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Effect of Ethylene on Protease Activity in Petals and in Ovaries from Cut Roses

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Abstract. **Ethylene treatment affected protease activity in ovaries, but not petals, of cut roses (*Rosa hybrida* L.) 'Sonia'. Protease activity in ovaries of ethylene-treated flowers decreased within the first 3 days and then remained at the same level until the 12th day. In ovaries from controls, protease activity decreased from day 6 until day 9 and then remained constant until day 12. Protease activity of petals did not differ significantly between the ethylene-treated and control flowers; it was constant until day 6 and then increased steadily until day 12. Differences between ethylene-treated and control flowers were visible immediately after treatment (day 1) and until day 3, as the sepals of the ethylene-treated flowers reflexed. Later (day 6), this difference disappeared as the sepals of the control flowers also reflexed. On day 9 the outer petals of the ethylene-treated flowers were bluish, whereas the controls were not. There were no other differences in development between treated and control flowers.**

Gorin et al. (1) reported an increase in protease activity in petals from cut 'Sonia' roses. Lukaszewska et al. (3) reported similar results in hydroponically grown 'Sonia'. They treated flowers with ethylene, but found no clear relationship between protease activity in petals or ovaries. We studied protease activity in both organs from soil-grown roses to determine if these activities were correlated and if an ethylene treatment would increase protease activity. Possibly ethylene is involved in protease activity, since (2-chloroethyl)phosphonic acid (ethephon) accelerated senescence of 'Carina' roses (2).

The methods used were those outlined by Lukaszewska et al. (3), except a sample for analysis was derived from three flowers instead of two and triplicate (instead of single) samples were collected at 3-day intervals (Fig. 1) to estimate protease activities. Initial date (day zero) was 29 May 1985.

Roses were harvested in the nursery of A.A. Pouw (De Kwakel, near Aalsmeer, Netherlands) at an early stage (before normal harvest). The sepals were not yet reflexed, and the corolla was tightly folded. The stems were cut to a length of 50 cm. Flowers were transported to Wageningen within 90 min and placed in double-distilled water in vases. One half of the lot was placed in a large cylinder for ethylene treatment. The remaining half was placed into a 2nd cylinder without added ethylene. Both cylinders were part of the installation described by Woltering and Sterling (5). The gas concentration in the first cylinder was ethylene, 11.8 ppm; CO_2, 0.08%; O_2, 20.8%; and the remainder was N_2. Gas concentration in the 2nd cylinder was similar except there was a residual level of ethylene of 0.01 ppm. Flowers were treated in darkness at 20°C for 24 hr. Subsequently, flowers were placed in 1-liter jars (three flowers/jar) containing 0.5 liter of water and held continuously in fluorescent light (Philips TLD 36 W/8) with an illuminance of 1 klx at 20° and 60% RH. The water was changed and the stem ends were recut each day.

The preparation of acetone powders from petals and ovaries, the respective enzyme extracts, and the estimation of specific catalytic activity were carried out as described by Lukaszewska et al. (3). The only difference was the measure absorbance (ΔA) at 406 nm per 10 min per milligram of protein was changed to micromoles of *p*-nitroaniline (*p*NA) released per second from the *p*-nitroanilide tripeptide (*H*-D-prolyl-L-phenylalanyl-L-arginine-*p*-nitroanilide dihydrochloride, S-2302, KabiVitrum, Sweden) per kilogram of protein ($\mu mol \cdot s^{-1} \cdot kg^{-1} = \mu kat \cdot kg^{-1}$) (4). For this purpose, the molar absorption coefficient of *p*NA was estimated under the same conditions in which protease activities were determined (1); i.e., 25.0°C and in the sample cuvette, a solution of phosphate buffer (0.093 M), EDTA-Na_2 (0.483 mM), cysteine-HCl (2.414 mM), S-2302 (0.286 mM), and *p*NA (0.023 mM), and, in the reference cuvette, the same solution without *p*NA. In this medium, ϵ at 406 nm was 9.96 (± 0.15) $\times$ $10^3 \cdot M^{-1} \cdot cm^{-1}$ (mean of triplicates and SD, respectively). Pure *p*NA (recrystallized) was from KabiVitrum (S-11287).

There was a linear relationship between catalytic activity (y) (5–17 pkat) and the amount of enzyme extract (x) (0.2–0.7 ml) as indicated from petals of control flowers on day 9; $y = 0.04 + 24.2857\ x$, $r = 0.999$. The data on specific catalytic activity are shown in Fig. 1. Separation of means was by analysis of variance (ANOVA) fol-

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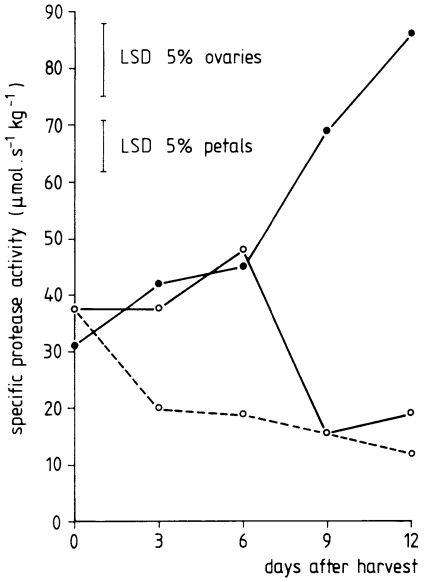

Fig. 1. Specific catalytic activity of protease in ovaries (○) from cut 'Sonia' roses treated with ethylene at a concentration of 12 ppm (---), and from untreated controls (—) for a 12-day period after harvest. Protease activity in petals (●) from treated and untreated flowers (which did not differ). Means were calculated from triplicate samples.

lowed by a least significant difference (LSD) test at the 5% level of probability. ANOVA showed a significant effect of ethylene treatment and of days after harvest, and an interaction of these two factors on protease activity in ovaries. LSD for ovaries was computed on the basis of the interaction. Thus, the LSD test was used for separation of means from both classes of ovaries pertaining to a given day after harvest and for separation of means within the same class of ovaries on different days after harvest. An F test did not indicate any significant effect of ethylene treatment of cut roses on protease activity in petals, but it indicated a significant effect of days after harvest. Thus, the LSD test for petals was computed for separation of means pertaining to different days after harvest.

Ethylene treatment of cut roses affected protease activity in ovaries but not in petals. Protease activity in ovaries from treated flowers decreased from cutting until the 3rd day, and then remained at the same level until the 12th day. The activity from the controls was constant during the first 6 days, decreased until day 9, and remained at that level until day 12.

Protease activity in petals from both treatments was constant during the first 6 days and increased steadily until the 12th day. We cannot explain why the ethylene treatment affected protease activity in ovaries but not in petals.

In the control flowers, an increase in protease activity in petals coincided with a decrease in activity in ovaries. Such simultaneous changes in activity were not detected in ethylene-treated flowers nor in either treated or control cut roses from plants grown hydroponically (3). Thus, it appears that there is no causal relationship between activities in the two parts of the flower.

In ethylene-treated flowers, the sepals were reflexed immediately after treatment. On day 6, the control flowers also had reflexed sepals. On day 9, the outer petals from ethylene-treated flowers were bluish. Otherwise, no differences were observed between treated and control flowers. Petal abscission occurred after 12 days.

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Synthesis and Biological Activity of [^{14}C]IAA Ethyl Ester

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Abstract. **A simple procedure for synthesizing and purifying the [^{14}C]ethyl ester of IAA (Et-IAA) is described. This auxin has been found to stimulate parthenocarpic fruit set in day-neutral strawberries (*Fragaria ×ananassa* Duch. 'Fern'), which are nonresponsive to various other auxins. Et-IAA may prove useful in eliciting physiological responses in systems shown previously to be auxin-nonresponsive. Chemical name used: 1*H*-indole-3-acetic acid (IAA).**

Application of auxins to receptacles of June-bearing and ever-bearing strawberry cultivars induce parthenocarpic fruit set. The range of auxin compounds capable of stimulating parthenocarpy in these cultivars include IAA (7), 1*H*-indole-3-butanoic acid (IBA) (7, 11), 1-naphthaleneacetic acid (NAA) (1, 2, 7), and 2-naphthoxyacetic acid (2-NOA) (7, 10, 11). Experiments performed (unpublished results) indicate that day-neutral strawberry cultivars ('Hecker', 'Brighton', 'Fern', and 'Selva') do not set fruit in response to the previously mentioned auxins. The only auxin found to date that induces parthenocarpy in these day-neutral strawberries is Et-IAA.

Early work on identification of auxins in plant tissues indicated that Et-IAA was a natural constituent of both corn and apple endosperm, which may be the factor responsible for fruit set (8, 9). Later, it was reported that the presence of Et-IAA in the plant extracts was an artifact of the extraction procedure, and there was no endogenous Et-IAA present (3). There have been no further reports, to our knowledge, of the presence of this auxin in plant tissues.

In this study, a simple method for synthesizing [^{14}C]Et-IAA is described. The demonstration of an auxin response with Et-IAA in day-neutral strawberries, combined with the availability of the radiolabeled compound, may stimulate new investigations of auxin-mediated physiological responses in previously auxin-nonresponsive systems.

Synthesis of [^{14}C]IAA ethyl ester. The procedure followed was a modification of Jackson's tryptophol synthesis method (6). A total of 250 μCi (1 Ci = 37 GBq) of 2-[^{14}C]IAA (Amersham, specific activity 2.04 GBq/mmol) in 5.0 ml toluene was dried under a stream of N_2 prior to the addition of 3.0 ml of 75% ethanol. The resulting solution was brought to 1.0 N with HCl and stirred in the dark for 24 hr. The reaction was stopped by the addition of 0.35 ml of 10.0 N NaOH (bringing the pH to 6.0) and the solution was dried under vacuum. Distilled water was added to the reaction vial, and the products were partitioned three times against H_2O-saturated ethyl acetate. The aqueous phase was discarded, and the ethyl acetate phases containing [^{14}C]Et-IAA were combined and dried under vacuum.

Purification, identification, and quantification. The products were resuspended in 100% methanol, sonicated, and brought to 20% methanol with the addition of distilled H_2O. The solution was centrifuged in an Eppendorf centrifuge for 10 min at 15,600× *g*, and the supernatant was decanted and subjected to fractionation on a 10 × 150 mm

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