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were consistently higher for the citrus pectin fractions than for corresponding fractions of the commercial low methoxyl pectin or the PE-treated pectins. Although NaH_2PO_4 elution chromatography separates pectin by ester content (Smit and Bryant, 1967), results of the present study show that a given molarity of NaH_2PO_4 would not invariably elute pectin of a particular ester content. This phenomenon may be due to differences in the molecular weights of the parent pectins.

When pectin fractions were tested for clarifying properties, fractions having DE values of 21% or higher did not destabilize orange juice cloud, but fractions with DE values of 14% or less did. Prior work relating esterification level to clarification measured juice pectin ester content; thus, the results may well have been biased because of nonuniformity in the DE of the pectins. For example, Rouse (1949) observed a 25% "separation" of cloud in 15 min when the methoxyl contents of juice pectins declined to between 4.56 and 5.87%, expressed on a calcium pectate basis. Expressed on a pectinic acid basis, his data show clarification was initiated as average DE values declined to between 30 and 38%. In the present work, PE-treated pectins and commercial low methoxyl pectin had average DE values between 35 and 38%. Of the fractions derived from these pectins, only those with DE values of 14% and below clarified the test juice.

A further difficulty in relating the DE of unfractionated pectin to clarification becomes evident when Figures 2 and 3 are compared. Both low methoxyl pectins had similar DE values (36 and 38%), yet only 8% of the commercial low methoxyl pectin could clarify juice, as compared with 32% of the PE-treated pectin. Krop et al. (1974) obtained an 85% cloud reduction in juice when juice pectin DE was reduced to 27.4%. The present work showed that cloud was not precipitated by a fraction consisting predominantly of pectins that were only 21% esterified. Therefore, in any study relating pectin esterification to clarification, pectins as homogeneous as possible with respect to ester content should be used.

Table II shows that as the esterification level of pectin fractions approached the critical level, they could support more cloud than the amount normally present in juice. A similar phenomenon has been reported by a number of investigators, who found that cloud density in whole juice increased shortly before the onset of clarification (Joslyn and Pilnik, 1961). The increase in turbidity may be due to the initial precipitation of pectate floc prior to its complexing with cloud particulates.

In conclusion, several pectins differing in ester content were fractionated on DEAE-cellulose and were assayed both for degree of esterification and juice clarifying potential. Pectin fractions with degrees of esterification as low as 21% did not destabilize cloud in the test system employed. Clarification was initiated by pectin fractions with DE values of 14% or less, indicating that the critical DE for clarification is between 14 and 21%.

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Daminozide Residues on Orchard-Treated Apples

Terminal residues of daminozide were determined on apples after application of Alar as a dilute or concentrated spray. Thirty-five days after treatment, uncorrected results for daminozide residues were 5.2 and 7.1 ppm for the dilute and concentrated sprays, respectively, and after 71 days the concentrations were 5.0 and 5.8 ppm, respectively. Control apples showed apparent residues of 2.9 and 4.4 ppm on the early and late harvest dates, respectively. No significant differences (*t* test, <98%) were found between the residue from either spray type and control apples at the later harvest date.

Daminozide (Alar; succinic acid 2,2-dimethylhydrazide) is used in Ontario (Publication 360, 1978) and in other apple-producing areas mainly as a growth regulator to control preharvest drop of apples. There has been an indication that daminozide-treated fruit shows a higher incidence of physiological disorders than untreated fruit

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(Sharples, 1973). Recently some concern has been expressed that apple fruit treated with Alar as a concentrated spray may be more susceptible to "soft McIntosh" problems (Blanpied et al., 1977). However, because Alar is such a useful material and because it has been difficult to attribute a direct deleterious effect upon the fruit at recommended rates, Alar continues to be recommended for use on apples and, in fact, is widely used as a stop drop spray. It has also been shown that daminozide and substituted hydrazines could be inimical to health (Toth and Wilson, 1971; Toth et al., 1977). Residue of daminozide on apples have previously been reported (Edgerton et al., 1967). The present study reports on terminal residues of daminozide on apples after application of Alar as a dilute (1×) or concentrated (5×) spray.

MATERIALS AND METHODS

Alar-85 was applied as an aqueous foliar spray to 29-year-old McIntosh trees on *Malus robusta* no. 5 rootstalks planted 10.7 × 10.7 m apart at the Agriculture Canada Smithfield Experimental Farm, Trenton, Ontario. All sprays were applied on July 27, 1977. Some trees received a dilute spray (1×) of 0.34 kg in 454.6 L of water applied until run-off occurred using a spray-broom applicator. Alternatively, a concentrated spray (5×) was applied with an air-blast sprayer at 1.36 kg in 273 L of water/0.405 ha. Five trees were used as replicates in each treatment (control, dilute, and concentrated spray); random samples of ten apples were taken per replicate.

Daminozide was determined according to the method of Edgerton et al. (1967) in which residues were determined by alkaline extraction and hydrolysis of Alar to dimethylhydrazine which was distilled and measured colorimetrically based on its reduction of phosphomolybdic acid to the heteropoly blue. Ten apples were composited in a Hobart food chopper and a 10-g subsample was hydrolyzed with 20 mL of 50% NaOH in a small jar. After standing overnight at room temperature, the solution was filtered through cheese cloth into a 125-mL round-bottom flask. The residue was rinsed with 10 mL of base and the cloth squeezed to remove all excess liquid. The flask was attached to a 75° connecting tube, Liebig condenser and a receiving adapter, and the apparatus was placed on a heating mantle. The solution was refluxed until 8 mL of distillate was collected in a 15-mL graduated test tube. One milliliter of aqueous 5% phosphomolybdic acid was added to the tube which was then placed in a steam bath for 1 h. The tube was cooled and the solution diluted to exactly 10 mL with distilled water. The absorbance was determined at 625 nm using a 1-cm cell against a reference of distilled water. Turbid samples were centrifuged prior to quantitation.

A standard curve was generated by adding daminozide (0–100 μg) in water to a flask and adding water so that all flasks contained 4 mL. Sodium hydroxide was added, and the standards were hydrolyzed and analyzed as above. Linear calibration curves were obtained with standards; the y intercept was essentially 0.000 absorbance units and the slope of the curve was 0.0025 absorbance units per microgram of daminozide. Solutions having high absorbance readings could be determined after dilution with water. The mean recovery from fortified apple homogenates was 82%.

RESULTS AND DISCUSSION

Samples were taken August 31 (day 35) some 2–3 weeks before the normal harvest date, and on October 6 (day 71), 1 week after the harvest would usually be finished. As noted by Edgerton et al. (1967) control fruit showed "apparent" daminozide residue; in our case, control values

Table I. Daminozide Residues on Apples Sampled on Two Dates after Treatment with Alar

treatment[a]	daminozide residues[b] remaining on	
	Aug 31	Oct 6
control	2.9 ± 0.37	4.4 ± 0.52
dilute spray (uncorrected)[c]	5.2 ± 0.56	5.0 ± 1.2
concentrated spray (uncorrected)	7.1 ± 1.4	5.8 ± 1.6
dilute spray (corrected)	2.3 ± 0.56	0.6 ± 1.2
concentrated spray (corrected)	4.2 ± 1.4	1.4 ± 1.6

[a] Treated on July 27, 1977. [b] Mean ± standard deviation of five sample replicates. [c] Corrected for control values.

ranged from 2.4 to 5.2 ppm. Apparent residue in the controls increased between the two sampling dates presumably due to the development of a natural product that interfered with the analysis (Table I). Daminozide residues on the treated apples decreased with time as expected. The variation in residue concentration was greater at the longer harvest interval. All residues were well below the current Canadian tolerance of 30 ppm.

The significance of the difference between the means of the residue data for the two sampling dates and the three treatments were analyzed using the t test on unpaired results. Significant differences (>98%) were found between the control apples on both sampling dates and between the treated apples and controls at the early harvest. Corrected residues from the concentrated spray on the two sampling dates were just significantly different (t = 2.91 vs. 2.90). No significant differences were found between either spray treatment and controls at the later date and between the dilute and concentrated spray residue on both dates.

It was hoped that both the dilute and concentrated spray application would apply the same amount of material to the apples so that apple quality parameters could be compared for the two treatments. At the two sampling dates, the same amount of residue was present from the dilute and concentrated spray application. A dissipation of residue was evident between the two sampling dates based on the corrected residue results and by day 71 no significant difference was found between the treated apples and controls. The apple quality results will be reported elsewhere.

Alar-treated apples were also examined after freeze-drying to about one-tenth the fresh weight. Approximately ten times the concentration of daminozide was found in treated apples compared to the fresh fruit results and the background interference similarly increased; controls indicated 58 ppm and the treated apples contained 78 ppm uncorrected daminozide residue. Obviously the freeze-drying concentrated both the daminozide residue as well as the background contaminant, and this might lead to erroneous results for concentrated substrates such as in apple pomace.

No effort was made in this study to remove substituted hydrazines from daminozide prior to analysis or to eliminate the "background" levels of "apparent" daminozide in the control apples. Use of the Edgerton et al. (1967) procedure results in high control values for apple substrate, and the analyst must be aware of this problem when analyzing unknowns. Furthermore, it appears that the background level may increase with increased spray-harvest intervals. While it is possible to distinguish between residues near tolerance (30 ppm) and control apples (2–5 ppm), this study indicates that determination of actual harvest residues may be difficult. Further studies are being conducted with other methods and substrates to determine

the nature and concentration of terminal daminozide residues.

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A Change in the Degradation Pathway of Parathion after Repeated Applications to Flooded Soil

The degradation pathway of parathion shifted to hydrolysis from reduction after repeated applications of parathion or its hydrolysis product, p-nitrophenol, to a flooded soil. This shift occurred as a result of the proliferation of parathion-hydrolyzing microorganisms that utilized p-nitrophenol as the energy source. This is probably the first report of the enrichment of a population capable of degrading a parent molecule upon application of the primary product of its metabolism.

The major pathways in the degradation of parathion in soil and water environments are nitro group reduction (Lichtenstein and Schulz, 1964) and hydrolysis (Sethunathan et al., 1977), both mediated essentially by microorganisms. It is well established that microbial populations with pesticide-degrading potentials readily build up in soil and water systems following repeated applications of a wide array of pesticides which serve as sources of carbon and energy for growth (Waid, 1972). But a shift from one pathway to another following repeated applications of a pesticide characterized by more than one degradation pathway as in parathion is not known. We report here a clear shift in the degradation pathway of parathion from nitro group reduction to hydrolysis after the repeated application of parathion or its hydrolysis product, p-nitrophenol, to a predominantly anaerobic flooded soil.

EXPERIMENTAL SECTION

At 15-day intervals, 1.72 μmol of parathion was added to 20 g of alluvial soil contained in sterile test tubes (200 × 25 mm) and the soil was flooded with 25 mL of sterile distilled water. At periodic intervals after each parathion addition, parathion and its predicted degradation products, aminoparathion and p-nitrophenol, were analyzed in duplicate soil samples. For quantifying nitro group reduction, parathion and aminoparathion were extracted from soil samples with methanol–acetone–benzene (1:1:1) and the residues in the benzene fraction were analyzed in a Perkin Elmer gas chromatograph Model 3920 equipped with flame photometric detector specific for phosphorus. Using this procedure, the recoveries of parathion and aminoparathion immediately after application to the soil ranged between 85 and 95%. In studies dealing with the hydrolysis of parathion, parathion and p-nitrophenol were extracted from the soil samples with chloroform–diethyl ether (1:1)

and then analyzed after separation by thin-layer chromatography. p-Nitrophenol from the chromatogram was eluted directly in 0.1 N NaOH while parathion was converted first to p-nitrophenol by alkaline hydrolysis (Sudhakar-Barik and Sethunathan, 1978). p-Nitrophenol was measured at 400 nm. The recoveries of known quantities by this method ranged from 72 to 86% for parathion and from 78 to 90% for p-nitrophenol.

RESULTS AND DISCUSSION

The rate of degradation of parathion in a flooded soil increased progressively after each successive application (Figure 1). Interestingly, aminoparathion was recovered as the major product after the first addition of parathion, both aminoparathion and p-nitrophenol after the second addition and p-nitrophenol alone after the third addition (Table I). Thus the data clearly demonstrated that the pathway in the degradation of parathion shifted from reduction to hydrolysis after its repeated application to a flooded soil. The p-nitrophenol formed was metabolized subsequently as indicated by its disappearance, in agreement with an earlier report (Siddaramappa et al., 1973). The degradation of parathion in flooded soil followed first-order kinetics for all applications irrespective of the pathway involved (Figure 1), but the kinetic constant gave the indication that hydrolysis of parathion proceeded at a remarkably faster rate than reduction.

Tenfold dilutions of the soil were prepared at 5 days after each addition of parathion and 1 mL of each dilution was added to 15 mL of a sterile mineral salts medium (Sethunathan, 1972) supplemented with 0.69 μmol of parathion for most-probable-number (MPN) estimates of the population capable of hydrolyzing parathion. Uninoculated medium served as control. Direct assay of p-nitrophenol formed in the medium was not possible because of