tion concluding the rebuttable presumption against registration of pesticide products containing ethylene dibromide. April 22, 1981 report. EPA, Washington, D. C.
15. USDA/State/EPA. 1978. The biologic and economic assessment of the pesticide ethylene dibromide. USDA/State/EPA EDB Assessment Team. June 1980. Hyattsville, MD.
16. U. S. Environmental Protection Agency. 1980. Ethylene dibromide position document 2/3. December 1980. Office of Pesticide Programs, Washington, D. C.

Proc. Fla. State Hort. Soc. 94:266-267. 1981.

A RAPID METHOD FOR ANALYSIS OF CITRUS FRUIT FOR RELEASE®[1,2]

R. HILTON BIGGS
University of Florida, IFAS, Fruit Crops Department, Gainesville, FL 32611

SUSAN V. KOSSUTH
USDA Forest Service SE Experimental Station, P. O. Box 70, Olustee, FL 32072

Additional index words. Citrus sinensis (L.) Osbeck, abscission, growth regulators, harvest aid.

Abstract. **Citrus fruit can be analyzed for 5-chloro-3-methyl-4-nitro-1H-pyrazole (Release®), a potential chemical aid for fruit harvest, in less than 2 hours. The method employs the use of a Sep-pac, followed by quantitative Gas-liquid Chromatography (GLC). Samples are silylated for the latter. Uses of the technique were applied to analyzing immature and mature citrus peel for Release® and it was demonstrated that immature fruits metabolized the compound faster.**

Release® (5-chloro-3-methyl-4-nitro-1H-pyrazole) is an effective chemical aid for harvesting citrus fruits (1, 3, 9). It apparently stimulates citrus peel tissue to produce ethylene, thus promoting fruit abscission (1, 5). Ethylene production depends on the concentration of Release® in the peel (2) and this, in turn, is influenced by internal and external factors (4, 6, 7, 8), particularly temperature (6), and the turnover rate at which the parent compound is metabolized to components non-active in stimulating ethylene synthesis (1, 2).

This study was undertaken because it was apparent that a simpler, more efficient method for purification of Release® was needed. The easiest method to reduce losses and decrease the analysis time is to reduce the steps required for partial purification. This was done by using a small cartridge packed with silica gel produced by Waters Associates (Framingham, Mass.). These cartridges (Sep-pac) allowed us to decrease the number of steps before Release® could be quantified easily by GLC-FID.

Materials and Methods

Plant Material. Mature and immature fruits of *Citrus sinensis* (L.) Osbeck cv. 'Valencia' were treated with 300 ppm of Release®. Samples were taken periodically for the purification procedure to follow tissue levels of parent compound.

Purification. A summary of the separation procedure is as follows: one-gram quantities of freeze-dried flavedo portions of the orange peel were homogenized for 1 minute in 100 ml of 80 percent acetone. To each extract was added 6 pg of ^{14}C-labelled 5-chloro-3-methyl-4-nitro-1H-pyrazole with the ^{14}C at the 3-position in the pyrazole ring to allow for the estimation of the final recoveries of parent compound and initially to validate the procedure. This mass of Release® was less than could be detected in the aliquot analyzed by GLC. Cellular debris was filtered from the extracts using Whatman No. 1 filter paper in a Büchner funnel with suction. The filtrates were taken to dryness *in vacuo* at 35°C followed by freeze-drying. Two ml of methylene chloride was added to the dry extracts and sonicated. One ml of the resulting extract was then placed, using a glass syringe, onto a Sep-pak prewashed with 5 ml methylene chloride. A series of organic solvent mixture were used to partially purify Release® before it was eluted. The sequence of solvents used for this fractionation procedure is as follows: the original extract of 1 ml methylene chloride displaced 1 ml of methylene chloride from the Sep-pak cartridge. The loaded cartridge was then washed with a series of 5-ml aliquots of organic solvents. A flow rate of 60 s/5 ml fraction was maintained throughout. Faster or slower flow rates will alter retention time. Five aliquots of freshly distilled n-hexane dried over Na_2SO_4 were followed by 5 aliquots of methylene chloride which was followed by 3 aliquots of 5% diethyl ether in methylene chloride. Release® is normally eluted in the first and second aliquots of the latter. Occasionally it will start eluting in the last aliquot of methylene chloride and its altered retention time seems to be related to the amount of terpenoids in the samples. The elution position was established by radioactive tracer techniques.

These steps using the Sep-pak require less than 30 minutes for completion. Fractions 10, 11, and 12 containing Release® were then combined and quickly taken to dryness. Addition of 10 ml of 0.5 M phosphate buffer (pH 8.0) to the dried residue will dissolve the Release®, leaving in the residue much of the pigmented materials. The Release® was then partitioned back into ethyl acetate by lowering the pH to 1 with 1 N HCl. The ethyl acetate fraction was dried using anhydrous Na_2SO_4 before ethyl acetate was removed using a stream of dry N_2 gas. The residue can be silylated and analyzed using a gas chromatograph equipped with a 1.50 m glass column x 3.4 mm od and packed with OV-17 at 3 percent on 100/120 mesh Gas Chrom Q. The gas chromatograph equipped with a FID can be operated isothermally at 250°C or programmed from 100° to 300°C with helium as the carrier gas at 30 ml min^{-1} at 2.10 bar of column inlet pressure. In the programmed mode, the silylated Release® elutes as 2 isomeric forms with retention times of 10.5 and 10.7 minutes.

To validate the above initial, fast clean-up procedure, isotopic dilution analysis was applied to each step. Radioactivity was determined using scintillation techniques and a Packard Tri-carb scintillation counter.

[1]Release® is a trademark registered by Abbott Laboratories.

[2]Florida Agricultural Experiment Stations Journal Series 3546.
A portion of this work was done under a contract from the Florida Department of Citrus, Harvesting Research and Development Committee to Dr. R. H. Biggs.

Results and Discussion

With the use of the Sep-pac technique, small samples can be annalyzed and the cumbersome and time-consuming solvent partitioning clean-up procedure (10) can be eliminated. It is estimated that the initial clean-up procedure can be less than 30 minutes with a single sample and less than this if several samples are being prepared at one time. Also, the amount of solvents needed is much less and percent recovery of parent compound is better (Table 1). The solvent partitioning procedure (10) required several hours to prepare the sample for GLC analyses. The residue is also suitable for quantitative HPLC separations and GLC/mass spectrometer analyses (2).

The data in Figure 1 was obtained by using the Sep-pac clean-up procedure, isotopic dilution analysis and GLC. Areas under the eluting curves as matched to recovery of ^{14}C was used to determine relative amounts extracted from the flavedo portion of the peel. As shown previously (4), this portion of the peel retains over 90 percent of the Release® applied to an orange as a chemical aid for harvest. It can be seen from this graph that the half-life of parent compound is much less in immature fruits than in mature fruits even though immature fruits seem to have a higher initial uptake of the material. The calculated half-life in this series of tests was approximately 19 hours for the immature fruit and 30 hours for the mature fruit. These data support the concept that the differential response between green-immature and orange-mature fruit is highly dependent on the rate of metabolism of Release® by the tissue and is a critical factor controlling ethylene synthesis, the agent stimulating fruit abscission. As reported previously (1, 2), Release® *per se* is the active component and not the metabolites of the parent compound.

Thus, the advantages of this technique are 4-fold. It is rapid, taking 2-3 hours from grinding the sample to GLC. It is simple and inexpensive, with good rates of recovery even with complex, highly pigmented tissues. Also, it requires less than 150 ml of solvent per sample. These factors allow analytical analysis of Release® to be made as related to physiological responses and to efficacy tests.

Table 1. A testing of the Sep-pac method for partial purification of Release® from citrus peel tissue (flavedo portion).

Fraction	Radioactivity (dPM)	Percent[z] Recovery
Release® added	452,000	
Methylene chloride	443,520	98 ± 2
Sep-pac fraction:		
1-5	97,064	8.2 ± 3
6-9	4,720	1.1 ± 1
10-12	402,280	89 ± 6
13	5,876	1.3 ± 1
Residue for analysis	388,700	86 ± 5[y]

[z]Percent recovery based on amount added and 5 reps.
[y]Percent recovery for the solvent partition procedure (10) was 83 ± 4%.

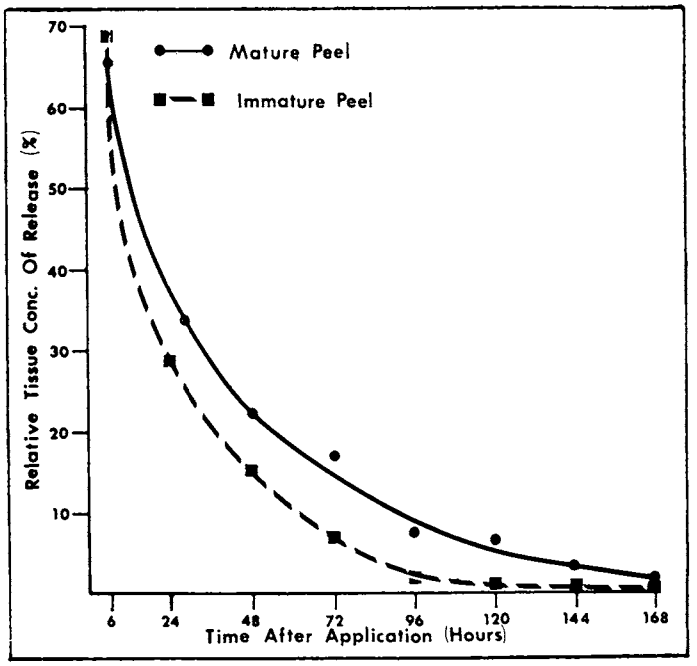

Fig. 1. Relative changes in concentration of Release® in immature and mature orange peel tissue with time after application.

Literature Cited

1. Biggs, R. H. and S. V. Kossuth. 1980. Modeling the effectiveness of Release as a citrus harvest aid for 'Valencia' fruits. Proc. Fla. State Hort. Soc. 93:301-305.
2. ———— and ————. 1980. Metabolism of Release. Proc. of Pl. Growth Reg. Soc. 1:81-82.
3. Kenney, D. S., R. K. Clark, and W. C. Wilson. 1974. ABG 3030: An abscission chemical for processing oranges: Biological activity. Proc. Fla. State Hort. Soc. 87:34-36.
4. Kossuth, S. V. 1978. Uptake of ^{14}C-Release in 'Valencia' oranges as affected by its location on the fruit, temperature, humidity, treatment duration and fruit position on the tree. J. Amer. Soc. Hort. Sci. 103:561-564.
5. ———— and R. H. Biggs. 1977. Fruit removal force, cellulase and ethylene production in Release and ethephon-treated oranges.
6. ————, ————, and F. C. Martin. 1979. Effect of physiological age of fruit, temperature, relative humidity and formulation on absorption of ^{14}C-Release by 'Valencia' orange. J. Amer. Soc. Hort. Sci. 104:323-327.
7. ————, ————, and V. W. Winkler. 1978. Uptake and distribution of ^{14}C-labeled 5-chloro-3-methyl-4-nitro-1H pyrazole in 'Valencia' and 'Hamlin' oranges. J. Amer. Soc. Hort. Sci. 103:20-22.
8. Murphy, S. K., R. H. Biggs, and V. W. Winkler. 1976. Uptake and distribution of "Release" in 'Valencia' and 'Hamlin' oranges. Proc. Fla. State Hort. Soc. 89:43-45.
9. Wilson, W. C. 1973. A comparison of cycloheximide with a new abscission chemical. Proc. Fla. State Hort. Soc. 86:56-60.
10. Winkler, V. W., W. C. Wilson, D. S. Kenney, and J. M. Yoder. 1974. ABG-3030: Abscission chemical for processing oranges: Analytical residue and environmental considerations. Proc. Fla. State Hort. Soc. 87:321-324.