Extraction, Purification, and Quantitation of Paclobutrazol from Fruit Tree Tissues

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β-[4-(chlorophenyl) methyl]-α-(1, 1-dimethylproplyl)-1-H-1,2,4-triazole-1-ethanol (paclobutrazol) is one of a new class of plant growth regulators that affect both vegetative and reproductive components of fruit tree growth (1–3). The movement and fate of absorbed paclobutrazol must be established to better understand its persistence and mode of action. Accordingly, we have developed an analytical method for quantifying low levels of paclobutrazol extracted from plant tissue.

One to 5 g of freeze-dried plant material ground to pass a 20-mesh screen was extracted with cold 80% MeOH [10 ml·g−1 of tissue (dry weight)] for 5 min with a Sorvall Omni-mixer. The extract was filtered and the extraction process repeated. Methanol was removed from the combined filtrates with reduced pressure and the aqueous residue adjusted to pH 11 with NaOH. The aqueous fraction was then extracted twice with equal volumes of methylene chloride and the combined organic phase back-washed once with a half volume of water buffered at pH 7. The methylene chloride was taken to dryness under reduced pressure and the dried extract redissolved in MeOH. Buffer [0.005 M (NH₄)₂HPO₄ adjusted to pH 6.5 with H₃PO₄] was added to make the MeOH 80% before introduction onto a column of C₈ Porasil B (Waters Associates). Two grams of C₈ Porasil B in a 10-ml plastic syringe barrel plugged with glass wool were used. Pressure was used to move the extract through the column at about 5 ml·min⁻¹. Two washes of 2 to 3 ml each of the evaporation flask with buffer 80% MeOH were used to wash paclobutrazol through the column. Most of the pigments remained on the column. The column was easily regenerated for use with washes of acetone, MeOH, and 80% MeOH buffered at pH 6.5. The eluate was evaporated under reduced pressure and redissolved in methylene chloride for further purification on Florisil.

One gram of Florisil (100- to 200-mesh Florisil activated at 120°C for 2 hr) in a 3-ml plastic syringe barrel plugged with glass wool was used. Pressure was used to move the extract through the column at about 2 ml·min⁻¹. Three washes of about 2 ml each of methylene chloride; the column was next washed with 5 ml of 1 anhydrous ether: 1 hexane (v/v) before paclobutrazol was eluted with 97 anhydrous ether: 3 MeOH (v/v). The first 5 ml of ether-MeOH containing paclobutrazol was collected, evaporated under reduced pressure, and redissolved in 50 acetonitrile: 50 water (v/v) prior to separation by high-pressure liquid chromatography (HPLC). HPLC separation was done isocratically on a 300 x 3.9 mm Bondapak C₁₈ column (Water Associates) with 50 acetonitrile: 50 water (v/v) and a flow rate of 1 ml·min⁻¹. A 2-ml fraction eluting in the area of authentic paclobutrazol (Rᵣ = 11 min) was collected.

With the procedure outlined, GC baseline separation of paclobutrazol was obtained from leaf extracts of trees treated with paclobutrazol (Fig. 1). There were no peaks in the paclobutrazol or diclobutrazol area of the chromatogram when untreated tissue was analyzed. With the ability to detect small quantities of paclobutrazol, contamination of samples during processing or during analysis may occur, so we carried an untreated control sample through the procedure to monitor this aspect. Diclobutrazol was added immediately prior to GC as an internal standard for increased precision of GC quantitation. Chromatography on C₁₈ Porasil B and Florisil were rapid and effective purification steps; the HPLC step was more time-consuming, but was generally necessary for well resolved GC chromatograms. We found no evidence that paclobutrazol was lost during the extraction process.

evaporation under reduced pressure, but all glassware used for this purpose was routinely silylated.

After the procedure was developed, it was tested and a recovery curve developed by spiking quadruplicate 2-g aliquots of freeze-dried apple leaves with 0, 0.4, 0.8, and 1.6 µg of paclobutrazol when the extraction process was started. Recoveries were greater than 90% (Fig. 2), as determined by direct measurement of paclobutrazol.

**Literature Citations**


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**Semi-automated Pit Cracking Machine for Rapid Seed Removal**

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Germination of stone fruit seeds is accelerated at an elevated percentage if the stony endocarp (pit) is removed prior to planting. Stone fruit breeders and those who screen seed populations usually remove pits with bench vice jaws that manually compress pits either end-to-end or edge-to-edge to affect seed release. The vice was effective but production was low and seasonal production was limited. Consequently, efforts were initiated to find means to alleviate the problem of low seed release productivity while maintaining the effectiveness of the vise.

**Pit cracker development.** Investigations were started by measuring the pit lengths, widths, required compression forces, and the allowable over-travel after cracking. The range of lengths and widths, plus allowable over-travel, indicated a need to size-sort and provide machine adjustments. To make the machine versatile, the adjustable range needed to accommodate dimensions from small olive pits to large peach pits (about 6 to 38 mm). Required compression forces (maximum observed) were found to range from 3570 N for peaches to 1785 N for olives.

A powered reciprocating plunger was selected to duplicate the performance of the vise procedure and minimize physical effort. By allowing the plunger to cycle automatically and having the operator concentrate on feeding/positioning pits between the plunger and anvil, it was anticipated rates of 60 pits per min per plunger could be achieved.

The design selected for this machine used a constant torque, variable speed 3/4 horsepower motor direct connected to a 20:1 worm drive gear box with output shafts on 2 sides. Crank wheels attached to both shafts permitted a 2-plunger system. Crank pins offset 9.5 mm from the center of the crank wheels provided 19 mm of stroke. Timing of the crank wheels with the crank pins 180° apart yielded alternating plunger travel between the pair.

An integral direction and speed control module mounted on the end of the drive motor provided infinite adjustment up to 125 cycles per min. The 2.14 NM of constant motor torque yields 4452 N potential force, at all speeds, at the plungers.

The machine (Fig. 1) consists of: a base

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Fig. 1. Pit-cracking machine as seen from several vantage points. The indentations shown in the bolts (lower right) help position pits and avoid slippage.