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.

Literature Cited


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

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Abstract. A simple procedure for synthesizing and purifying the [14C]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 non-responsive to various other auxins. Et-IAA may prove useful in eliciting physiological responses in systems shown previously to be auxin-nonresponsive. Chemical name used: 1H-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 parthenocarpic in these cultivars include IAA (7), 1H-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 Experiments performed (unpublished results) indicate that day-neutral strawberry cultivars ('Hecker', 'Brighton', 'Fern', and 'Selva') do not set fruit in response to the previously mentioned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 induce parthenocarpic 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 [14C]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 [14C]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-[14C]IAA (Amersham, specific activity 2.04 GBq/mmol) in 5.0 ml toluene was dried under a stream of N2 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 H2O-saturated ethyl acetate. The aqueous phase was discarded, and the ethyl acetate phases containing [14C]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 H2O. 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
Physiological activity. One-year-old day-neutral strawberry plants were planted in 15-cm pots in the greenhouse in 1 soil : 1 peat : 1 sand (by volume), and fertilized with 1 g of Osmocote (14N-6P-12K) slow-release fertilizer. After florescence development the plants were moved to a growth chamber equipped with mercury halide and fluorescent lamps maintained at a light intensity of 440 μmol·s⁻¹·m⁻². The temperature in the chamber was held at 21.6° ± 0.3°C during the 13-hr day cycle and at 17.7° ± 0.2°C during the night cycle. Flowers were emasculated 1 day prior to opening and the following treatments applied: 1) hand-pollinated, with pollen of the same cultivar; 2) nonpollinated control; or 3) Et-IAA. Et-IAA (2.5 × 10⁻³ M) in aqueous solution containing 2% dimethyl sulfoxide (DMSO) and buffered at pH 6.8 was applied to the receptacles by dipping for 10 sec. The nonpollinated control treatment consisted of dipping receptacles in a 2% DMSO aqueous solution buffered at pH 6.8. Fruit set and growth in the three treatments were determined by measuring receptacle length.

The synthesis of [¹⁴C]IAA ethyl ester is simple, fast, and requires only standard laboratory glassware and reagents. HPLC analysis revealed four UV absorbing peaks, of which the presumptive Et-IAA peak constituted the majority of the area. GC–MS spectra of the standard Et-IAA and the synthesized Et-IAA were identical (Fig. 1 A and B). Quantification of the product using UV absorption indicated a reaction yield of 43%. This yield was confirmed by liquid scintillation spectrometry.

Preliminary time course studies indicated that the reaction can continue in the dark at room temperature for up to 48 hr without decreasing yield. After that time, however, yield of Et-IAA begins to decrease. After 72 hr in the dark, yield of Et-IAA is ~24%.

Various synthesis methods were tried, but discarded on the basis of lower yield. Among these was the use of thionyl chloride (SOCl₂) to convert IAA into the acid chloride, followed by reaction of the acid chloride with ethanol to form the ethyl ester. Theoretically, these are essentially irreversible reactions that go to completion and the use of SOCl₂ is convenient for the formation of the acid chloride, since other products formed are gases and therefore easily separated from the acid chloride (5). However, HPLC analysis of the IAA–Cl derivative revealed the presence of more than 40 peaks in the reaction mixture. Further reaction of the acid chloride with ethanol to form the ester also resulted in numerous peaks upon HPLC analysis, rendering preliminary identification of the desired product extremely difficult. This synthesis method was then discarded in favor of direct esterification of the acid, which has the advantage of being a single-step synthesis.

Various manipulations of the direct esterification method were performed. Initially, the reactants (IAA and ethanol, with HCl as an acid catalyst) were refluxed at 90°C, and aliquots taken hourly to determine yield. Refluxing for 6 hr gave the maximum yield of Et-IAA (22%). Refluxing in the dark had no effect on yield. Since heating is known to enhance the rate of degradation of acidic IAA solutions (4, 12), direct esterification of IAA at room temperature was tried. This method resulted in the highest yield (43%, as reported earlier) and was therefore the method of choice.

Et-IAA induces fruit set and growth in day-neutral strawberries (Fig. 2). Fruit set of the Et-IAA-treated receptacles ranged from 75% to 100%, while set in the pollinated treatment ranged from 95% to 100%. Growth of the auxin-treated fruit keeps pace with growth of the pollinated fruit during the early part of the developmental cycle. However, growth of the auxin-treated fruit begins to slow between 6 and 9 days after treatment, although these fruit mature and ripen simultaneously with pollinated fruit. This decrease in the growth rate of the Et-IAA-treated fruit is in agreement with work done on auxin-induced set and growth of June-bearing and everbearing strawberry cultivars (1, 10), where auxin-induced fruit growth ceased 10–12 days after the initial auxin application. Growth could be restimulated by additional applications of auxin to the receptacle, suggesting that a continuous supply of auxin is needed for continued growth.

The present study demonstrates an easy method for synthesizing and purifying...
Fig. 2. Effects of pollination, nonpollination, and Et-IAA on fruit growth of ‘Fern’ strawberry. (SE is indicated, n = 10)

$^{14}$C]Et-IAA. It also demonstrates that Et-IAA is a biologically active auxin that is capable of inducing parthenocarpic fruit set in strawberry cultivars that were thought to be auxin nonresponsive with respect to set. Whether the ester itself is biologically active or the ester conjugation prevents the degradation of IAA, thereby allowing the free acid to reach the sight of action, is unknown.

**Literature Cited**