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The Liverwort, Marchantia polymorpha, L. as a weed problem in Horticulture; its extent and control

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CONTENTS

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SUMMARY

The liverwort, Marchantia polymorpha L. can be a troublesome weed in certain situations. No completely satisfactory control method is yet in use. Gemmae appear to be the most important agents involved in spread. A circulated inquiry produced observations on liverwort/crop situations, competitive effects and current means of control employed. Experiments with the dithiocarbamate fungicide thiram (tetramethyl-thiuram disulphide or TMTD) confirmed its phytotoxicity to M. polymorpha when present at high rates on surface tissue. Concentrations of approximately five times those employed for fungicidal action (i.e. spore germination inhibition) were required and complete surface cover was needed. Applications either as a dust (50% a.i.), a dust/sand mixture or 20% a.i. aqueous spray were effective in destroying thalli but complete initial kill was rarely achieved. Regeneration occurred as a result of survival of gemmae, a phenomenon thought to be due to physical protection within the receptacle. Isolated gemmae proved susceptible to 0.5% a.i. thiram indicating the absence of any inherent tolerance at rates phytotoxic to the thalli.

Foliar application of 1.5% and 2.5% a.i. thiram sprays to 37 species of pot-grown crop and weed plants caused negligible growth inhibition. Only Papaver rhoeas L., Sinapis arvensis L., Avena fatua L., sugar beet and dwarf bean, were inhibited by 25% or more. Prevailing environmental conditions and watering regimes influenced the degree of inhibition.

INTRODUCTION

Distribution and habitat of M. polymorpha

Although terrestial weed problems caused by non-vascular plants are seldom of any great magnitude, unicellular green algae, lichens and mosses prove troublesome in particular situations and liverworts, notably the species Marchantia polymorpha L. can do likewise. Marchantia is mainly a tropical and subtropical genus and M. polymorpha is the only British species and the only one with a wide distribution in temperature regions. It is a plant of damp situations to be found along river banks and on wet moorlands (Watson, 1955). It has been found to be an early coloniser of land after burning, e.g. after forest fires (Graff, 1936), and on burnt heathland (Watson, 1955) where it is found in association with mosses, particularly Funaria hygrometrica, Hedw. Watson (1955) also records M. polymorpha as being a common weed in greenhouses and near buildings, although other liverworts are also to be found in these situations. Watson mentions Lunularia cruciata (L) Dum, which like Marchantia is thallose and gemmiferous but possesses protective ridges instead of cup-shaped receptacles.

M. polymorpha has been the subject of many investigations. Anatomical, ecological and morphological aspects are well documented in several standard works (Fritsch and Salisbury, 1961; Macvicar, 1926; Watson, 1955). Physiological research has been much pursued in Germany and North America. As a weed problem however little work has been done.

Reproduction, spread and growth of M. polymorpha

The thallus which is haploid can reproduce asexually either by fragmentation or by the development of new thalli from the special reproductive 'buds', the gemmae. Sexual reproduction produces a diploid sporophyte which develops and is parasitic on the female plant. Haploid spores released by the sporophyte complete the life cycle by developing into new thalli.

Gemmae in M. polymorpha occur only within the receptacles or gemmae-cups. Their production on the thallus is thus localised. They are the primary reproductive mechanism, for gemmae production commences early in the life of the new gametophyte and presents a mode of increase much preceding that of spore production. Gemmation as a mode of reproduction has the following advantages:

a) it is direct without the need of an 'intermediary', i.e. alternating, generation;

b) it is prolific;

c) it is rapid in terms of the growth and establishment of new thalli.

Conversley, being a vegetative method, it does not present the opportunity for inherent variation such as arises from character recombinations during meiosis and sexual fusion.

Gemmae-cup production by the thalli varies according to several factors. Voth and Hamner (1940) found that a nutritional balance with low phosphates and high nitrates and a short photoperiod of 8 hours encouraged maximum gemmae-cup production. Later work (Voth, 1943) showed that although maximum vegetative growth was achieved by the use of solutions having relatively low osmotic potentials from 0.18 - 0.21 atmospheres, maximum gemmae-cup production was favoured by a higher potential of 0.59 atmospheres.

The gemmae are multicellular structures and arise at the base of the receptacle. They are flattened and bi-lobed with one single-cell meristem at the base of each of the two notches. Prior to independent growth the gemmae are bi-dorsiventral and the rhizoid initials present may emerge from either side. The direction taken is determined by the direction of incident light; the rhizoids being negatively phototropic. Unlike the thallus gemmae do not appear to be geotropically influenced (Miller and Voth, 1962).

Germination of the gemmae is inhibited while they are still within the receptable but inhibition ends rapidly when the receptacle and thallus dies or when the gemmae are removed from the receptacle. Taren (1958) has suggested that two independent inhibitory processes or substances are involved, one affecting rhizoid elongation and the other affecting meristematic growth.

Mature gemmae escape from the receptacle in rain splashes or may be pushed out by the development of younger gemmae below them.

Under favourable conditions the developing gemma gives rise to a dorsiventral thallus with initially two lobes expanding in opposite directions. These lobes are produced only if the apical cells concerned are in contact with the substrate (Halbsguth, 1953). They continue growth, produce rhizoids and branch dichotomously. Gemmae-cups and eventually gametangiophores are produced; the latter being produced more freely under long day conditions (Voth and Hamner, 1940).

THE WEED PROBLEM

The results of an inquiry summarised below show that M. polymorpha is a not uncommon plant in and around greenhouses and accompanying buildings, and recently an infestation at Begbroke has given rise to some concern with the invasion of pots of plants being used for herbicide evaluation experiments.

The presence of the liverwort is undesirable for several reasons: with ornamental plants it is unsightly, while with experimental plants it represents an illdefined source of error. Complete removal of the thalli by hand or mechanical means is difficult and with potted plants such removal necessarily entails a reduction in the volume of compost left for the plant to grow in, for adhering to the thallus is an attendant rhizoid/compost mass. Further the development of a complete thallus cover renders judging of moisture status and water application difficult and impedes fertilizer application.

Extent of the Problem

An inquiry was circulated to ten Botanic Gardens and similar establishments in England, Scotland and Eire with the object of ascertaining the frequency of occurrence of M. polymorpha as a weed and the seriousness of the problem it presented. Nine replies were received.

In three cases (Oxford, Cambridge, Edinburgh) a serious problem was not encountered although difficulties due to liverwort development over slow germinating seeds were mentioned (Edinburgh).

Situations where liverworts were regarded as being particularly troublesome were:

a) among collections of filmy ferns (Kew, Glasnevin);

b) among young ericas and related plants (Kew, Wisley);

c) on pans of slow germinating seeds (Chelsea, Glasgow, Glasnevin, Liverpool);

d) among slow growing seedlings (Kew, Liverpool);

e) in propagating frames, and mist propagation units (Glasnevin):

f) in cool greenhouses generally (Glasgow).

In addition the liverwort has proved troublesome both under glass and outside at Begbroke where both pots and outside standing grounds have become infested.

Competitive effects

The long period taken by certain ornamental plants to emerge allows the liverwort to establish itself as a thick surface mat. This can considerably hinder seedling emergence and development and this aspect and various measures to combat it were frequently referred to.

Severe competitive effects to seedlings, mainly those with slow initial growth were described: erica seedlings had been 'swamped' in one instance (Kew) and species of dwarf rhododendrons (Kew, Liverpool), Lilium and Ramonda spp. (Liverpool) also suffered severely in the seedling stage.

Inhibition of the rooting of cuttings requiring a long period in the propagating frame was reported (Glasnevin). Infestation of potted ornamentals remaining in frames or plunge beds for long periods undisturbed was a common occurrence. While in most cases there was no observable detrimental effect to established plants there was no doubt that such an effect was possible, and it was reported that 'less vigorous plant varieties' could be so affected (Glasnevin). Such plants included azaleas, conifers, Gentiana and Primula spp. (Glasnevin); and erica and Calluna spp. (Glasnevin, Wisley).

Ecological requirements

Various observations, which were generally in agreement, shed interesting light on conditions favourable to liverwort growth and spread. M. polymorpha was considered to be mainly a glasshouse weed, but it could be equally troublesome out-of-doors when conditions were favourable (Chelsea). It was to be found mainly in cool greenhouses or in frames, although it could appear and spread at high temperatures under very wet or waterlogged conditions (Kew).

A degree of shade was mentioned as being favourable; thus north-facing frames (Edinburgh) and shaded glasshouses and frames (Glasnevin) were frequently infested. Low light intensity together with acidity was considered to be responsible for encouraging growth over seed-pans (Glasnevin).

Although M. polymorpha is known to tolerate a wide range of pH values (Voth, P.D., 1943) its frequent occurrence on acid media and among acid loving plants (Glasnevin, Kew, Wisley, Liverpool) appears to indicate a preference. The deterrent value of lime and cement wash used on greenhouse brickwork was reported (Kew).

High humidity is a factor generally recognised as being of importance in the development of the sporophyte and the dispersal of gemmae. Water supplies were twice mentioned as possible sources of infection (Oxford, Kew).

Substrate type and texture were also thought of importance, although here somewhat divergent views were expressed. Weathered boiler ash, employed commonly as a plunging medium was suited to rapid liverwort establishment (Oxford), but conversely panning by winter rains was considered to be one pre-requisite to liverwort spread over seed pans (Glasnevin). Some difference was noted in the relative speed of colonisation by liverworts of soils from differing localities (Edinburgh).

The association of liverworts with other low plant forms was mentioned, particularly mosses but lichens were also involved (Glasnevin).

Control measures practised

Preventative measures were frequently adopted. Besides general hygiene and soil pasturisation these included the employment of surface layers of g rit or granite chippings over seed pans (Chelsea, Liverpool), or alternatively the use of black polythene covers (Liverpool).

Hand removal was felt to be sufficiently practicable in some cases (Cambridge), but difficult and unsatisfactory in others (Glasnevin, Edinburgh). Resurfacing and renewal of compost had frequently to be resorted to (Glasnevin) while surface peat dressings had in some instances, led to temporary suppression of the liverworts (Liverpool).

Various types of chemical control were employed to a minor extent but generally with unsatisfactory results. These included watering with a strong solution of potassium permanganate, and use of "Panasand" (5,5-dichloro-2,2-dihydroxy diphenylmethane) (Chelsea) and dusting with calomel (Wisley). The results of the first treatment were described as a 'limited success' and calomel failed to give a complete control. Results following the use of "Panasand", however, were reported to be encouraging.

Conclusion

Thus M. polymorpha has been found to infest potting soils, greenhouse borders, frames, plunge beds, pots and other containers, and also outside pot standing areas and seed beds. The increasing practice of growing nursery stock for direct sale in containers, and their irrigation by overhead spraylines has provided another situation in which M. polymorpha can thrive (Clements, private communication 1966). It would therefore appear that it is under conditions of high humidity, high soil moisture status and minimal plant competition, that establishment can occur and spread become rapid.

Thus, although not a major problem, when M. polymorpha infestations occur they are not dealt with easily, and the difficulties created by their presence are sufficient to warrant a control method being developed.

Previous work on control

Control of Marchantia has attracted little attention although on the Continent some work on control amongst flower crops, nursery stock and forestry seed beds has been done (Netherlands, 1962, 1964; Van der Laar, 1964).

The residual herbicide chloroxuron achieved selective control of Marchantia sp. and also the moss Funaria hygrometrica, Hedw. among an annual carnation crop (Netherlands, 1964), when applied at 6 kg/ha to soil with a 25% organic matter content. Van der Laar (1964) also found simazine (using a 2.0% granular formulation at $7 g/m^2$ in 200 ml sand) to give a very good control. The further use of these materials for liverwort control in other crop/soil situations requires careful consideration in view of their persistence.

Materials in use for the control of mosses and/or algae are obvious candidates as 'hepaticides'. Preliminary observations at Begbroke showed cupric sulphate ($CuSO_4.5H_2O$) to be relatively ineffective against well developed plants of M. polymorpha; concentrations of 10,000 ppm was required to kill existing thalli. A concentration of 1,590 ppm $CuSO_4.5H_2O$ however proved fatal to the M. polymorpha cultures of Voth (Voth 1945), when applied with the nutrient solution to ascertain algicidal and anti-bacterial activity. The plant material in these cultures consisted of 1 cm^2 thalli segments and was more susceptible due to isolation and complete exposure to the solutions applied than intact plants.

Other copper salts used by Voth (1945) proved yet more toxic. Thus 20 ppm of either cupric tartrate ($CuC_4H_4O_6$) or acetate ($Cu[C_2H_3O_2]_2H_2O$) resulted in death of the segments after four days. Repeated additions of the tartrate at 1 ppm caused death of the segments after a period indicating it to be cumulative.

Other metal salts have been frequently recommended for moss control (Woodford and Evans, 1965) but the main one amongst those, calomel (HgCl) has been reported as failing to give full control of liverworts although it was the most promising of several fungicidal materials tried by Brooks, (private communication, 1966). Potassium permanganate ($KMnO_4$) is another material used for moss control, although some species are resistant (Woodford and Evans, 1965). Used as a saturated solution in Voth's cultures (1945) it caused initial necrosis but the apices survived and regrew. When used against thalli in situ, it has proved of limited use (Mackenzie, private communication 1966).

Manganese sulphate ($McSO_4.5H_2O$) at 1,600 ppm caused death of M. polymorpha culture segments within a week (Voth, 1945). Utilization of the copper salts, manganese sulphate and potassium permanganate may be limited by their toxic effects on other plants.

The dithiocarbamate fungicide thiram (tetramethyl-thiuram disulphide) has been employed in Holland and Germany for control of both Marchantia spp. and mosses, against conifer seedlings and nursery stock. A mixture of sand and a thiram powder applied to form a surface cover 3 mm thick was effective in controlling Marchantia spp. in frames (Netherlands, 1962). Thiram was also used successfully as a spray application for moss control. Van der Laar (1964) reports that application of 2.0% a.i. aqueous solution at 400 ml per m^2 gave complete control by September.

Effects of 'conventional' foliar-acting herbicides on M. polymorpha have not been described but it might be expected that the general effects would differ from those shown by vascular plants. The author has observed that M. polymorpha can survive treatment with paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride), and although the mode of survival has not been closely investigated, it would appear to be the result of inadequqte spray cover and lack of contact with the gemmae.

EXPERIMENTS WITH THIRAM

As a candidate material for liverwort control thiram offers the advantage of being of low toxicity to both animals and vascular plants. Its widespread use as a fungicide (Martin, 1965) has occasioned little comment regarding phytotoxicity or other hazards. This is partially explained by the insolubility of the material and by the low concentrations which suffice for effective fungicidal action.

The experiments conducted were designed to a) test effectiveness of the material on mature thalli; b) test effectiveness of the material on the gemmae; and c) determine the degree of phytotoxicity of the material, at the high concentrations necessary, on a range of temperate plant species.

Materials and Methods

Experiments with thalli

Material of M. polymorpha was obtained from plants infesting an outside pot-standing area at Begbroke. Both male and female plants were present. The plants were characterised by being dark green, possessing a distinct 'mid-rib', and producing an abundance of gemma-cups.

Plants were established in 3.5 inch (9 cm) diameter plastic pots by taking segments of thalli approximately 2 cm^2 and planting one per pot. A peat/sand compost based on University of California formula UC II C was used as a growing medium after an initial trial had shown it to induce much faster growth than either loam, or a John Innes potting compost.

All plants were grown in a heated greenhouse with a minimum temperature of 10°C. Day temperatures did not exceed 18°C.

Plants were selected for treatment before the development of gametangiophores, and when a complete cover of the surface had been achieved.

The plants were watered overhead where dust or dust-sand treatments were applied. Sprayed plants were generally sub-irrigated on capillary beds to avoid disturbing surface deposits of thiram.

Thiram dust treatments were applied by means of a compressible plastic container with pin size apertures, and dust/sand mixtures were distributed by hand. Spray treatments were applied by either a "Solo No. 27" double action hand sprayer with a swirl nozzle, or by a special pot spraying machine employing a "Teejet 8002E" fan nozzle. This moved along a track at a constant speed twelve inches above the plants and delivered 24.7 gpa at one application. Higher volume rates were achieved by several successive applications.

Damage symptoms were recorded using a subjective scoring scale as follows:

0 = no damage; as controls
1 = slight damage; small proportion of thalli affected
2 = moderate damage; approximately 50% of thalli affected
3 = severe damage to majority of thalli, but regeneration possible. Gemma clusters or receptacles surviving.
4 = very severe damage; regeneration unlikely or impossible. All receptacles and almost all thalli affected - no gemmae apparent, or if present, few and of unhealthy appearance.
5 = all tissue dead.

Half scores were permitted, and these scores were later used for conversion into percentages representing degree of growth or 'vigour' depression (inhibition).

Experiments with gemmae

Various techniques have been employed successfully for growing isolated gemmae in vitro (Miller and Voth 1962, Voth and Hamner 1940, Taren 1958). A filter paper substrate was used in the present investigations to allow for immersion of the gemmae or established gemmalings into solutions without disturbance or damage.

Mature ungerminated gemmae were removed from the receptacle and placed ten per 9 cm filter paper disc in petri dishes. Three ml. of distilled water was added and 48 hours allowed to elapse prior to the application of nutrients in order to encourage enhanced rhizoid growth (Miller and Voth, 1962). The dishes were placed in controlled environment rooms with 16 hours of light per day with an intensity of 150 lumens/ft^2 provided by 80 watt 'daylight' fluorescent tubes. Temperatures were 24°C during the light period and 19°C during the dark period.

On the third day after planting nutrient was added to the dishes using a complete nutrient solution (Hoagland and Arnon, 1950).

Gemmae and gemmalings were treated either by pipetting thiram suspensions onto individual plants or by immersing the whole disc into the suspension for 15 seconds, followed by 15 seconds draining before replacing in the dish. Gemmae within the receptacles were treated by both immersion and pipetting using excised 2 cm^2 segments of thalli, each bearing an apex and two or more mature receptacles. Following treatment the segments were replanted and placed in the glasshouse. Samples of gemmae were then taken from the treated receptacles and tested for viability by petri dish culture.

Experiments on selectivity of thiram treatments

A range of temperate annual and perennial crop and weed plants were raised from seed sown directly into 3.5 inch plastic pots. Exceptions were Agrostis stolonifera L. and Agropyron repens (L) Beauv., which were propagated vegetatively from stolon and rhizome sections respectively. M. polymorpha was grown as previously described. All plants were grown in a peat/sand compost and thinned to a fixed plant number per pot varying according to species. Planting and sowing dates were staggered in order that all species could be at a roughly equivalent stage of growth at the time of treatment. Additional nutrients were supplied at intervals but to avoid possible interference with treatments no insecticide or other pesticides were applied.

All plants in experiment 1 were grown throughout in a greenhouse with a 10°C (50°F) minimum temperature. After treatment they were watered by capillarity. Plants in experiment II were germinated in the glasshouse and then transferred out-of-doors. All watering was applied overhead, apart from a 24 hour period immediately after treatment when no watering on the foliage was allowed. The thiram sprays were applied overhead, apart from a 24 hour period immediately after treatment when no watering on the foliage was allowed. The thiram sprays were applied until 'run-off' using the 'Solo' sprayer.

Damage was assessed using a 7 point subjective scoring scale with the highest score representing the least damage. The scores recorded were converted to percentages and a figure for vigour depression was obtained.

Results

Experiments with thalli

In an initial experiment thiram dust and dust/sand mixtures were applied to plants growing in three composts. Two volumes of the sand mixture were applied to each compost. Higher rates were applied to the peat/sand compost in which greater growth had been made. The results are shown in Table Ia and Ib. All treatments severely affected the thalli and only the 1.25% a.i. dust/sand mixture applied at 22 ml per pot to the large plants in the peat/sand compost failed to reduce vigour by more than 75%. Sand alone caused a setback but apical growth continued and new thalli were eventually produced above the sand layer and at the pot edge.

Table I

The effects of thiram dust and dust/sand mixture on
<u>M. polymorpha</u> grown in three composts

a) Dust/sand mixtures

% a.i.	Factor assessed	No. of days after treatment	Compost					
			Loam		John Innes potting		Peat/sand	
			Volume of sand mixture per pot (ml)					
			11	22	11	22	11	22
1.25	⌿ Damage score	12	4.5	4.5	4.5	4.5	3.5	4.4
	* No. of replicates with live gemmae	21	2	1	0	1	1	0
		35	0	0	0	1	0	0
2.50	⌿ Damage score	12	5.0	4.0	5.0	5.0	4.5	4.0
	* No. of replicates with live gemmae	21	0	0	0	0	1	2
		35	0	0	0	0	1	1
5.00	⌿ Damage score	12	5.0	5.0	5.0	5.0	5.0	5.0
	* No. of replicates with live gemmae	21	0	0	0	0	0	0
		35	0	0	0	0	0	0

b) Dust (50.0% a.i. applied as a light surface film)

Factor assessed	No. of days after treatment	Compost		
		Loam	John Innes potting	Peat/Sand
≠ Damage score	12	5.0	5.0	4.5
* No. of replicates with live gemmae	21	0	1	2
	35	0	0	0

≠ 0 = as control 5 = complete kill

values in table are means of two replicates

* maximum of two

Although thalli were severely affected by the thiram treatments, any surviving gemmae presented a possible source of regeneration. Clusters of still-green gemmae became prominent in a proportion of the dying receptacles which were exposed but their numbers declined with time (Table I). None were observed on any pots treated with 5.0% a.i. dust/sand mixture.

The application of thiram as an aqueous foliar spray was next investigated. An 80.0% a.i. wettable powder formulation was applied at high volume to 'run-off' at three concentrations and compared with the dust treatment. The results are presented in Table 2. Only the 2.0% a.i. spray caused an inhibition exceeding 75% followed by a complete kill with no subsequent regrowth. The dust treatment compared unfavourably with its previous performance and the receptacles were not prevented from producing further gemmae.

Table 2

The effects of thiram spray and dust treatments on M. polymorpha

Treatment % a.i.	Estimated % area of thallus killed	Number of days after treatment		
		6	15	35
Sprays			scores*	
0.4	19.0	1.5	1.6	1.6
0.8	80.0	2.8	3.3	3.5
2.0	90.0	4.0	4.8	5.0
Dust				
50.0	78.0	2.8	3.4	3.2

* means of ten replicates 5 = complete kill
0 = as control

Since the above results of spraying were obtained with sub-irrigated plants it was possible that overhead watering might reduce the activity of thiram by washing off the spray deposits. Plants were therefore treated with three concentrations of thiram and watered either overhead or by sub-irrigation. The method of watering had little effect on the resultant damage (Table 3). There was also little difference due to the dose employed.

Table 3

The influence of watering method on susceptibility of M. polymorpha to thiram sprays

Treatment % a.i. spray	Mode of water application		Means
	Overhead	Sub-irrigation	
	scores*		
1.5	3.2	3.4	3.30
2.0	3.5	3.4	3.45
2.5	3.4	3.4	3.40
Means	3.36	3.40	

* means of five replicates

The effect of the stage of development and amount of growth made by the thalli on the ease of control was investigated in a further experiment employing sub-irrigation. The results are presented in Table 4. Where less than 15% of the available surface area of the pot was covered at the time of treatment a complete control was obtained. The degree of control was little affected once thallus growth exceeded this. The difference in surface area occupied reflected differences in development and degree of overlapping of tissue. Thus the smaller plants (5-15% surface cover) possessed few mature gemmae-cups and were thus less able to reproduce in this way. All other categories possessed many gemmae-cups, the number increasing with the area covered.

Application of thiram suspensions by droplets onto thalli showed the resultant damage to be very localised. An initial paling preceded complete 'bleaching' of the treated area only, without adjacent tissues being affected.

In the foregoing experiments dosage has been a function of concentration; a volume of liquid being applied in excess of the retention properties of the tissue. To determine response in terms of a.i. per unit area (i.e. lb/ac or gal/ac of n% spray applications of up to 198 g.p.a. were made with several concentrations of thiram suspensions using the special pot spraying machine described above. When well developed plants were treated, it was found that

Table 4

The influence of stage of development on susceptibility of M. polymorpha to a 1.5% a.i. thiram spray

% area of 3½" pot estimated to be covered by thalli	Mean vigour ≠ Score 7 days after treatment (5 reps)	% area estimated to be covered by regenerated tissue 37 days after treatment
5 - 15	4.8	nil
30 - 50	3.4	18
60 - 75	3.2	11
80 - 100	3.4	12

≠ Score 5 = all dead; 0 = as control

198 g.p.a. of a 2.0% a.i. proved insufficient to achieve adequate control (Fig. 1). A greater degree of inhibition was recorded on thalli at an early stage of development (developing thalli). On these doses of 148 to 198 g.p.a. gave a control comparable to that achieved by hand spraying. The amount of regrowth made in the pots 33 days after treatment is indicative of the level of this control (Table 5).

Table 5

Regeneration of M. polymorpha after treatment with thiram sprays (after 33 days)

+Gallons 2% a.i. spray per acre	74	95	124	148	173	197	'hand spray'
% pot surface covered (Means of 5 replicates)	23.3	10.0	5.0	2.5	5.5	2.5	nil

Such applications by maintaining spray concentration, differed in both dose of a.i. in lb/ac and in volume of liquid carrier. To further investigate the relationship between these two factors, thalli were treated with a standard dose of 19.8 lb/ac a.i. in 25, 49, 74 and 99 gal/ac water. The results (Table 6) for two experiments performed were somewhat dissimilar but their means indicate that there was essentially little difference in growth inhibition as a result of the treatments, and that increasing the volume rate between 25 - 100 gal/ac did not result in increased toxicity.

Fig. I

The toxicity of T.M.T.D. sprays to M. polymorpha

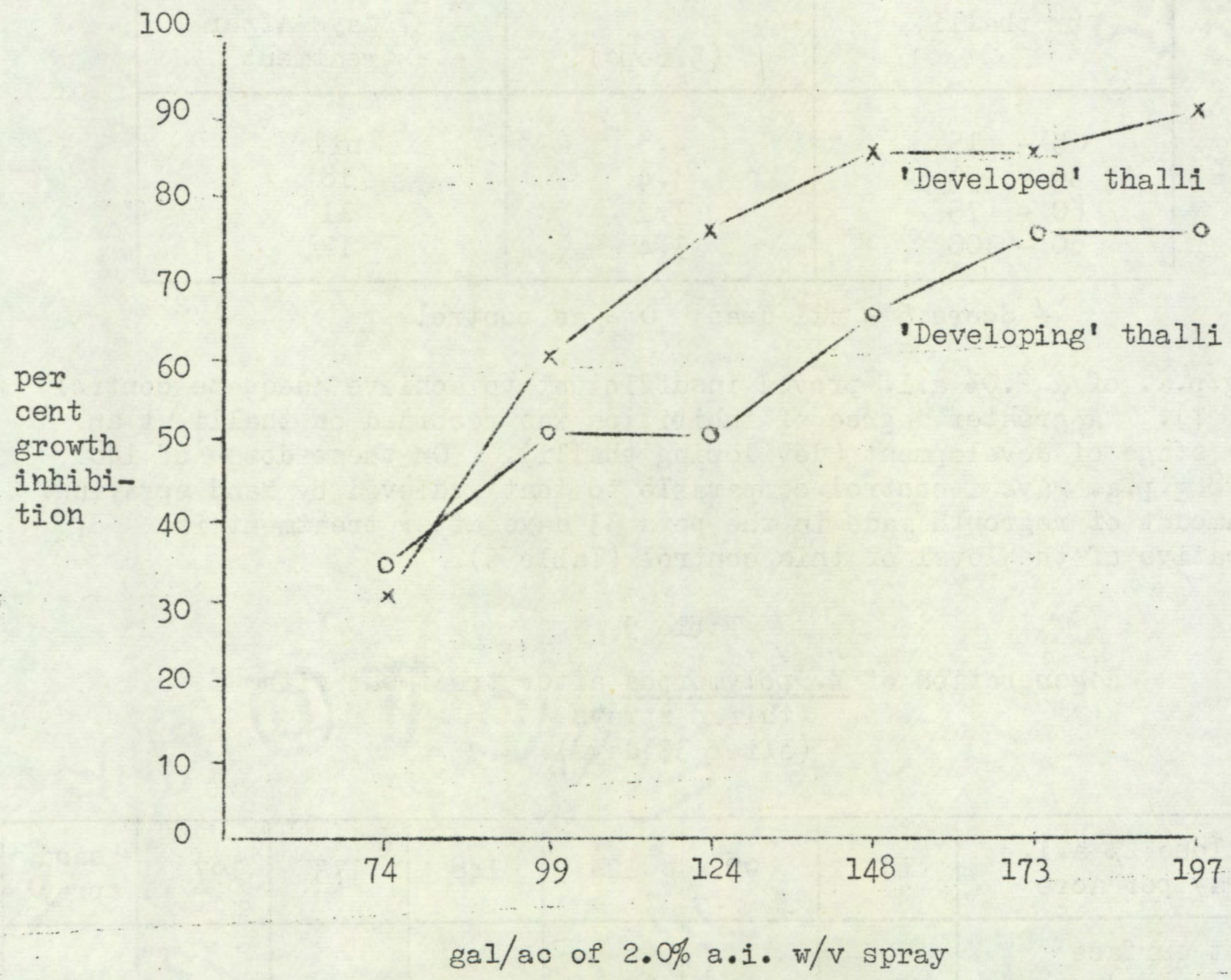

Table 6

The phytotoxicity of thiram to M. polymorpha at 19.8 lb/ac with concentration/volume product a constant

Treatment		8.0% a.i. at 25 gal/ac	4.0% a.i. at 49 gal/ac	2.66% a.i. at 74 gal/ac	2.0% a.i. at 99 gal/ac
% growth inhibition (Mean of five)	Expt. I	60.0	36.0	44.0	52.0
	Expt. II	30.0	28.0	34.0	26.0
Mean		45.0	32.0	39.0	39.0

New thalli established in pots from surviving gemmae after previous treatment were subjected to a further spraying by hand of a 1.5% a.i. suspension. In all cases complete control was obtained provided gemmae-cups had not matured

Experiments with gemmae

Several possibilities are raised by the ability of the gemmae to survive thiram treatments which cause death to the thalli. Survival could be due to either a) the gemmae being sheltered from the toxic material due to their position in the receptacles; or b) the gemmae being inherently tolerant to the material. Any inherent tolerance might be connected with the inhibitory activity of the receptacle so that germinating gemmae would differ from non-germinating (inhibited) gemmae in susceptibility.

To examine the reaction of gemmae to thiram, applications of suspensions were made to both gemmae in situ within the receptacle, and to isolated gemmae and gemmallings growing in petri-dishes on filter paper as previously described. Treatments were applied 10 days after removal from the receptacle in the case of the gemmallings and after 24 hours, in the case of the gemmae. Since inhibition of germination by the receptacle has been found to end rapidly (Taren, 1958), the gemmae were probably thus in the process of germinating, having been supplied with water and exposed to light immediately after removal. The gemmae were examined immediately before treatment and no external rhizoid growth was apparent, although it is probable that meristematic growth had commenced.

Concentrations of 0.5%, 1.5% and 4.5% a.i. thiram were applied by both immersion ('dip') and pipette ('drop') methods as previously described. Observations of treated receptacles showed the presence of white deposits on the upper achlorophyllaceous regions, and on a proportion of the exposed green gemmae. These deposits were present with all treatments but were more abundant with increasing concentrations of the suspension used, and with the 'drop' application method. Such deposits were present initially and also at the time of a second sampling of gemmae 13 days after treatment. Some gemmae were directly killed within the receptacles when treated with the 4.5% a.i. suspension of thiram, and further dead gemmae were seen at the time of the second sampling in both the 1.5% a.i. and 4.5% a.i. treatments. No live gemmae were present, and receptacles were completely dead after 33 days, with the 1.5% a.i. 'drop' treatment and with both 4.5% a.i. treatments.

Gemmae taken from treated receptacles were selected to be representative and thus in some cases they possessed adhering deposits, but otherwise all were green and apparently healthy. Table 7 shows the degree of survival recorded for three samples of gemmae each grown in petri-dish culture for 12 days. Increasing dosage caused increased mortality, and 'drop' applications tended to be more lethal than the 'dip'. This was despite the more general damage to the thallus tissue which the latter treatment induced. Mortality decreased at the second sample, but tended to increase slightly at the third.

Table 7

The effects of thiram on gemmae treated <u>in vivo</u>

Treatment	% living gammae+		
	1st sample (after 3 days)	2nd sample (after 13 days)	3rd sample (after 33 days)
Control A ≠	100	100	90
Control B ≠	100	100	100
0.5% a.i. dip	75	100	85
0.5% a.i. drop	10	100	85
1.5% a.i. dip	50	80	80
1.5% a.i. drop	15	45	*
4.5% a.i. dip	nil	65	*
4.5% a.i. drop	nil	nil	*

\+ out of 20
* no living gammae present for sample
≠ A received a distilled water dip and
B a distilled water drop treatment

Table 8 shows the isolated gemmae and gemmallings to have been more susceptible than those treated in situ, for all treatments had produced complete mortality by 12 days in the case of gemmae, while only with a 0.5% dip had any degree of resistance been offered by the gemmallings. Treatments therefore differed only in the rapidity of their effects and not in the end result. Gemmae were more quickly affected than gemmallings.

<u>Experiments on selectivity of thiram treatment</u>

The effects of thiram on higher plants when applied at concentrations required to control <u>M. polymorpha</u> were investigated using a range of 37 species commonly employed as herbicide test plants. Concentrations of 1.5% a.i. and 2.5% a.i. thiram were applied to plants both in the glasshouse (experiment I) and outside (experiment II). There were many instances of marked differences in degree of inhibition between the two experiments. These are attributable to the large differences in the environment of the experiments and in watering methods used.

Table 8

The effects of thiram on gemmae and gemmallings treated *in vitro*≠

Treatment	Gemmae		Gemmallings	
	After 2 days	After 12 days	After 2 days	After 12 days
Control A*	0	0	0	0
Control B*	0	0	0	0
0.5% a.i. dip	1.8	3.0	1.0	2.4
0.5% a.i. drop	1.6	3.0	1.2	3.0
1.5% a.i. dip	3.0	3.0	1.8	3.0
1.5% a.i. drop	2.4	3.0	1.2	3.0
4.5% a.i. dip	3.0	3.0	2.6	3.0
4.5% a.i. drop	3.0	3.0	1.6	3.0

* see Table 7

≠ Figures are mean scores of 5 replicate dishes; a 0-3 subjective scoring scale being used;
0 = green, turgid as controls
1 = some obvious growth inhibition compared to controls
2 = some damage, tissue-water soaked or partially bleached
3 = dead

The majority of species were not adversely affected by the treatments. The degree of inhibition of growth resulting from the 2.5% a.i. treatments is shown in Table 9.

Only *Papaver rhoeas* L. treated with a 2.5% a.i. spray showed appreciable growth inhibition in both experiments. *Sinapis arvensis* L. and dwarf bean showed a 30% and 45% inhibition respectively in the first experiment with sub-irrigation, whilst sugar beet and *Avena fatua* L. showed 27.5% and 35% inhibition when watering was overhead. These adverse effects generally took the form of scorch, but in the case of dwarf bean the foliage assumed a hardened and brittle condition and both tri- and unifoliate leaf expansion was arrested.

With the exception of *Alopecurus myosuroides*, Huds., and *A. fatua*, all grasses and cereals used were most affected in experiment I (sub-irrigation in the greenhouse). Other groups (e.g. Crucifers) shown no such uniformity of response.

Of the 37 species treated with 2.5% a.i. only 5 suffered a depression in excess of 15%, a further 13 being depressed by 15% and 8 more by less than 15%. With the 1.5% a.i. treatment three species (dwarf bean, sugar beet and *P. rhoeas*) were depressed by more than 15%. The following species were depressed by 15% with the 1.5% a.i. treatment; lucerne, perennial ryegrass, *Gallium aparine* and *Stellaria media* L.

Table 9

The effects of a 2.5% a.i. thiram foliar spray on
37 species of crop and weed plants

Species	% vigour depression		
	Expt. I	Expt. II	Mean
Crops:			
Cabbage	nil	nil	nil
Carrot	nil	7.5	3.75
Clover S100	nil	nil	nil
Clover S123	15.0	nil	7.5
Dwarf bean	45.0	nil	22.5
Field bean	nil	nil	nil
Kale	nil	nil	nil
Lettuce	nil	nil	nil
Lucerne	15.0	7.5	11.25
Onion	nil	15.0	7.5
Parsnip	15.0	7.5	11.25
Pea	nil	nil	nil
Sugar beet	nil	27.5	13.75
Swede	nil	7.5	3.75
Barley	7.5	nil	3.75
Cocksfoot S37	nil	nil	nil
Oat	7.5	nil	3.75
Perennial ryegrass S 23	15.0	7.5	11.25
Timothy S50	7.5	nil	3.75
Wheat	7.5	nil	3.75
Weeds:			
Chenopodium album L.	7.5	7.5	7.5
Chrysanthemum segetum L.	15.0	7.5	11.25
Galium aparine L.	nil	15.0	7.5
Papaver rhoeas L.	25.0	35.0	30.0
Polygonum lapathifolium L.	nil	15.0	7.5
Raphanus raphanistrum L.	15.0	nil	7.5
Rumex crispus L.	nil	nil	nil
Senecio vulgaris L.	nil	nil	nil
Sinapis arvensis L.	30.0	nil	15.0
Spergula arvensis L.	15.0	nil	7.5
Stellaria media L.	nil	15.0	7.5
Tripleurospermum maritimum L. sub. sp. inodora	nil	nil	nil
Agropyron repens (L) Beauv.	7.5	nil	3.75
Agrostis stolonifera L.	nil	nil	nil
Alopecurus myosuroides Huds.	nil	15.0	7.5
Avena fatua L.	nil	35.0	17.5
Poa annua L.	15.0	7.5	11.25
Liverwort			
Marchantia polymorpha L.	80.0	85.0	82.5

DISCUSSION

It can be seen from the foregoing review that M. polymorpha is a special weed problem occurring only under a limited set of environmental conditions, but that control is not easily achieved. If the structure of the gametophyte is considered from the viewpoint of its reproductive potential then the properties required of any suitable 'hepaticide' are quite considerable.

Practical application of results

The results obtained with thiram indicate that a practicable degree of control of 80%, can be achieved either by use of a dust in a sand carrier, or with an aqueous foliar spray. The requirements for success of the latter are a)the use of a relatively high concentration of 1.5-2.5% a.i. and b) the application of a sufficiently complete cover. The concentrations required are approximately five times those needed for the inhibition of fungal spore germination. A good cover is also important owing to the relatively localised contact action although no advantage is gained by increasing volume rates above the capacity of the tissues for retention.

The time of application in relation to thallus development is important. Applications of thiram before mature gemmae have formed will prevent regeneration, for a complete kill of non-gemmiferous thalli is easily achieved. Mature thalli killed by one thiram application are likely to leave behind some gemmae capable of growth and development. A second spray applied after the old thalli have died and some growth of new thalli has occurred, but before the commencement of gemmae production should result in control. Reinfestation, particularly by means of spores may occur and require further treatment.

The relative lack of phytotoxicity of thiram to higher plants enables it to be used for selective control of M. polymorpha. Many annual species tested in the present trial were unaffected by the material. Van der Laar (1964) found seedlings of Berberis cult atropurpurea to be unharmed by 2.0 a.i. thiram. Thiram sprays of 14.5% a.i. did no damage when applied to rhododendron seedlings (Netherlands 1962).

Allowing for two consecutive applications of 200 g.p.a. at 2.0% a.i. conc., 80 lb/ac thiram will be applied. The long term effects of such a dose require consideration. From a short-term economic viewpoint, the material cost is in the order of £50/acre for two applications, using an 80% a.i. wettable powder at 10/- per lb. Although expensive the treatment may be justified if other methods prove impracticable, for the areas treated will generally be small and the plants infested of high value.

Survival of gemmae

The susceptibility of the gemmae within the receptacle to thiram has been observed and their susceptibility in isolation has been shown by experiment. This indicates that survival of gemmae is due to operation of a protection mechanism. This is most likely to take the form of the uppermost (older) gemmae sheltering those beneath from any deposits. This infers a lack of penetrating power by thiram which may be associated with its low water solubility.

Subsequent establishment of surviving gemmae is possibly quite hazardous due to several factors. Firstly the gemmae are likely to become more susceptible to thiram once they start active growth. (Death of the thallus removes the source of germination inhibition). Secondly the cessation of further gemmae production following death of the thallus removes one mechanism of escape from the receptacles, although this may be negated due to decomposition of the dying receptacle walls. Thirdly any surrounding areas otherwise suitable for establishment may contain thiram deposits. The toxicity of such 'weathered' deposits requires to be established. Despite these factors gemmae do become established and provide a source of reinfestation.

Activity of thiram

From observation, the gross effects of thiram on M. polymorpha is to cause tissue necrosis by direct contact action. Tissues not directly contacted may also be eventually killed once the surrounding connective tissues are disorganised, although this may not be the case if such 'escaping' areas possess apices.

Other materials for control

The usefulness of materials chemically related to thiram requires to be investigated. High doses of thiram are required for control and other more potent compounds which would reduce the cost and increase efficiency of control are needed.

The precise mode of action involved in the growth inhibitory properties of thiram and related compounds is not fully understood (Thorn and Ludwig, 1962). Thiram and DMDC (dimethyl dithiocarbamate) are considered to be equivalent fungicidally and the direct derivatives of sodium DMDC are available commercially as fungicides ('Ferbam' and 'Ziram'). These effects of these materials on M. polymorpha require to be evaluated.

Penetration of the tissue

Structure, thickness and composition of the plant cuticle effect the entry and thus influence activity of toxic materials (Martin, 1966). The cuticle on the dorsal surface of M. polymorpha is described as thick and impermeable, being instrumental in reducing water loss. Although stomata are absent, a mode of entry to the underlying parenchymatous tissues exists in the form of the air pores which are rigid structures allowing for gaseous interchange. These are of small diameter (circa. 0.056 mm) and thus not easily penetrated. The success of materials, in controlling M. polymorpha will however depend to a great extent on their ability to penetrate into the gemmae cups and kill the gemmae in situ, in addition to affecting the non-reproductive parts of the thalli. Such penetration might be aided by the use of additional surface active agent in the spray.

Conclusions

The adoption of new growing methods such as the use of peat/sand composts, mist propagation and overhead irrigation systems and the increasing tendency to grow plants in containers provides more situations in which liverworts may appear. Similarly increasing use made of residual herbicides may present open sites free from competition in which liverworts may occur and survive, as have mosses. It is therefore of importance to gather information and have some means of control available for these events. The use of thiram offers at least an interim method of control.

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