Phytochemistry, 1975 Vol 14, pp 2559–2562 Pergamon Press Printed in England

ETHYLENE AND EXTRANEOUS MATERIAL FORMATION IN WOODY TISSUES

W. E Hillis

Division of Building Research, CSIRO, Highett, Victoria 3190, Australia

(Received 14 May 1975)

Key Word Index—*Eucalyptus astringens*, *E calophylla*, *E sieberi*, *E. sideroxylon*, *Rhus* spp, ethylene, polyphenols, kino

Abstract—Chloroethyl phosphonic acid (CEPA) was administered to the cambial region of *Eucalyptus astringens*, *E. calophylla*, *E. sieberi* and *E. sideroxylon*. The kino which exuded has been associated with the liberation of ethylene from the CEPA in the sap stream. The kinos from *E. astringens* and *E. calophylla* were chemically identical with those obtained naturally from these species. The composition of the polyphenols formed in the sapwood of *Rhus* after administration of CEPA had a similar chromatographic pattern to that obtained from the heartwood. In both these situations, the induced polyphenols differ from those normally present in the tissues in that the former contain acetate engendered moieties. CEPA produced copious amounts of carbohydrate gum from an apricot branch, and eucalypt sapwood blocks ventilated with ethylene contained a greater proportion of tyloses than the controls. The evidence supports the view that ethylene is an active intermediate in the formation of extraneous components Under natural conditions, ethylene could arise as a result of injury or physiological stress such as water shortage.

INTRODUCTION

Several workers have found a considerable proportion of the gas in woody stems to be CO_2 and Höll [1] examined the extent of its refixing in the stem and partial conversion to heartwood constituents No evidence was found to support the view that this fixing and conversion occurs adjacent to the heartwood periphery in material collected during winter.

Under *in vitro* conditions the innermost sapwood (or transition zone) of *Robinia pseudoacacia* [2] and *Pinus radiata* [3] shows, particularly during the dormant season, enhanced respiration compared with adjacent sapwood zones. The high CO_2 content in the heartwood could be due to this increased respiration and storage in a region of a low capacity for CO_2 fixation.

Under certain physiological conditions, a situation is established which abruptly triggers off a series of reactions resulting in the production of extraneous materials (i.e. extractives and tyloses) found in heartwood and some damaged tissues [4]. Earlier work carried out under *in vitro* conditions indicated ethylene played an active role in this situation [5,6]. The transition zone of *P. radiata* produces enhanced amounts of ethylene during the dormant season [6]. Ethylene production was demonstrated also in the sapwood of *P. radiata* which was mechanically injured and particularly after *Sirex-Amylostereum* attack [5]. When blocks of living *P. radiata* sapwood were ventilated *in vitro* with air containing 5 ppm of ethylene, polyphenols were formed, although not in the same ratio as in the heartwood.

This paper reports the changes observed when (2-chloroethyl)-phosphonic acid (CEPA, "Ethrel") was administered to growing woody plants. CEPA liberates ethylene under pH conditions of 4 and above [7]. One of the systems studied was that of kino formation and this is usually associated with injury (e.g insects, fire, knots) to the cambium [8] The injuries could liberate ethylene to act as the intermediate in kino formation When formed, kino is an aqueous solution of polyphenolic compounds almost entirely of a flavonoid nature

RESULTS

Effects of the Administration of CEPA

(a) *Rhus succedanea and an Acacia sp* About 5 ml of a granular liquid began to exude from the injection holes in the treated branches of *R succedanea* within 5 days of adding the CEPA solution in mid-November. At the same time, a dark brown gummy material collected around the periphery of the hole Neither material was formed by the control drillings

When the treated branches were removed from the tree after 4 months, yellow zones had extended 6–10 cm into the wood, with the greatest volume being above the injection hole The 20·4 and 21·4% of methanol extractives contained components previously found in the heartwood of the same tree [9] but absent from, or present in small amounts in, the sapwood Dihydrofisetin was the major component, appreciable amounts of fisetin were present as well as small quantities of garbanzol and 3,7,4′-trihydroxyflavone The control drillings had only small amounts of fluorescent polyphenols immediately surrounding the hole The unaffected sapwood samples contained 21·5 and 15·3% methanol soluble material which contained very small amounts of fluorescent components or substances which were resolved by GLC The major extractable components of the sapwood were gallotannins [9].

The amount of polyphenols formed by CEPA in the sapwood of an *Acacia* species, on the other hand, was only slightly more than that formed in control drillings and was insufficient for examination

(b) *Apricot* Copious amounts of a colourless carbohydrate gum exuded from the injection holes bored in a branch of apricot (*Prunus armeniaca*) after administration of CEPA solution and smaller amounts exuded from the apices of the branch Small amounts of gum also appeared on branchlets of the two neighbouring branches Discoloured tissue occupied a zone 1 cm radius around the treated drilling hole and polyphenols were present The control drillings showed no response

(c) *Eucalyptus spp* The regions of the tree stems to which the CEPA solution had been added yielded kino in a greater number of cases (90% with *E. sieberi*, 60% *E astringens*, 60% *E sideroxylon* and 100% with *E. calophylla*) than the control regions (10, 5, 0 and 60% respectively). The period elapsing before kino exuded was 14–17 days, and the flow continued for 1–4 weeks, except in the case of *E calophylla* when exudation continued for 3 months and recommenced without artificial initiation in the following spring season.

Whereas most eucalypts yield kinos composed almost entirely of polymeric flavans, *E. astringens* and *E. calophylla* are 2 species forming kinos containing a number of monomeric polyphenols which result in characteristic 2-D paper chromatograms Kinos resulting from administration of CEPA gave the same chromatographic pattern as those obtained naturally

Solutions of IAA and lanolin pastes containing IAA or NAA added to exposed cambial regions on *E sieberi* and *E. astringens* trees (12 regions for each species) during the spring season resulted in the formation of kino in 3 cases with the first species and none with the second

Ethylene analyses

Similar sized blocks containing the cambial region and part of the phloem and sapwood were taken in the spring season from freshly cut *E obliqua, E. rubida and E sieberi*. Pairs of blocks contained kino veins and control pairs without veins in the cambial region were analysed for ethylene. The blocks containing kino veins yielded after 1 day about double the amount of ethylene yielded by the controls. Specifically the amounts ($\times 10^{-9}$ l/g wood per hr) were for *E obliqua* 5 8 as against control 3·7, 10·8 (4·4), 7·1 (4 6), *E rubida* 10·0 (5 2) and *E. sieberi* 12 3 (5·7). The ethylene production ($\times 10^{-9}$ l/g dry wood per hr) of the transition zone of *E tereticornis* was highest in late autumn and early winter period The amounts were November 1 25, December 3·33, January 1 66, March 5 0, April 17 9, May 7·1, June 1·25, July 1·25, September 5·0.

Sections taken from the blocks of *E. tereticornis* which had been ventilated with ethylene contained visually more tyloses than the dried control blocks.

DISCUSSION

It was found the transition zone in *E. tereticornis* behaved similarly to that in *P. radiata* [6] and yielded significant amounts of ethylene although in smaller amounts, and to the greatest extent in the late autumn-early winter period. Injection of CEPA into eucalypt sapwood did not have a marked effect on the extractives. However injection into *Rhus* sp. sapwood produced notable amounts of polyphenols previously found in heartwood [9].

The cambial regions of *E. sieberi*, *E. obliqua* and *E. rubida* containing kino yielded larger amounts of ethylene than adjacent regions without kino. The administration of CEPA to the cambial region of *E. sieberi* and other eucalypts resulted in kino formation. Similarly the injection of "Tordons" resulted in copious kino exudations from a number of eucalypts. This is also probably due to ethylene as the "Tordons" contain picrolam alone or together with 2,4-D or 2,4,5-T and these chemicals are known to form ethylene in living tissues [10].

The observations made in this work support the view that ethylene plays a dominant role in the formation of exudates. The constant composition of the kinos from *E. astringens* and *E. calophylla* indicates that other hormones have little effect or are available in the same proportion in the cambial region. However, the situation is complex and this is shown by the work of Dowden and Foster (personal communication, 1973) in that they found the application of NAA to the bark of 1–2 yr old eucalypt seedlings will produce kino, whereas injury or treatments of other types rarely forms kino in eucalypts of this age. NAA is an unnatural auxin which can however stimulate ethylene formation when applied to plant tissues [10].

Attention is drawn to the situations of this present study in that not only is the amount of extraneous material formed enhanced by the presence of ethylene but that the nature of the compounds is considerably different from those previously present in the tissues. Ellagitannins are present in undamaged cambial tissues of eucalypts, gallotannins in *Rhus* sp. sapwood [9] and monobenzenoid compounds in *P. radiata* sapwood [11]. These compounds originate from the pentose phosphate shunt and shikimic acid. The compounds formed when ethylene is present include flavonoids and stilbenes requiring, in addition, acetate units from the TCA cycle.

Ethylene increases the activity of a range of enzymes [10]. Ethylene (30 ppm in air) promotes phenolic biosynthesis and the formation of a lignin-like compound in swede root tissue [12]. The recent work of Brown and Leopold [13] supports the viewpoint that ethylene increases radial growth stems of pine and other tree species when mechanical stress is applied. On the other hand, kino formation is accompanied by a temporary cessation of formation of woody tissues [14], (Dowden and Foster, personal communication). Also, ethylene (50 ppm) inhibits xylogenesis and completely prevents fibre lignification in etiolated pea seedlings [15]. The complexity of the influence of ethylene is further shown in this work in that preliminary studies associated the presence of ethylene with the formation in eucalypt sapwood of tyloses (mainly composed of carbohydrates) which normally are formed in heartwood. This association has been reported in other species [10]. Also the copious exudation of carbohydrate gum from apricot that is probably induced by ethylene [4] has also been observed by others in this tree and in peach and cherry trees [10].

Variations in the amounts of kino found in representatives of the one species indicate genetic control. However a recent study of 26 open-pollinated families of *E. regnans* suggested that variation in the degree of their development is due largely to environmental rather than genetic factors (J C. Doran, personal communication, 1974).

Stresses of different types can produce ethylene which could be responsible for polyphenol formation in kino and heartwood. The enzyme inhibitors arsenite and iodoacetic acid used to block the utilisation of acetate in the TCA cycle and promote the formation of flavonoid compounds [9, 16], may have had their effect due to ethylene production [10]. The inter-relationship between water stress, ethylene production and polyphenol formation warrants further study. Water deficit leads to greatly increased ethylene concentrations or production in intact cotton petioles [17] and *Vicia faba* [18]. Rewatering can reduce production rates to prestress levels. Day [19] found that eucalypts growing on the poorer, drier sites were the most severely affected by kino formation and he considered the latter due to water deficiency during periods of active growth. Also, water stress may be a primary factor influencing resin pocket formation in pines [20]. Many damaged woods and heartwoods have a narrow intermediate zone which has a lower moisture content than the adjacent sapwood and yields a significantly higher amount of ethylene [5,6]. A change in the balance between crown size, transpirational capacity, and diameter of the tree may initiate changes leading to heartwood formation.

MATERIALS AND METHODS

Plant material. The trees of *Eucalyptus astringens*, *E. calophylla*, *E. sideroxylon*, *E. sieberi*, *Rhus succedanea* and an *Acacia* sp. and *Prunus armeniaca* (apricot) were garden specimens grown in Melbourne and older than 15 yr, *E. sieberi* seedlings of 3 yr age or less were grown in a glass house or in the open. The other tree specimens *E. tereticornis*, *E. marginata*, *E. pilularis*, *E. grandis*, *E. maculata* and *E. regnans* were at least 15 yr age and were treated or sampled in their natural habitat.

Treatment methods. The (2-chloroethyl)-phosphonic acid (CEPA, "Ethrel") was diluted immediately before use to 0·5% in H_2O. Injections using the dil soln adjusted to pH 2 with NaOH were tried but with no noticeable difference in behaviour. In these injections and those mentioned below, the CEPA solns would be dil in the sap stream. Control administrations were done using H_2O or diluted CEPA neutralised to pH 4–7 and allowed to stand for 7 days. Solns of 1 mM 3-indolyl acetic acid (IAA) were also injected, and 1% pastes of IAA or naphthalene acetic acid (NAA) in lanolin were applied. Solns were injected into 2 mm diam. holes drilled into the wood or into incisions made through the bark to the cambial region. The pastes were applied over incisions. Adminstrations were done throughout the year for 3 yr. *E. marginata*, *E. pilularis*, *E. grandis*, *E. maculata*, *E. regnans* etc. which had been injected with the arboricides "Tordon" and "Tordon 50D" yielded copious amounts of kino.

Chromatography. The ethylene in the wood samples was analysed by the method previously described [5]. Polyphenols in different materials were examined by 2-D PC using BuOH–HOAc–H_2O (6 : 1 : 2) followed by 6% HOAc. Chromatograms were examined by methods previously described [11]. The GLC method used for the *Rhus* heartwood polyphenols involved the separation of silylated compounds using a liquid phase of 1·5% SE-30 or 1·0% OV-1 with the column temp. at 200° [21].

Extractions. Treated branches of *Rhus* and *Acacia* were removed from the tree 4 months after treatment. Coloured areas were spread 1–2 mm around the control drilling holes, and 3-4 mm around the treated holes in the *Acacia* sp. and 6–10 cm in the *Rhus* sp. Coloured and surrounding tissues were separated, dried, ground, weighed and extracted with MeOH in a continuous extractor and the extract evaporated and weighed. The extracted coloured wood was still coloured after 25 hours extraction. Cambial scrapings from *E. sieberi*, *E. calophylla*, *E. astringens* and *E. sideroxylon* were extracted with Me_2CO and the extract freeze-dried and examined by PC.

The effect of ethylene on the production of tyloses. Blocks of *E. tereticornis* were treated and ventilated with ethylene as previously described [6]. Sections were taken from the blocks and examined microscopically for tyloses.

Acknowledgements—The author wishes to acknowledge discussions with Dr. L. Shain, the assistance of Mr. A. Rozsa and Mr. L. Lau, and to Ciba-Geigy Australia Ltd. for the supply of Ethrel. A summary of this work was given at the IUFRO-Division V Meeting in South Africa, September 1973.

REFERENCES

1. Höll, W. (1974) *Can. J. Botany* **52**, 727.
2. Höll, W. and Lendzian, K. (1973) *Phytochemistry* **12**, 975.
3. Shain, L., Mackay, J. F. G. (1973) *Can. J. Botany* **51**, 737.
4. Shigo, A. L., Hillis, W. E. (1973) *Ann. Rev. Phytopath.* **11**, 197.
5. Shain, L. and Hillis, W. E. (1972) *Phytopathology* **62**, 1407.
6. Shain, L. and Hillis, W. E. (1973) *Can. J. Botany* **51**, 1331.
7. Edgerton, L. J. and Blanpied, G. D. (1968) *Nature* **219**, 1064.
8. Jacobs, M. R. (1955) *Growth Habits of the Eucalypts*, Forestry and Timber Bureau; Canberra, Australia.
9. Hillis, W. E. and Inoue, T. (1966) *Phytochemistry* **5**, 483.
10. Abeles, F. B. (1973) *Ethylene in Plant Biology*, Academic Press, New York.
11. Hillis, W. E., Inoue, T.: (1968) *Phytochemistry* **7**, 13.
12. Rhodes, M. J. C. and Wooltorton, L. S. C. (1973) *Phytochemistry* **12**, 107.
13. Brown, K. M. and Leopold, A. C. (1973) *Can. J. Forest Res.* **3**, 143.
14. Skene, D. S.: (1965) *Aust. J. Botany* **13**, 367.
15. Apelbaum, A., Fisher, J. B. and Burg, S. P. (1972) *Am. J. Botany* **59**, 697.
16. Hillis, W. E. (1964) *Biochem. J.* **92**, 516.
17. McMichael, B. L. Jordan, W. R. and Powell, R. D. (1972) *Plant Physiol.* **49**, 658.
18. El-Beltagy, A. S. and Hall, M. A. (1974) *New Phytol.* **73**, 47.
19. Day, W. R. (1959) *Empire For Rev.* **38**, 35.
20. Cown, D. J. (1973) *N.Z. J. Forestry* **18**, 233.
21. Hemingway, R. W., Hillis, W. E. and Bruerton, K. (1970) *J. Chromatog.* **50**, 391.