Weed Research, 1979, Volume 19, 225–230

Ultrastructural effects of formulated picloram on leaflets of velvet mesquite and catclaw acacia*

C. A. BLECKMAN†, H. M. HULL, AND H. L. MORTON *U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, 2000 East Allen Road, Tucsan, Arizona 85719*

Received 1 December 1978

Summary: Résumé: Zusammenfassung

The effects of picloram formulated with a nonionic surfactant (X-77) and of the surfactant alone on the ultrastructure of leaf cells of velvet mesquite *(Prosopis velutina* Woot.) and catclaw [*Acacia greggii* var. *arizonica* (Gray) Isely] were examined. The surfactant induced temporary protrusions from chloroplasts in both species. A proliferation of rough endoplasmic reticulum (RER) was noted in velvet mesquite within 8 h of application of the herbicide and in catclaw within 27 h. By 72 h after treatment, both species exhibited distortions of organelles with more severe symptoms in catclaw, the species more sensitive to the herbicide. Leaf abscission occurred subsequently and was more pronounced in catclaw than in mesquite. It is known that RER proliferation is induced by ethylene and that ethylene evolution is stimulated by picloram. The present study suggests that the interaction between these two chemicals was similar in the two plant species studied.

Effets ultrastructuraux du piclorame formulé sur les folioles de Prosopis velutina *et d'*Acacia gregii.

Les auteurs ont examiné les effets du piclorame formulé avec un agent de surface non ionique (X-77), ainsi que les effets de l'agent de surface seul, sur l'ultrastructure des cellules foliaires de *Prosopis velutina* Woot. et d'*Acacia gregii* var. *arizonica* (Gray) Isely. L'agent de surface a induit temporairement des protrusions à partir des chloroplastes chez les deux espèces. Une prolifération du réticulum endoplasmique grossier (REG) a été constatée chez *P. velutina* dans les 8 heures qui ont suivi le traitement herbicide et au bout de 27 heures pour *A. gregii*. Soixante-douze heures après le traitement, les deux espèces montraient des distorsions des organites, avec des symptômes plus graves chez *A. gregii*, espèce plus sensible à l'action de l'herbicide. Une abscission foliaire s'est manifestée ensuite et elle a été plus prononcée chez *A. gregii* que chez *P. velutina*. Il est connu que la prolifération du REG est induite par l'éthylène et que cette évolution de l'éthylène est stimulée par le piclorame. La présente étude suggère que l'interaction entre ces deux composés a été similaire chez les deux espèces étudiées.

Die Wirkung von formuliertem Picloram auf die Ultrastruktur der Blätter von Prosopis velutina *Woot. und* Acacia greggii *(Gray) Isely*

Es wurde die Wirkung von Picloram, das mit einem nichtionischen Netzmittel formuliert worden war, und die des Netzmittels alleine, auf die Ultrastruktur der Blattzellen von *Prosopis velutina* Woot. und von *Acacia greggii* var. *arizonica* (Gray) Isely untersucht. Das Netzmittel löste bei beiden Arten vorübergehend ein Hervortreten der Chloroplasten aus. Bei *Prosopis* trat innerhalb von 8 Stunden und bei *Acacia* in 27 Stunden eine Vermehrung des Rauhen Edoplasmatischen Reticulums (RER) ein. Bei beiden Arten traten nach 72 Stunden Veränderungen an den Organellen auf, die bei *Acacia*, der empfindlicheren Art, stärker ausgeprägt waren. Der darauffolgende Blattfall war bei *Acacia* stärker ausgeprägt als bei *Prosopis*. Es ist bekannt, daß die Zunahme an RER durch Äthylen induziert wird und daß die Äthylenbildung durch Picloram stimuliert wird. Die dargestellten Untersuchungen legen den Schluss nahe, daß die Wechselbeziehung zwischen den beiden Verbindungen in den zwei untersuchten Pflanzenarten ähnlich war.

Introduction

Picloram (4-amino-3,5,6-trichloropiclonic acid) is used in the Southwestern United States in efforts to control members of the genera *Prosopis* (mesquite) and *Acacia* (catclaw, huisache). Bovey & Meyer (1974) found that picloram killed about 75% of honey mesquite *(P. juliflora)* seedlings up to 8 weeks after emergence but killed over 90% of huisache *(A. farnesiana)* seedlings of the same age. Baur & Morgan (1969) demonstrated that both honey mesquite and huisache treated with picloram lost the ability for leaf movement and exhibited epinastic curvature of leaves and stems; but only huisache was defoliated. In a field evaluation, Morton Hull & Martin (1974) found picloram to be more than three times as effective on catclaw (*Acacia greggii* var. *arizonica* (Gray)

* From a dissertation submitted by C.A.B. in partial fulfillment of the requirements for the degree of doctor of philosophy in botany, University of Arizona, Tucson.

† Research Services Division, Continental Oil Company, Ponca City, Oklahoma 74601.

0043-1737/79/0800-0225$02.00

Isely) as on velvet mesquite (*Prosopis velutina* Woot.). The present work reports on the comparative ultrastructural responses of velvet mesquite and catclaw to foliar-applied picloram.

Materials and Methods

Leaflets were obtained from 35 day old seedlings grown in a plastic covered greenhouse at Tucson, Arizona. A commercial formulation of the potassium salt of picloram, Tordon* 22K, was applied to the adaxial surface of leaves in a water carrier at a concentration of 2500 ppmw a.e. The solution also contained 0·5% (v/v) of the nonionic surfactant Multifilm† X-77 to enhance spreading of the herbicide on the leaf surface. The mesquite seedlings ranged in development from sixteen to twenty-one nodes and were 25–35 cm tall. Three leaves per plant (No. 9, 10, and 11 from the base) were treated with 40 μl of the solution per leaf to give 300·0 μg of herbicide per plant. The catclaw seedlings had sixteeen to nineteen nodes and were 15–20 cm tall. Two leaves (No. 9 and 10) were treated to give 200 μg of herbicide per plant. Other seedlings of both species were treated as above using only a 0·5% surfactant solution.

Samples for ultrastructural observations were collected 1, 3, 8, 27, and 72 h after treatment. Leaflets from the 10th leaf (treated) were taken at each collection along with comparable leaflets on untreated plants. Longitudinal sections about 0·5 mm wide were cut from the leaflets, fixed for 4 h in 5% glutaraldehyde, postfixed for 2 h in 2% OsO_4, dehydrated with acidified 2,2-dimethoxypropane (Muller & Jacks 1975), and embedded in Spurr's epoxy resin. Thin sections were cut with a diamond knife on a Porter-Blum MT-2-ultramicrotome, stained with uranyl acetate and lead citrate, and photographed with a Hitachi HS-7S electron microscope.

Results

Observations of mesquite ultrastructure were generally confined to cells of the palisade parenchyma. These cells were typically 6–8 μm wide and 20-40 μm long. Chloroplasts were positioned around the perimeter of the cells and the nucleus was more or less centrally located (Fig. 1a). A large vacuole was usually visible adjacent to the nucleus toward either end of the cell. Endoplasmic reticulum and dictyosomes were observed infrequently in mature untreated tissue. Mitochondria and microbodies were common and had no unusual features.

Within 1 h of treatment, chloroplasts of both species exhibited large, grana-free protrusions (Fig. 1b). These protrusions developed in cells of both herbicide/surfactant- and surfactant-treated plants and can probably be ascribed to the action of the surfactant alone (Bleckmann, 1977). Chloroplast protrusions persisted for at least 3 h, but had largely disappeared by 8 h after treatment.

With the exception of the chloroplast modifications described above, no unusual characteristics were noted in the herbicide treated mesquite until 8 h after application. At that time the only change observed was an extensive proliferation of rough endoplasmic reticulum (RER) within the cells, most evident near the nucleus (Fig. 1c). By 27 h after treatment the amount of RER had greatly decreased and by 72 h severe symptoms appeared (Fig. 1d). Some organelles, especially the chloroplasts, were shrunken, and the vacuoles had enlarged.

The ultrastructure of untreated catclaw cells was generally very similar to that of untreated mesquite cells. However, an extensive membrane system, much like that reported in diseased oats (Easton & Hanchey, 1972), was found in the vacuoles of many cells of both treated and untreated catclaw plants (Fig. 2a). A proliferation of RER, comparable to that in treated mesquite, was also observed in treated catclaw; however, it was not evident until 27 h after treatment. As in mesquite, the RER was concentrated near the nucleus, but in catclaw it also extended into the surrounding cytoplasm (Fig. 2b).

By 72 h after treatment, cells of catclaw (Fig. 2c) were more severely disrupted than those of mesquite. The grana membranes within the chloroplasts had separated and the osmiophilic bodies had enlarged.

Leaflets from near the apex of plants whose lower leaves had been treated were harvested at each collection time, but few or no ultrastructural anomalies were observed in most samples. However, cells from the final (72 h) collection of cat-

* This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation by the USDA nor does it imply registration under FIFRA.

† Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the University of Arizona, and does not imply its approval to the exclusion of other products that may also be suitable.

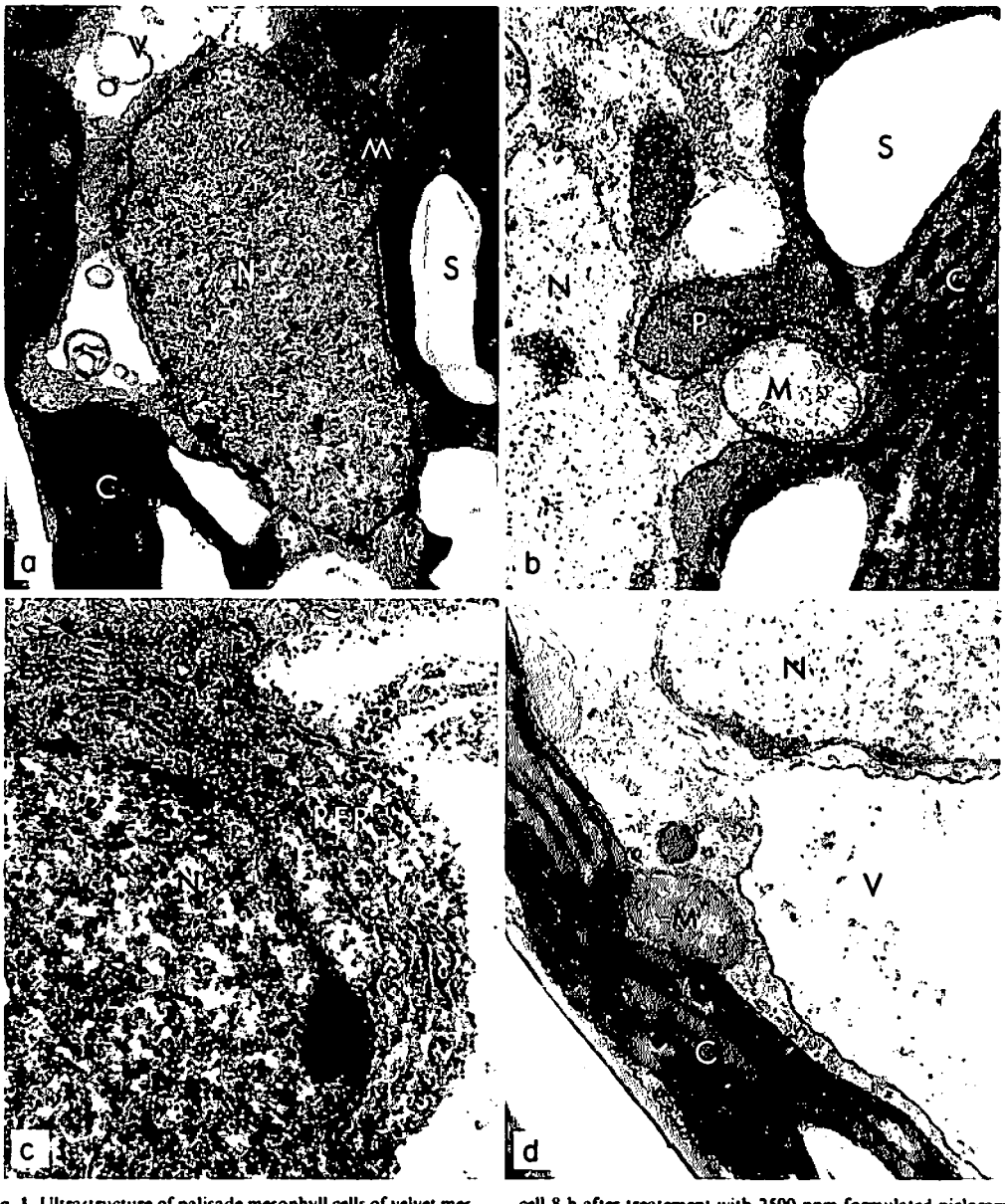

Fig. 1. Ultrastructure of palisade mesophyll cells of velvet mesquite. (a) Untreated control N- nucleus, C- chloroplast, S- starch granule, M- mitochondria, V- vacuole. × 10,900. (b) Palisade cell treated with surfactant alone. P- chloroplast protrusion. × 21,000. (c) Rough endoplasmic reticulum (RER) in cell 8 h after treatement with 2500 ppm formulated picloram. × 33,900. (d) Palisade cell, 72 h after treatment with 2500 ppm formulated picloram. Chloroplasts are shrunken, starch granules are reduced and vacuole is enlarged. × 20,500.

claw apical leaves (Fig. 2d) retained the characteristics of immature cells. Such cells had not elongated as much as typical mature cells; they had an unusually large amount of cytoplasm in proportion to the organelles; their chloroplasts and mitochondria were small; and their RER was concentrated near the cell wall. Apparently the herbicide caused these cells to maintain a juvenile form.

Discussion

Alteration of internal membrane structure of chloroplasts has been one of the most commonly

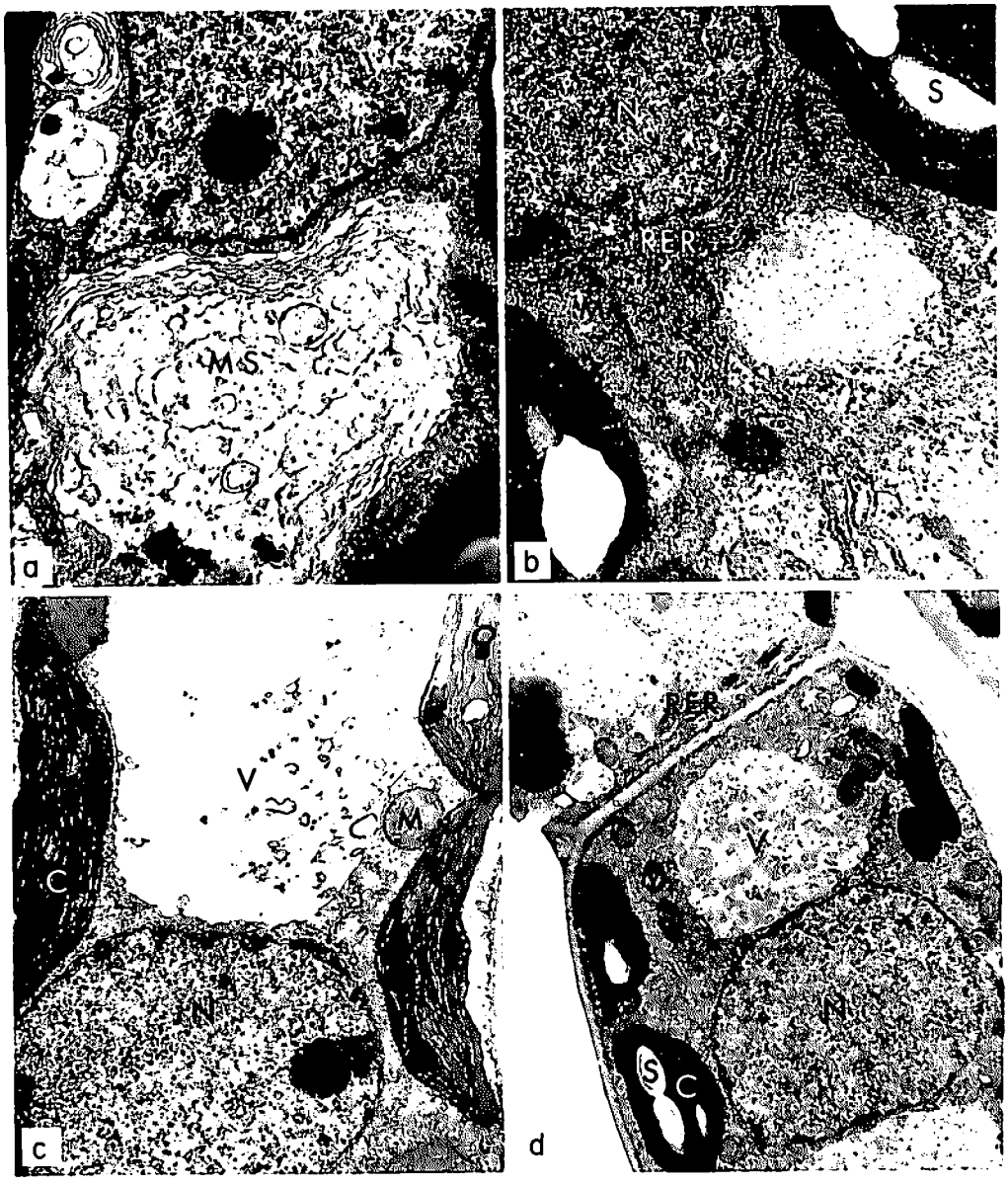

Fig. 2. Ultrastructure of palisade mesophyll cells of catclaw. (a) Untreated cell with an extensive membrane system (MS) within the vacuole. × 17,200. (b) Cell 27 h after treatment, with a proliferation of rough endoplasmic reticulum (RER). × 20,100. (c) Cell 72 h after treatment, with enlarged vacuole and internal disruption of chloroplasts. × 12,500. (d) Cell from leaflet near apex 72 h after treatment, exhibiting juvenile morphology. × 17,200.

reported ultrastructural effects of herbicides (Anderson & Thomson, 1973). However, neither velvet mesquite nor catclaw cells showed large modifications of the grana and fret system until 72 h after treatment. Symptoms were more severe in catclaw than in velvet mesquite.

Bonzi & Fabbri (1975) reported chloroplast protrusions virtually identical to those described here, of apparently natural origin in cells of *Arisarum proboscideum* leaves. Freeman & Duysen (1975) reported similar chloroplast modifications, induced by water stress in wheat cells. Whether the protrusions observed in this study are the direct result of the surfactant or a more

general response to stress cannot be determined from the information available. In any case, the effects of surfactants on modifications of cell ultrastructure clearly should be considered in any study in which these compounds are used in conjunction with other active ingredients (Parr & Norman, 1965).

The stimulation of an increase in RER by picloram has not been previously reported, although such effects have been noted with other growth regulators. Gibberellic acid stimulated an increased RER within 10 h of application to barley aleurone cells (Jones, 1969 a,b). Stimulation of protein production and increase in RER have also been shown in beans treated with *s*-triazine herbicides (Singh, Campbell & Salunkhe, 1972).

Baur & Morgan (1969) and Morgan & Baur (1970) studied the effect of picloram on ethylene production, epinasty, and abscission in honey mesquite and huisache. They found that root-applied picloram stimulated the production of ethylene in both species and also noted that plants exposed to ethylene responded much like those exposed to picloram. Mesquite stem tissue had the highest rate of ethylene production, although epinasty and ethylene synthesis also occurred in the leaves. Ethylene production rates remained high in both stems and leaves of huisache for about 30 h after treatment. Later, production approximately doubled in the leaves but not in the stems. Abscission occurred only in huisache. It is of interest to compare these differences with the differences in timing of RER proliferation observed between the two species in the present study (8 h after treatment for velvet mesquite vs. 27 h for catclaw). Morgan & Baur (1970) reported increased ethylene production in huisache leaves at 30 h after treatment, while in the present study RER increase occurred in catclaw 27 h after treatment.

Picloram is an auxin-type growth regulator (Kefford & Caso, 1966; Eisinger & Morré, 1971). Auxin/ethylene interactions have been studied for many years (Crocker, Hitchcock & Zimmerman, 1935). Ethylene formation is induced by IAA (Abeles, 1966; Burg & Burg, 1966) and 2,4-D (Morgan & Hall, 1962; Holm & Abeles, 1968). Ethylene was required for epinasty of tomato petioles and auxin stimulated ethylene synthesis (Stewart & Freebairn, 1969).

Valdovinos, Jensen & Sicko (1971, 1972) showed a 30-fold increase of the ethylene induced RER development in abscission cells of tobacco flower pedicels over that of controls 5 h after exposure. Similar effects were noted by Sargent & Osborne (1975), and Osborne (1976) in epidermal and cortical cells from the hook region of etiolated pea seedlings exposed to ethylene. They proposed that ethylene stabilized or reorganized, rather than proliferated the RER. Osborne & Sargent (1976) also showed that abscission cells of *Sambucus nigra* were much more sensitive to ethylene than were the surrounding cells. Ethylene/RER relationships have been demonstrated for cells of the abscission zone of both petioles and pedicels. The present study suggests that such a relationship also exists in the leaf parenchyma of velvet mesquite and catclaw acacia.

Acknowledgments

This work was a result of cooperative investigations by the Agricultural Research, Science and Education Administration, U.S. Department of Agriculture and the Arizona Agricultural Experiment Station, University of Arizona, Tucson 85721 (Publication No. 2897).

References

ABELES F.B. (1966) Auxin stimulation of ethylene evolution. *Plant Physiol.* **41**, 585–588.

ANDERSON J.L. & THOMSON W.W. (1973) The effects of herbicides on the ultrastructure of plant cells. *Residue Rev.* **47**, 167–189.

BAUR J.R. & MORGAN P.W. (1969) Effects of picloram and ethylene on leaf movement in huisache and mesquite seedlings. *Plant Physiol.* **44**, 831–838.

BLECKMANN C.A. (1977) Leaflet ultrastructure of *Prosopis velutina* Woot. and *Acacia greggii* var. *Arizonica* (Gray) Isely and responses to the hebicide picloram and the surfactant X-77. Ph.D. Dissertation, University of Arizona.

BONZI L.M. & FABBRI F. (1975) Chloroplast protrusions in *Arisarum proboscideum* (L.) Savi. Caryologia. **28**, 407–426.

BOVEY R.W. & MEYER R.E. (1974) Mortality of honey mesquite and huisache seedlings from herbicides and top removal. *Weed Sci.* **22**, 276–279.

BURG S.P. & BURG E.A. (1966) The interactions between auxin and ethylene and its role in plant growth. *Proc. natn. Acad. Sci., U.S.A.* **55**, 262–269.

CROCKER W. HITCHCOCK A.E. & ZIMMERMAN P.W. 1935. Similarities in the effects of ethylene and the plant auxins. *Contrib. Boyce Thomson Inst.* **7**, 231–248.

EASTON C.Z. & HANCHEY P. (1972) Localization of crystals in diseased oats treated with uranyl acetate. *Plant Physiol.* **50**, 706–712.

EISINGER W.R. & MORRÉ D.J. (1971) Growth regulating properties of picloram, 4-amino-3,5,6-trichloropicolinic acid. *Can. J. Bot.* **49**, 889–897.

FREEMAN T.P. & DUYSEN M.E. (1975) The effect of imposed water stress on the development and ultrastructure of wheat chloroplasts. *Protoplasma* **83**, 131–145.

Holm R.E. & Abeles F.B. (1968) the role of ethylene in 2,4-D induced growth inhibition. *Planta* 78, 293–304.

Jones R.L. (1969a) Gibberellic acid and the fine structure of barley aleurone cells. I. Changes during the lag-phase of α-amylase synthesis. *Planta* 87, 119–133.

Jones R.L. (1969b) Gibberellic acid and the fine structure of barley aleurone cells. II. Changes during the synthesis and secretion of α-amylase. *Planta* 88, 73–86.

Kefford N.P. & Caso O.H. (1966) A potent auxin with unique chemical structure -4-amino-3,5,6-trichloropicolinic acid. *Bot. Gaz.* 127, 159–163.

Morgan P.W. & Baur J.R. (1970) Involvement of ethylene in picloram induced leaf movement response. *Plant Physiol.* 46, 655–659.

Morgan P.W. & Hall W.C. (1962) Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorghum. *Physiol. Plant.* 15, 420–427.

Morton H.L., Hull H.M. & Martin R.D. (1974) Evaluation of soil-applied herbicides for control of woody plants in southern Arizona. *Research Progress Report, West. Soc. Weed Sci.* 34–35.

Muller L.L. & Jacks T.J. (1975) Rapid chemical dehydration of samples for electron microscopic examinations. *J. Histochem. Cytochem.* 23, 107–110.

Osborne D.J. (1976) Control of cell shape and cell size by the dual regulation of auxin and ethylene. In: *Perspectives in experimental biology. Vol. 2.* (ed. Sunderland N.) pp. 89–102. Pergamon Press, Oxford.

Osborne D.J. & Sargent J.A. (1976) the positional differentiation of ethylene-responsive cells in rachis abscission zones in leaves of *Sambucus nigra* and their growth and ultrastructural changes at senescence and separation. *Planta* 130, 203–210.

Parr J.F. & Norman A.G. (1965) Considerations in the use of surfactants in plant systems: a review. *Bot. Gaz.* 126, 86–96.

Sargent J.A. & Osborne D.J. (1975) An effect of ethylene on the endoplasmic reticulum of expanding cells of etiolated shoots of *Pisum satirum* L. 124, 199–205.

Singh B., Campbell W.F. & Salunkhe D.K. (1972) Effects of *s*-triazines on protein and fine structure of cotyledons of bush beans. *Am. J. Bot.* 59, 568–572.

Stewart E.R. & Freebairn H.T. (1969) Ethylene, seed germination, and epinasty. *Plant Physiol.* 44, 955–958.

Valdovinos J.G., Jensen T.E. & Sicko L.M. (1971) Ethylene-induced rough endoplasmic reticula in abscission cells. *Plant Physiol.* 47, 162–163.

Valdovinos J.G., Jensen T.E. & Sicko L.M. (1972) Fine structure of abscission zones. IV. Effect of ethylene on the ultrastructure of abscission cells of tobacco flower pedicels. *Planta* 102, 324–333.