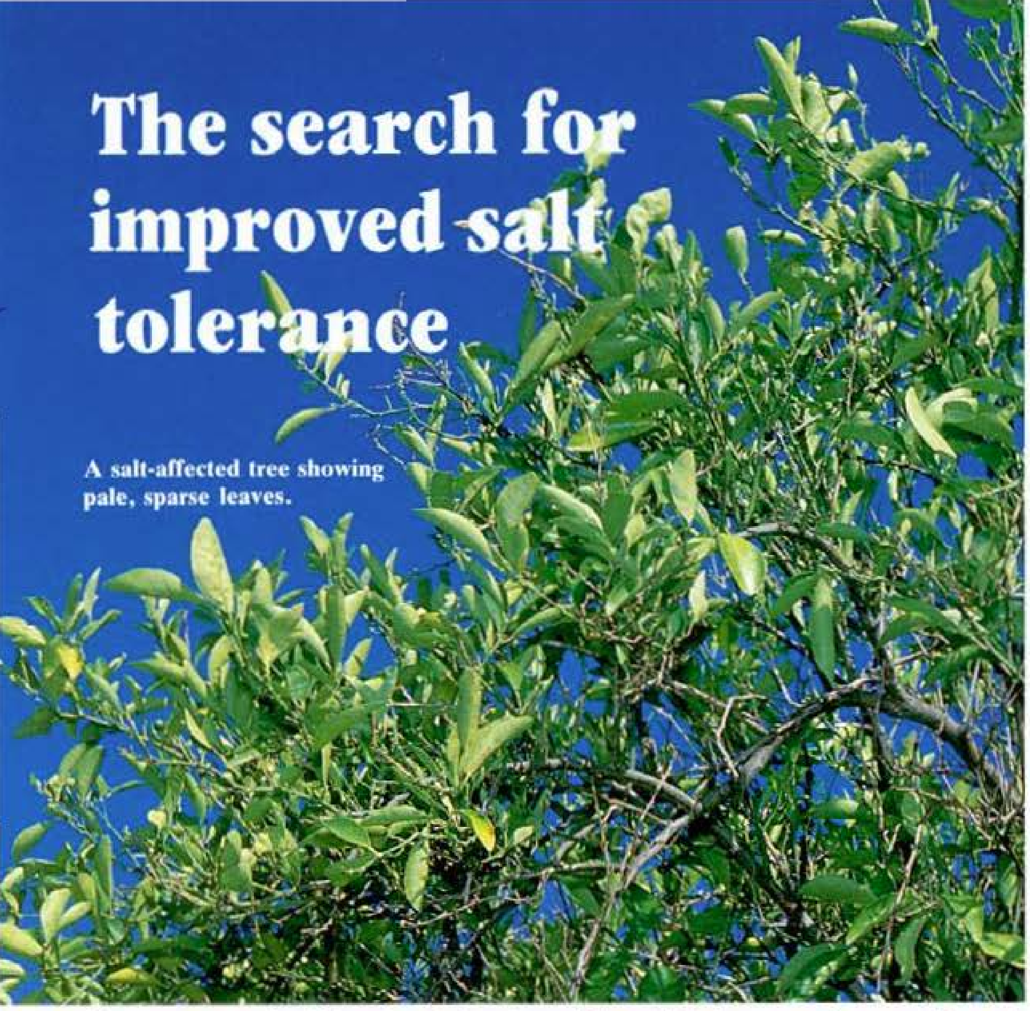
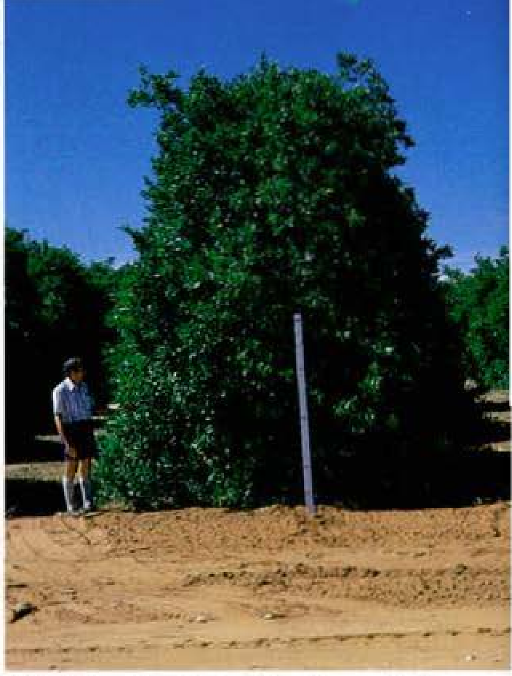
The search for improved salt tolerance



A salt-affected tree showing pale, sparse leaves.



A healthy citrus tree with a dense, dark green canopy.

Some people like to take tequila with lemon in one hand and salt in the other. On the whole, though, citrus and salt never pair happily — either in the bar or in the kitchen — and nor do they in the field. But in the Sunraysia and Riverland districts of the Murray Valley, salinity is forcing the citrus and grapevine industries into a search for new plant varieties that can cope with salt.

South Australian citrus- and grapevine-growers are already losing more than $4 million a year due to salt stress. The problem is that the commonly used commercial rootstocks have not been selected for salt tolerance. Rather, horticulturists have in the past selected rootstocks for resistance to major pests and diseases and for their effect on fruit size and quality, tree size, and drought resistance.

A rootstock can be of a different variety or even species from the scion — the 'top half' of the tree grafted onto the rootstock. Familiar names such as navels or Valencias refer to fruit produced by scions. However, rootstocks can have some effect on the fruit; scions grafted onto Cleopatra mandarin, which is more salt-tolerant than the other common citrus rootstocks (sweet orange, rough lemon, and *Trifoliata*) produce smaller fruit.

Researchers at the CSIRO Division of Horticultural Research at Merbein, Victoria, and the Departments of Agriculture of three States — New South Wales, South Australia, and Victoria, based at Dareton, Loxton, and Tatura respectively — are carrying out a combined attack on the salinity problem.

The CSIRO strategy for improving the salt tolerance of citrus and grapevines is based mainly on breeding and selecting rootstocks that combine salt tolerance with as many as possible of the other desirable rootstock traits. One aspect of the work is the identification of biochemical 'markers' that indicate whether or not a plant is salt-tolerant. This will enable scientists to screen for salt-tolerant plants more rapidly — those plants with the marker will be tested and evaluated further.

Breeding program

The basic requirement for the improvement of salt tolerance is the existence of genetic variability for this trait within, and between, species. The Division of Horticultural Research's Merbein group, led by Dr Rob Walker, is currently incorporating salt tolerance into hybrid citrus rootstocks, and then screening and evaluating them for other qualities.

Dr Steve Sykes, the team's geneticist, is responsible for the breeding program. Hybrids are screened for salt tolerance by subjecting the seedlings of a cross to several weeks of salt stress in the glasshouse. By looking for the healthiest seedlings and analysing the chloride levels in leaf tissues after salt treatment, Dr Sykes can select the most salt-tolerant types, which are known as salt-excluders.

Because citrus rootstocks are themselves very variable — and salt tolerance appears to be due to the action of many genes — Dr Sykes cannot predict what characteristics the offspring of experimental crosses will have. The process is empirical — the greater the number of hybrids screened, the greater is the chance of producing one plant with all the desired characteristics in the right combination. More than 3000 hybrids have been produced from this small-scale breeding program, and Dr Sykes has screened all of them, setting aside about 150 promising ones for further testing.

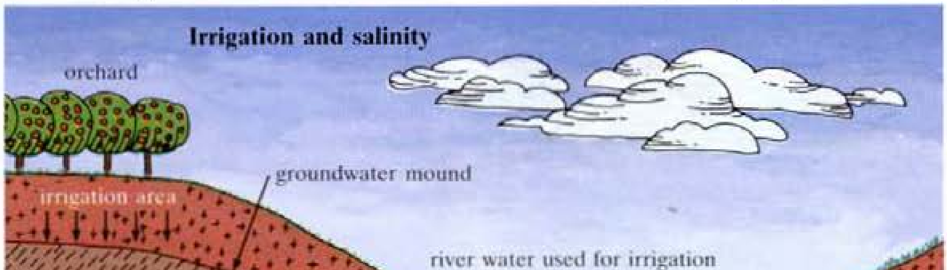
The CSIRO scientists have joined forces with various State Departments of Agriculture in forming a National Citrus Rootstock Evaluation Program. They expect it will be at least 15 years before the first salt-tolerant varieties are made available for commercial evaluation.

The information on salt tolerance that Dr Sykes extracts from his breeding trials is being used by Dr Walker and Dr Trevor Douglas, also of the Division, in their search for a 'biochemical marker' for salt exclusion. The information exchange is reciprocal. Successful identification of a biochemical marker that 'flags' salt tolerance in citrus could be incorporated into the screening programs to sort out promising rootstock seedlings that can exclude salt from the plant. We will return to this research after a look at how salt affects the leaves and photosynthesis of citrus trees.

Leaves, photosynthesis, and salt

Salt-tolerant citrus rootstocks are known as salt-excluders because they restrict the amount of salt entering the shoot from the root. This strategy differs from that used by the salt-tolerant plants known as halophytes (such as saltbush), which can accumulate sodium and chloride ions to high levels — particularly in their leaves — and still grow normally. Citrus trees can't stand too much salt in their leaf tissue.

Water from irrigation of orchards or vineyards drains into the soil, building up the water table underneath to form a groundwater 'mound'. A result is increased saline seepage into the river, which is the source of irrigation water.



Experiments conducted by Mr Phil Cole of the South Australian Department of Agriculture have shown that salinity levels that produce no apparent damage such as leaf burn can cause substantial yield reductions in navel oranges. Consequently, many apparently normal trees being irrigated with low-salinity water are probably suffering salt stress, leading to reduced productivity.

Dr Paul Kriedemann, of CSIRO but based at the Waite Agricultural Research Institute in Adelaide, and Mr Jon Lloyd, a postgraduate student at Waite, are collaborating with the New South Wales Department of Agriculture's team — led by Dr Alistair Grieve — to investigate the effect of salt on leaf photosynthesis. By using a technique based on the natural emission of fluorescence from chlorophyll in living leaves, they have identified some of the invisible effects of salt stress in citrus leaves. The strength of emission depends on the photosynthetic use of absorbed light, and so changes in emission give an early indication of loss in photosynthetic effectiveness.

Dr John Downton, at the Division of Horticultural Research in Adelaide, has also been using this method to study the effects of salt on photosynthesis in a number of other food plants.

Fluorescence occurs in plants when some of the light absorbed by the leaves is re-emitted as light of a longer wavelength, rather than being trapped in the photosynthetic pathway of energy conversion. So changes in the fluorescence of citrus leaves indicate early that photosynthesis has become less efficient.

What Dr Kriedemann found was that salt treatment exacerbated damage caused to citrus leaves by strong light. Further, a reduction in photosynthetic efficiency occurred well before the appearance of visible leaf damage. Dr Kriedemann observed the same result in all salt-treated trees, regardless of rootstock. He is collaborating with the Departments of Agriculture in both New South Wales and South Australia to develop fluorescence measurement as a technique for gauging the extent of salt stress to which commercial crops are being exposed.

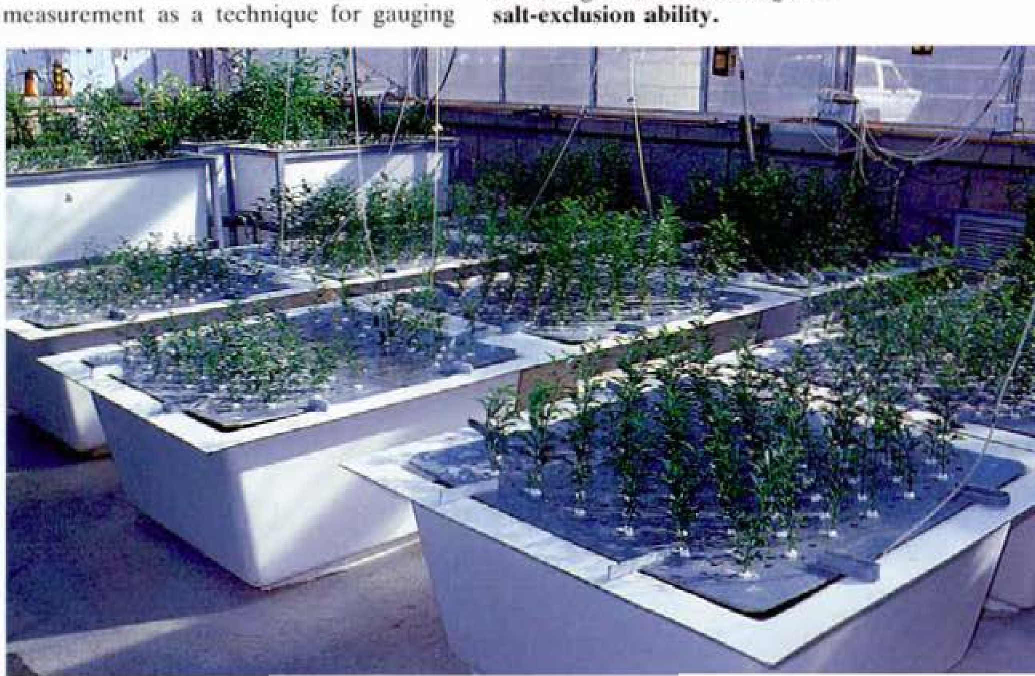
How does salt affect photosynthesis? Dr Walker, Dr Downton, and Mrs Edith Torokfalvy, also of the Division, grew seedlings of a good salt-excluder, Rangpur lime, and a non-commercial rootstock susceptible to salinity damage, Etrog citron, in salty conditions. They found that salt treatment progessively reduced photosynthesis in mature leaves of both cultivars. The photosynthetic rates of 6-month-old leaves of Rangpur lime, the salt-excluder, were particularly affected by salt treatment.

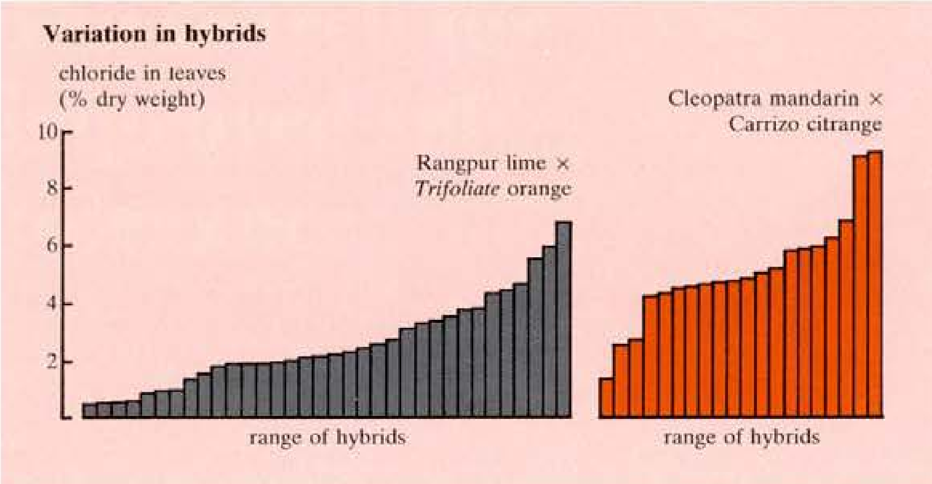
Under normal conditions, the leaf cells of most plants contain concentrations of ions and organic substances that maintain the potential for water to flow into the cell, rather than outwards. As a result, leaves maintain 'turgor' — they remain turgid rather than flaccid. Think of the difference between a fresh crisp lettuce and a stale wilted one. The 6-month-old Rangpur lime leaves tended to become less turgid during salt treatment. Some citrus scions on salt-excluding rootstocks may be better able than others to counteract this tendency.

Dr Walker and Mrs Torokfalvy, together with Dr Grieve and Ms Lynda Prior, studied the ability of the leaves of an important scion variety in Australia — Valencia orange — to adjust and maintain turgor when grafted to salt-excluding rootstocks grown in relatively high salinities. They found that the leaves of the Valencia orange adjusted to salt stress, and maintained turgor pressures at or above the values measured for leaves on plants that had not been salt-treated.

The turgor effect has important consequences for transpiration and photosynthesis through its effect on the tiny pores

Hydroponic tanks used in glasshouse screening of citrus seedlings for salt-exclusion ability.





The variation in chloride-exclusion ability of citrus hybrids from two different crosses. The scientists measured chloride levels in the hybrids' leaves after 8 weeks' growth in moderately salty conditions.

concentrated on the undersurface of leaves, called stomata. Stomata allow gases such as carbon dioxide (for photosynthesis) and oxygen to move into and out of the plant. They also permit the release of water vapour from the leaf through transpiration, which sets up an internal 'suction' inside the plant causing the upward movement of water from the soil through the stem.

Each stoma consists of two guard cells, which move away from each other when turgid, to allow gases to move through the opening. The pores close when the guard cells lose water and collapse against each other.

The Merbein team found that the leaves of salt-treated Etrog citron plants were affected differently from those of Rangpur lime. Salt treatment did not reduce the turgor of Etrog citron leaves. Instead, the high levels of chloride these contained appeared to have been responsible for the reduction in photosynthesis.

Salt-excluding rootstocks are a means of reducing the extent of chloride accumulation in leaves, giving the plant some protection against the harmful effects of chloride on photosynthesis. However, the selection of a salt-excluding rootstock, although a major aim of the program, is not the only one. Suitability of the scion also has to be considered. The scion needs to be compatible with the rootstock and should possess an ability to maintain normal growth and development when the rootstock is under even moderate salt stress.

Back to the roots

We mentioned earlier that citrus rootstocks are roughly divided into two types — salt-excluders such as Rangpur lime and Cleopatra mandarin, and salt-accumulators such as Etrog citron. However, where in the plant exclusion takes place is still not entirely clear. Studies elsewhere on soybeans have indicated that the process involves the restriction of uptake of salt from the soil, and possibly restriction of salt transport between the root and shoot.

Dr Grieve and Dr Walker studied the leaves, stems, and roots of various citrus rootstocks to determine the differences in sodium chloride uptake between the plant organs. They found little or no difference in root chloride and sodium concentrations between the rootstocks. But the existence of different patterns of chloride and sodium concentration in the young leaves of various ones suggested that separate mechanisms were operating to limit the transport of the two ions from the roots.

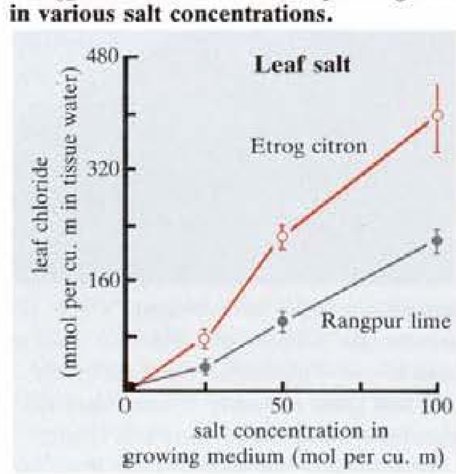
Dr Walker and Dr Douglas also studied the effects of salinity level on the uptake and distribution of chloride, sodium, and potassium ions in Rangpur lime, Etrog citron, and another citrus variety, Kharna khatta. They found differences in the chloride concentrations in leaves and, to a lesser extent, the stems between these rootstocks. Further, an external salinity existed for each rootstock beyond which no further increase occurred in root chloride concentrations, suggesting a limit to the chloride-accumulating capacity of available 'salt sinks' within the roots. The roots of all three rootstocks took up similar amounts of chloride, even though differences existed in the extent of chloride accumulation in leaves.

These findings provided evidence that variations in salt-exclusion ability of some rootstocks are due to differences between their uptake and root-to-shoot transport of chloride. Indeed, the scientists found that the roots of Rangpur lime can restrict chloride entry to shoots even at quite high salinities.

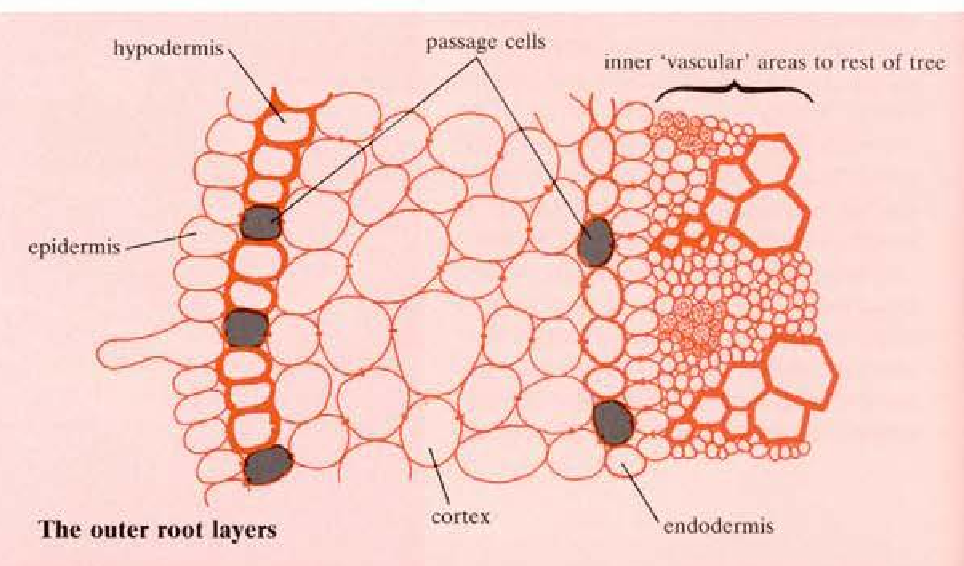
In woody plants such as citrus, the first barrier to the entry of water and solutes into the root is the outer epidermis. In citrus species so far studied, this layer is ineffective in salt exclusion and the layer beneath the epidermis — the hypodermis — assumes a more important function in keeping out salt.

Beneath the hypodermis lies a layer of cortical cells and, further in, the endodermis adjoins the central vascular bundle that transports water to the stem and leaves. Endodermal cells are surrounded by a strip

The chloride concentrations in leaves of Etrog citron (salt-susceptible) and Rangpur lime (salt-tolerant) plants grown in various salt concentrations.



In citrus roots, water and solutes are transmitted through the passage cells of the hypodermis and endodermis, the cell layers that may play an important role in salt exclusion.



The outer root layers

Irrigation and salinity

Salinity is not only a 'modern' environmental problem. Historians have found evidence that the most advanced civilization in the thirteenth century B.C. — the Sumerians in Mesopotamia — changed their major food crop from wheat to the more salt-tolerant barley as the fertile, but poorly drained, soils of the Tigris–Euphrates basin became increasingly saline. The evidence also indicates that a subsequent decline in the yield of barley was a major factor in the break-up of Sumerian civilization.

Today, the problem is extensive. Rising salt is causing agricultural havoc in America's Colorado Basin and Egypt's Nile Valley, along the Indus in Pakistan and the Yellow River in China, and on the Steppes of Russia. In Australia, it has devastated nearly two million hectares of our most productive land, and another million hectares are under threat. The worst-hit areas are the Murray Valley and the wheat belt of Western Australia.

Following planting of shallow-rooted crops in place of the native trees, more rainfall percolates through the root zone, allowing the water table to rise. We have also put more water into the soil through irrigation, accelerating the rise. Saline irrigation water draining into rivers and creeks adds to the problem.

In Australia's salt-affected citrus-growing areas, researchers are providing producers with advice on how to manage the irrigation of crops with minimum damage to trees. Dr Grieve, Dr Cole, and their colleagues in the various State Departments of Agriculture have been developing better irrigation techniques for orchards and vineyards.

Past irrigation technology has been rather crude, involving the use of furrows to distribute water. This results in the part of a row of vines or trees that receives too much or too little water looking stunted. Horticulturists resorted to overhead sprinklers, but the ineffective distribution of these again presented a problem. A further problem is that trees can take in salt through their leaves far more rapidly than through their roots. Dr Grieve has estimated that one-half to two-thirds of the salt load accumulated by citrus trees irrigated by overhead sprinklers is absorbed through the leaves.

He has found that the best method of irrigating is the use of sprinklers placed below the foliage. This eliminates the uptake of salt through leaves and can save up to 25% of water by reducing evaporation losses. An important benefit here is that the less water irrigators use, the lower the risk of land and river salinization from escalating water tables becomes.

Besides the question of how to irrigate, Dr Grieve is investigating the problem of timing. Currently, growers usually irrigate infrequently, using large amounts of water each time. The problem here is that the more water that leaves the soil between irrigations, the higher the average salt concentration in the soil water rises. And the more salt a soil contains, the more salt the plants take up. Dr Grieve has shown that smaller, more frequent irrigations are more effective.

The methods he has recommended have been tested in field trials. Citrus trees irrigated with water having a salinity seven times that of the present 'low' level in the Murray River at Sunraysia ended up with less salt in their tissues than neighbouring trees on commercial properties irrigated with river water using conventional techniques.

Dr Grieve plans to provide a computer-based scheduling service for farmers, incorporating information on the rate at which crops use water derived from temperature, radiation, wind, and evaporation data.

Mr Cole and his colleagues at the South Australian Department of Agriculture are developing a mathematical model to simulate the river water–soil–plant system to provide answers on how best to use irrigation management to reduce salinity in plant tissue. When completed and verified, the model will also provide horticulturists with a management tool that can predict appropriate management strategies for different conditions.

of waterproofing material called suberin, which prevents water and ions from moving into the vascular bundle through cell walls and intercellular spaces.

Finding a 'biochemical' marker

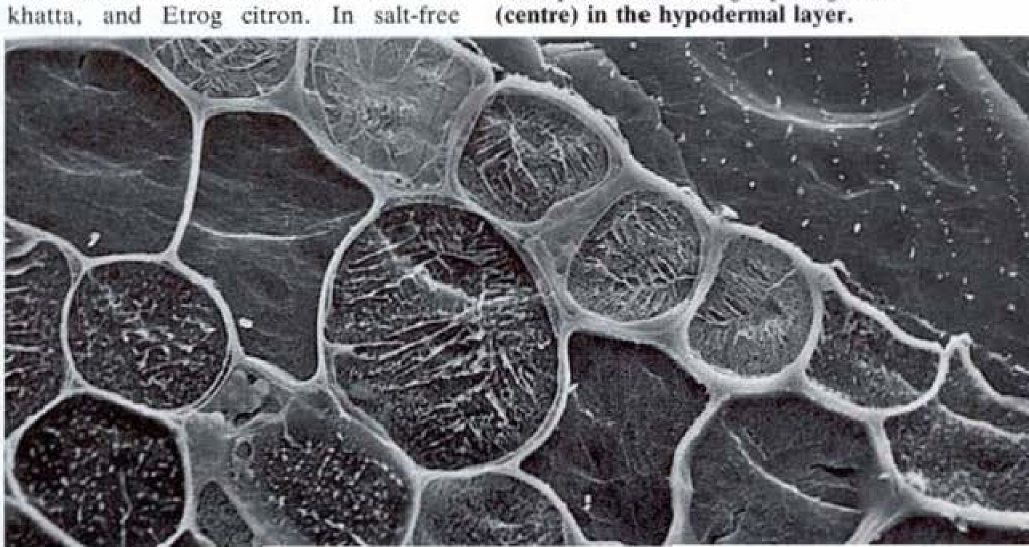
Dr Walker and Dr Douglas, together with Dr Margaret Sedgley and Ms Meredith Blesing, also of the Division, grew seedlings of Rangpur lime and Etrog citron in a range of salinities. To observe how salt affected the anatomy and ultrastructure of roots, they examined cross-sections of the root under the microscope.

No differences in root anatomy between the two rootstocks occurred in the absence of salt. When grown under salty conditions, the hypodermis and endodermis of both citrus rootstocks suberized nearer the root tip than when grown in 'control' conditions. But what accounts for the difference in salt exclusion ability between the two? Dr Walker proposed that the key may be the living membrane of 'passage' cells in the hypodermal layer of the root.

The properties of plant cell membranes, like those of animal cells, depend on their constituents — largely phospholipids, proteins, and sterols such as cholesterol. Plant physiologists already knew that sterol composition in plants is affected by a number of environmental stresses, including extreme temperature, drought, and salinity.

Dr Douglas and Dr Walker examined the sterol compositions of root preparations from specimens of Rangpur lime, Kharna khatta, and Etrog citron. In salt-free conditions, the researchers found no clear-cut differences in sterol composition related to a rootstock's ability to exclude excess salt from leaves. The only consistent biochemical change the two scientists observed in all three salt-treated rootstocks was a decrease in the ratio of one plant sterol, sitosterol, to another one, stigmasterol, mainly as a result of increased levels

A cross-section of a frozen Rangpur lime root specimen showing a passage cell (centre) in the hypodermal layer.



of the latter. Dr Douglas and Dr Walker suggested that, as these two sterols are related, increased salinity might have stepped up the conversion of sitosterol to stigmasterol.

This phenomenon has been documented during temperature increases, aging of plant tissue, germination, exposure to light, and treatment with plant growth hormone. As the ratio between the two decreases with tissue age, the decreases noted by the CSIRO researchers might have been related to the salt-induced reduction in root growth rate.

The sitosterol:stigmasterol ratio was highest in Rangpur lime and lowest in Etrog citron, correlating well with salt-exclusion capacity. But the best correlation between sterol content and salt tolerance showed up when the scientists looked at the structure of the sterols. They found the highest ratio of 'more-planar' to 'less-planar' sterols in the salt-excluders, under saline conditions.

How does the structure of plant sterols regulate membrane stability and permeability?

The molecular structure of the sterols allows them to be classified as 'less planar' if they have a bulky substituted side chain that lies outside the flat plane of the steroid ring structure or 'more planar' if their side chain is less bulky and lies within the plane of the steroid ring structure (see the diagram).

The volume filled by a 'less planar' sterol (such as sitosterol and stigmasterol) will be much greater than that filled by a 'more planar' one (for example, cholesterol and campesterol) due to the bulky side chain in the former. 'Less planar' sterols cannot pack between phospholipid molecules in plant cell membranes as efficiently as the 'more planar' sterols, and they disrupt the close packing of the phospholipid molecules, causing the membrane to become more permeable to ions such as chloride.

Dr Douglas and Dr Sykes also looked at the phospholipids in the root preparations of various citrus hybrids and found that plants that took up salt readily when grown in salty conditions had a lower ratio of phospholipid to sterol in their roots when grown in salt-free conditions than did salt-excluders. When salt treatment increases sterol levels, thus reducing the phospholipid:sterol ratio, the better chloride-excluders keep a balance of 'more planar' to 'less planar' sterols that maintains a low degree of permeability to ions.

Has the Merbein team found its biochemical marker for salt exclusion? The ratio of

Variation in salt-exclusion ability shows up clearly in these salt-treated citrus seedlings.

'more planar' to 'less planar' sterols in root membrane preparations will not do, since the changes in these only occur after salinization. In citrus, the most likely candidate appears to be the phospholipid:sterol ratio, since this ratio in the control plants grown by the CSIRO team was a good indicator of the capacity of roots to regulate the uptake of ions — especially chloride — during salinization. In other species so far examined, no similar marker has been identified, so the use of the phospholipid:sterol 'marker' is presently confined to citrus.

Despite this progress, the team has yet to understand fully the mechanics of chloride movement into, and through, roots to the stem and leaves and to localize the major regulatory site for this process. Dr Richard Storey has recently begun using an X-ray microprobe to search for such sites in snap-frozen root tissue preparations.

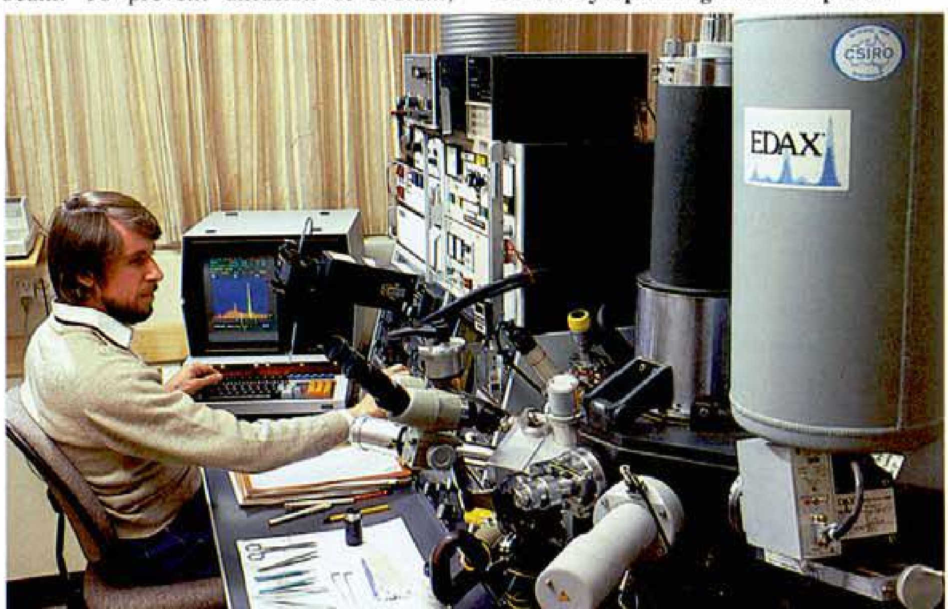
The microprobe at Merbein is based upon a scanning electron microscope; it can analyse the X-rays emitted from the specimen when it is scanned with the electron beam. To prevent diffusion of sodium, chloride, and potassium ions within and from root tissue during 'fixation' Dr Storey freezes the root — less than 1 mm in diameter — in melting nitrogen, which has a temperature of −196°C. The frozen root, supported in a small copper stub, is transferred to a 'preparation chamber' linked to the microscope.

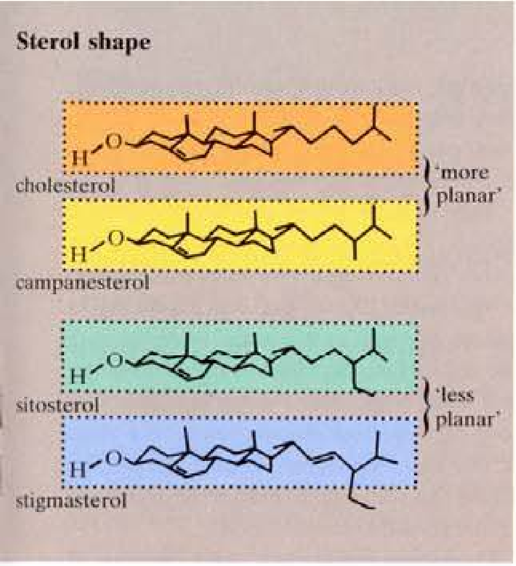
At this stage, Dr Storey cross-sections the specimen at −100°C. This leaves an ice-like surface on the root section that reveals little detail of cell interiors. Dr Storey reveals subcellular features like membranes and organelles by lightly 'etching' the specimen — subliming a thin layer of ice from the surface at the relatively warm temperature of −86°C. He makes the specimen conductive by evaporatively coating the etched surface with a thin film of chromium.

The X-ray spectrum, a visual representation of the X-rays emitted by the specimen, allows Dr Storey to determine which elements are present, and to calculate the concentrations, through calibration with standard salt solutions. The electron beam can scan over a large part of the specimen or over a small segment, such as a cell vacuole. This method allows Dr Storey to localize areas of sodium, potassium, and chloride accumulation within roots, and may eventually enable the team to identify the location of the 'cellular gate' or 'valve' that regulates the movement of chloride into and through roots.

Apart from all of the research on citrus, the Division of Horticultural Research has studied the effect of salinity on other fruit crops, such as avocados and grapes. Dr Downton, Dr Walker, and Dr Sykes have completed a number of studies on salt-treated grapevines. Like citrus, grapevines are sensitive to chloride, and salt stress can occur at leaf chloride concentrations below the level that causes visible damage. Dr Sykes is experimenting with salt-excluding

Dr Storey operating the microprobe.





The molecular structure of four plant sterols. The ratio of 'more planar' to 'less planar' sterols in cell membranes is related to salt-exclusion ability.

rootstocks for breeding salt-tolerant commercial cultivars.

Avocados form the basis of a rapidly expanding industry in Australia. Planting is being carried out on a large scale in the Murray Valley, but salinity is beginning to affect production. Dr Downton's work on avocados has shown that one particular race of rootstock — called Guatemalan — can be more effective at salt exclusion than others in saline soils. At the moment, farmers are using any seedling material they can find for rootstocks, since the demand for trees is high.

Meanwhile, the citrus research continues to expand. The CSIRO team at Merbein is currently screening a range of citrus hybrids sent by the United States Department of Agriculture's Horticultural Research Laboratory in Orlando, Florida, as part of a co-operative program between the two organizations on breeding better citrus rootstocks. The CSIRO and the New South Wales Department of Agriculture are also planting hybrids selected from glasshouse screening tests into the field for a much more rigorous evaluation of their salt tolerance.

Mary Lou Considine

More about the topic

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The Murray — more salt coming?

Mallee vegetation adjacent to land cleared for crops.

Each time they turned on a tap during the 1978–79 South Australian drought, the residents of Adelaide were reminded of what the Murray River carries to the sea — not fresh, but salty water.

The Murray River and its tributaries — the largest river system in Australia — drain more than a million sq. km of land, or about one-seventh of the total area of the continent. Although its salinity has always been a problem, the river is becoming even more salty, a cause for concern among the people of South Australia. In years of moderate rainfall, it provides up to 30% of Adelaide's water supply. In dry years, the figure can climb to 80%.

Where does the salt come from? Much comes in groundwater flowing into the river from the surrounding country. The level of the water table in the lower Murray has always been higher than river level, so the aquifers have slowly spilt their salty load into the river.

However, scientists are uncertain about the source of salt in Murray Basin aquifers. Some think it comes from salt left behind in marine limestones when these emerged from the ocean. Much of the Murray valley was once submerged below sea water forming an arm of an ancient ocean that reached as far upstream as Swan Hill in Victoria.

Dr Graham Allison of the CSIRO Division of Soils in Adelaide argues, instead, that much of the salt is derived from rainfall. He has calculated that enough time has elapsed since the seas withdrew from the region for the trapped salt to have been flushed away.

Whatever the origin, Dr Allison is more concerned with the immediate problem of how land-clearing has changed the rate of movement of the salty water through the soil and consequently into the river.

Roots and recharge

Most studies of salinity in the Murray have focused on the effect of irrigation on salt inflow. But clearing of the native vegetation can also contribute to the problem. A great deal of clearing has occurred; for example, during the past 50 to 80 years, land-owners in the counties of Albert and Alfred, which