משרד החקלאות - דו"ח לתוכניות מחקר
לקרן המדען הראשי

קוד זיהוי	א. נושא המחקר (בעברית)
14 - 0924 - 256	**<u>פיתוח טכנולוגיה חדשה ליישום הורמון חדש להפחתה או עידוד הסתעפויות צדדיות לחסכון בימי עבודה</u>**

ב. צוות החוקרים		
	שם משפחה	שם פרטי
חוקר ראשי	קולטאי	חננית
חוקרים משניים		
1	קפולניק	יורם
2	בן שבת	שמעון
3	בן דרור	עמירם
4		
5		
6		
7		

ג. כללי			
מוסד מחקר של החוקר הראשי			
מינהל המחקר החקלאי			
סוג הדו"ח	**תאריכים**		
	תקופת המחקר עבורה מוגש הדו"ח		**תאריך משלוח הדו"ח למקורות המימון**
מסכם	התחלה	סיום	
	שנה חודש	שנה חודש	שנה חודש
שנת המחקר: שנת מחקר/ סהכ שנים	11 / 2012	1 / 2016	/

ד. מקורות מימון עבורם מיועד הדו"ח		
שם מקור המימון	קוד מקור מימון	סכום שאושר למחקר בשנת תיקצוב הדו"ח
		בשקלים

ה. תקציר שים לב - על התקציר להיכתב בעברית לפי סעיף ה' שבהנחיות לכתיבת דיווחים

- הצגת הבעיה: אחת מהבעיות המרכזיות בעיצוב ייחורים מושרשים, שתילים, וכנות, במשתלה ובגידול בשדה, במגוון רחב של גידולים חקלאיים היא בעית ה"שוצים" – ההסתעפויות הצדדיות שדורשות השקעה רבה של ידיים עובדות להסירן. הורמון הסטריגולקטון הוכר זה לאחרונה כהורמון צמחי חדש בעל תפקיד מכריע בשמירה על השלטון הקודקודי.
- מטרות המחקר: ההצעה הנוכחית מציעה לפתח טכנולוגיה חדשה המבוססת על יישום חד פעמי של "חבק" המכיל את ההורמון למניעת הסתעפות של ענפים צדדיים במגוון גידולים חקלאיים. 1. בחינת פעילות ביולוגית של אנאלוגים של סטריגולקטונים בשילוב עם חומרים ביולוגיים או כימיים אשר ירכיבו את החבק. 2. כיול ולימוד השפעת החבק בצמחי מודל (זיתים). 3. יישום החבק על מגוון גידולים חקלאיים ובחינת פתרונות לציפויי החבק.
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- תוצאות עיקריות: בשנה הראשונה הצלחנו בפיתוח הבוחן הביולוגי ליישום סטריגולקטונים, שהראה תוצאות דומות לאלו של יישום סטריגולקטונים בעציצים. מצאנו אנאלוג פעיל לסטריגולקטונים, EGO10 , בעל עלות נמוכה יחסית, שהינו בעל פעילות מובהקת בצמצום התפתחות הענפים הצדדיים. כמו כן מצאנו כי חומרי התווך

EGO10 הביאו לעליה בעמידות ובפעילות החומר הפעיל. בשנה השנייה מצאנו כי HPC+glycerin ו-PVA פעיל בתנאי משתלה וקבענו את הריכוז האופטימאלי לפעילותו. כמו כן בחנו כיסויי חבק שונים, נמצא כי כיסוי פלסטי קשיח עדיף על אחרים עבור החבק. בשנה השלישית נוסה החבק על מגוון גידולים חקלאיים, נקבעו דרכי יישום וריכוזים וכן נמצאו כיסויי חבק יעילים.

- מסקנות: פיתחנו טכנולוגיה חדשה המבוססת על יישום של "חבק" המכיל את ההורמון למניעת הסתעפות של ענפים צדדיים בשתילי זיתים. קבענו את הפעילות ביולוגית של אנאלוגים של סטריגולקטונים ומצאנו שילובי חומרים כימיים אשר מביאים לייצוב והגברת חדירות החומר הפעיל. תרכובת חומרים אלו מרכיבה את החבק. כמו כן בחנו כיסויים שונים לחבק שיאפשרו את פעילות החומר הפעיל וייצובו. במחקר שנערך גם כיילנו את השפעת החבק בצמחי מודל (זיתים) וגידולים חקלאיים נוספים, ומצאנו כי פעיל בתנאי משתלה מסחרית וחממה עבור מספר גידולים חקלאיים. כמו כן פיתחנו ציפוי לחבק שיכול לשמש מסחרית. פיתוח ביוטכנולוגי זה נמצא כעת בפיתוח מסחרי וצפוי להביא ליתרונות חקלאיים-כלכליים של חיסכון בידיים עובדות, הגדלת הפוריות, איכות המוצר והרווחיות למגדל.

ו. אישורים

הנני מאשר שקראתי את ההנחיות להגשת דיווחים לקרן המדען הראשי והדו"ח המצ"ב מוגש לפיהן

חוקר ראשי	מנהל המחלקה	מנהל המכון (פקולטה)	אמרכלות (רשות המחקר)	רשות המחקר	תאריך (שנה) (חודש) (יום)

דו"ח מסכם לתכנית מחקר מספר 256-0924-14

שנת המחקר: 3 מתוך 3 שנים

פיתוח טכנולוגיה חדשה ליישום הורמון חדש להפחתה או עידוד הסתעפויות צדדיות לחסכון בימי עבודה

Development of new technology for strigolactones application by collars

מוגש לקרן המדען הראשי במשרד החקלאות ע"י

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תקציר

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מעריכים מומלצים לבדיקת הדוח המדעי

1. **שבתאי כהן (מדריך חקלאי sab@inter.net.il**
2. **דורון הולנד (חוקר, מינהל המחקר החקלאי vhhollan@volcani.agri.gov.il)**
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הצהרת החוקר הראשי:

הממצאים בלוח זה הינם תוצאות ניסויים.

הניסויים מהווים המלצות לחקלאים: לא

Introduction and description of the problem:

The current proposal offers to develop new technology for preventing or minimizing the formation of lateral branches in woody plants. Different levels of apical dominancy ensures continuous growth and plant design necessary for agriculture. This is true of vegetables, ornamental woody plants and fruit trees. In many of these plants one of the problems is vegetative growth of side branches, termed axillary branches, which require removing constantly. In olives and many other fruit trees apical dominance is essential in the first stage of growth in the nursery (following rooting and hardening), for the development of one bud only to apical branch. However in most cases (97%!) multiple buds develop simultaniously, which requires the investment of manpower for pruning of these axillary branches. Other plants such as ornamental plants and fruit tree require defined architecture, which may add value to the determined product; in most of these plants it is necessary to create a section without axillary branching (a "feet"), at the base of the stem. Prevention of branching of the plant requires considerable investment and farm work using valuable manpower.

Strigolactones (SLs) are plant hormones shown to act as long-distance branching factors, suppressing the outgrowth of axillary shoot buds. Hence, they are prominent effectors of apical dominance in plants. Present in a wide variety of plant species, SLs are derived from carotenoids and are biosynthesized through several steps. Previously, we have found that root development of seedlings is regulated by SL activity (reviewed by Brewer et al., 2013). They are also involved in plant communication in the rhizosphere, and act as stimulants of parasitic plant (*Striga* and *Orobanche*) seed germination (reviewed by Xie et al., 2010) and as stimulants of arbuscular mycorrhizal fungi hyphal branching (reviewed by Koltai et al., 2012).

Here we have develop an agro-technologies for a novel usage of SLs in different crops for suppression of axillary buds growth, for the prevention of axillary branch development and for reduction in the need for manpower in agriculture. Commercialization of this new technology may result in agricultural and economical benefits: labor savings (less pruning and trimming), increasing product quality and increased profitability. This principle of developing a new approach for longer-term design of the plant may be applied for a variety of crops and agricultural uses. As stated above, SLs suppress the outgrowth of preformed axillary buds in the shoot. Therefore, the contribution of this hormone to agriculture in Israel will be reflected in savings in manpower for pruning , shaping, thinning and removal of axillary buds and branches. In addition, the study will develop an infrastructure for implementation of additional materials that will have future economic significance.

Research aims

This study proposed to develop a new technology, based on the application of synthetic SL plant hormone in collars, which will be applied on a variety of crops for repressing the development of axillary branches. The technology will enable a reduction in manpower and associated cost required to perform manual work for the design of plant architecture in nurseries and in the field.

Specific aims:

1. Development of active collar components while examining the biological activity of SL analogues combined with biological or chemical substances which will make up the collar.
2. Examine the extent of the effect of a collar prototype on model plants (olive), on strengthening the apical dominance and finding the optimum effect of the materials.
3. Development of collar application on a variety of crops:
    a. determination of concentrations, site and duration of use for different crops
    b. development of applicable collar covers to facilitate collar use in agriculture.

Experiments that were conducted during the project

FIRST YEAR

Examination of the effect of application of synthetic SL analogs on olive seedlings

1. Application of SL analogs by irrigation

For an examination of the effect of different SL analogs on the epical dominance and axillary branching, we have chosen olives as a model plant. This is because one of the prominent labor-demanding issues with olive growth in nurseries is their need of pruning of axillary branches. Moreover, we have found in preliminary experiments (described below) that olives respond well to the applied SL analogs and therefore- may be used for development of SL-based collars for increased apical dominance. As a first step, we have examined the effect of application of various analogues of SLs on olives, given to roots by irrigation. Three different SL analogs were examined, GR24, EGO10 and ST362. Analogs were applied at a concentration of 10^{-8} M during vegetative growth, in seedlings that were obtained from Agrogold nursery. These seedlings were rooted from cuttings and hardened. At the time of the experiment they were about 6 months old. Growth soil mixture was used as in the nursery. Application of the SL analogs was carried for 6 weeks, using 3 dosages: 0, 0.03 and 0.3 µM. Solution was injected to the roots twice a week and all the growing buds were counted. For each treatment (treated and control groups) 10 plants were measured. The results show that application of GR24 lead to a significant reduction in number of lateral branches (Figure 1). The effect was most prominent at a concentration of 0.03 µM.

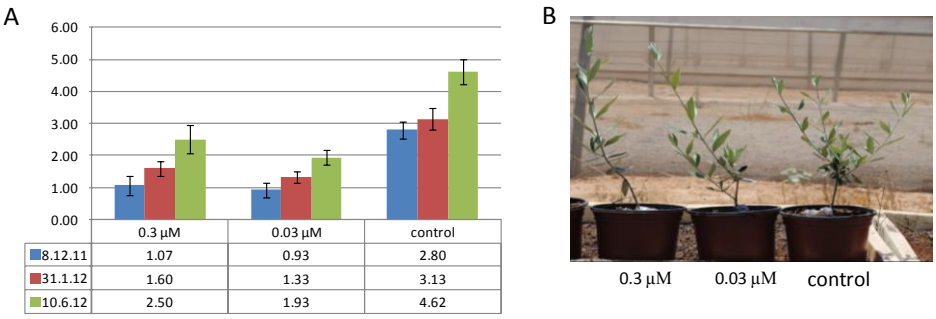
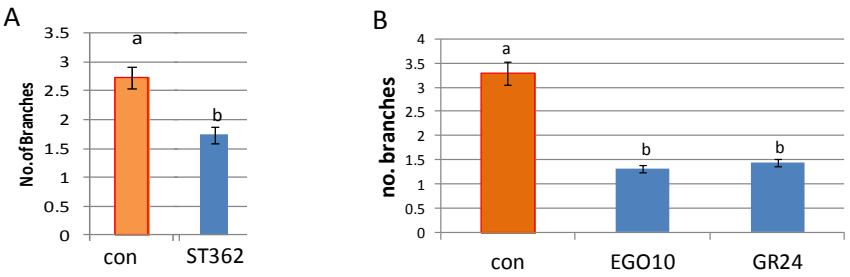


Fig 1: Effect of application by irrigation of synthetic SL analog (GR24) on apical dominance in Picual olives. Analog was applied at a concentration of 0, 0.03 and 0.3 µM twice a week. (A) Number of growing buds following GR24 treatments and control. For each treatment (treated and control groups) 10 plants were measured. (B) A representative example of treated and control olives seedlings.

Nevertheless, despite the wide usage of GR24 in laboratory experiment it is costly and therefore may not be suitable for agricultural use. Hence, as a second stage we have examined the activity of two other SL analogs, EGO10 and ST362, which are much less costly and easier to synthesized, and amended for high mass production. A similar, albeit less potent effect was evident upon the application of ST362 to plants (Figure 2), whereas EGO10 showed a repressing effect on axillary branch growth similar to that of GR24 (Figure 2).

Fig 2: Effect of application by irrigation of synthetic SL analogs (ST362 [A]; EGO10 [B]) on apical dominance in Picual olives. Analog was applied at a concentration of 0, 0.03 and 0.3 µM twice a week. Number of growing buds is shown. For each treatment (treated and control groups) 10 plants were measured.



2. Increasing SL analogs stability

The implementation of the SL analogs necessitates a degree of material stability, to ensure their activity in the soil. To cope with this requirement, first, we examined the stability of the SL analog GR24 under different environmental conditions. Results show that GR24 loses biological activity and is molecularly degrading following long term exposure (over 5 days) to temperatures above 40◦C, under acidic pH solutions and following exposure to UV irradiation. Secondly , we tried to stabilize it with HPC (Hydroxypropyl Cellulose), a hydrophobic medium. Stability was tested by both biological activity and by assessing the molecule using LC-MS . HPC medium stabilized GR24 and increased both its biological activity and intact structure under the examined conditions. Hence HPC may be used as a stabilizing medium to extend GR24 biological activity under the abiotic stress conditions. These results are being published in the attached article (performed **before** the commence of this research; Enzer et al., enclosed).

Next, we examined the stabilization ability of HPC on EGO10 at different pH and temperature around 60^0C and examined *Orobanche* (parasitic weed) germination in the presence of EGO10 and HPC (Figure 3; more details on this bioassay are in Enzer et al., enclosed). Based on the experimental results we observed that HPC stabilize EGO10 under basic pH and at 60 ^{0}C.

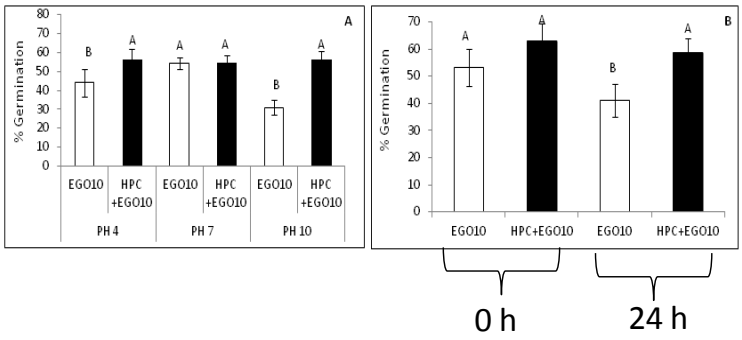


Fig 3: Orobanche germination in the presence of EGO10-HPC at (A) different pH (B) temperature 60 ^{0}C for 0 h and 24 h incubation time

3. Application of SL analogs directly to stems

3.1. Development of the bioassay

Next, we sought to examine whether the active SL analog may be used as an effective repressor of shoot branching by its application on the stem itself. For that purpose we have established a bioassay for examination of SL analogs effect on shoot branching in vitro. For that purpose, olive seedlings were grown in greenhouse under controlled temperature of 23 C. We have prepared 1/2 Murashige and Skoog (MS) solidified with 1.5% w/v agar in partitioned plates. After solidification we cut down the center plastic area (2 cm) with the help of scalpel. Olive seedlings were cut between the second and fourth node. The cuttings were surface-sterilized by using 70% ethanol and 1% sodium hypochlorite treatment and kept on the plates containing agar media. EGO10 was mixed with 1/2MS in agar, cubes were cut (1.5cm area) and positioned at the base of the cutting in the plates (Figure 4). Experimental controls for the olive seedling was with acetone at the concentrations used in the respective EGO10 treatments. Plates were sealed only in two side with the help of saran wrap to prevent accumulation of gases, and positioned in an upright 90^o position and incubated at 22°C under a photoperiod of 16 h light/8 h dark. We have taken photographs every 3 days till 12 days by using binocular. We have kept eight replicates in each treatment. We have measured the bud outgrowth using Image J software and calculated the length of the bud minus its length at the first day following application. Means of replicates were subjected to statistical analysis by using the JMP statistical package.

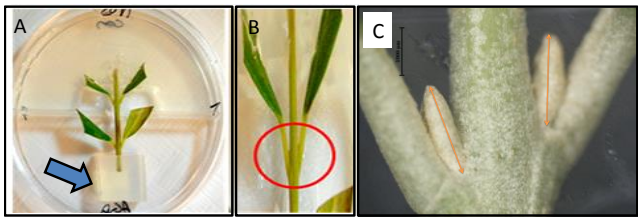


Fig 4: An example of the plate bioassay we have developed for examination of bud outgrowth in olive cuttings. (A) The divided plate with olive cutting, arrow points to the agar cube that contains SL analog (or acetone control). Two upper buds and two downward bud are present on each segment (B) Bud position in the node. (C) The way bud outgrowth was measured, bar denotes 1000 µm.

3.2. Effect of EGO10 on bud outgrowth supplied in agar cube at the base of the cutting

Previously, other research groups showed that application of 5 µM of GR24 is sufficient to acknowledge reduction in shoot branching in in vitro systems (Ward et al., 2013). Hence, we have applied EGO10 at a concentration of 5 µM to the cuttings. Once we have applied EGO10 to the base of the cutting of the olive seedling a marked reduction was observed in bud outgrowth in comparison to control (results of delta of bud growth at day 12; Figure 5). These results suggest that EGO10 may be used as an SLs analogue for the inhibition of olive bud outgrowth.

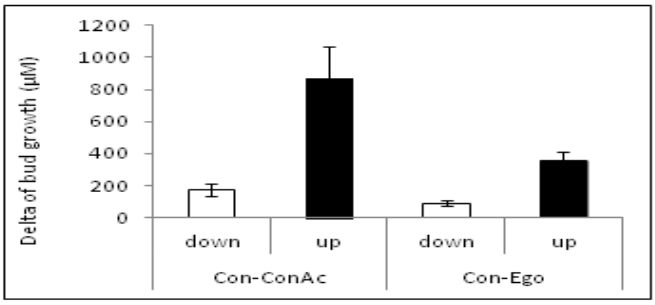


Fig 5: Effect of EGO10 (5 µM) supplied in agar cube to the base of the cutting of olive seedling on bud growth. Y axis- the length of the bud minus its length at the first day following application. X axis- down or up refer to bud position (see Fig 3); Con-Con Ac, upper part of plate contain 1/2MS water, lower part (agar cube) contain 1/2MS acetone control; Con-Ego, upper part of the plate contain 1/2MS water agar, lower part (cube) 1/2MS EGO10.

3.3 Effect of EGO10 on bud outgrowth supplied in agar cube on the cutting bark

For practical application of EGO10, it will need to be applied through the bark of the stem. For that purpose we have examined whether EGO10 application on the bark may reduce bud outgrowth. Therefore, an agar tube that contains EGO10 was placed this time on the bark of the cutting. Also, for increased penetration of the hormone analog we have scratched the bark and examined the ability of Ego10 to reduce bud outgrowth, in comparison to EGO10 application to non-scratched bark. The results showed that first, application of EGO10 (5 µM) in agar cube of 1.0 cm on the bark led to a significant reduction in bud outgrowth in comparison to control (Figure 6). These results suggest that the EGO10 is diffusing through the bark stem of olive seedling and lead to bud outgrowth inhibition.

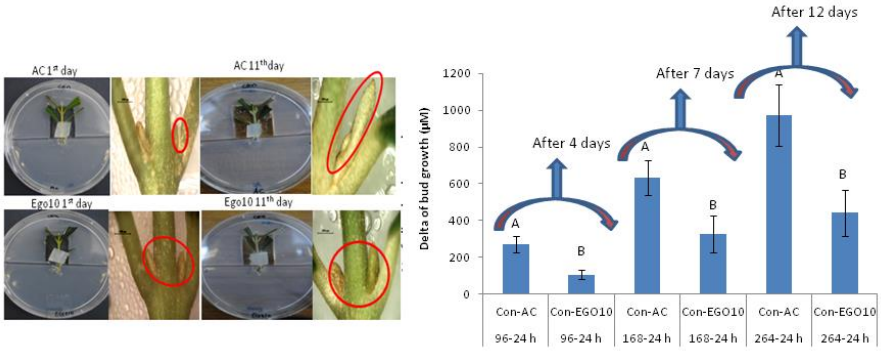


Fig 6: Effect of Ego10 (5 µM) on bud growth applied as agar cube on the bark of the cutting of olive seedling. (A) example for the plates-experiment in which agar cube was placed on the cutting bark. Circled-examined buds. (B) Results of bud outgrowth following 96h, 168h and 264h. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Con- Ac, plate contains 1/2MS water agar, agar cube contains 1/2MS acetone control; Con-Ego10, plate contains 1/2MS water agar, agar cube contains 1/2MS EGO10.

Second, scratching the bark led to callus development on the nodes (Figure 7) and alterations in bud outgrowth (Figure 8).

Fig 7: Effect of scratches on the olive seedling cuttings.

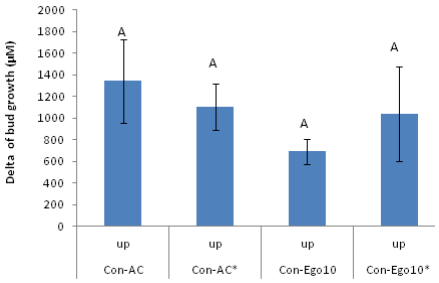


Fig 8: Effect of EGO10 (5 µM) on bud growth applied as agar cube on the bark of the cutting of olive seedling. Results of bud outgrowth at 264 h. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Con- Ac, plate contains 1/2MS water, agar cube contains 1/2MS acetone control; Con-Ego10, plate contains 1/2MS water agar, agar cube contains 1/2MS EGO10. *, bark was scratched.

Taken together, the results suggest that scratching of the olive bark is not needed, and that EGO10 is able to penetrate the cutting bark without the need of scratching.

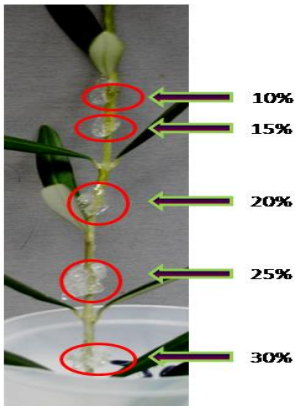
3.4. Effect of EGO10 application on the cutting bark in stabilizing substances

As a next step in the development of SL application technology, we have examined different substances as media that support hormonal activity. Agar examined above contains usually nutritional compounds. As a result bacteria and fungi can easily contaminate agar cubes; therefore these are not suitable for field application. Therefore, other substances were examined, as described below. Experiments were done similar to as described in 2.3, by application of EGO10 in different media on the cutting bark, in plates. Bud outgrowth was monitored as above.

3.4.1 Strigolactone mixed with hydroxy propyl cellulose (HPC)

We have examined the use of Hydroxypropyl cellulose material (HPC) instead of agar as a media for EGO10 application on the bark. Notably, we have previously found that HPC leads to stabilization of SL analogs, including EGO10 (detailed above in section 2). Following, we have determined which percentage of HPC might be suitable for field application. For that purpose we prepared the different percentage of HPC and examined its capability to stick to the stem. We found that 30 % of HPC in water was suitable for sticking to the stem (Figure 9). Lower percentages of HPC in water remained liquefy and were not suitable for field application.

Figure 9: Different percentage of HPC applied on olive stem.



Based on these results we have used 30% HPC and mixed it with two different concentrations of EGO10: 5 and 10 µM (Figure 10). Bud outgrowth was monitored as described above and was directly compared to bud outgrowth using the agar tube application (described above), as a positive control. The results showed that in the presence of

HPC and both concentrations of EGO10 bud outgrowth was reduced till 4 days, however at day 4 post application bud outgrowth resumed, whereas application using agar cube reduced bud outgrowth through the experiment period (Figure 11). These results suggest that HPC is not a suitable material for the application of SLs to olive seedling. We speculated that HPC became dry following 4 day of application and hence could not support EGO10 diffusion through the stem. Therefore we had to find another material which can be stable for a longer period of time and support diffusion of the hormone though the bark.

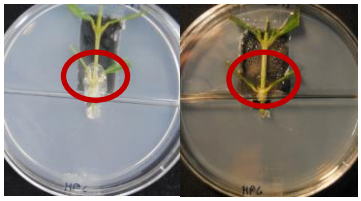


Figure 10: An example for application of HPC and EGO10 on olive cutting stem.

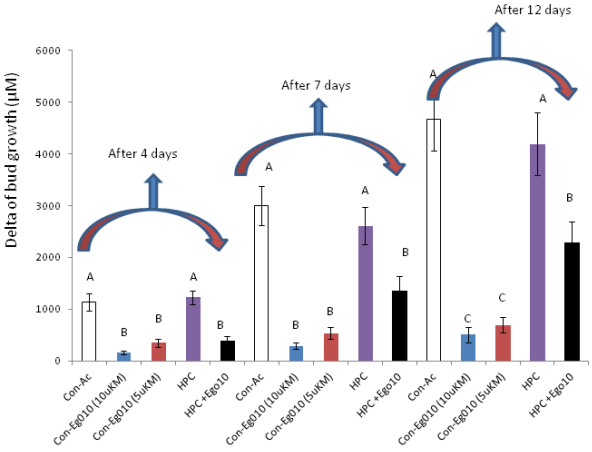


Figure 11: Effect of Ego10 (5 µM and 10 µM) on bud growth supplied in agar cube or HPC on the bark of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Con- Ac, plate contains 1/2MS water agar; HPC- plate contains 1/2MS water agar and HPC applied with acetone; HPC+Ego10, plate contain 1/2MS water agar and HPC applied with EGO10; Con- Ac, plate contains 1/2MS water, agar cube contains 1/2MS acetone control; Con-Ego10, plate contains 1/2MS water agar, agar cube contains 1/2MS EGO10. Results of 96h (4 d), 168h (7 d) and 264h (12 d) are shown.

3.4.2 Strigolactone mixed with hydroxy propyl cellulose (HPC) and glycerin

To improve HPC performance we used a mixture of HPC and glycerin in ratio of 3:1. Glycerin, as a known humectant, has the capability to moist materials. Bud outgrowth was monitored as described above. The results show that using HPC mixed with glycerin and EGO10 increased HPC performance as media for EGO10 application (Figure 12). Bud outgrowth was inhibited by EGO10-HPC-glycerine application throughout the experiment period.

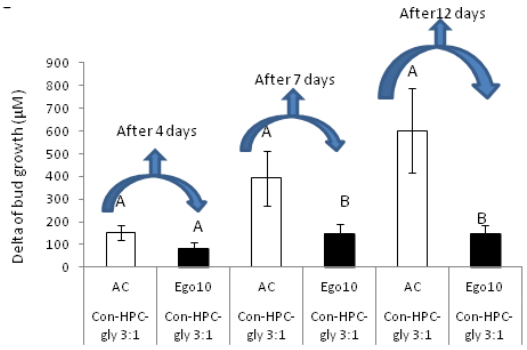


Figure 12: Effect of EGO10 (5 µM) on bud growth applied in HPC mixed with glycerin on the bark of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Plate contains 1/2MS water agar and HPC mixed with glycerine, with acetone (Ac) or EGO10. Results of 96h (4 d), 168h (7 d) and 264h (12 d) are shown.

3.4.3 Strigolactone mixed with polyvinyl acetate (PVA) polyvinyl pyrrolidone (PVP) and hydroxy ethyl cellulose (HEC)

To have a wider collection of possible substances as media for EGO10 application we have screened other polymers, including polyvinyl acetate (PVA) polyvinyl pyrrolidone (PVP) and hydroxy ethyl cellulose (HEC). We used 30% of HEC, PVA and PVP. 30% PVP was not solid and therefore could not be tested. However HEC and PVA were examined for their ability to support

EGO10 activity. Bud outgrowth was calculated as above. We found that PVA supported EGO10 activity of bud outgrowth inhibition, while HEC did not (Figure 13 and Figure 14).

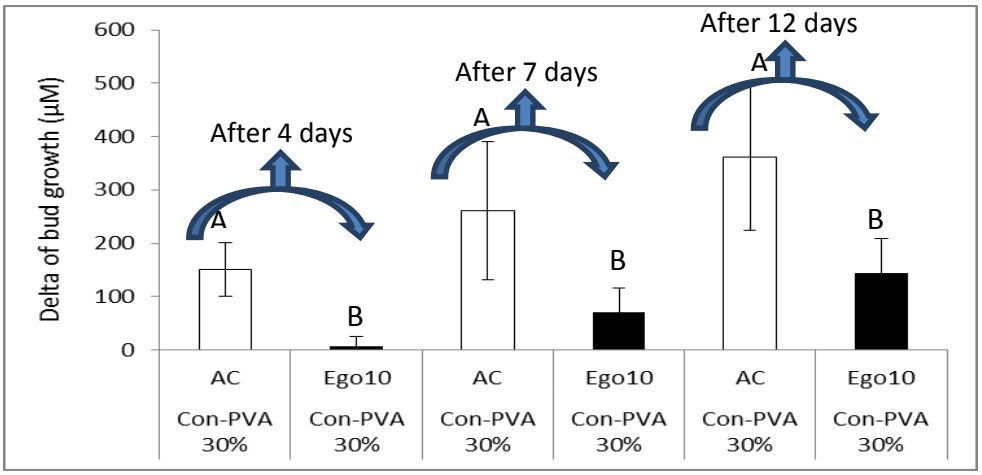


Figure 13: Effect of EGO10 (5 µM) on bud growth supplied in PVA on the bark of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Plate contains 1/2MS water agar and PVA, with acetone (Ac) or EGO10. Results of 96h (4 d), 168h (7 d) and 264h (12 d) are shown.

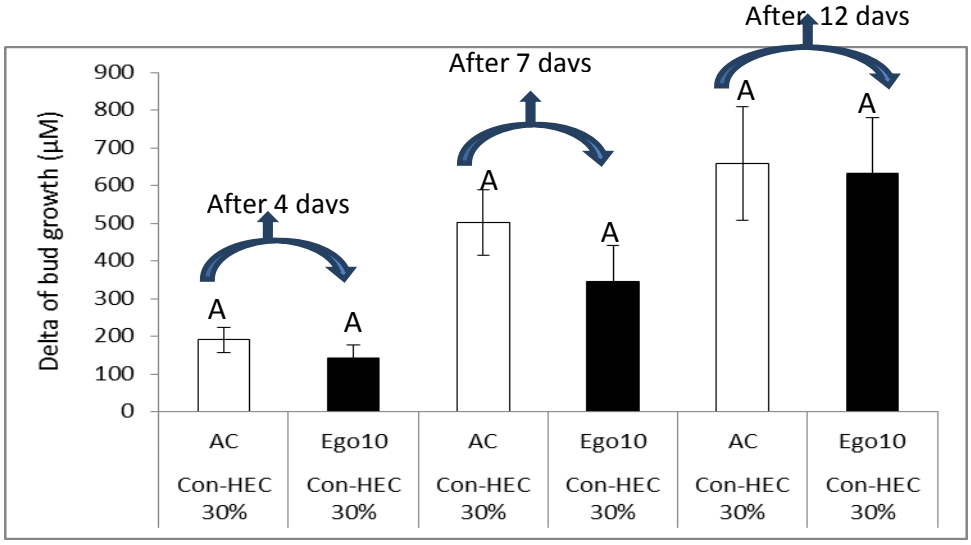


Figure 14: Effect of EGO10 (5 µM) on bud growth supplied in HEC on the bark of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Plate contains 1/2MS water agar and HEC, with acetone (Ac) or EGO10. Results of 96h (4 d), 168h (7 d) and 264h (12 d) are shown.

Following, we tried different mixtures of the polymers, applied as described above and monitored bud outgrowth. We found that a mixture of PVA with HEC or PVA and PVP did not support EGO10 ability to act to inhibit bud outgrowth (Figure 15 and Figure 16). This might be due to area of brown spots, interpreted as damage to the stem, upon application.

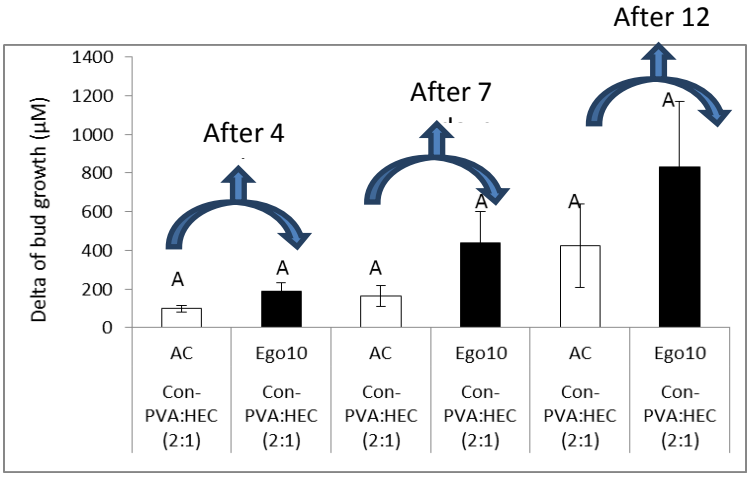


Figure 15: Effect of EGO10 (5 µM) on bud growth supplied in PVA:HEC (2:1) on the bark of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Plate contains 1/2MS water agar and PVA:HEC (2:1), with acetone (Ac) or EGO10. Results of 96h (4 d), 168h (7 d) and 264h (12 d) are shown.

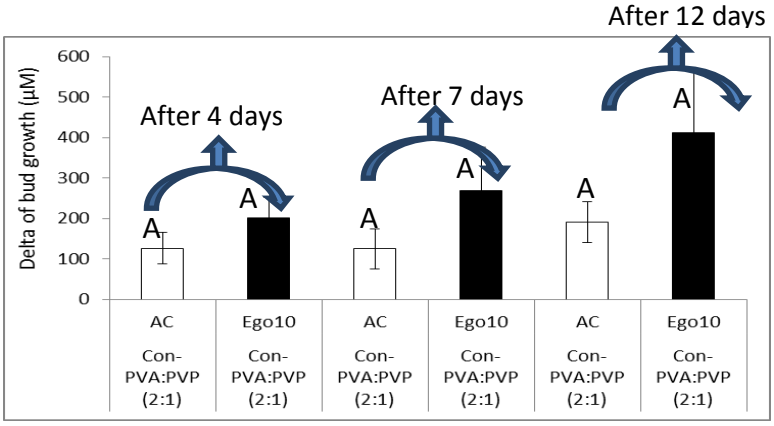


Figure 16: Effect of EGO10 (5 µM) on bud growth supplied in PVA: PVP (2:1) on the bark of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. X axis- Plate contains 1/2MS water agar and PVA: PVP (2:1), with acetone (Ac) or EGO10. Results of 96h (4 d), 168h (7 d) and 264h (12 d) are shown.

To summarize, during the first year of the project, while setting a bioassay for olive bud outgrowth we have identified the active compound EGO10 and determined that the most effective substances for EGO10 activity are HPC+glycerin and PVA (Fig 12) for application of SL analogs on stem, for inhibition of bud outgrowth. By this we have completed the first year goals.

SECOND YEAR

In the second year of the project we used the knowledge developed in the first year for application of EGO10 under greenhouse and commercial nursery conditions, while developing an apparatus for efficient application of EGO10 for reduction of axillary branches growth.

We have conducted experiments on olive seedlings in our greenhouse to determine a. Preferred apparatus coverage, b. Preferred substance to support EGO10 activity under greenhouse conditions.

Three different coverage were tested on the stem of Olive seedling

1. Parafilm Cover
2. Flexible plastic cylinder

3. Rigid plastic tube

1. Parafilm Cover

To examine the performance of the substances that were found in plates experiments to be most effective for EGO10 activity, we have applied those substances, namely HPC + glycerin and PVA on olive seedling stem under greenhouse conditions (Figure 17). In each experiment, for each treatment, 10 plants were used. Greenhouse conditions include controlled temperature room of 25 C. The substances were applied on the stem with the help of spatula and covered with parafilm (Figure 17 A and 17B). However, under the relatively warm conditions of the greenhouse, after 1day both HPC + glycerin and PVA liquefied and lost their adhesion to the stem (Figure 17 D, arrow denote some of the liquefied material).

Hence, we came to the conclusions that: 1. HPC + glycerin or PVA may not be suitable for application under greenhouse or nursery conditions. 2. Another cover is needed for the apparatus. Therefore we also decided to try and utilize different polymeric substances and different covers for EGO10 application under greenhouse conditions.

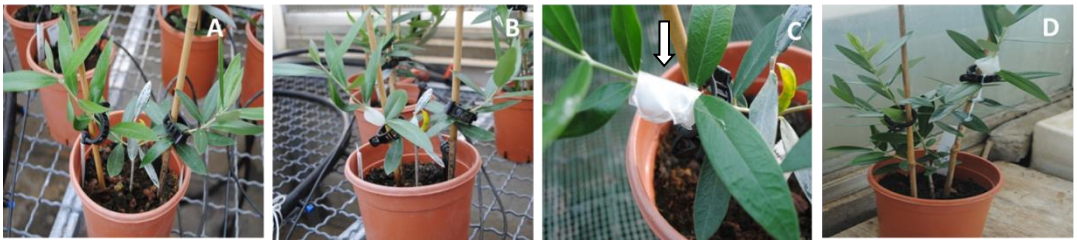


Figure 17: Application of EGO10 or Ac (acetone control) under greenhouse conditions (A) Control Olive Seedling ;(B) EGO10 and the substance (HPC + glycerin or PVA) are applied on the stem ;(C) cover with parafilm; (D) following one day only HPC+Glycerin liquefies on the stem.

2. Flexible plastic cylinder

To improve the performance of the apparatus, in this set of experiments we have used different substances and different cover. The cover consisted of flexible plastic tubes that was put on the stem and was sealed with transparent adhesive plaster. Four formulations were developed and tested: AN I, AN II, AN III, AN IV. These formulations are based on polysaccharide-sorbent-humidifier substances while the main difference between them is the viscosity of the final product and the ability to retain and release of the active ingredient (EGO 10). All formulations included preservatives against biological decay and were tested under lab condition and were proven to be less heat susceptible, and less likely to liquefy under greenhouse conditions. The combination of the humidifier in the formulation and the use of the plastic, flexible tube cover was expected to keep the material moisture over the treatment period. The different substances (AN-I to AN-IV) were applied in the plastic tubes and then the tubes were used to cover the seedling stem (Figure 18A 18B). The tubes were sealed with the transparent plaster (Figure 18C-18E). On both sides moist cotton was put to minimize water evaporation (Figure 18F). Bud outgrowth was measured for 30 days.

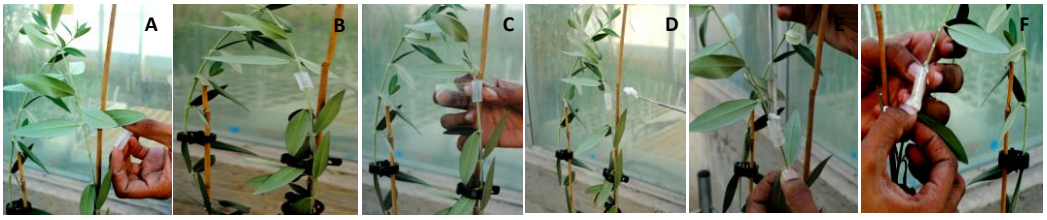


Figure 18: Application of EGO10 or Ac (acetone control) under greenhouse conditions (A) Tube was cut; (B) the tube was fitted on the stem; (C) The tube was sealed with transparent plaster; (D) substance and active compound filled the tube; (E) completed filled tube; (E) tube was sealed from both sides by cotton wool.

The results of application of AN-I and AN-II with EGO10 (10 µM) as described above are shown in Figure 19. Both compounds were not liquefied under greenhouse conditions (not shown), however, use of AN-I abolished EGO10 activity on bud outgrowth. Use of AN-II led to a marked decrease in bud outgrowth in the acetone control (that did not include EGO10). The AN-II plants lost their leaves and seemed to be hampered in their development as a result of the applied material.

Hence, we concluded that despite their better resistance to greenhouse conditions these subtances were not suitable for olive treatments because of their strong retention of the active ingredient and bad release pattern to the plant.

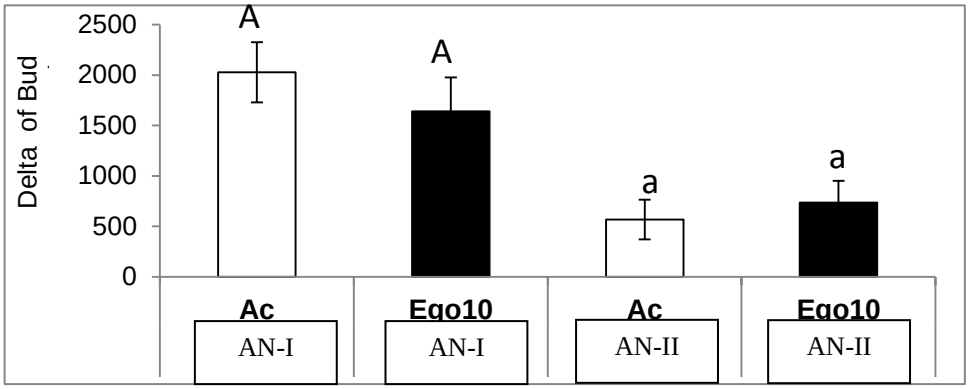


Figure 19: Effect of EGO10 (10 µM) or Ac (acetone control) on bud growth supplied in AN-I and AN-II on the stem of the olive seedlings. Y axis- the length of the upper bud minus its length at the first day following application. Results of 30 days are shown.

Following, in order to find improved substances that on the one hand support greenhouse usage and on the other, EGO10 activity, we have used the improved AN-III and AN-IV substances that contain less sorbents and enable higher release rates of the active ingredient to the plant. Using AN-III and AN-IV substances hampered plant development to some extent (Figure 20). Moreover, their use did not support EGO10 activity. This is since EGO10 used under these conditions did not inhibit bud outgrowth (Figure 20).

In conclusion, the ANs series of substances was found to be unsuitable for use as substances for EGO10 application.

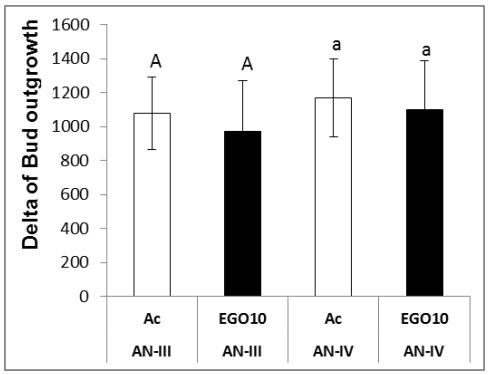


Figure 20: Effect of EGO10 (10 µM) or Ac (acetone control) on bud growth supplied in AN-III and AN-IV on the stem of the olive seedlings. Y axis- the length of the upper bud minus its length at the first day following application. Results of 30 days are shown.

3. <u>Rigid plastic tube</u>

Since the ANs series of substances was found to be unsuitable for use as substances for EGO10 application, we had to go back to the usage of the most suitable compounds that support EGO10 activity, namely HPC + glycerin or PVA, and find practical solutions to prevent their leakage from the used apparatus. This was done by using plastic tubes that are sealed on their bottom, for the prototype we used appendorf

tubes. The tube was cut in the middle and was fitted on the stem. We placed small moist cotton at the bottom of the tube and filled it with the examined material. The tubes were filled with HPC + glycerin or PVA and EGO10 (Figure 21). In each experiment, for each treatment, 10 plants were used. Greenhouse conditions include controlled temperature room of 25 C.

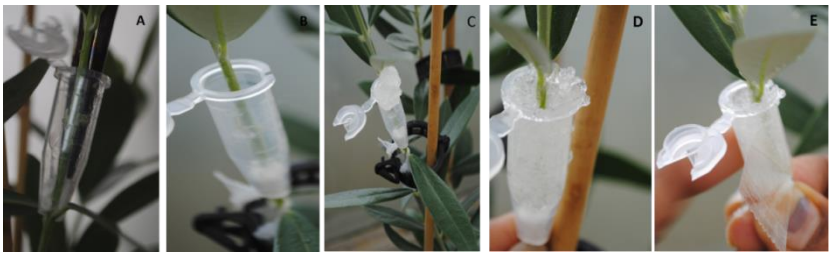


Figure 21: Application of EGO10 or Ac (acetone control) hormone in the field (A) tube was fitted on the stem; (B) cotton was placed at the bottom of the tube; (C, D) materials were put to fill the tube; (E) tube was cover with transperant plastic tape.

Application of EGO10 (10 µM) or acetone control in PVA under the examined conditions led to inhibition of seedling outgrowth (Figure 22). Therefore, despite its high performance in plates it is quite clear that PVA is not suited to be used as stabilizing substance for EGO10 under greenhouse conditions. On the other hand, use of HPC+glycerin under these conditions did not lead to inhibition of outgrowth (Figure 22). Moreover, HPC+glycerin with EGO10 led to some inhibition of bud outgrowth, without delaying plant growth (Figure 23). However control and treatment were not significantly different. Based on these results we decided to increase EGO10 concentration, and apply it in the HPC+glycerin substance, for inhibition of bud outgrowth.

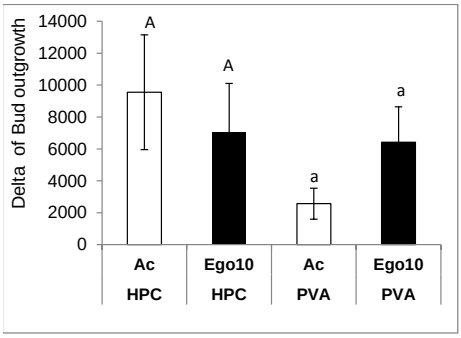


Figure 22: Effect of EGO10 (10 µM) or Ac (acetone control) on bud growth supplied in HPC + Glycerin and Polyvinyl alcohol (PVA) on the stem of the olive cuttings. Y axis- the length of the upper bud minus its length at the first day following application. Results of 30 days are shown here.

Higher concentrations of 50 µM of EGO10 or Ac (acetone control) were applied with HPC+glycerin using the rigid tubes. Bud outgrowth was measured till 30 days. We found that EGO10 in HPC+glycerin have the capability to inhibit bud outgrowth significantly in comparison to control (Figure 23). Also, all treated olive buds grew properly. These results suggest that the use of HPC + glycerin and EGO10 (50 µM) in rigid tubes can be examined under commercial nursery for axillary bud outgrowth inhibition.

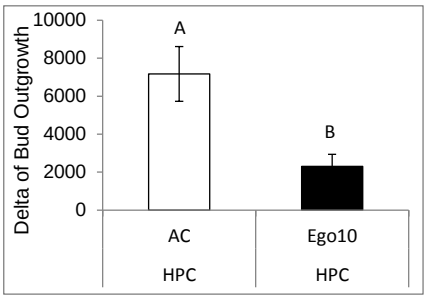


Figure 23: Effect of EGO10 (50 µM) or Ac (control) on bud growth supplied in HPC +glycerin on the stem of the olive cuttings. Y axis-the length of the upper bud minus its length at the first day following application. Results of 30 days are shown.

<u>Application in a commercial nursery</u>

Experiments were done in AGROGOLD nurseries. We applied HPC+glycerin with EGO10 (50µM) in rigid tubes, as described above (Figure 24). In each experiment, for each treatment, 10 plants were used. Commercial nursery conditions include nethouse, 40% shed without temperature control. The number of wake up buds was counted 75 days following application and the seedling architecture and general growth and development were examined.

Figure 24: Application of Ego10 hormone in the commercial nursery same way like green house. (A) fit the appendrof on the stem (B) cover with cello tope outside of the appendrof (C) filled the material inside the appendrof (D) completed filled by material (E) cover the cotton on the upperside of the appendrof (F) complete experiment set up.

We found that EGO10 in HPC+glycerin have the capability to inhibit bud outgrowth significantly in comparison to control, also under commercial nursery conditions (Figure 25). Importantly, seedling outgrowth at that time was not inhibited, and all treated olive buds grew properly (Figure 26). The control seedlings had a bushy appearance (not favorable by the farmer and necessitate cutting by hand). In contrast the EGO10 treated seedlings had one or two leading branches, a favorable plant architecture that does not require any investments of working hands (Figure 26). The application site, after removal of the apparatus appeared to be unharmed and functional (Figure 27).

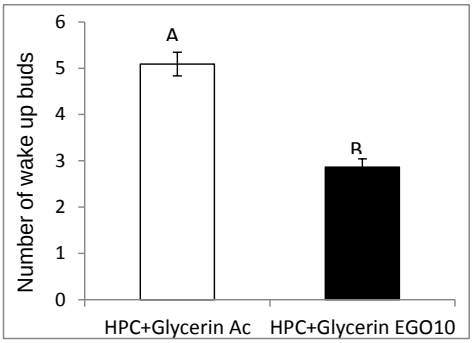


Figure 25: Effect of EGO10 (50 µM) or Ac (control) on bud growth supplied in HPC +Glycerin on the stem of the olive cuttings. Y axis-the number of wake up buds and results of 75 days are shown.

Figure 26: Effect of EGO10 treatment on seedling architecture and growth.

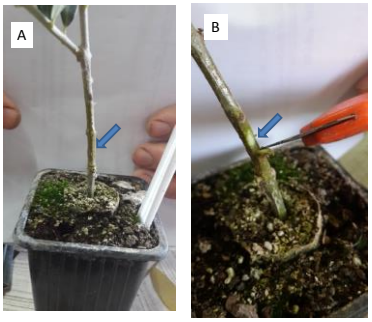


Figure 27: The apparatus application site (arrow). (A) Bark; (B) vascular system.

<u>These results suggest that the use of HPC + glycerin and EGO10 (50 µM) in rigid tubes might be suitable to be used in commercial nursery for axillary bud outgrowth inhibition.</u>

<u>THIRD YEAR:</u>

In the third year of the project we used the knowledge developed in the first and second years for development of application of EGO10-based collar for a variety of agricultural crops. This was done under greenhouse and commercial nursery conditions. Also, an advanced version of collar in terms of its envelop was developed, for promotion of a cost effective and efficient apparatus for agricultural use, for reduction of axillary branches growth.

Below are results of EGO10-collar (and granules) application to multiple crops

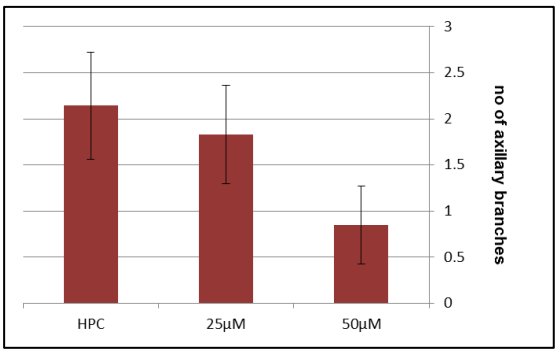
Roses (*Rosa hybrida*)

Three replicates on experiments were done in two commercial plots. EGO10 was applied at the indicated concentrations as a collar in Eppendorf tube (Figure 28).

Figure 28: an example to application of the collar on roses.

In all experiment a clear tendency of reduction of lateral branching was determined (Figure 29). Importantly, application of the collar did not hinder development of "water branches", which are important for development of branches of flowers.

Figure 29: Number Of axillary (side) branch in roses following treatments with EGO10 as collars of 25 and 50 µM EGO10.



Hypericum (*Hypericum perforatum*)

Two replicates on experiments were done in the greenhouse. EGO10 was applied at the indicated concentrations as a collar. Also, EGO10 was applied in another, new way of application- as granules of slow release of $3X10^{-8}$ M that were applied in the soil, close to the root zone. As for now, on Hypericum, this way of application did not result with substantial changes in the no. of axillary branches. In contrast, application of 50 µM EGO10 as collar (in Eppendorf tubes) led to a marked reduction in the no. of axillary branches (Figure 30). Although results were not significantly different, a clear tendency may be seen. An example to the results of the collar treatment is in Figure 31.

Figure 30: Number of axillary branches in Hypericum following treatments with EGO10 as granules and collars of 50 µM EGO10.

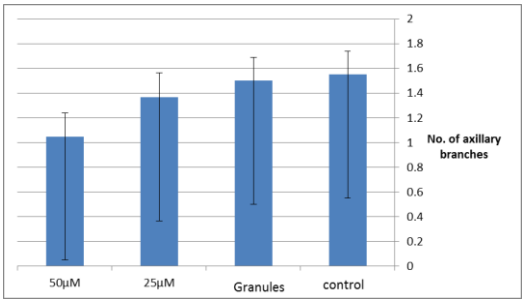


Figure 31: One example to the effect of treatment with EGO10 as collar of 50 µM (right) on side branching, in comparison to control (left)

Pomegranate (*Punica granatum*)

Two replicates on experiments were done in commercial nursery. Application of 50 µM EGO10 as collar (in Eppendorf tubes) led to a marked and significant reduction in the number of axillary branches (Figure 32).

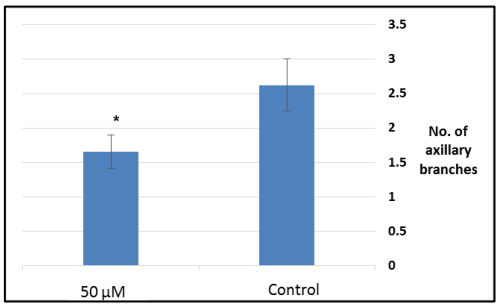


Figure 32: Number of axillary branches in Pomegranate following treatments with collar of 50 µM EGO10.

Almond (*Prunus dulcis*)

Two replicates on experiments were done in commercial nursery. Application of $3X10^{-8}$ M EGO10 as granules of controlled release (granules are based on sugar polymers), and not in collar, led to a marked and significant reduction in the number of axillary branches (Figure 33). Moreover, application of both granules and collar reduced the inhibitory effect of the granules on side branches. Example to the results is in Figure 34.

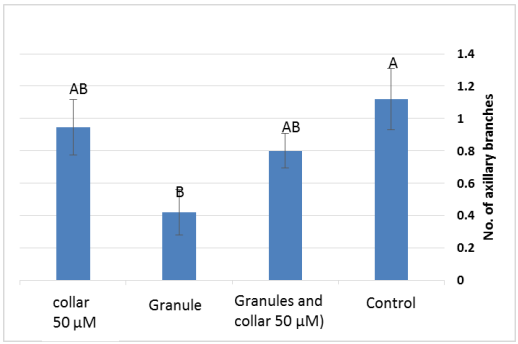


Figure 33: Number of axillary branches in Almond following treatments with EGO10 as granules and collar of 50 µM.

Figure 34: An example to Almond tree treated with EGO10 granules (left) and control (right)

Mandevilla (*Mandevilla sanderi*)

Two replicates on experiments were done in greenhouse. Application of 50 µM EGO10 as collar did not lead to a significant changes in the number of axillary branches (Figure 35).

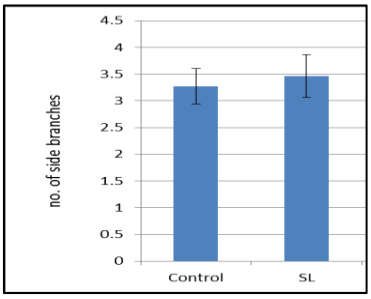


Figure 35: Number of axillary branches in Mandevilla following treatments with EGO10 as granules and collar of 50 µM.

Olives (Koroneiki olives cultivar)

Previously (during the first year of the project) all experiments on olives were done on Picual cultivar. Hence, and since Koroneiki olives is one of the most abundant cultivars worldwide, we have examined the effect of application of collar of 50 µM and granules that release $3X10^{-8}$ M on development of side branches in this cultivar. The results showed that application of collar was effective in preventing side branches development in Koroneiki olives (Figure 36).

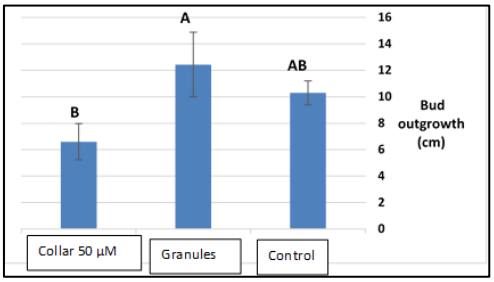


Figure 36: Branch length (cm) in Koroneiki olives following treatments with EGO10 as granules and collars of 50 µM EGO10.

Tomato (*Solanum lycopersicum*)

Greenhouse tomato (Shiren, Ikram and other cultivars) are one of the crops that necessitate vast investment in the constant trimming of side branches. Hand labor is needed for this trimming, on an everyday basis, to avoid significant yield loses. The constant trimming is a cause for infection at trimming sites. Hence, adapting collar application to tomato growth is at utmost importance, to reduce the need for the trimming of side branches. We have used for these experiments two cultivars of greenhouse tomato, Ikram and Shiren, both vigorously producing side branches that should be trimmed under commercial growth conditions. Collar was applied at 25 and 50 µM of EGO10, enveloped by parafilm (application of tube is not feasible on tomato, as it leads to scratching of the stem) as demonstrated in Figure 37. Also, treatments that include irrigation with $3X10^{-6}$ M (once a week for 6 weeks, 5 ml) and granules application (controled release at that same concentration of $3X10^{-6}$ M) were applied. Each treatment consisted of 20 plants, blocks of 4 plants were randomly distributed in the greenhouse.

Figure 37: Example for collar application (arrow) on tomato and the experiment in the greenhouse.

The results (shown here as side branch weight) demonstrated that in Shirez, treatments with EGO10 did not change significantly branching (Figure 38A). However, treatment with EGO10 collars reduced to some extent branching in Ikram (Figure 38B).

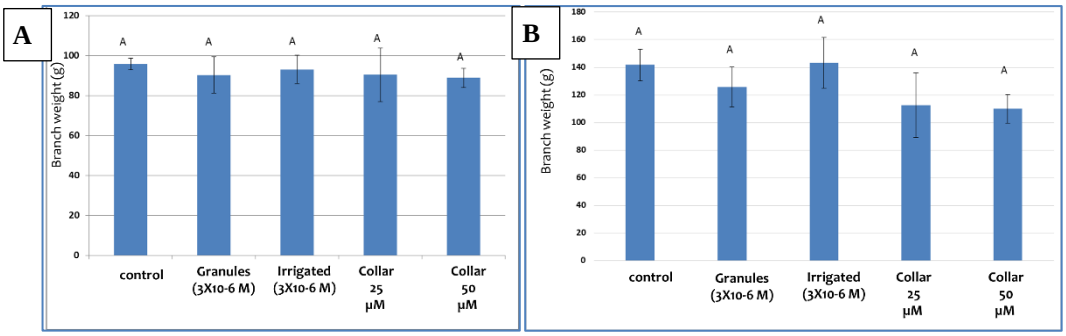


Figure 38: Branch weight (g) in tomato cultivars Shirez (A) and Ikram (B) following treatments with EGO10 as granules, via irrigation and collars of 25 and 50 µM EGO10.

Importantly, in Ikram, in the same experiment, yield determined as number of fruit was increased in the EGO10 treated plants (Figure 39).

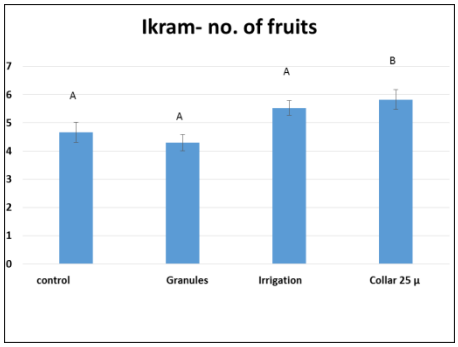


Figure 39: Number of fruits in tomato cultivars Ikram following treatments with EGO10 as granules, via irrigation and collars of 25 µM.

Following, and to increase the possible effect of EGO10 on tomato branching, additional experiment was done with tomato plants were treated with collar of 500 µM EGO10. Here, although Shiren did not respond to this treatment in Ikram a significant reduction in side branches was determined (Figure 40).

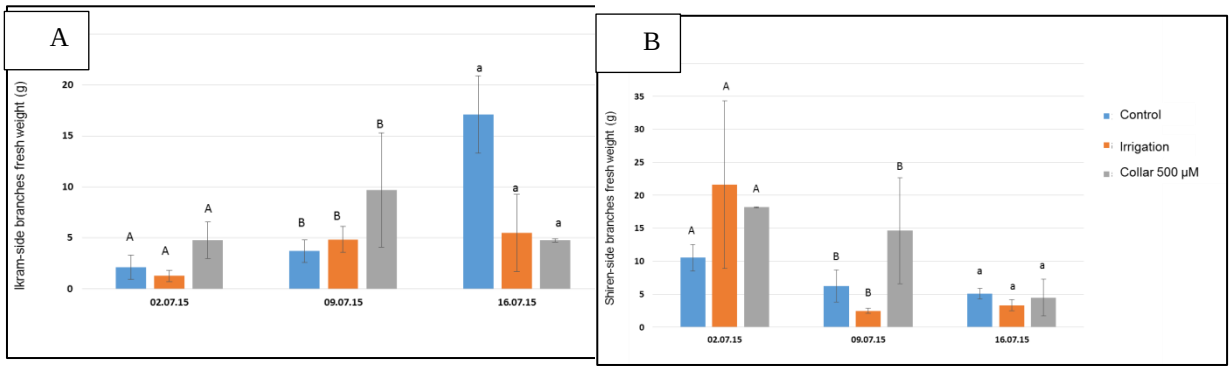


Figure 40: Branch weight (g) in tomato cultivars Ikram (A) and Shirez (B) and following treatments with EGO10 via irrigation and collars of 500 µM EGO10.

To summarize, by application of EGO10 as collar, in granules or via irrigation led to reduction of side branches in several agricultural crops, in some this reduction is with high economic importance.

<u>Development of collar cover (or envelope) for a cost effective application of EGO10</u>

Another aspect of collar development is development of its cover, or envelop. The hormone and its medium (HPC and glycerin) are needed to be covered with some substance to avoid drying or leaking of the compounds once applied on the stem. Moreover, we sought to develop a environmentally friendly collar, which is based on non-hazardous materials. For that purpose we have examined several polymers as collar covers. In Table 1 polymers that were tested and their assessment.

Polymer	Picture	Assessment
Mix of amylose and amylopectin: 8 g Glycerin, 200 µL acetic acid, 43 ml distilled water. All materials were mixed and heated gradulally from 50 to 150 C, with constant steering. When mixture is transperant it was poured into plates and dried at 90 C for 2 h.		Easy to apply on plant however flexibility is not sufficient. Cracks in outer layer were apparent few days following application.
Mix of amylose and amylopectin, 8 g Glycerin, 200 µL acetic acid, 43 ml distilled water. All materials were mixed and heated gradulally from 50 to 150 C, with constant steering. When mixture is transperant it was poured into plates and dried at 90 C for 45 min.		Improved performance in terms of flexibility however cracks in outer layer still apparent few days following application.
Mix of amylose and amylopectin, 8 g Glycerin, 200 µL acetic acid, 43 ml distilled water. All materials were mixed and heated gradulally from 50 to 150 C, with constant steering. When mixture is transperant it was poured into plates and dried at root temperature.		Improved version. Application is easy, flexibility is high, no cracks, seems to be suited as collar cover. May be used commercially.

This research paved the way to attract private entity that is willing to support further research and development, to develop EGO10 application products for a vast variety of crops, in agriculture. Recently a contract was signed with a private company for this purpose.

Based on this research a patent provisional was issued, which will soon become PCT application. This IP is highly important for the investing company.

We would like to thank the Chief Scientist foundation, the Chief Scientist office and the reviewer for their continuance support of the project and its successful outcomes. This support allowed the initiation of development of a new agri-device for improved farming.

רשימת ספרות מצוטטת

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סיכום עם שאלות מנחות

נא להתייחס לכל השאלות בקצרה ולעניין, ב-3 עד 4 שורות לכל שאלה (לא תובא בחשבון חריגה מגבולות המסגרת המודפסת).

שיתוף הפעולה שלך יסייע לתהליך ההערכה של תוצאות המחקר.

הערה: נא לציין הפנייה לדו"ח אם נכללו בו נקודות נוספות לאלה שבסיכום.

מטרות המחקר תוך התייחסות לתוכנית העבודה.
1. בשנה הראשונה מטרתינו היתה בחינת פעילות ביולוגית של אנאלוגים של סטריגולקטונים בשילוב עם חומרים ביולוגיים או כימיים אשר ירכיבו בהמשך את החבק.
2. בשנה השנייה מטרתינו היתה בחינת פעילות חומרי התווך, אנאלוגים, וחבק על צימוח ענפים צדדיים בשתילי זיתים תחת תנאי חממה ותנאי משתלה מסחרית.
3. בשנה השלישית מטרתינו היתה יישום החבק למגוון גידולים חקלאיים בהם יש צורך בהורדת מספר הענפים הצדדיים או ה"שוצים" הגדלים במהלך גידול מסחרי. כמו כן פיתוח מעטה לחבק שיאפשר יישומו באופן יעיל, כדאי כלכלית וידידותי לסביבה.
עיקרי התוצאות.
1. פתחנו מבחן ביולוגי בזיתים לבחינת מידת הסתעפות הנצר
2. מצאנו אנאלוג פעיל לסטריגולקטונים בעל עלות נמוכה יחסית, שהינו בעל פעילות מובהקת בצמצום התפתחות הענפים הצדדיים.
3. מצאנו חומרי תווך אשר הביאו לעליה בעמידות ובפעילות החומר הפעיל
4. מצאנו כי EGO10 פעיל בתנאי משתלה וקבענו את הריכוז האופטימאלי לפעילותו.
5. מצאנו כי HPC+glycerin היא התערובת העדיפה בתנאי חממה לשם ייצוב ה-EGO10.
6. כמו כן בחנו כיסויי חבק שונים, נמצא כי כיסוי פלסטי קשיח עדיף על אחרים.
7. בתנאי משתלה מסחרית נמצא כי החבק היה בעל פעילות של עיכוב צימוח ענפים צדדיים ללא עיכוב התפתחות השתיל.
8. כיילנו יישום החבק למגוון גידולים חקלאיים והראינו יעילותו למספר גידולים שיש בהם צורך בהפחתת מספר הענפים הצדדיים המתפתחים.
9. פתחנו מעטה לחבק שהינו קל להשמה ומתכלה. מעטה זה יתכן וישמש כמעטה החבק באופן מסחרי.
10. בחנו שיטה נוספת ליישום ההורמון – גרנולות של שחרור מבוקר. שיטה זו הייתה יעילה עבור חלק מהגידולים שנבחנו.
בעיות שנותרו לפתרון ו/או שינויים (טכנולוגיים, שיווקיים ואחרים) שחלו במהלך העבודה; התייחסות המשך המחקר לגביהן, האם יושגו מטרות המחקר בתקופה שנותרה לביצוע תוכנית המחקר?

בשלוש השנים האחרונות פיתחנו במימון המדען הראשי טכנולוגיה חדשה המבוססת על יישום חד פעמי של "חבק" המכיל את ההורמון למניעת הסתעפות של ענפים צדדיים בשתילי זיתים. קבענו את הפעילות ביולוגית של אנאלוגים של סטריגולקטונים ומצאנו שילובי חומרים כימיים אשר מביאים לייצוב והגברת חדירות החומר הפעיל. תרכובת חומרים אלו מרכיבה את החבק. כמו כן בחנו כיסויים שונים לחבק שיאפשרו את פעילות החומר הפעיל וייצובו. במחקר שנערך גם כיילנו את השפעת החבק בצמחי מודל (זיתים), ומצאנו כי פעיל בתנאי משתלה מסחרית. החבק יושם על מגוון גידולים חקלאיים שיש בהם צורך בהפחתת יצירת הענפים הצדדיים והראה בחלקם היתכנות. כמו כן בחנו ציפויים שונים עבור החבק. יישום ההורמון נבחן גם בצורה של גרנולות. כעת, בידינו מספר אבות טיפוס של חבק (וגרנולות) המבוססים על אנאלוג כימי פעיל וזול של ההורמונים הצמחיים. המשך פרויקט פיתוח זה ממומן כעת על ידי חברה מסחרית, שפועלת לפיתוח מסחרי של פרויקט החבק לשימוש רחב בחקלאות.
הפצת הידע שנוצר בתקופת הדו"ח: פרסומים בכתב - <u>ציטט</u> ביבליוגרפי כמקובל בפרסום מאמר מדעי;
הידע לא הופץ בכתב. זאת מכיוון שבימים אלו, בטיפול קידום ומשרד עורכי פטנטים (לוצאטו), מוצא PCT על הטכנולוגיה לשם הגנה על הקניין הרוחני הפותח במחקר, הכל בכפוף להנחיות המדען הראשי ומשרד החקלאות. כמו כן, כאמור, המשך פרויקט פיתוח זה ממומן כעת על ידי חברה מסחרית, שפועלת לפיתוח מסחרי של פרויקט החבק לשימוש רחב בחקלאות.
פרסום הדו"ח: אני ממליץ לפרסם את הדו"ח: (סמן אחת מהאופציות)
➢
➢ הידע לא הופץ בכתב. זאת מכיוון שבימים אלו, בטיפול קידום ומשרד עורכי פטנטים (לוצאטו), מוצא PCT על הטכנולוגיה לשם הגנה על הקניין הרוחני הפותח במחקר. כמו כן, כאמור, המשך פרויקט פיתוח זה ממומן כעת על ידי חברה מסחרית, שפועלת לפיתוח מסחרי של פרויקט החבק לשימוש רחב בחקלאות.
האם בכוונתך להגיש תוכנית המשך בתום תקופת המחקר הנוכחי?
לא- המשך פרויקט פיתוח זה ממומן כעת על ידי חברה מסחרית, שפועלת לפיתוח מסחרי של פרויקט החבק לשימוש רחב בחקלאות.

*יש לענות על שאלה זו רק בדו"ח שנה ראשונה במחקר שאושר לשנתיים, או בדו"ח שנה שניה במחקר שאושר לשלוש שנים