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FLOWERING RESPONSE OF THE OLIVE (OLEA EUROPAEA L.) TO CERTAIN GROWTH REGULATORS APPLIED UNDER INDUCTIVE AND NONINDUCTIVE ENVIRONMENTS

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ABSTRACT

Olive trees grown in containers received outdoor winter chilling until February 27, 1969, then were moved to a greenhouse where several growth regulators were applied by dipping shoots into test solutions. Flowering was promoted by a growth retardant, B-9 (Alar-85) at 100 ppm, and was inhibited by either GA_3 at 25 or 50 ppm or ABA at 10 or 50 ppm; in addition, ABA at 10 ppm stimulated lateral buds—normally flower buds—to grow vegetatively. NAA at 50 ppm caused total inhibition of bud development. Cytokinin (6-benzylamino purine) at 50 or 250 ppm had no effect. Under noninductive conditions (trees held in a heated greenhouse throughout the winter months), repeated applications (twice a week for 10 weeks) of IAA, GA_3, IAA + GA_3, a mixture of GA_4 and GA_7, or ABA failed to substitute for the low-temperature requirement for flower induction, although GA_3 strongly stimulated shoot growth.

Introduction

There is growing evidence indicating that plant hormones, gibberellins in particular, play a significant role in flower induction and development in several plant species (LANG 1965; CHAILAKHYAN 1968). However, generalizations cannot be made as to the flowering response of different species to the different types of growth regulators. For example, while LANG (1957) found that gibberellins may substitute for the low temperature or long photoperiods (but not for short photoperiods) required to induce flowering in several species, NANDA et al. (1967) reported that GA_3 induced flowering in a short-day species grown under noninductive long photoperiods. Moreover, gibberellins may also inhibit flowering in both short-day (HARDER and BÜNSOW 1958; GUTTRIDGE 1963) and long-day plants (SACHS, KOFRANEK, and SHYR 1967; CLELAND and BRIGGS 1969). Similarly, contradicting data have been reported by EL-ANTABLY and WAREING (1966), EL-ANTABLY, WAREING, and HILLMAN (1967), and CATHEY (1968) in regard to the effect of abscisic acid on flowering in several plant species, including short-day, long-day, and temperature-sensitive plants. The same conflicting situation exists for gibberellin (MICHNIEWICZ and LANG 1962; CLELAND and ZEEVAART 1970) and for other plant growth regulators.

The olive is a subtropical, woody plant which must be exposed to low temperatures for flower induction to occur (HARTMANN 1953; HARTMANN and PORLINGIS 1957; HACKETT and HARTMANN 1963, 1964, 1967; BADR and HARTMANN 1971). It has recently been found that flower induction in the olive is associated with qualitative and quantitative changes in endogenous gibberellins and growth inhibitors (BADR, HARTMANN, and MARTIN 1970).

The purpose of the present investigation was to determine the effect of applications of gibberellins and other growth regulators on the flowering response of olive trees when treated under both noninductive and inductive environmental conditions.

Material and methods

In one experiment, two olive cultivars, known to have obligate low-temperature requirements for flowering, were used to determine possible responses to treatments with auxin, gibberellin, and abscisic acid, applied under noninductive environments.

1. Four-year-old 'Ascolano' trees grown in 2-gal containers were moved into a heated greenhouse on September 26, 1968, where the minimum temperature was maintained above 15 C (noninductive conditions). Aqueous solutions of GA_3 (potassium salt of gibberellic acid) and IAA (indoleacetic acid), alone or in combination, were applied with a hand sprayer to whole trees to the point of slight drip. For combination treatments, GA_3 was applied first, with sufficient time allowed for absorption (18 hr); then IAA was applied. Each growth regulator was used at 100, 250, and 500 ppm; distilled water applications were used as controls. All solutions contained 0.01% Tween-20 as a surfactant. Treatments were made on October 1, 1968, with repeat applica-

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tions to the same trees on October 4; three replicate trees were used for each treatment.

2. On October 25, 1968, 12 5-year-old 'Manzanillo' olive trees grown in 10-gal containers were moved into a heated greenhouse (noninductive conditions). Three main branches were selected on each tree; on these branches uniform 12-inch shoots of the previous summer's growth were tagged for subsequent treatment. Ten shoots on the same branch of each of three different trees were used per treatment (total of 30 shoots per treatment). The materials and concentrations used were as follows: (*a*) GA_3 at 100, 250, and 500 ppm; (*b*) GA_4 + GA_7 (a mixture of 60% GA_4 + 40% GA_7) at 50, 100, and 250 ppm; (*c*) abscisic acid at 50, 100, and 250 ppm; and (*d*) distilled water. All solutions contained 0.01% Tween-20 as a surfactant.

A second experiment was designed to study the influence of several growth regulators on flower bud development on trees which had been subjected to inductive environmental conditions and which had already initiated flower parts.

Eighteen 6-year-old 'Sevillano' olive trees, grown in 10-gal containers, were maintained outdoors, where they were subjected to the natural winter chilling at Davis, California, until February 27, 1969. The trees were then moved into a heated greenhouse with a minimum temperature of 18 C. The materials and concentrations used were as follows: naphthaleneacetic acid (NAA) at 10 and 50 ppm; gibberellic acid (GA_3) at 25 and 100 ppm; abscisic acid (ABA) at 10 and 50 ppm; a cytokinin, 6-benzylamino purine, at 50 and 250 ppm; a growth retardant, succinic acid 2,2 dimethylhydrazide—(Alar-85) (B-9), at 100 and 500 ppm; and distilled water.

Each solution contained 0.01% X-77 as a surfactant. Application was made by dipping individual shoots for 1 min into a 100-ml cylinder containing the test solution. Treatments were made on February 28 and repeated on March 2; three replicates, each on a different tree, with 20 shoots per replicate, were used per treatment.

Results

EFFECT OF GROWTH REGULATORS ON TREES UNDER NONINDUCTIVE ENVIRONMENTS.—None of the growth regulators used induced flowering of olive trees maintained under a noninductive environment. However, certain morphological responses to the different regulators indicated that the compounds were absorbed and physiologically active within the trees. Gibberellins promoted shoot elongation mainly through internode extension. They inhibited terminal bud development in 'Manzanillo' but not in 'Ascolano.' This may have been due to a difference between the two cultivars in the physiological condition of the terminal buds at the time of GA application. These responses are shown in figure 1.

EFFECT OF GROWTH REGULATORS ON TREES WHICH HAD BEEN SUBJECTED TO AN INDUCTIVE ENVIRONMENT.—Trees which were exposed to the natural outdoor winter chilling until February 27, 1969, then brought into a warm greenhouse, produced inflorescences on which the flowers attained full bloom by April 4. Figure 2 shows the effect of the different growth regulators on the percentage of lateral buds which developed into inflorescences (flower buds) and those which developed into lateral shoots (vegetative buds). Statistical analysis of the data showed that flowering was slightly, but not significantly, promoted by NAA at 10 ppm; however, at 50 ppm NAA caused certain toxic effects, such as leaf desiccation, and total inhibition of bud development. Both GA and ABA significantly (1% level)

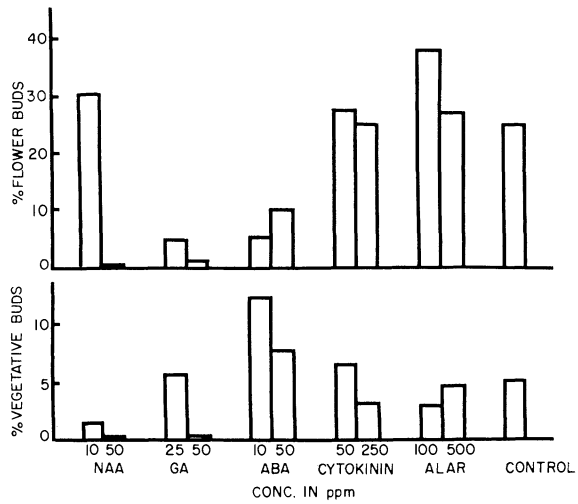

FIG. 2.—Effect of application of several growth regulators on type of bud development in 'Sevillano' olive trees which had been exposed to winter chilling until February 27, 1969. *Flower buds*: lateral buds which developed into inflorescences; *vegetative buds*: lateral buds which developed into shoots. L.S.D.: flower buds—0.05 = 10.6%; 0.01 = 15.0%; vegetative buds—0.05 = 4.2%; 0.01 = 5.9%.

FIG. 1.—Effect of gibberellin (GA_3, 250 ppm) applications on 'Manzanillo' olive shoots. Control on left; treated on right. Arrows show position of shoot terminal at time of application. *Above,* promotion of terminal growth by GA_3, including stimulation of lateral shoots. *Below,* lateral bud development following injury to terminal bud.

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inhibited flowering, while cytokinin had no effect in this respect. The percentage of lateral buds forming inflorescences was significantly (1% level) increased by the growth retardant, Alar, at 100 ppm but not at 500 ppm. None of the growth regulators had an effect on lateral buds except that ABA at both concentrations caused a significant (1% level) increase in the percentage of such buds developing vegetatively.

Discussion

Several reports have indicated that gibberellins may substitute for the long photoperiods or low temperatures required for flower induction in a number of unrelated species (LANG 1956, 1957, 1965; CARR, MCCOMB, and OSBORNE 1957; CHAILAKHYAN 1957, 1968; HARADA and NITSCH 1959; MICHNIEWICZ and LANG 1962). In some cases, they were effective only when the plants were held at temperatures slightly higher than the critical inductive temperatures (WITTWER and BUKOVAC 1957). Most of the species that respond to GA are characterized by rosette growth patterns where the plants develop an elongated floral axis prior to actual floral initiation. Accordingly, it has been suggested that gibberellins induce flowering in these species indirectly through promotion of shoot elongation (BUKOVAC and WITTWER 1957; LANG and REINHARD 1961; LANG 1965; CHAILAKHYAN 1968).

In the present study with the olive, where flower induction and initiation do not involve prior shoot elongation, gibberellins (GA_3 or a mixture of $GA_4 + GA_7$) alone, or in combination with an auxin (IAA), failed to induce flowering in trees held under noninductive conditions. Morphological (fig. 1) and anatomical (BADR, BRADLEY, and HARTMANN 1970) responses to applied GA_3 show that this material is absorbed by the olive in sufficient amounts to be physiologically active. Thus, the failure of gibberellins to induce flowering in the olive under noninductive environments cannot be attributed to insufficient treatments or lack of absorption, as has been suggested by LANG (1965) and others as a possible cause for lack of response in certain other species.

It has recently been shown that flower induction in the olive resulting from chilling during the winter was associated with an increase in the endogenous GA level in the lateral (potential flower) buds (BADR, HARTMANN, and MARTIN 1970). Therefore, one would expect that applied GA might substitute for the low temperature requirements for flower induction in the olive. The failure of GA_3, or $GA_4 + GA_7$, to do so in this study may indicate that endogenous gibberellins in the olive include types other than, or in addition to, those applied exogenously under nonchilling conditions. The specificity of the different types of gibberellins in their effect on flowering was demonstrated by several workers for different species (LANG and REINHARD 1961; MICHNIEWICZ and LANG 1962; WITTWER and BUKOVAC 1962), although data to contradict this were reported for the long-day species *Silene armeria* (CLELAND and ZEEVAART 1970).

Another possible explanation for the failure of exogenous gibberellins to induce flowering in the olive may be based on CHAILAKHYAN's concept of the nature of the flowering hormone(s) (CHAILAKHYAN 1964, 1968). It may be assumed that both gibberellins and "anthesins" are formed in the olive in response to low-temperature treatments and subsequently induce flowering. Under noninductive environments (i.e., a heated greenhouse) perhaps neither of these compounds is formed; thus the fact that exogenously applied gibberellins failed to induce flowering may be due to a lack of the concomitantly required "anthesins."

Relationships of abscisic acid to flowering in plants under noninductive environments are known to be somewhat questionable (CATHEY 1968). The evidence reported here suggests that it has no effect in the case of the olive.

The fact that GA_3 inhibited flowering in olive trees which had been exposed to inductive temperatures may be attributed to either or both of the following reasons: (*a*) GA_3 may not be a part of the endogenous GA-complex. (*b*) It has been shown (BADR, HARTMANN, and MARTIN 1970) that the level of endogenous GA in olive buds was decreasing at the time exogenous application of GA_3 was made (February); treatment at this time may have caused excessive accumulation of gibberellins in the buds, disturbing the balance of native hormones and leading to inhibition of flowering. This is further supported by the fact that application of the growth retardant, Alar, at this same time promoted flowering (fig. 2). Later applications of GA, in March or April, had very little or no influence on the percentage of lateral buds forming inflorescences; however, such late applications certainly affect inflorescence development and, in some instances, GA induced leaf development on the inflorescence axis (BADR 1970), which is normally leafless. Similar results were reported by WARDELL and SKOOG (1969) on tobacco stem segments grown in vitro.

Gibberellin applications have inhibited flowering in pear (GRIGGS and IWAKIRI 1961) and apple (GUTTRIDGE 1962); however, the environmental requirements for flower induction in these species

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are not well defined. Moreover, GA_3 caused total inhibition of development of both flower and vegetative buds in several *Prunus* species (BRADLEY and CRANE 1960).

Although abscisic acid did not affect flowering of olive under noninductive environments, it did inhibit flowering in trees which had been exposed to inductive temperatures during the winter. Thus, both gibberellic acid and abscisic acid inhibit flowering in induced olive buds but possibly through different mechanisms. Abscisic acid increased the percentage of lateral buds (potential inflorescences) which developed into lateral shoots (i.e., ABA caused reversion from reproductive to vegetative development), while GA caused almost total inhibition of lateral bud development.

Cytokinin applied to induced olive trees had no influence on the percentage of buds developing into inflorescences or vegetative shoots. This reaction to cytokinin is similar to results reported by WARDELL and SKOOG (1969) with tobacco stem segments grown in vitro.

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WATER STRESS IN KRUMMHOLZ, WASATCH MOUNTAINS, UTAH

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ABSTRACT

The krummholz form of Engelmann spruce was found to develop water potentials below —90 bars during the winter. Changes in sugar concentration associated with cold hardiness could not account for such low water potentials. These low water potentials may reflect a frozen substrate and windy conditions.

Introduction

Desiccation has been shown to be an important factor in the formation of krummholz[1] near timberline (KLIKOFF 1965; WARDLE 1968). However, few precise measurements of water stress in krummholz forms have been made (LINDSAY 1967), and little attention has been paid to the significance of low water potentials in this life form.

Since water potential, a useful index of water stress, incorporates the effects of osmotic (solutes), turgor, and matric potentials as components of the total measure (TAYLOR 1968), an increase in sugars should have a direct effect on water potential in winter-hardened plants.

Increase in free sugars has been correlated with cold-temperature tolerance by PARKER (1962*a*, 1962*b*), STEPONKUS and LANPHEAR (1968), and BILLINGS and MOONEY (1968). STEPONKUS and LANPHEAR (1968) found sugar concentrations as high as 30%, dry-weight basis, under cold acclimation. The question then arises as to whether the increase in sugar concentration can be linked with high water stress (lower water potential) of krummholz forms during the winter.

Methods

In the course of analyzing microclimatic effects on krummholz formation in the Wasatch Mountains, Utah (HANSEN 1969), we measured the water potential and sugar content of krummholz needles. The study area near the upper terminus of a ski lift at Alta, Utah, was chosen so that measurements could be made during the winter with relative ease. Alta, approximately 29 km southeast of Salt Lake City, Utah, is located in a high mountain valley. The valley is almost completely surrounded by ridges that bear krummholz forms of Engelmann spruce, *Picea engelmannii* (Parry) Engelm., and subalpine fir, *Abies lasiocarpa* (Hook.) Nutt. In the study area most of the krummholz forms consisted of Englemann spruce with small amounts of subalpine fir skirting some of the islands. The vegetation of the area is described by REAM (1963).

From early January to mid-May 1969, measurements of water potential and sugar content were made of Engelmann spruce krummholz with special attention to one representative plant. Numerous other randomly selected plants were also measured as checks on the representative plant. Rarely was the water potential of any one check plant measured more than a few times during the study.

Needles were collected randomly from all branches exposed above the snow for both water potential and sugar determinations as composite samples from a given plant. Water-potential determinations were made at approximately 10-day intervals, with six determinations at each sampling date. Water potential was determined using the Shardakov method as outlined by KNIPLING (1967). The steep rise in molarity versus osmotic pressure at lower water potentials (higher molarity) made it

[1] "Krummholz" is used here in the sense of WARDLE's (1968) "cushioned krummholz," i.e., "cushion of contorted stems and needles."

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