8. Hughes, K., Bell, S.L. and Caponetti, J.D. 1975. Anther derived haploids of the African violet. *Can. J. Bot.*, 53:1442.
9. Langhans, R.W., Horst, R.K., and Earle, E.D., 1977. Disease-free plants via tissue culture propagation. *HortScience*, 12:25.
10. McCure, I.G. Education and training of the plant propagator in the 1980's. *Proc. Inter. Plant Prop. Soc.* 30:605.
11. Morel, G.M. 1964. Tissue culture, a new means of clonal propagation in orchids. *Am.-Orchid Soc. Bull.*, 33:473.
12. Morel, G.M. 1960. Producing virus-free cymbidium. *Am. Orch. Soc. Bull*, 29:495.
13. Murashige, T. 1974. Plant propagation through tissue culture. *Annu. Rev. Plant Physiol.* 25:135.
14. Murashige, T., Personal Communication. Flow Laboratories.
15. Murashige, T. 1973. Sample Preparations of Media Tissue Culture. Methods and Applications, in Eds. Krose and Patterson, Academic Press Inc. 698-703.
16. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant*, 15:473.
17. Pearson, A.E., and Hughes, K.W. 1978. Propagation of *Episcia* by tissue culture. in abstr. 4th Int. Cong. Plant Tissue and Cell Culture, Thorpe, T.A. Ed., University of Calgary, Canada.
18. Scowcroft, W.R. and Larkin, R.J. 1981. Some clonal variation, A cell culture option for plant improvement. *Proc. of 1st. Australian National Plant Tissue Conference.*
19. White, P.R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. *Plant Physiol.* 9:585.

REFERENCES

1. de Fossard, R.A. 1976. Tissue Culture for Plant Propagators. Univ. New England Printery, Armidale, NSW.
2. Gamborg, O.L. and Wetter, L.R. 1975. Plant Tissue Culture Methods. NRC-CNRC Ottawa, Canada.
3. Conger, B.V., 1981. Cloning Agricultural Plants via In Vitro Techniques. CRC Press Inc. 2000 NW 24th Street, Boca Raton, Florida, 33431.
4. Street, H.E. 1974. Tissue Culture and Plant Science. Academic Press, New York, N.Y.

PASTEURIZATION OF GROWING MEDIA BY MICROWAVE RADIATION

G.M. MOORE, R.G. HALL and E.A. JAMES
Burnley Horticultural College
Swan Street, Richmond, Victoria. 3121

Abstract. The pasteurization or disinfestation of growing media is now an accepted part of nursery hygiene. Media can be pasteurized by physical or chemical means, but the preferred method of treatment involves steaming the medium at 60°C for 30 minutes. Such methods are essentially a disinfestation, as some organisms survive the treatment.

Methods of pasteurization suffer from disadvantages such as high cost, time consumption, and a limitation in the volume treated. Thus a new

method of media treatment has been investigated — the exposure of the media to microwave radiation.

Although the method is restricted in the volume of material that can be treated, it is a very rapid technique and because the material is heated directly it is also energy efficient. The method can be used to either completely sterilise media, or to disinfest them so that at least some of the bacteria and saprophytic fungi survive.

It is envisaged that the technique could be used to treat a continuous supply of a medium moving along a conveyor, thereby eliminating a major bottle-neck in nursery production.

INTRODUCTION

A system of nursery hygiene is normally implemented to promote healthy plant growth by minimising the outbreak of disease. This system emphasises the prevention of disease rather than its cure and has been widely accepted because nurseries simply cannot afford high plant losses. In Australia, hygiene programs are based upon the views of Baker (1), which require the use of clean stock, clean soil, and a sanitary method of growing plants. It is the preparation of clean growing media that is the subject of this paper.

The use of pasteurized media means that plants are grown in material which is either free from pests and diseases that may be detrimental to plant growth, or that the incidence of pests and diseases has been reduced to an acceptable level (4). Of the many methods used to treat media for pasteurization, only two have achieved wide acceptance — chemical treatment of the medium, and the use of heat and/or steam.

The chemical treatments involve the use of gases, volatile liquids, or fungistatic drenches (2). The most widely used of these methods involves the injection of a liquid into the medium, allowing the chemical to vaporise.

The medium is sealed under plastic, which traps the gas, and is allowed to stand for 24 to 48 hours. Depending upon the chemical used, it may not be possible to use the medium for several days or even weeks, as the sterilant remains in the medium. Other problems associated with chemical pasteurization include the non-specificity in what is killed, the danger to the user and inadequate penetration of the medium.

The use of dry heat to pasteurize media invariably reduces moisture levels and, because higher temperatures are required than for steam or steam-air techniques, they are often fuel inefficient. Media are generally slow both to heat and to subsequently cool. Furthermore, since the conduction of dry heat through the medium is relatively poor, these techniques also suffer from the problem of inadequate penetration.

Thus the use of steam or steam-air mixtures remains as

the most widely used and preferred technique for media pasteurization. The steam is passed through the medium, raising its temperature to 60°C, at which temperature it is maintained for 30 minutes. The medium is then allowed to cool to atmospheric temperatures before it is used. Properly controlled, steam pasteurization eliminates harmful organisms, but beneficial micro-organisms survive. The technique is more effective than the other methods described because the use of steam enables a rapid and even conduction of heat through the whole volume of the medium. The use of steam is more effective than dry heat because the specific heat of water is about four times that of air.

All of the methods so far described suffer from some common problems. They are all comparatively expensive procedures, either to operate or to establish. They handle only small volumes of media and they are time-consuming. Thus media pasteurization often becomes the bottleneck of the nursery production line. Furthermore, there is often an uneven treatment of the medium and the nutrient status of the medium may be altered. Thus a cheaper, simpler, and more rapid system of media pasteurization has been sought.

The use of microwave radiation to pasteurize media seems to overcome many of these problems. Preliminary studies on the technique have shown that it effectively kills micro-organisms and that killing is very rapid (3). The radiation heats the water molecules inside organisms, as well as those inside the medium, and so it kills directly. It is a very rapid treatment and, despite limitations in the volumes of media that could be treated, it appears to be an energy efficient process.

MATERIALS AND METHODS

So that the results could be applied to a variety of growing media, two soil-based and two soil-less media were treated (Table 1). Each of the media was sterilised by either steam or microwave methods. Steam sterilisation involved heating at 60°C for 30 minutes, while samples to be irradiated were placed inside a Litton or Sharp domestic microwave oven with a radiation frequency of 2450 MHz. The media were exposed to the radiation for durations of 0.25, 0.50, 1.0, 2.0 and 6.0 minutes. Three replicates were used for all treatments.

After sterilisation, 50.0g samples of the media were placed in 50.0 ml of sterile water. The water was then filtered from the media and diluted to one in 10, 10^2, 10^3, 10^4, or 10^5 times in sterile water; 2.0 ml samples of each of these dilutions were then placed on plates containing malt extract agar, with the pH adjusted to 3.5 with lactic acid. At most dilutions there was so

Table 1. Media treated by steam or by microwaves.

Soil Based Media		Soil-less Media	
A.	30 parts sandy loam 15 parts mountain soil 6 parts coarse sand	B.	1 part sawdust 1 part scoria 1 part sand
D.	1 part sandy loam 1 part fine bark 1 part sawdust	C.	1 part brown coal 1 part coarse sand 1 part scoria

much fungal growth that identification was not possible, but the one in 10^5 dilution provided individual colonies, that could be easily separated and identified. The low pH of the agar deterred the growth of bacteria and the plates were incubated in the dark at 20°C for ten days before the colonies were counted and identified.

Because there were no pathogenic fungi present (or at least recovered by the technique described above) in any of the media, the microwave technique had not been shown to be effective against them. Accordingly, water-agar plates were inoculated with isolates of *Pythium* spp., *Phytophthora cinnamomi*, *Fusarium oxysporum*, *Rhizoctonia* spp. and *Penicillium* spp. These plates were then exposed to radiation for durations ranging from 3 to 24 seconds. The exposure times are so brief because the agar boils very quickly. The subsequent growth of fungi was then observed after incubation for four days in the dark at 20°C. It is interesting to note the responses of water, agar and potting media to exposure to microwave radiation (Table 2). The technique can be used to sterilise both water and agar, which may be of importance for tissue culture work.

Table 2. Duration time — temperature (°C) for different media after exposure to microwave irradiation

Duration (min.)	1	2	4	6	10	20
Soil mix	88	89	97	108	128	183
Agar (20g/l)	74	99	100	—	—	—
Water	66	100	100	100	100	—

In addition to the effects that microwave radiation has on microorganisms, they alter the medium itself.

Consequently the effects of microwave radiation on soil temperature and moisture content were monitored. The pH and conductivity of the media were also measured to determine whether the microwaves altered the nutrient status. These changes were then compared with the changes wrought by steam sterilisation. For these studies the media were fertilised with either Osmocote or Nutricote while they were being mixed. A more thorough mineral analysis was made by Consolidated Fertilizers, Ltd.

Finally the microwave oven itself was investigated to determine its reliability as a source of microwave radiation. The time versus power relationship to cause the boiling of agar was also established. The use of domestic microwave ovens for this study was far from ideal, but they did allow the study to proceed and valuable information to be obtained.

RESULTS

The microwave ovens used provided a consistent and controllable level of radiation (Figure 1). Treatment with the microwave radiation raised media temperatures very rapidly. (Figure 2), and consequently dried the media (Figure 3). Neither steam nor microwave sterilisation of the media caused significant changes in their pH (Table 3), although there were some minor changes in pH with time after treatment. Both pasteurization techniques increased the conductivity of fertilised media (Table 4), but it is worth noting that the microwave radiation had a less drastic effect than the steam. Again the conductivities increased with time after the pasteurization procedure.

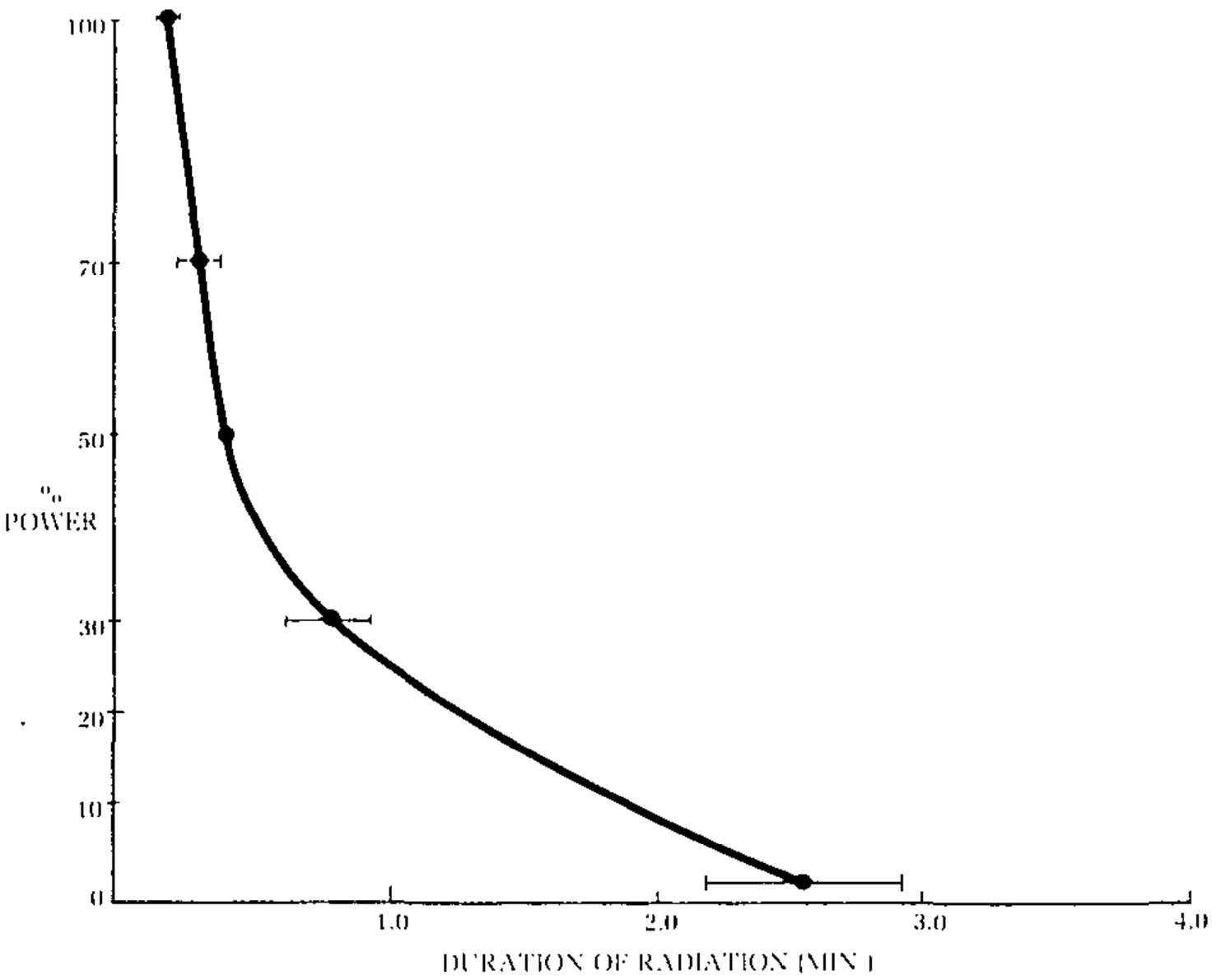

Figure 1. Duration of radiation versus power required to boil agar (P.D.A.)

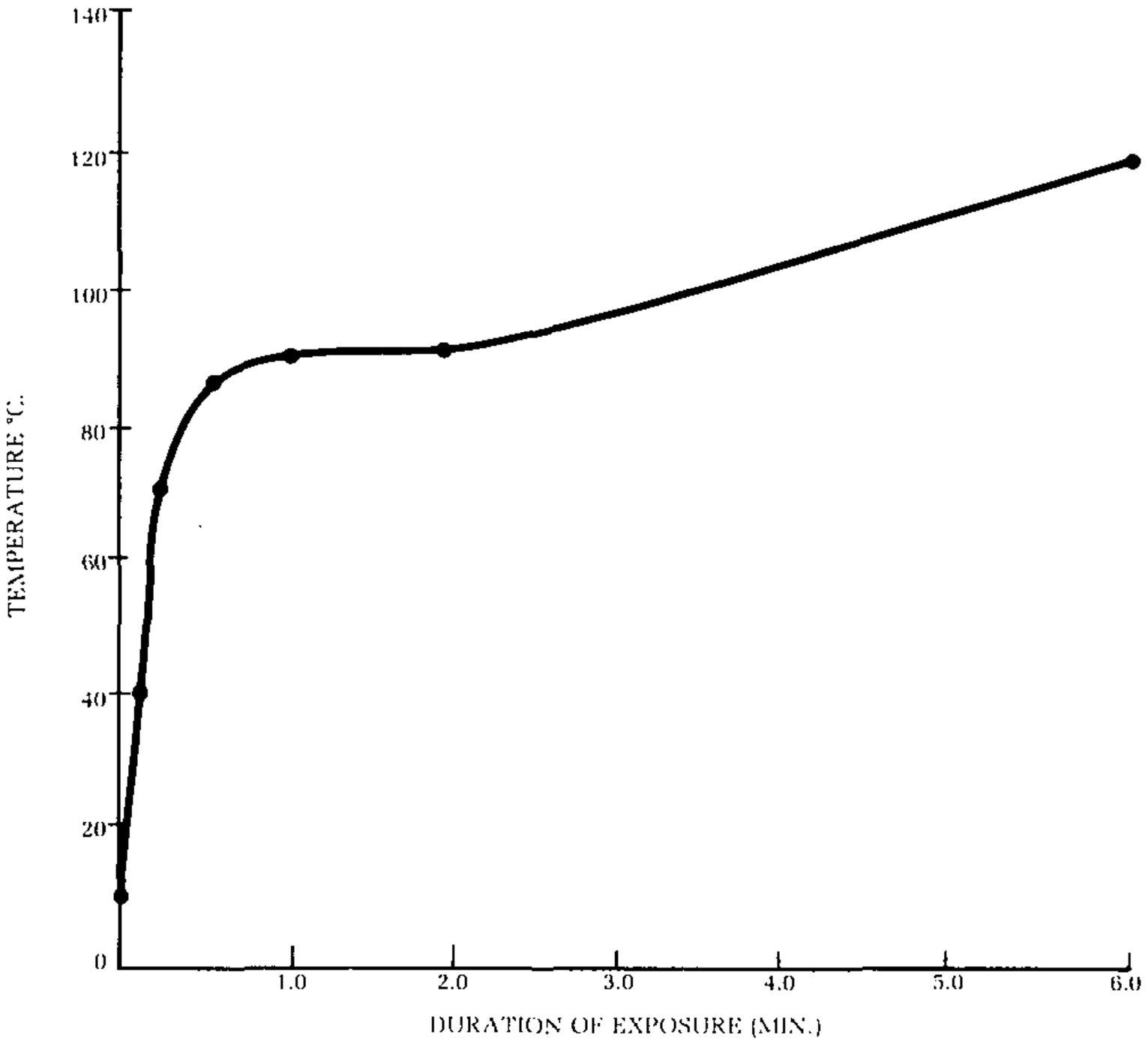

Figure 2. Media temperature versus duration of exposure to microwave radiation

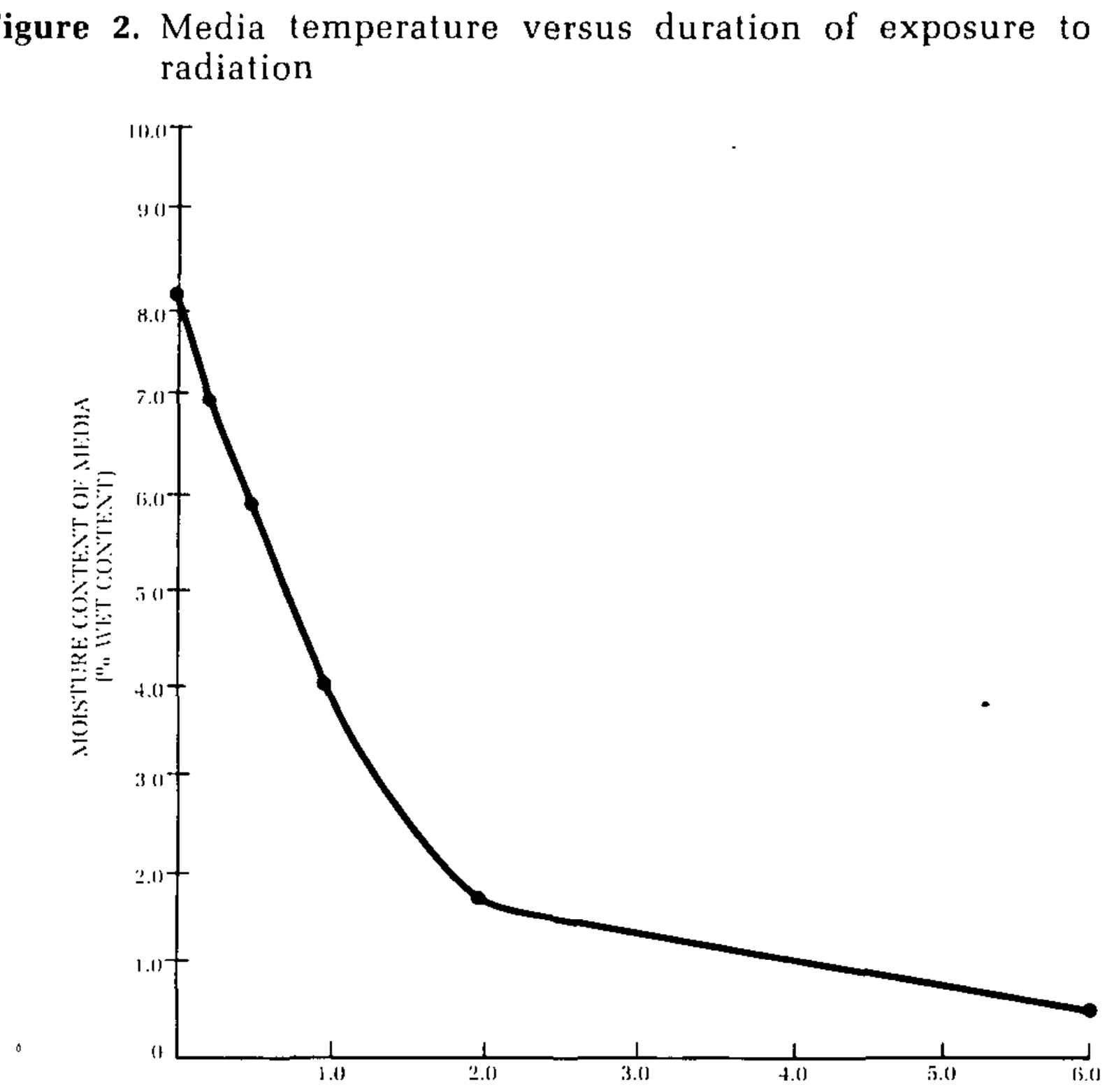

Figure 3. Moisture content of media versus duration of microwave radiation.

Table 3. Effects of steaming and microwave treatment on the pH of media

Media type	Control	Steaming	Microwave (5 Min.)
Soil-based + Osmocote	5.1	5.0	4.9
Soil-based + Nutricote	5.1	4.8	4.9
Soil-less + Osmocote	5.2	5.1	5.4
Soil-less + Nutricote	5.3	5.2	5.4

Table 4. The relative effects of steam and microwave treatment on the conductivity of various media

Media type	Steam treatment	Microwave
Soil-based + Nutricote	0.30	0.33
Soil-based + Osmocote	0.25	0.27
Soil-less + Nutricote	0.36	
Soil-less + Osmocote	0.49	

Note: Conductivities measured in $MS.CM^{-1}$

The mineral analysis of microwaved media showed the same type of result (Table 5), where there are only slight increases in the microwaved media in comparison with untreated media. Many of the differences can be regarded as insignificant.

Table 5. Mineral analysis in parts per million.

Media: — Microwaved — Oven-dried	NO_3	P	K	Ca	Mg	Fe	Cu	Mn	Zn	Na	Cl
Soil-based +	54.5	98	53	628	135	94	8.4	36	19.2	39	10
Osmocote	60+	80	97	523	103	92	9.9	49	20+	25	10
Soil-less +	60+	200+	1023	3244	1570	200+	15.1	101	20+	297	135
Osmocote	60+	200+	640	3321	1590	182	14.6	100+	20+	316	105
Soil-based +	34.5	56	39	537	114	111	10.4	48	20+	24	10
Nutricote	42.5	102	44	566	225	68	6.9	36	17.1	34	10
Soil-less +	60+	200+	442	3056	1372	200+	5.7	66	18.0	266	80
Nutricote	60+	200+	524	3030	1394	200+	3.6	74	13.5	298	60

Both steam and microwave pasteurization reduce the number of fungi and bacteria present in the media (Tables 6 and 7). Microwave treatment has the greater effect of the two and may sterilise the media completely. If short durations of microwave radiation are applied then both fungal and bacterial populations may be reduced without being entirely eliminated. Microwave irradiation of agar plates inoculated with fungi shows that the fungi may be killed and the plates sterilised very rapidly (Table 8). Thus the radiation effectively kills known plant pathogens.

Table 6. The effects of steam-treatment on fungal and bacterial populations

Medium	Steam-Treated	Untreated
A. Soil-based	Fungi: 18 Bacteria: 2 Total: 20	Fungi: 14 Bacteria: 9 Total: 23
B. Soil-less	Fungi: 21 Bacteria: 1 Total: 22	Fungi: 45 Bacteria: 11 Total: 56
C. Soil-less	Fungi: 21 Bacteria: 11 Total: 32	Fungi: 24 Bacteria: 1 Total: 25
D. Soil-based	Fungi: 16 Bacteria: 8 Total: 24	Fungi: 32 Bacteria: 2 Total: 34

Table 7. Effects of microwave exposure on microbial populations.

			Exposure to radiation (minutes)					
Medium	Microorganism	Control	0.25	0.50	1.0	2.0	6.0	10.0
A.	Fungi	23	2	8	1	2	2	—
	Bacteria	3	—	—	1	—	—	—
	Total	26	2	8	2	2	2	—
B.	Fungi	45	3	—	1	—	—	—
	Bacteria	11	4	—	—	1	—	—
	Total	56	7	—	1	1	—	—
C.	Fungi	24	2	—	—	—	—	—
	Bacteria	1	2	2	1	1	1	1
	Total	25	4	2	1	1	1	1
D.	Fungi	32	—	—	—	—	—	
	Bacteria	2	1	1	1	1	1	—
	Total	34	1	1	1	1	1	—

Table 8. Effect of microwave exposure on fungal cultures.

	Power rating (%)										
	0	50				70			100		
Duration (sec.)	0	6	12	18	24	6	12	18	3	6	12
Pythium spp.	✓✓✓	✓✓✓	✓xx	xxx	xxx	✓✓✓	xxx	xxx	✓✓✓	✓✓✓	xxx
Phytophthora cinnamomi	✓✓✓	✓✓✓	xxx	✓xx	xxx	✓✓✓	xxx	xxx	✓✓✓	✓✓✓	xxx
Fusarium oxysporum	✓✓✓	✓✓✓	✓✓✓	✓✓✓	xxx	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓x
Rhizoctonia spp.	✓✓✓	✓✓✓	✓xx	✓xx	xxx	✓✓✓	xxx	xxx	✓✓✓	✓✓✓	xxx
Penicillium spp.	✓✓✓	✓✓✓	✓✓✓	✓✓✓	xxx	✓✓✓	✓✓✓	✓xx	✓✓✓	✓✓✓	✓✓✓

NOTE: ✓ Indicates culture activity growing
x Indicates culture has been killed

In summary, the results show that the pasteurization of potting media and agar plates by microwave radiation is more rapid and effective than the use of steam. It also appears that microwave radiation has fewer effects on the physical proper-

ties of the media treated. Microwave radiation may be used to either pasteurize or sterilise media.

DISCUSSION

Although somewhat limited, this study shows the potential of microwave radiation as a viable alternative to the other forms of commercial media pasteurization. The technique clearly provides an efficient means of raising media temperatures. This is because the radiation acts directly upon the water molecules held within the medium, and does not rely on heating and conducting heat from other components therein.

Indeed, provided there is sufficient water in the medium, microwave radiation pasteurization is essentially a steaming process, with the steam being generated within the medium itself.

Unlike conventional heat systems, which heat the surface of a mass first and then the rest of the mass is heated by conduction, microwaves penetrate the mass according to an inverse square law. Thus the medium is heated simultaneously both at the surface and within. This explains why media temperatures rose so dramatically, and why there is such an even penetration of heat through the whole medium. For the microwave radiation to raise the temperature, the media must have relatively high moisture content. It would seem that moisture contents of between 10 and 15% are suitable for pasteurization; most potting and growing media would contain about these levels of moisture. Because exposure times are so brief it is unlikely that the technique would dry the media to any significant extent.

Both steam and microwave pasteurization techniques alter the physical and chemical properties of the media. Most of these alterations are small, but the changes in conductivity may be important. Steam does appear to cause an initial release of nutrients from the fertilizers, while the release due to microwave radiation is smaller. The release of nutrients by steam is probably due to the combination of high temperature and moisture level. It is also worth noting that soil-less media consistently have higher conductivities than soil-based media. The relatively low release of nutrients by microwave radiation is regarded as another advantage of the technique.

There is no doubt that microwave radiation represents an effective means of killing living organisms. Both fungal and bacterial populations may be reduced or eliminated entirely by this technique. By choosing the appropriate exposure times it is possible to eradicate pathogens, while leaving at least some

of the beneficial micro-organisms alive. Since microwave radiation acts directly on water molecules, and because most organisms contain high levels of water, many microbes will be killed directly by the radiation rather than by the heat conducted from the media. This is a far more efficient means of achieving pasteurization than any of the other techniques. For this reason too, many of the troublesome weed seeds that can survive steam sterilisation would be killed by the microwave technique.

The microwave technique, however, is not without its problems. No large volume trials have been performed to determine the efficiency and economy of the technique in a commercial situation. The radiation is dangerous and adequate operator-safety must be assured. Such problems do not seem to be insurmountable; it has been suggested that the power of commercial units could be as high as 60 kw with a frequency of 896 or 915 MHz, which would be cheaper to manufacture and allow deeper penetration (see acknowledgements). Large microwave generators are already in use in other industries and the safety problems have been solved, so this no longer represents a limitation to the use of microwave radiation in nursery sterilisation techniques.

Microwave radiation can be used for many other laboratory sterilisation procedures. It has been used for the preparation and sterilisation of agar plates in this study, and may replace the costly and time-consuming exercise of autoclaving in many laboratory and tissue culture procedures. Although this work concerns only the sterilisation of growing media, other work is proceeding that investigates the use of microwave radiation as a more general sterilising technique.

The system of pasteurization described in this paper is not suitable for commercial use. The system envisaged for this purpose (Figure 4), involves the use of a continuous supply of mixed media passing along a conveyor, through a "tunnel microwave generator" to a work bench. This would allow the pasteurization of large volumes of media and, because it is a continuous process, the usual production bottleneck would be eliminated. The variable speed at which the conveyor may operate provides a means of altering the exposure of the media to the radiation.

In conclusion, it can be seen from the results reported here that microwave radiation provides a rapid and efficient means of pasteurizing growing media. Although full commercial studies have not been done, it would seem that this process may be more economic than other processes because exposure times are so brief. The direct killing of organisms rather than relying on the conduction of heat contributes to

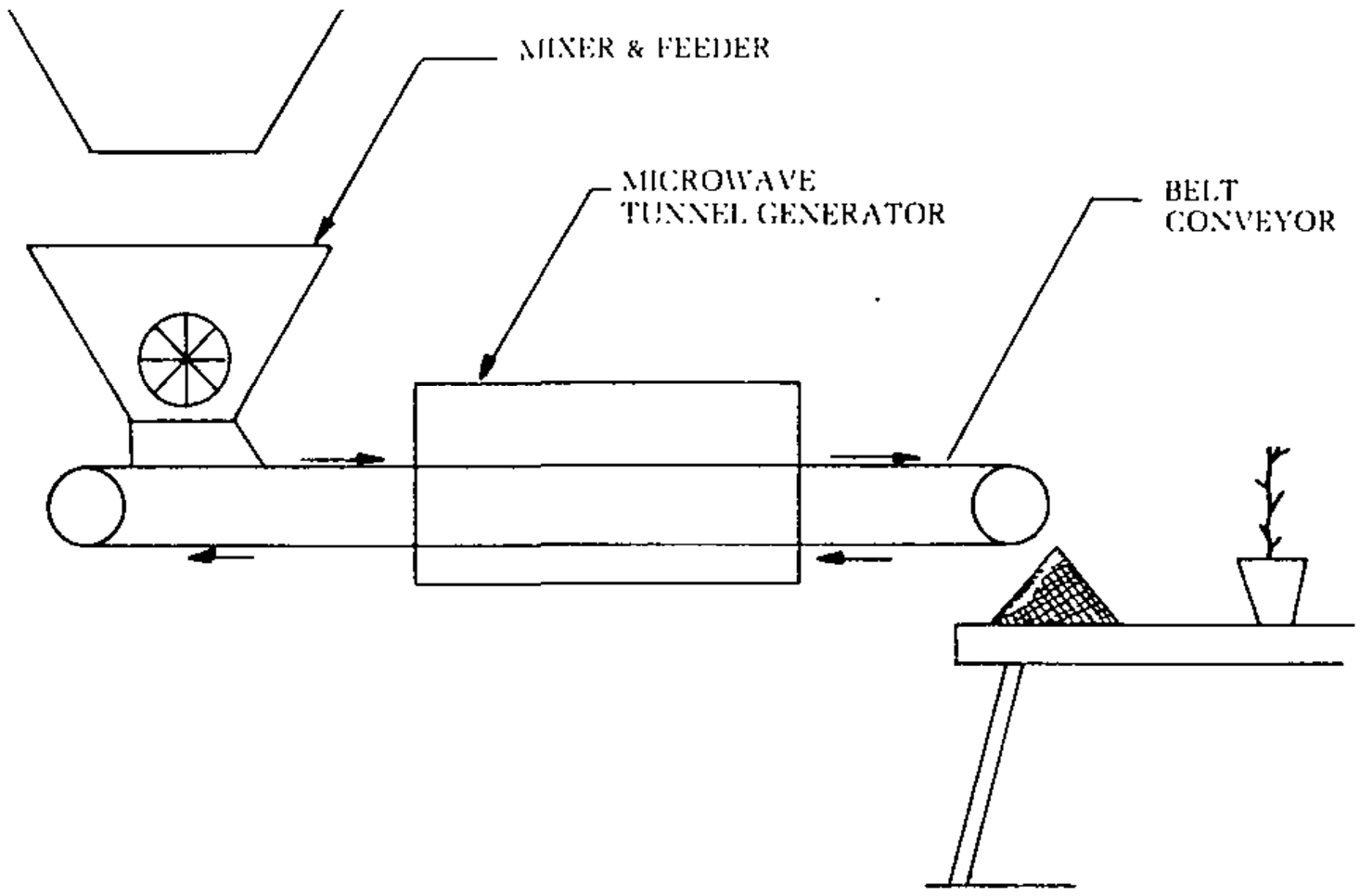

Figure 4. Schematic diagram of a microwave sterilisation technique.

both the speed and the efficiency of the process. The application of the microwave technique to other pasteurization or sterilisation processes should not be ignored.

Acknowledgements The authors thank Ms. A. Williamson and Mr. H.Y. Yip, Botany School, Melbourne University, for the identification of fungi, Ms. L. Smith for her technical assistance, and Mr. G. Kitney of the S.E.C. for making resources available. Mr. L. Cooper of Toptron Microwave is thanked for his technical advice, and Consolidated Fertilizers for the mineral analysis.

LITERATURE CITED

1. Baker, K.F. 1957, ed. The U.C. System for Producing Healthy Container-Grown Plant. University of California, Division of Agricultural Sciences.
2. Hartmann, H.T. and Kester, D.E. 1975. Plant Propagation: Principles and Practices. 3rd ed., Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
3. Moore, G.M., Hall, R.G. and Price, J. 1981. Sterilisation of Potting Media by Microwave Radiation. *Aust. Hort.* 79(10):45-53.
4. Mulder, D. 1979, ed., Soil Disinfestation. Elsevier Scientific Publishing Co., Amersteram.

GROWING CLEMATIS JACKMANII HYBRIDS

RUTH E. AULD and ARTHUR CARRALL
Castle Hill Nursery Pty. Limited
Kellyville, New South Wales

Except for the higher areas away from the coast, clematis plants do not usually grow really well around Sydney, Australia. The climate during late summer is very humid. Often considerable rain is experienced; such weather is conducive to phytophthora development. However, if the planting site is