. 11). Not all the identified GAs were validated by camparisons with stundards. We did not have standards for all the GAs identified. Therefore we had to establish their authenticity only by comparisons with GAs' data in the literatue based on their KRI values, the appearance of all the ions at the same Rt, the ions abundance and the relative ratios of these ions (Gaskin and MacMillan 1991). This validation is not as solid as the one described above, but it is accepted by all scientific journals because nobody has standards for all 86 known GAs. In all the comparisons made when authentic standards were available, it was found that KRI values, ions' masses and the relative ratios of these fractures, were enough for accurate GAs identifications. The comparisons with standards were only an additional validation which never contradicted the identifications based on the other 3 parameters.

It was found that "OFF" buds contained a large quantity of GA_4 during October (Fig. 12) which gradually decreased during November (Fig. 13), December (Fig. 14) and January (Fig. 15), and completely disappeared in February (Fig. 16). While in extracts of "ON" buds it was mainly epi-GA_4 in October and November (Figs 12 and 13) which decreased during December and January (Figs 14 and 15) and disappeared in February (fig. 16). The "ON" extracts also contained minute quantities of GA_4 in October and November (Figs 12 and 13). Another consistent gibberellin was GA_{37} which prevailed in most of the extracts no matter whether originated from "ON" or "OFF" buds. GA_1 was present in October, decreased in November and completely disappeared in December of both "ON" and"OFF" buds (Figs 12, 13 and 14). It had reappeared in January and grew even larger in "OFF" buds from February (Figs 15 and 16) but

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Misc Info: 1991 NUCLEOSIL
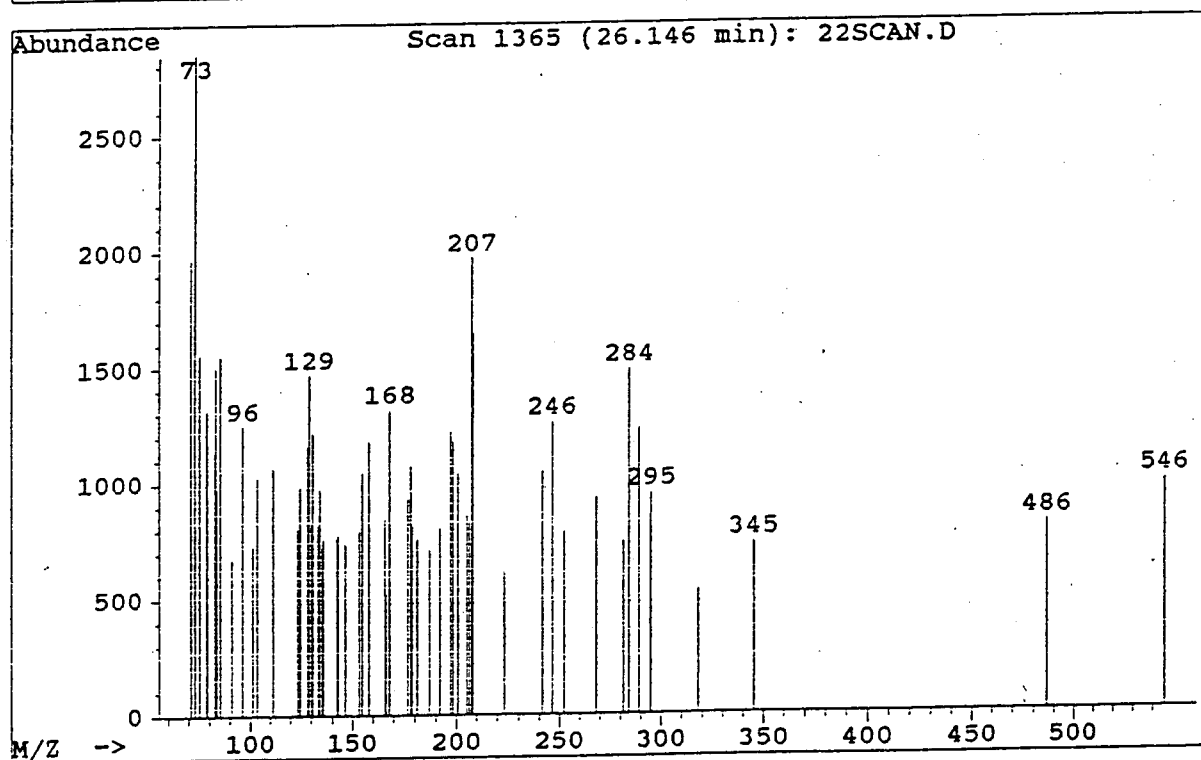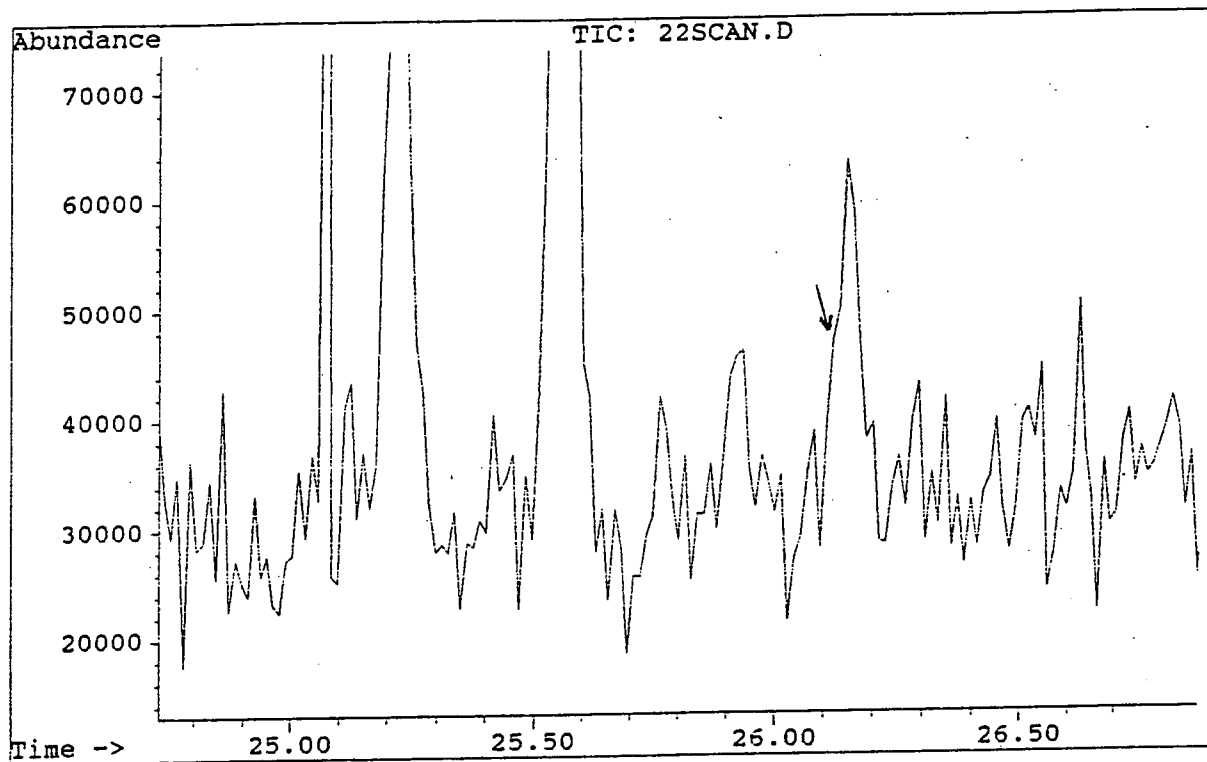ALS vial: 1

Fig.9. Total scan of an October "OFF" fraction that contained a large peak of GA-like activity.
A ristricted region of a GC-MS total scan of a fraction that included the Rt of a standard GA4 marker. The scan indicates the ions 289, 284, 224 and 129 at KRI of 2499. Typical of GA4.
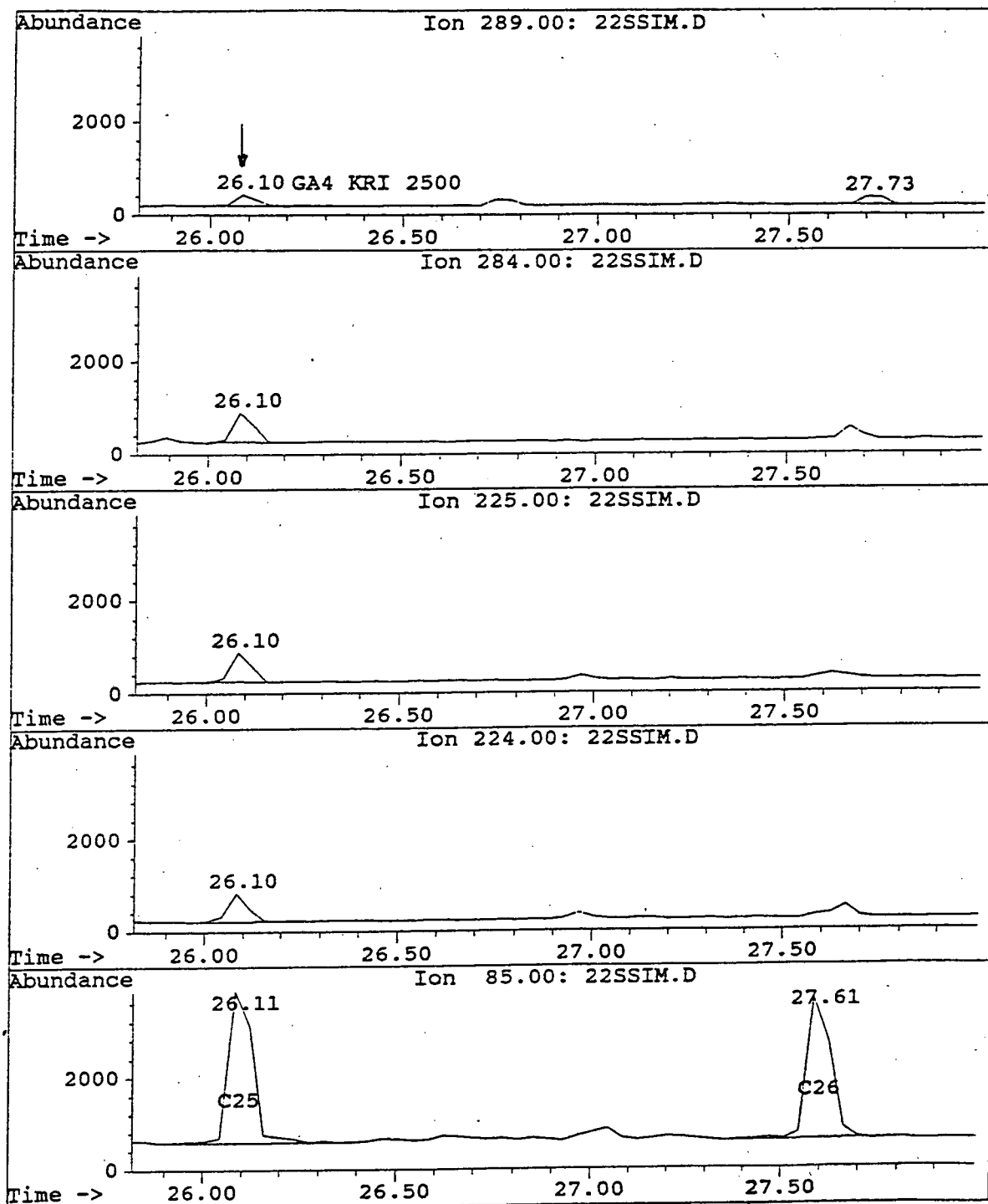
Fig. 10. SIM check of the same fraction including hydrocarbons.
The characteristic GA4 ions plus ion 85 (indication of hydrocarbons) were selected and the same sample was re-injected.

Fig. 11. Comparison of the total scan results with a library standard of GA4.
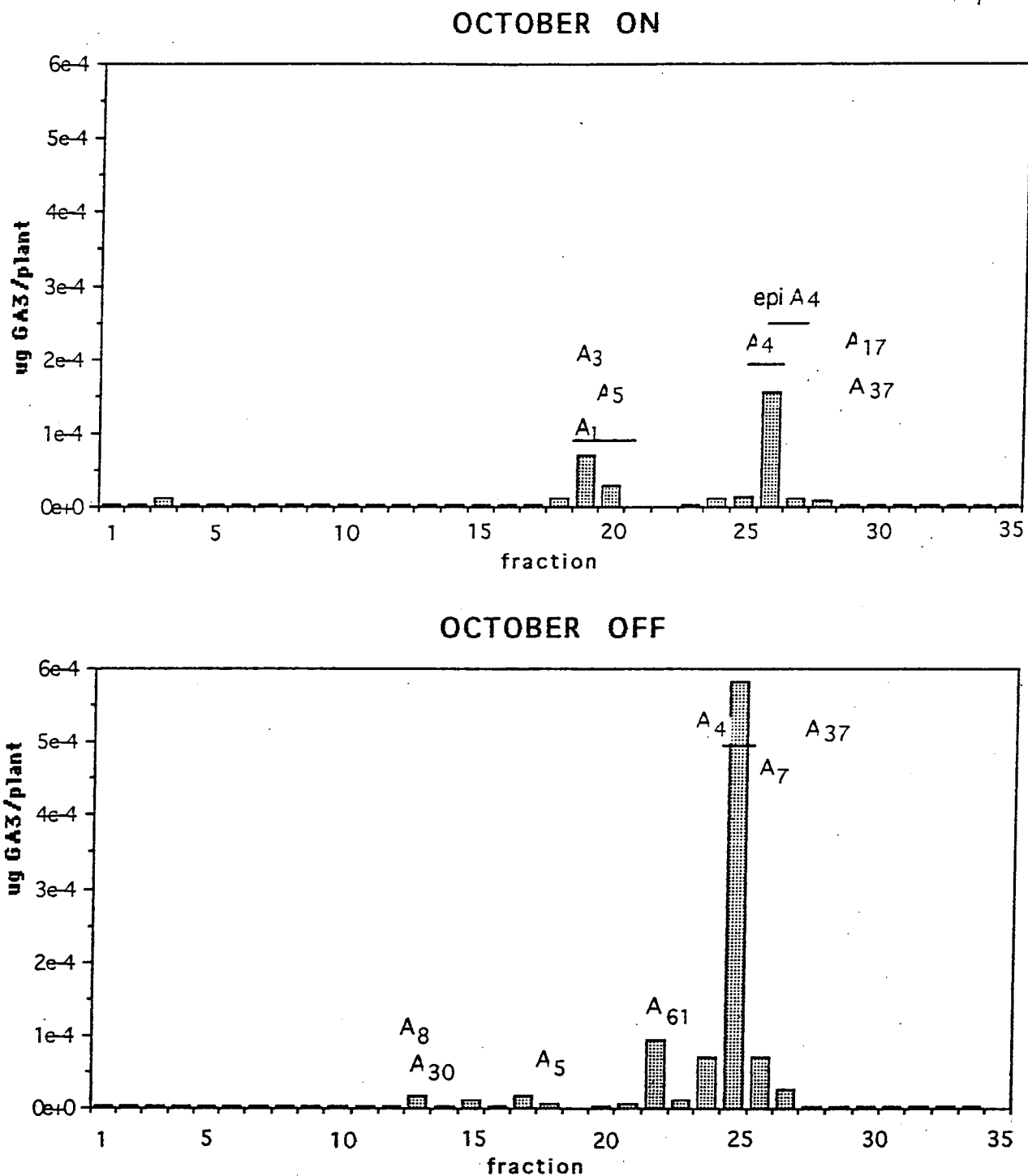
OCTOBER ON

OCTOBER OFF

Fig. 12. GAs identified by GC-MS in olive buds' extracts.
Buds' extracts were subjected to 2 - 3 different HPLC separations. However, the fractions marked above as containing the GAs are always the first HPLC run of which further separations were made as indicated in figure 8.
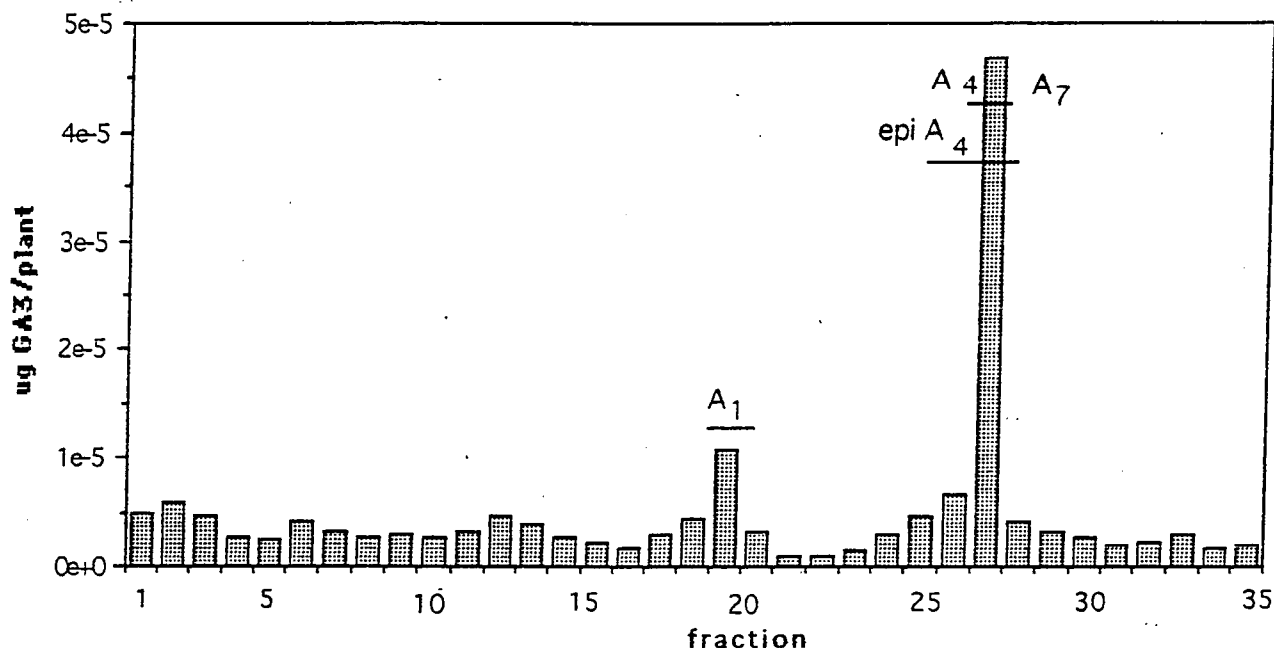
Fig. 13. GAs identified by GC-MS in olive buds' extracts.
Buds' extracts were subjected to 2 - 3 different HPLC separations. However, the fractions marked above as containing the GAs are always the first HPLC run of which further separations were made as indicated in figure 8.
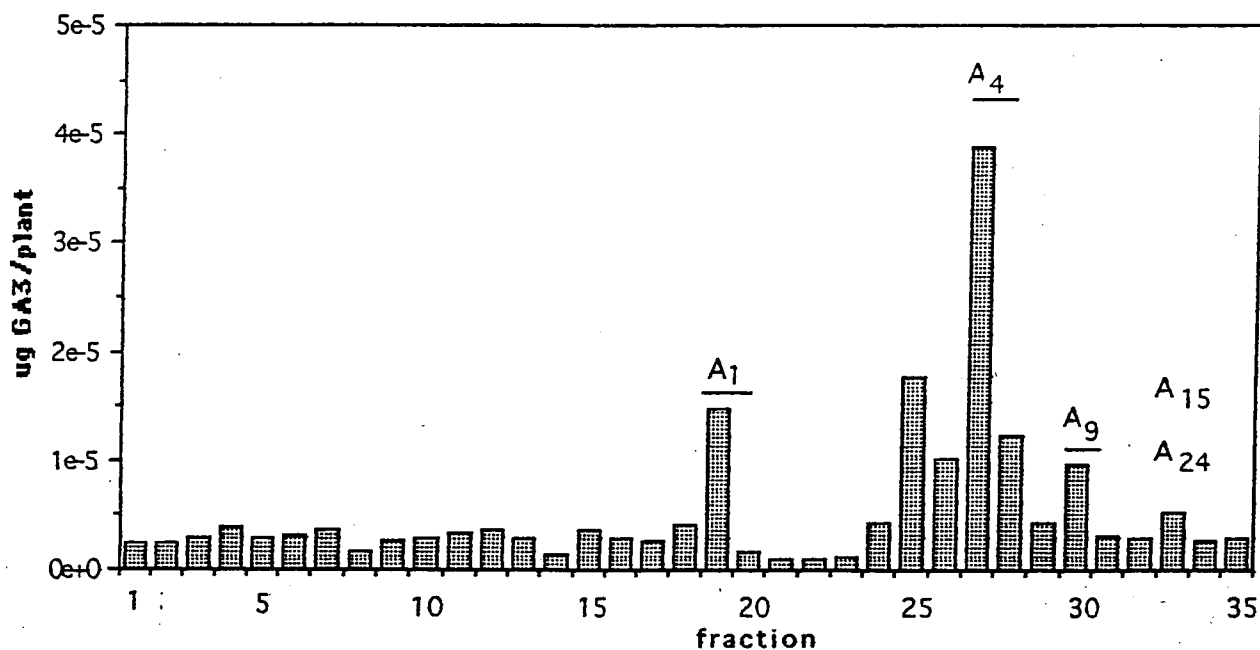
DECEMBER ON

DECEMBER OFF

Fig. 14. GAs identified by GC-MS in olive buds' extracts.
Buds' extracts were subjected to 2 - 3 different HPLC separations. However, the fractions marked above as containing the GAs are always the first HPLC run of which further separations were made as indicated in figure 8.

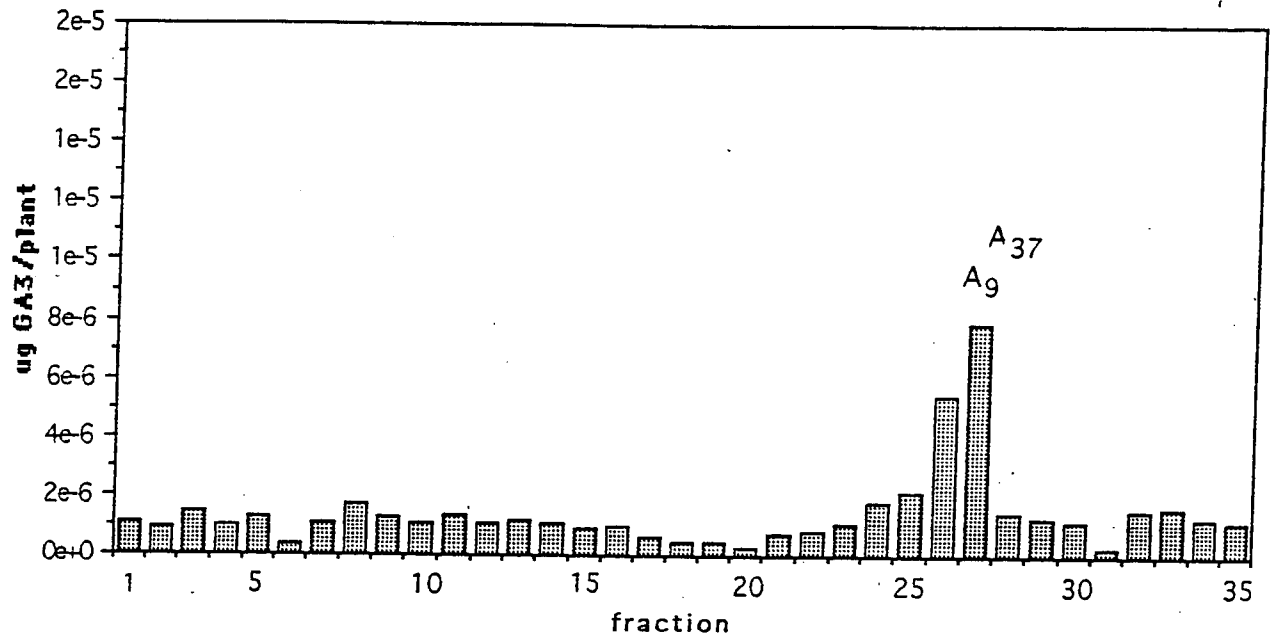## JANUARY ON

JANUARY OFF

Fig. 15. GAs identified by GC-MS in olive buds' extracts.
Buds' extracts were subjected to 2 - 3 different HPLC separations. However, the fractions
marked above as containing the GAs are always the first HPLC run of which further
separations were made as indicated in figure 8.
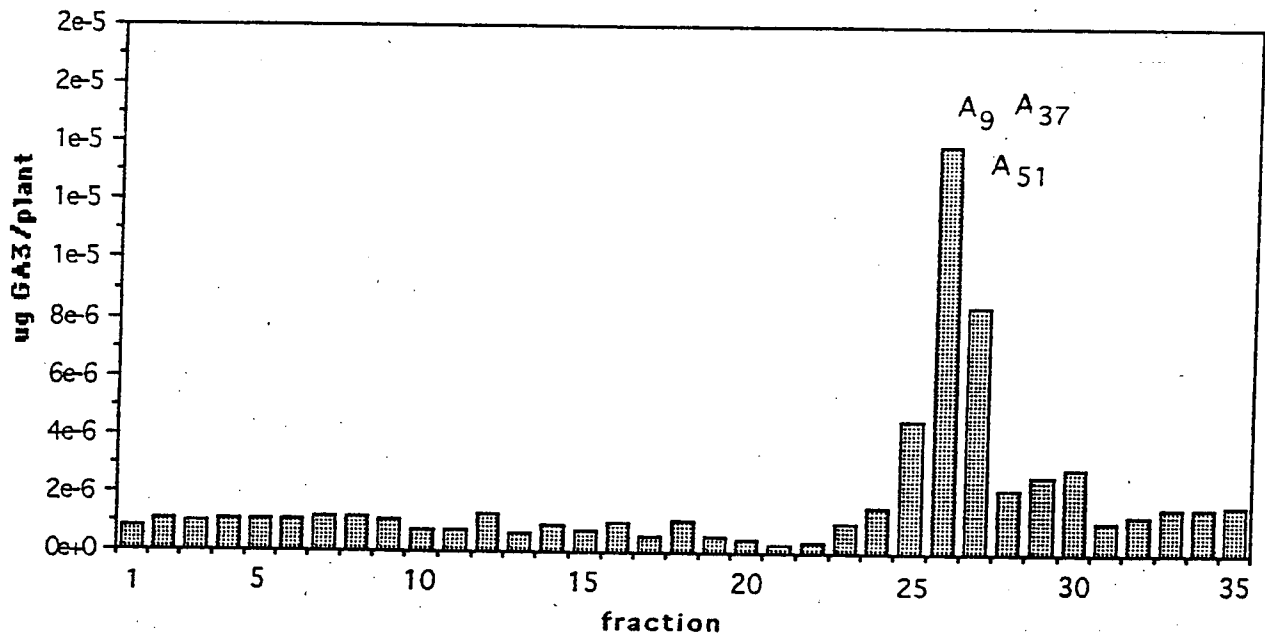
FEBRUARY ON

FEBRUARY OFF

Fig. 16. GAs identified by GC-MS in olive buds' extracts.
Buds' extracts were subjected to 2 - 3 different HPLC separations. However, the fractions marked above as containing the GAs are always the first HPLC run of which further separations were made as indicated in figure 8.
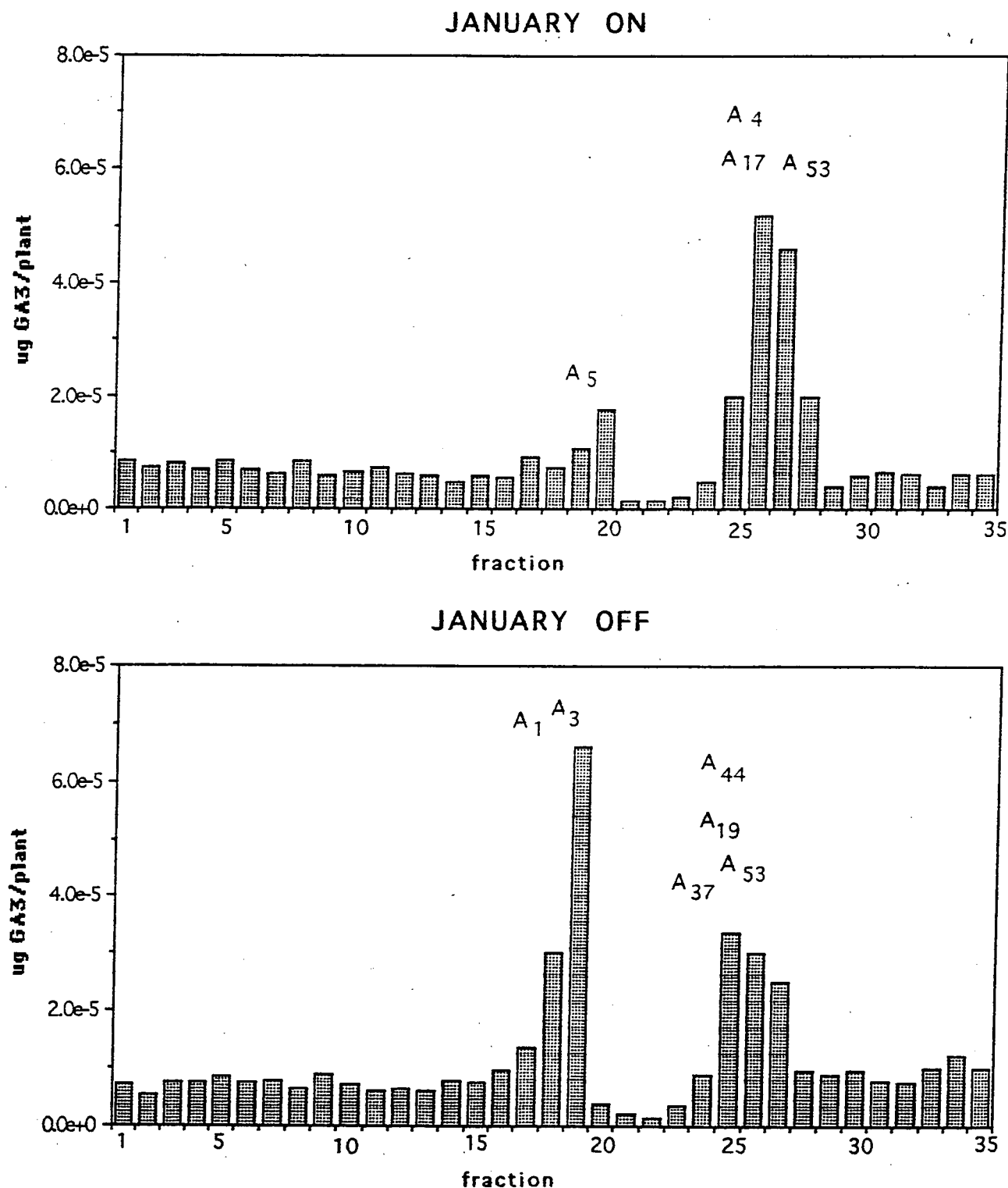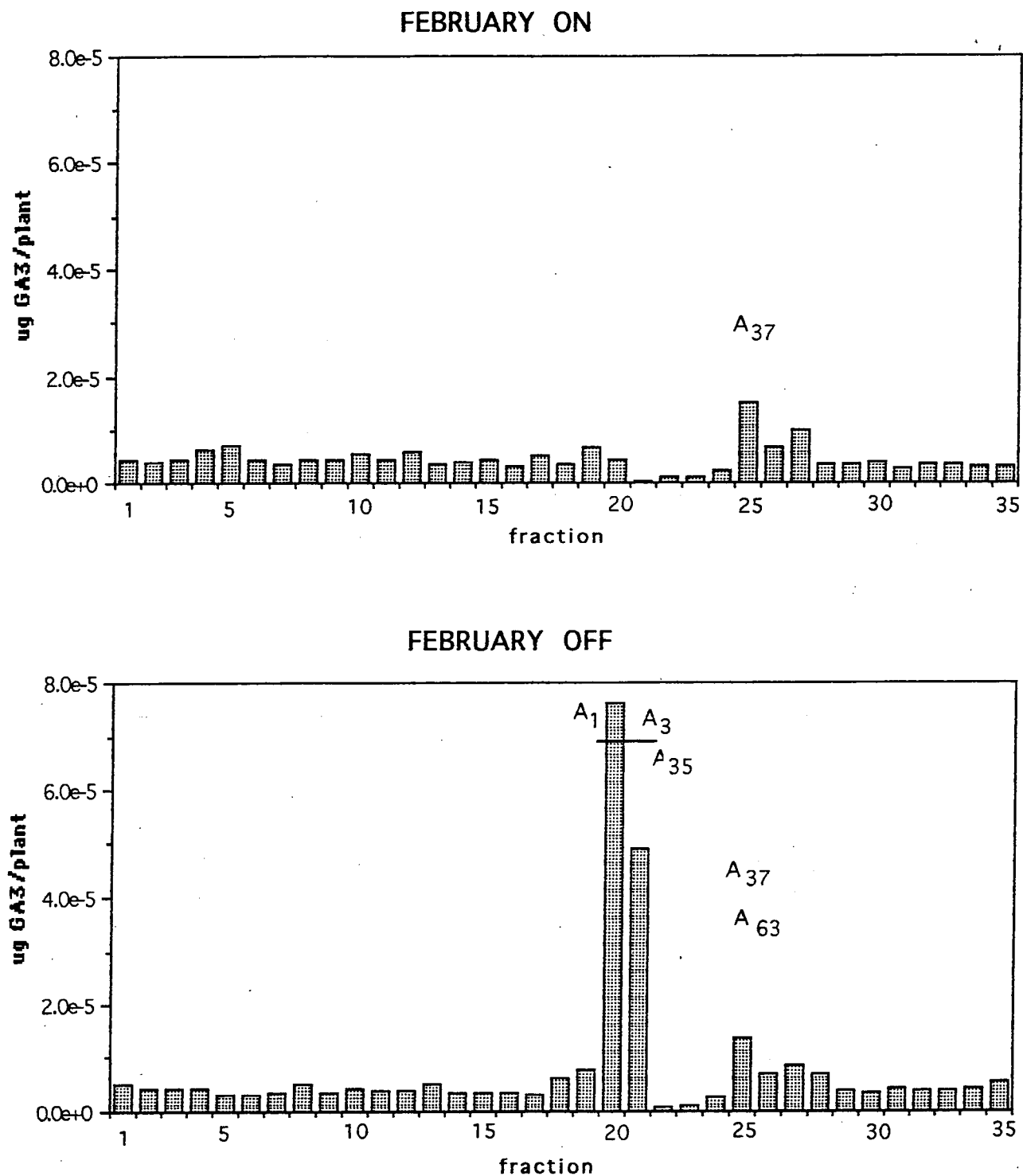
was relatively small in January and almost did not exist in February in extracts taken from "OFF" buds (Figs 15 and 16).

Discussion

The results of this investigation clearly indicated that there were significant differences between endogenous GAs in olive buds during the differentiation period depending whether these buds were originated from growth occured following "ON" or "OFF" years. These differences already existed in October both quantitatively and qualitatively. They might have resulted from two separate reasons. The first; "ON" trees were still loaded with fruits and their seeds might have been a source of different GAs which did not exist in trees without fruits. The second; due to an "OFF" year the vegetative growth of these trees was more vigorous and therefore might have resulted in larger quatities of GAs which are known to be associated with vegetative growth (Koshioka et al 1985, Fujioka et al 1988, Junttila and Jensen 1988, MacMillan 1990, Talon and Zeevaart 1990) and as we actually found.

In November and Decmber vegetative growth ceased and differentiation occurred. The "vegetative" GAs decreased and new GAs appeared. These new GAs were probably associated with flowering processes, like GA_4 and its precursors (Bonnet-Masimbert and zaerr 1987, King et al 1987, Lin and Stafford 1987, Pharis et al 1987). They appeared almost only in the "OFF" buds which were suppose to differentiate into inflorescences during the winter. GA_4 was found to be associated with flowering in other plants like apple (Looney et al 1985), $Lolium$ $temulentum$ (Pharis et al 1987, Evans et al 1990), $Pharbitis$ (King et al 1987) and others.

In January, and more so in February, the GA_4 disappeared from extracts of "OFF" buds and a new GA appeared - GA_1 - due to the beginning of vegetative development of these buds (Phinney 1985, Fujioka et al 1988).

The fact that GAs are involved in flowering processes is not new (Zeevaart 1976). In many fruit trees GA_3 is known to inhibit flowering (Sachs and Hackett 1969, Jackson and Sweet 1972).

Yet, during the last decade, since specific GAs became available, it was found that there is a wide range of GAs' influences on physiological processes which are controlled by different GAs. Looney et al (1985) demonstrated that GA_4 increased flowering in apple, while GA_3 inhibited it (Luckwill and Silva 1979). Moore et al (1986) extracted GA_1, GA_3 and iso-GA_3 from flowering apices of sugarcane while GA_{36} and GA_{19} were present only in vegetative apices. Dunberg (1980) showed that $GA_{4/7}$ promoted female flowering in *Picea abies* while GA_3 had no effect. Ingram et al (1985) listed 5 crops from which they isolated different GAs from vegetative parts as compared with GAs isolated from developing seeds. Lin and Stafford (1987) found in flowering (vernalized) wheat less C-20-GAs (GA_{53}, GA_{44}, GA_{19}) and more C-19-GAs (GA_1, GA_3, GA_{20}) and the opposite in non-vernalized wheat. It was suggested that conversion from C-20-GAs to C-19-GAs in wheat was controlled by vernalization, and the flowers development in this system is controlled by C-19--GAs. A single long day treatment induced flowering in the long day plant *Lilium temulentum*. As a result, extracted polar GAs were increased 3-5 fold 24 h after the completion of the single long day exposure. Rood et al (1989) demonstrated that different GAs were extracted from *Brassica napus* whether it was vegetative or not. Exogenous treatments with GA_{32} (a very polar GA) caused intensive flowering and almost no elongation, while GA_1 (much less polar) caused mainly elongation. Both treatments were applied under non-inductive conditions (Pharis et al 1987). On the other hand the same investigators found that in *Pharbitis nil* less polar GAs (GA_4 and GA_1) promoted flowering when applied before the inductive photoperiod (King et al 1987).

All these information support the hypothesis that specific GAs are required to promote flowering in different plants. Sometimes, the unique GA that promote flowering in one plant is inhibitory in another. Both promotion and/or inhibition might be controlled by the same GA in two different plant systems. The theory that less polar GAs promote flowering as was suggested by Pharis and Ross (1986) was proven not general by Pharis and co-workers in Pharbitis (King et al 1987). It is more likely that under different evolutionary conditions - day length, temperature, draught, different gases ratios in the atmosphere, anaerobic conditions etc. - plants had

developed different "right" GAs for controlling flowering. The biosynthesis pathway of the GAs had probably been "stopped" at different oxidation levels, or was diverted to other routes under different evolutionary growing conditions, which had affected rates and levels of oxidation along the biosynthesis pathway.

Our results indicated larger quantitative expression of GA-like activity in buds after "OFF" years. This difference was associated mainly with GAs of the GA_4 pathway like GA_{37}, GA_{15}, GA_{24} and GA_{51}. Later in the winter, January and February, the stronger GA-like activity was expressed mainly as GA_1 and others of its route ($GA_{53, 44, 19, 8}$ and GA_3).

It is likely that during the early stages of the winter, when differentiation occurs, the GA_4 route is dominating in the buds that have the potential to differentiate into inflorescences and possibly enables them to materialize as flowering buds. After differentiation was completed and the buds were ready to develop, the GA_1 route took over. In the "ON" buds, although the total GA-like activity was almost equal in October and slightly smaller in November, the major GA identified was 3-epi-GA_4 which probably interfered with the differentiation process during these months. Later in the winter, both the quantity and the number of GAs identified in "ON" buds, were reduced dramaticaly. This might be an indication that all these GAs in the "OFF" buds, resulted from differentiation which began earlier in the winter or even during the previous summer. The large number and quantities of GAs in the "OFF" buds toward the end of the winter also fits the fact that differetiated buds start to develop earlier than vegetative buds. The GAs that were identified in these buds at the end of winter belonged to the GA_1 pathway which is another indication of growth. These vegetative GAs had vanished from both kinds of buds in the early winter when growth ceases and differentiation was suppose to occur, and reappeared after differentiation, when vegetative growth was resumed. While GAs from the GA_4 route were dominant in buds during their differentiation, it was also observed that applications of GA_4 early in the winter on "OFF" trees, were much less inhibitive to flowering than GA_3 treatments (Ben-Tal, unpublished data). Figure 17 presents a scheme of the possible biosynthesis route of GAs in plants. The GAs that were identified in this investigation are marked. GAs 30, 35, 61 and 63

were not included because we were not sure how they were connected into this scheme. It is

based on a scheme presented by Rebers *et al* in a poster in the 14th international conference of

the International Association of Plant Growth Substances (1991), and other partial pathways

suggested by Koshioka *et al* (1988), Junttila and Jensen (1988), Nakayama *et al* (1989,1990)
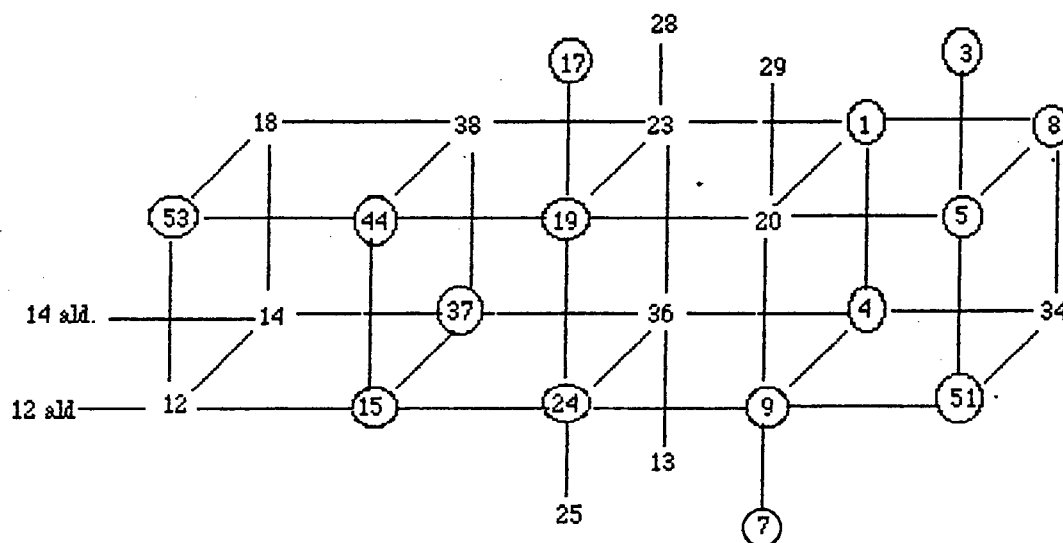
and Talon *et al* (1990).

Fig. 17. Possible metabolic connections among GAs in plants.
Adopted from Rebers et al (1991) with some additions suggested by others (see text).
GAs in circles were identified in the olive buds.

In conclusion we assume that this is the order of events:

1) In the fall, buds after "OFF" season, contain large quantities of GAs from the GA$_4$ route.

The "ON" buds contain mostly 3-epi-GA$_4$ which is interfering with the differentiation process.

Both kinds of buds still contain GAs of the GA$_1$ route that are left overs from the end of the last

growing season. This GA$_1$ is probably responsible for the major part of the GA-like activity

observed in the bioassys of October and November.

2) In the early winter, the GAs of the GA$_1$ route disappear in both kinds of buds and only GAs

from the GA$_4$ route are present. But these are "right" GAs required for the differentiating buds

("OFF"), while the 3-epi-GA$_4$ in the non-differentiating buds ("ON") is interfering with the

ability of these buds to differentiate.

3) In late winter the GAs of the GA_4 route start to subside in both kinds of buds. But large quantities of GAs from the GA_1 route start to accumulate in the differentiated buds ("OFF"), while the "ON" buds are still dormant and hardly any GAs can be detected. This GA_1 accumulation probably pushes forward the early development of the differentiated buds (after "OFF" years).

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Cooperation:

During the first year there was some information exchange and both principal investigators have presented a joint annual report. In the first year (1989) Ben-Tal visited Brenner's laboratory for a few weeks and used Brenner's GC-MS for identification of certain olive gibberellins. Further cooperation did not develop although Ben-Tal did send his 2nd year results for a joint annual report. The final report is related only to the Israeli project and is limited to biennial bearing in olive trees.

Project Achievements:

This project enlighted the role of GAs in the biennial bearing of olive. It was found that GA_3 is inhibitory to inflorescence differentiation when applied exogenously. But, it was found that GA_1 was necessary for inflorescence evocation and development. In "on" buds the total quantity of GAs was smaller and bud differentiation was delayed. However, it is not clear whether vegetative differentiation of buds after "on" years was due to lack of GAs of the GA_1 route that were supposed to be responsible for buds' evocation and development, or the fact that differentiation itself was inhibited because GA_4 type GAs were missing during the early phase of the winter.