Absorption and persistence of 2,4,5-TP in Packham’s Triumph pear trees

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Fruit set of the pear cultivar Packham’s Triumph is significantly improved by a post-harvest application of 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP), implying a persistence long enough to prevent fruitlet abscission the following spring. Since no literature regarding the persistence of 2,4,5-TP in higher plants could be found, it was decided to study its persistence in Packham’s Triumph pear tissue following post-harvest applications. Residual 2,4,5-TP in the tissues was determined by electron capture gas chromatography. It was found that 2,4,5-TP did not persist in detectable levels in the tissues of potted saplings until spring. However, significant amounts were found in buds of full-grown trees until bud break, as well as in leaves and fruitlets developing from these buds.

Na-oesbespuiting met 2-(2,4,5-trichlorofenoksie)-propioonsuur (2,4,5-TP) het tot betekenisvolle verhogings in vrugset by die Packham’s Triumph peer-kultivar geleë. Dit impliseer die nablywende werkings van 2,4,5-TP tot die volgende lente om vrugval dan te voorkom. Aangesien geen literatuur oor die nawerking van 2,4,5-TP in hoër plantes gevind kon word nie, is besluit om die nawerking daarvan in Packham’s Triumph peerweefsel na na-oesbehandelings te bestudeer. Residuele 2,4,5-TP in die weefsel is met behulp van elektronvangs-gaschromatografie bepaal. Daar is bevind dat 2,4,5-TP nie in naspeurbare hoeveelhede in die weefsel van jong boompies tot die volgende lente voortbestaan nie. Betekenisvolle hoeveelhede het egter in knoppe van volgroeide bome tot bottyd voorgekom, asook in blare en vruggies wat uit dié knoppe ontwikkel het.

Keywords: Persistence, 2,4,5-TP, pear trees.

Introduction

It is well known that the pear cultivar Packham’s Triumph sets poorly in the Western Cape region of South Africa, even under optimal conditions regarding compatible cross-pollination (Strydom et al. 1973). Fruit set can, however, be improved by spraying with 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) (Strydom et al. 1966). Optimum set is achieved by a post-harvest application during the latter half of April. Spraying during full bloom also results in large increases in set, but smaller fruit which are not suitable for marketing, are produced (Van Zyl & Strydom 1968).

Chemical control of fruit drop has received little attention during the past twenty years since combinations of 2,4,5-TP, naphthaleneacetic acid and daminozide effectively prevents fruit abscission (Marini et al. 1988). This led to the virtual absence of recent literature on the subject. However, we were interested in the mechanism whereby 2,4,5-TP improves fruit set, a matter about which little is known. According to several investigators, improvement in fruit set may be related to higher endogenous hormone levels in the fruit, enabling the latter to act as stronger metabolic sinks (Crane 1969; Luckwill et al. 1969). However, a previous study (Watts & De Villiers 1979) indicated that 2,4,5-TP, whilst improving fruit set by sixty percent, had no significant effect on source–sink relationships. It was deduced that 2,4,5-TP had a more direct effect by preventing the formation of abscission layers, implying its persistence in the tree from the time of application (April) to the time of fruit set (November). However, the ability of leaf tissue of Packham’s Triumph pears to decarboxylate 2,4,5-TP (Watts & De Villiers 1981) argues against such a long persistence. This study was therefore undertaken to determine the persistence of 2,4,5-TP, applied at different periods, in Packham’s Triumph tissues until fruit set.

Materials and Methods

One hundred Packham’s Triumph pear saplings with quince rootstock and Beurre Hardy pear interstocks were planted in large earthen pots in acid-washed sand. They were kept in the open and a complete Hoagland nutrient solution (Hoagland & Arnon 1950) was supplied twice a week, with irrigation as necessary inbetween. The sand was rinsed thoroughly with tap water immediately before each addition of nutrient solution.

Three days before each treatment [31 March, 30 April, 30 May and 7 October (full bloom)], 16 trees were placed in a greenhouse with a day temperature of 25 ± 2°C and ambient night temperature. On each treatment date the trees were sprayed with a solution containing 10 mg dm−3 2,4,5-TP (as the triethanolamine salt) and 60 mmol dm−3 Agral 90° as a wetting agent. After 3 days the trees were returned to the open. In a separate experiment 20 trees in a commercial orchard were sprayed with the above-mentioned solution during the middle of April.

On set dates after each treatment, 4 trees were removed from the pots and the sand was rinsed from their roots. Each tree was divided into leaves, stems and roots. The stem fraction consisted of all branch stems and the bark of the main stem, the latter being too thick to process. Buds from the orchard trees were collected 4 and 2 weeks before full bloom whilst leaves and fruitlets were collected 2 and 4 weeks after full bloom. All samples were thoroughly rinsed with running tap water to remove unabsorbed 2,4,5-TP, blotted dry, oven-dried at 105°C for 72 h and ground to a fine powder in a high-speed laboratory hammer mill. The
dried powders were kept at −15°C over CaCl₂ in closed containers.

The extraction, purification, esterification and gas chromatographical determination of total 2,4,5-TP (viz. free acid, bound and esterified forms) were performed according to procedures outlined by Meagher (1966). All solvents were purified by distillation.

The following preliminary determinations were carried out: Firstly, a recovery study involved the addition of a known amount of 2,4,5-TP to an unsprayed leaf sample and the determination of the amount of 2,4,5-TP that could be recovered. Secondly, parallel extractions were performed on fresh unsprayed, as well as fresh and oven-dried sprayed leaf samples to determine the effect of drying on the recovery of 2,4,5-TP.

Dried samples were extracted by stirring 100 g material in acetone (45°C) for 5 min with an electrically driven stirrer. Fresh material (± 500 g) was homogenized for 2 min in a ‘Waring’ blender with enough acetone to form a slurry. The homogenates were filtered in vacuo through Whatman No. 1 filter paper. Further extraction, purification and esterification were performed strictly according to Meagher (1966). Samples were finally taken up in 5 cm³ hexane. Ten-mm³ samples of the hexane solutions were used for gas chromatographical analyses, which were performed on the same day as the esterification.

A standard curve for 2,4,5-TP was obtained as follows: Samples containing 0.05 – 10 mg 2,4,5-TP (analytical standard, Amchem Products) in chloroform were transferred to 25-cm³ Erlenmeyer flasks and evaporated to dryness under a stream of cold air. The residues were esterified and further treated as described above.

The quantitative determination of the butoxyethyl esters of 2,4,5-TP was done on a Packard Model 7400 gas chromatograph fitted with a Packard Model 880 ⁶⁵Ni electron capture detector, a Vidar 6300 digital integrator and a glass column (1.6 m × 3 mm) packed with 3.8% SE-30 on Chromosorb W (HMDS, 60 – 80 mesh). The column temperature was 200°C, with the injector block and detector at 250°C. The detector potential was set at 2.5 V and the carrier gas (N₂) flow rate at 60 cm³ min⁻¹.

**Results and Discussion**

The butoxyethyl ester peak of authentic 2,4,5-TP had a retention time of 8.5 min (Figure 1). Extracts of unsprayed leaves yielded no peak at that retention time, but extracts of sprayed leaves (fresh or oven-dried) yielded a peak co-chromatographing with authentic 2,4,5-TP (Figure 1). It is therefore acceptable to employ oven-dried material for the determination of 2,4,5-TP residues in plant material. It was found that the relationship between peak area and 2,4,5-TP concentration is linear in the range 0.05 – 10 ng. In the recovery experiment, 92% of the initial amount of 2,4,5-TP was recovered.

Table 1 indicates the levels of 2,4,5-TP in the leaves, stems and roots of potted trees at different intervals following spraying with 2,4,5-TP.

No significant differences between the different leaf samples were found two days after the relevant treatments. Assuming similar rates of translocation and metabolic degradation for all samples during this period, it appears that the time of application had no significant effect on the absorption and translocation of 2,4,5-TP.

Decreases in the concentration of 2,4,5-TP in leaf samples following the March, April and October treatments may be ascribed to its translocation to stems as well as possible metabolic degradation. It is, however, also possible that 2,4,5-TP was directly absorbed through the stems. According to Weaver (1972), the absorption of hormone-type compounds is limited to young green stems. This aspect was not investigated and it is assumed that absorption through stems could have occurred to a limited extent.

Significantly less 2,4,5-TP was translocated to stems following the May treatment, as compared to the March and April treatments. The latter two treatments therefore led to the retention of higher levels of 2,4,5-TP in the tree, levels that may persist long enough to improve fruit set the following spring. This view is substantiated by results showing that treatments during April led to a much better improvement in fruit set than later treatments during May (Strydom et al. 1966).

The relatively low levels of 2,4,5-TP in stem tissue 30 days after the treatment at full bloom may be due to the fact that actively growing young leaves and fruits acted as metabolic sinks at this stage. According to Weaver (1972), such sinks serve as mobilizers of hormone-type compounds. It is also possible that the translocation of 2,4,5-TP occurred to a limited extent during this period.

![Figure 1 Gas chromatographical separation of 2,4,5-TP in fresh and oven-dried Packham's Triumph pear leaves. A. Solvent (hexane). 1. 2,4,5-TP (butoxyethyl ester). 3. Butoxyethanol. (Other peaks are unknown components.) B. Fresh (sprayed). C. Oven-dried (sprayed).](image-url)
As a result of dilution due to increased amounts of leaves, the levels of 2,4,5-TP to bridge the period between autumn and spring. Despite the observation that it can be decarboxylated by these tissues (Watts & De Villiers 1981). This persistence may be ascribed to the fact that side-chain metabolism of phenoxy acids is blocked by (a) meta substitution and (b) a methyl group on the side chain (Brian 1964). Both these criteria are met by 2,4,5-TP. The results of the current study are in concurrence with those of Weintraub et al. (1954), viz. that although detoxification of 2,4-dichlorophenoxyacetic acid does occur in dormant tissues of cherry trees, significant amounts persist to affect growth the following spring. It is, however, impossible to deduce whether the amount of 2,4,5-TP persisting up to full bloom and fruit development, is sufficient to prevent fruit abscission as such.

According to the literature, chlorophenoxy acids can persist for long periods in plants (Weintraub et al. 1954; Leopold 1955; Bradley & Crane 1957). No literature could be found regarding the persistence of 2,4,5-TP in higher plants. The results of this study indicate that 2,4,5-TP does persist in pear tissue from autumn to spring, despite the observation that it can be decarboxylated by these tissues (Watts & De Villiers 1981). This persistence may be ascribed to the fact that side-chain metabolism of phenoxy acids is blocked by (a) meta substitution and (b) a methyl group on the side chain (Brian 1964). Both these criteria are met by 2,4,5-TP. The results of the current study are in concurrence with those of Weintraub et al. (1954), viz. that although detoxification of 2,4-dichlorophenoxyacetic acid does occur in dormant tissues of cherry trees, significant amounts persist to affect growth the following spring. It is, however, impossible to deduce whether the amount of 2,4,5-TP persisting up to full bloom and fruit development, is sufficient to prevent fruit abscission as such. It may be possible that the 2,4,5-TP only supplements the endogenous hormone levels in the fruits to create and maintain a favourable auxin gradient across abscission zones.

### Table 1 Concentration of 2,4,5-TP in leaves, stems and roots of Packham’s Triumph pear trees following sprays at different periods

| Date of spraying | Tissue | Concentration of 2,4,5-TP (ng g⁻¹ dry mass) | Number of days after spraying |
|------------------|--------|-------------------------------------------|-------------------------------|
| 31 March         | Leaf   | 102.4 ± 14.3                             | 2                             |
|                  | Stem   | b                                         | 30                            |
|                  | Root   | b                                         | 60                            |
| 30 April         | Leaf   | 91.2 ± 10.6                               | 2                             |
|                  | Stem   | b                                         | 30                            |
|                  | Root   | b                                         | 60                            |
| 30 May           | Leaf   | 94.6 ± 11.8                               | 2                             |
|                  | Stem   | 8.1 ± 2.0                                 | 30                            |
|                  | Root   | b                                         | 60                            |
| 7 October        | Leaf   | 125.4 ± 16.2                              | 2                             |
| (full bloom)     | Stem   | 9.2 ± 2.8                                 | 30                            |
|                  | Root   | b                                         | 60                            |

* Mean ± S.E. of four samples.
* No sample taken.
* No sample available due to leaf fall.

No 2,4,5-TP was detected in the roots. The following factors could have contributed to this: Firstly, hormone-type compounds harm their own translocation through the establishment of metabolic sinks in the plant parts where they have been applied (Weaver 1972). Secondly, according to Osborne and Wain (1950), compounds with α-propionic acid side chains (like 2,4,5-TP) are associated with poor transport in plants. Thirdly, roots act as poor metabolic sinks during periods of poor root growth (Weaver 1972). Fourthly, the leakage of phenoxy herbicides from roots following foliar applications has been reported by several authors (Long & Basler 1973; Sanad & Müller 1973). Lastly, it is also possible that some or all of the 2,4,5-TP that may have reached the roots, was metabolized.

These results indicate that 2,4,5-TP cannot persist in the different plant parts from an autumn application to the following spring in significant amounts. A possible reason for this may be that the available leaf area of the trees at this time was too small for the absorption of sufficient amounts of 2,4,5-TP to bridge the period between autumn and spring. This phenomenon could also be due to metabolism.

The levels of 2,4,5-TP in the buds, leaves and fruitlets of full-grown trees sprayed during autumn are presented in Table 2. It is clear that 2,4,5-TP was translocated to buds where it persisted until spring. Following bud break it was still present in developing leaves and fruitlets. With time the levels of 2,4,5-TP decreased sharply in the leaves, possibly as a result of dilution due to increased amounts of leaves, translocation and metabolic degradation. Decreases in the levels of 2,4,5-TP in fruitlets were smaller, as their number and size remained fairly constant during the period involved. The higher levels of 2,4,5-TP in fruitlets (as compared to leaves) may be due to the fact that fruit tissues attract and accumulate hormones from vegetative organs (Crane 1965).

According to the literature, chlorophenoxy acids can persist for long periods in plants (Weintraub et al. 1954; Leopold 1955; Bradley & Crane 1957). No literature could be found regarding the persistence of 2,4,5-TP in higher plants. The results of this study indicate that 2,4,5-TP does persist in pear tissue from autumn to spring, despite the observation that it can be decarboxylated by these tissues (Watts & De Villiers 1981). This persistence may be ascribed to the fact that side-chain metabolism of phenoxy acids is blocked by (a) meta substitution and (b) a methyl group on the side chain (Brian 1964). Both these criteria are met by 2,4,5-TP. The results of the current study are in concurrence with those of Weintraub et al. (1954), viz. that although detoxification of 2,4-dichlorophenoxyacetic acid does occur in dormant tissues of cherry trees, significant amounts persist to affect growth the following spring. It is, however, impossible to deduce whether the amount of 2,4,5-TP persisting up to full bloom and fruit development, is sufficient to prevent fruit abscission as such. It may be possible that the 2,4,5-TP only supplements the endogenous hormone levels in the fruits to create and maintain a favourable auxin gradient across abscission zones.

### Table 2 Concentration of 2,4,5-TP in buds, leaves and fruitlets of Packham’s Triumph pear trees at different periods after an autumn application

| Time of harvest [weeks before (-) or after (+) full bloom] | Concentration of 2,4,5-TP (ng g⁻¹ dry mass) |
|----------------------------------------------------------|---------------------------------------------|
| -4                                                       | -2                                          |
| Buds                                                     | 450 ± 30.2                                  |
| Leaves                                                   | 406 ± 26.7                                  |
| Fruitlets                                                | 94 ± 15.1                                   |
|                                                          | 16 ± 4.8                                    |
|                                                          | 183 ± 20.3                                  |
|                                                          | 81 ± 7.9                                    |

* Mean ± S.E. of three samples.
* No sample available.
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