HAMAMELIS SEED GERMINATION

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Abstract *Hamamelis virginiana* seed contains complex dormancy factors involving not only the embryo but also the endosperm and possibly the integument and testa Optimum natural conditions for breaking dormancy in fresh seed are at least 8 weeks at about 20°C, followed by at least 20 weeks below 4°C, both in moist peat Neither warm nor cold alone will break embryo dormancy, but warm in some way sensitises the embryo to react to subsequent cold While dormant, the embryo produces a lipase inhibitor which presumably diffuses into the endosperm and prevents premature mobilisation of the fatty storage reserves. For the warm treatment a soak in gibberellic acid can be substituted, but a substitute for cold has not been found.

INTRODUCTION

Hamamelis (witch hazel) seeds are shed in autumn and, under natural conditions, most of the survivors germinate 18 months later after enduring a natural cold-warm-cold cycle. Some germinate 12 months later, having had a further period of warm and cold. In the nursery, seeds of this species have been regarded as difficult to germinate because they are slow and unpredictable, and may often give very low germination percentage. However, our business demands planned production of tens of thousands of plants per annum from a limited seed supply, so a higher success rate and greater predictability are essential.

The work described in this paper began in 1979, when we made our first serious attempt to harvest and germinate seed from our own plantation. In early November about ¼ million fresh seeds were mixed in moist peat and put in a warm place; after about 8 weeks they were transferred to a domestic refrigerator, and 20 weeks later we had 90% germination. The following year a similar procedure, but with a reduced warm treatment failed to produce significant germination. A quick experiment with mixtures of gibberellic acid and Ethrel revealed a treatment which seemed capable of kicking the seeds into action. This treatment was applied to the bulk of seed and satisfactory results were achieved. But we did not know why or how it had worked and we had no confidence that it would work again next time.

We felt, therefore, that we must try to understand more about seed dormancy in *Hamamelis* and, through that understanding, develop seed treatments which would enable us to achieve some measure of control over germination. We thus embarked on two projects: 1) To look at chemical seed treat-

ments, and to see if it might be possible to shorten or eliminate either the warm or the cold stratification periods, or both, and 2) To test various combinations of warm and cold to try and find clues as to what the seeds may be doing during these periods, and thus to elucidate the nature of dormancy in this species.

A search of several computer data-bases, both in the U.K. and in the U.S.A., failed to reveal any previously published work on seed dormancy and germination in *Hamamelis*.

MATERIAL AND METHODS

1. Chemical Treatments. With seed that has been through warm and cold, yet shows no sign of germination, our results have been rather variable. We have always succeeded in stimulating germination, but best results have not always been with precisely the same treatment. In general we have found 2% Regulex[1] plus 0.02% Ethrel[2] (in water at room temperature for 24 hours) quite good. Higher concentrations of Regulex have been less effective, and there has been no advantage in increasing the soak to 48 hours. As for possible influence of Regulex soaks on subsequent seedling growth, we have found, perhaps surprisingly, no statistically significant effect.

With unstratified seed, we have found that Regulex can substitute for the warm treatment, but the normal period of cold is required thereafter. We have not found any substitute for the cold.

2. Warm and Cold Treatments. All seeds were from the same fresh seed-lot, harvested from our own plantation of *H. virginiana*, and were graded for uniformity of size. For each treatment at least 100 seeds were mixed with about 200 ml moist peat, and sealed with plenty of air in a marked polythene bag. Each bag was placed in either "warm" or "cold" as the treatment schedule required. "Warm" was in a thermostatically-controlled room with fluctuating day/night temperatures of 24/16°C; "cold" was in a domestic refrigerator at 0 to 4°C. In both environments, bags were turned and moved around at least once every two weeks; at the same time, in the warm only, bags were opened briefly for ventilation. Treatments were 0,2,4, or 8 weeks "warm", followed by 0,2,4,8, or 16 weeks "cold", followed by a further 0,2,4, or 8 weeks "warm", and a final 0,2,4,8, or 16 weeks "cold" — a total of 308 different treatments.

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[1] Regulex (ICI, Plant Protection Division) contains 10 g l^{-1} of a mixture of gibberellins A4 and A7

[2] Ethrel (Amchem Products, Inc) contains 2-(chloroethyl) phosphonic acid

At the end of each treatment we applied the following tests:

a. Fifty seeds were sown in moist peat to assess germination percentage.

b. Embryos were removed by dissection from 10 seeds and were placed on sterile oil-emulsion agar: 1) to assess their ability to grow without the influence of other seed tissues, and 2) to detect lipase enzyme activity (we thought that when the embryos were ready to germinate they might produce lipases to digest the fatty endosperm storage tissue).

c. The endosperm tissue from those 10 seeds was macerated in water which was then used, unfiltered, to irrigate 20 radish seeds on seed-test papers in Petri-dishes. This test was applied only after week 16.

d. The remaining seeds were weighed, dried at 105°C, and re-weighed to measure moisture content.

RESULTS

Treatments were identified by a series of four numbers which represent weeks in warm, cold, warm, cold, respectively: thus, 4,8,2,16 means 4 weeks warm, 8 weeks cold, 2 weeks warm, 16 weeks cold. Treatments such as 2,4,0,8, where the two cold treatments are consecutive may be referred to as 2,12. In either case, the first number indicates warm.

1. Whole Seed Germination.

a. Warm only and Cold only. Irrespective of duration, there was no germination in any of these treatments.

b. Warm/Cold. See Figure 1. At least 8 weeks cold were needed for any germination to occur. As cold increased, up to 24 weeks, germination increased, but 32 weeks appeared too long. For any given length of cold, longer warm gave better germination, although it is clear that where cold will be 20 weeks or more there can be little to be gained from more than 8 weeks warm. Where total seed-treatment time is limited, there is clearly an optimum division between warm and cold. For example, 28 weeks divided 12,16 gave 72% germination, whereas 8,20 gave 83%; 4,24 gave only 50%.

c. Cold/Warm/Cold. See Figure 2. As there is little germination below 16 weeks final cold, the results are illustrated here by 15 treatments all of which end in 16 cold. Initial cold causes greater fluctuations in germination as the intervening warm becomes longer, but there is no clear trend in the results. It is curious, however, that the top two curves are mirror-images of one another, and it is a matter for speculation

whether there may be conclusions of ecological significance to be drawn from the shape of either.

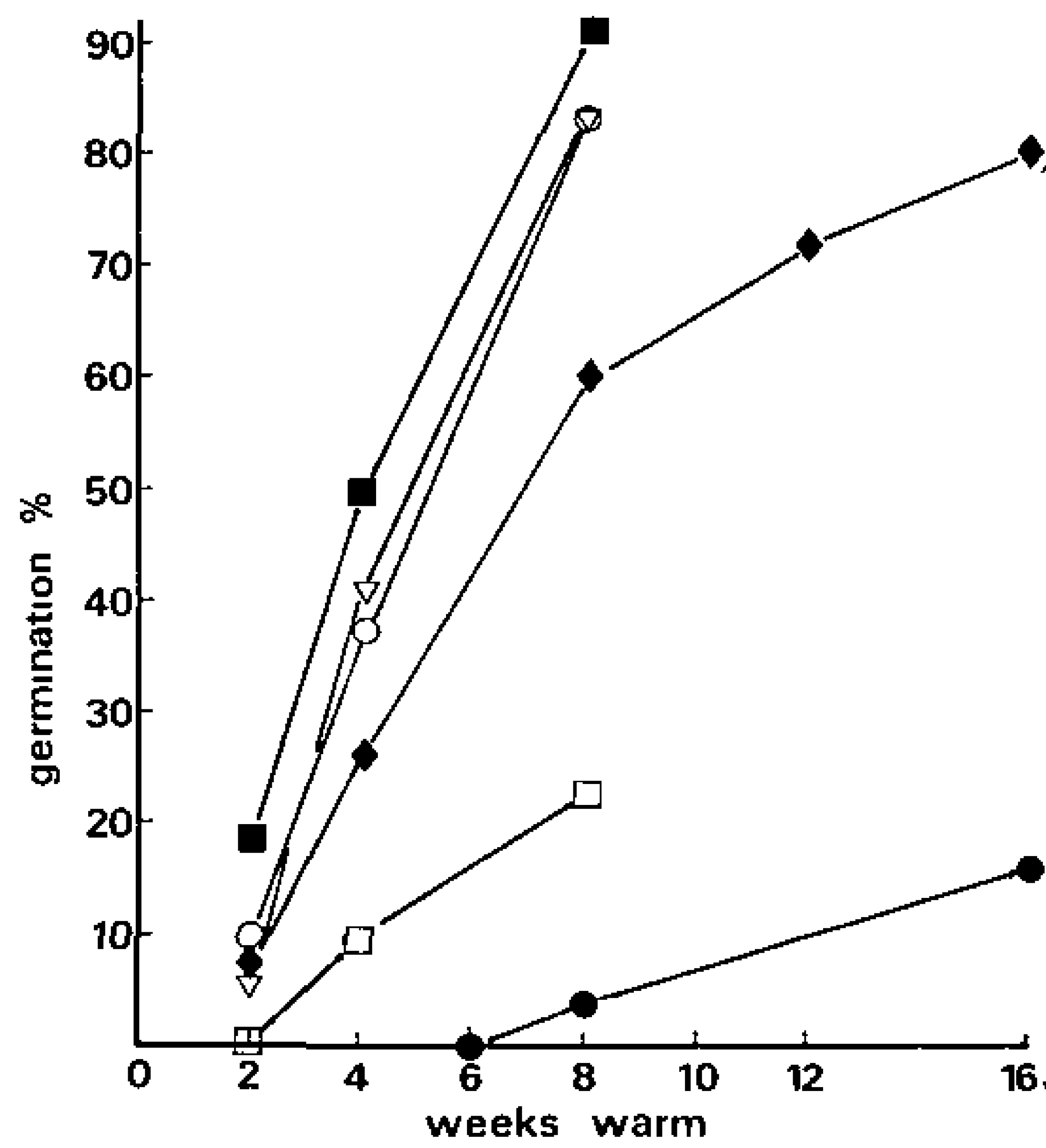

Figure 1. The effect on germination of 0 to 16 weeks warm, followed by 8(●), 12(□), 16(♦), 20(O), 24(■), 32(∇) weeks cold.

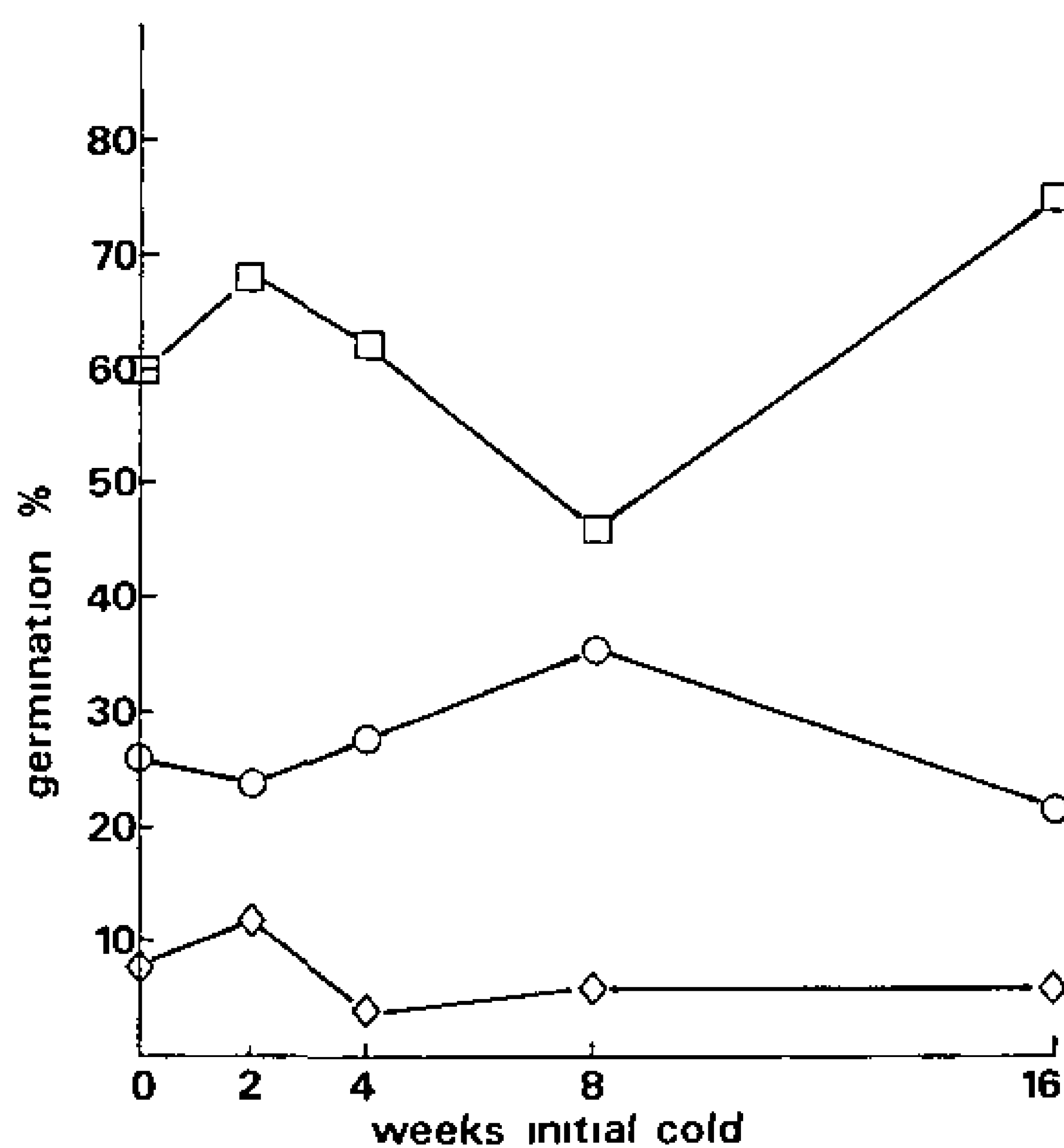

Figure 2. The effect on germination of 0 to 16 weeks initial cold, followed by 2,16(◇), 4,16(O), 8,16(□)

d. *Warm/Cold/Warm.* These treatments are not significant for whole-seed germination, since the final warm is equivalent to a germination test after a Warm/Cold treatment.

e. *Warm/Cold/Warm/Cold.* We have already seen that an extended cold period of at least 16, preferably 20 or 24, weeks is desirable, following at least 8 weeks warm. Thus this sequence of treatments effectively becomes a test of interrupting the cold with various lengths of warm at various points during the cold phase. For example, a treatment such as 8,24 can be

divided 8,8,-,16 or 8,16,-,8; while 8,20 can be 8,4,-,16 or 8,16,-,4.

The results are very clear (see Figure 3.). For 8,20 or 8,24, there is no effect on germination if the warm break occurs during the first half of the cold period, neither does the length of warm break matter. However, if the warm break is in the second half of the cold period there is a significant reduction in germination, which reduction tends to get greater as the length of warm increases. Treatment 8,16 can only be divided 8,8,-,8 (i.e. in the middle of the cold period), and here also there is serious loss of germination. Treatment 8,12 also shows reduced germination when the warm comes in the second half of the cold period, but is unique in showing increased germination from a 2 or 4 week warm break early in the cold period.

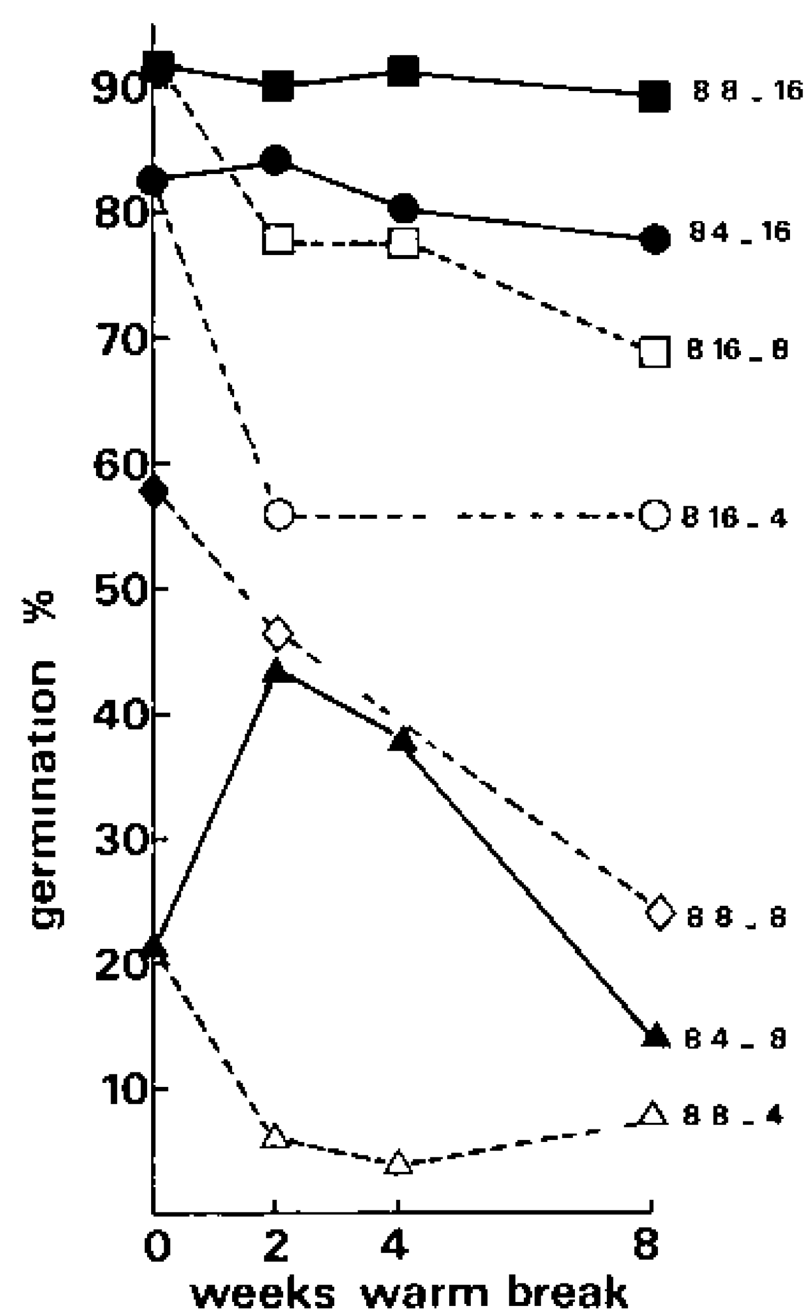

Figure 3. The effect on germination of 0 to 8 weeks warm break during the cold period in treatments 8,12(▲), 8,16 (◆) 8,20(●), 8,24(■) Warm break in either first half (——— and solid symbols) or second half (----- and open symbols) of cold period.

2. Embryo Tests.

a. Growth. With warm only; some embryos showed a slight tendency to greening of cotyledons and extremely limited radicle growth but there was no significant visible development, irrespective of length of treatment. With cold only, there was even less tendency to development. When 8 weeks warm were followed by increasing cold, the results generally paralleled those for whole seeds. There was little activity at 4 weeks cold, but with 8 weeks most embryos developed expanded green cotyledons and significant active root growth. After 16, 20 or 24 weeks cold, 80 to 100% of embryos were very active; but after 32 weeks cold, 40% appeared dormant, while others were much less vigorous.

b. *Lipase Activity*. Our search for lipase activity in the embryos was fruitless, but we did discover strong evidence for a lipase inhibitor. Because our working conditions were not microbially clean, we often got bacterial or fungal contaminants on our agar plates; many of these produced lipase which digested the oil emulsion and thus produced a zone of clearing around the colony. We observed that wherever this zone of clearing approached a dormant embryo, the clearing was inhibited; there was no such inhibition from active embryos (Figure 4.). The shape of the inhibition zone suggested that the cotyledons were the source of the inhibitor.

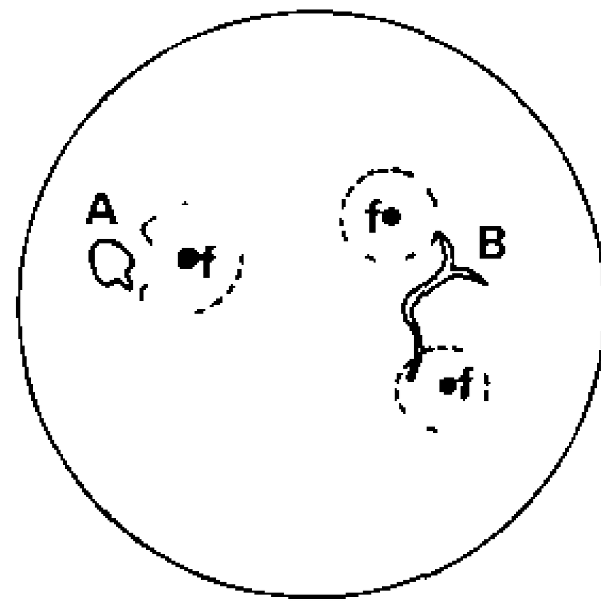

Figure 4. Diagram of one dormant (A) and one growing (B) embryo on oil-emulsion agar, with three lipase-producing fungal colonies (f) Dotted lines mark edge of cleared zones Clearing is inhibited by A, not by B.

3. Endosperm Test

This gave very clear evidence of a germination inhibitor, active against radish seeds, which was present in the *Hamamelis* endosperm after some treatments but not after others. A striking feature of this test was the very clear-cut nature of almost all the results: the radish seeds either germinated as well as the control (water only) seeds, or their germinaiton was completely inhibited.

We expected that the results of this test might correlate with those of the embryo test and the whole-seed germination test in such a way that germination would only occur if the embryo was ready for growth *and* the endosperm showed no inhibitor; either lack of readiness in the embryo, or inhibitor in the endosperm should prevent germination. In fact, this did not always happen (see Table 1.).

The first four examples in Table 1 show the expected results, where low germination correlates with either no embryo growth (2,16,2,4) or presence of inhibitor (0,16,8,4) or both (2,8,8,4), and high germination correlates with embryo growth and absence of inhibitor (8,8,8,16). The other two results appear anomalous: in 8,4,2,16 the inhibitor has failed to prevent germination, while in 8,4,8,4 there has been no germination despite positive embryo growth and absence of inhibitor. Possible explanations for these anomalies are discussed later.

Table 1. Whole seed germination, embryo growth, and endosperm inhibitor after various treatments

Treatment	Endosperm Inhibitor	Embryo Growth	Whole seed Germination
2,16,2,4	−	−	6%
0,16,8,4	+	+	4
2,8,8,4	+	−	2
8,8,8,16	−	+	88
8,4,2,16	+	+	84
8,4,8,4,	−	+	0

+ = presence of inhibitor or growth of isolated embryo
− = absence of inhibitor or no growth of isolated embryo

4. Moisture Content.

Moisture contents ranged from 45.5% to 70.0%. Excluding those treatmens which gave 5% germination or less, germination and moisture content were closely and positively related ($r=0.85$; $p<0.001$); see Figure 5. This correlation presumably relates to the conversion of osmotically inactive lipid storage materials to osmotically active carbohydrates.

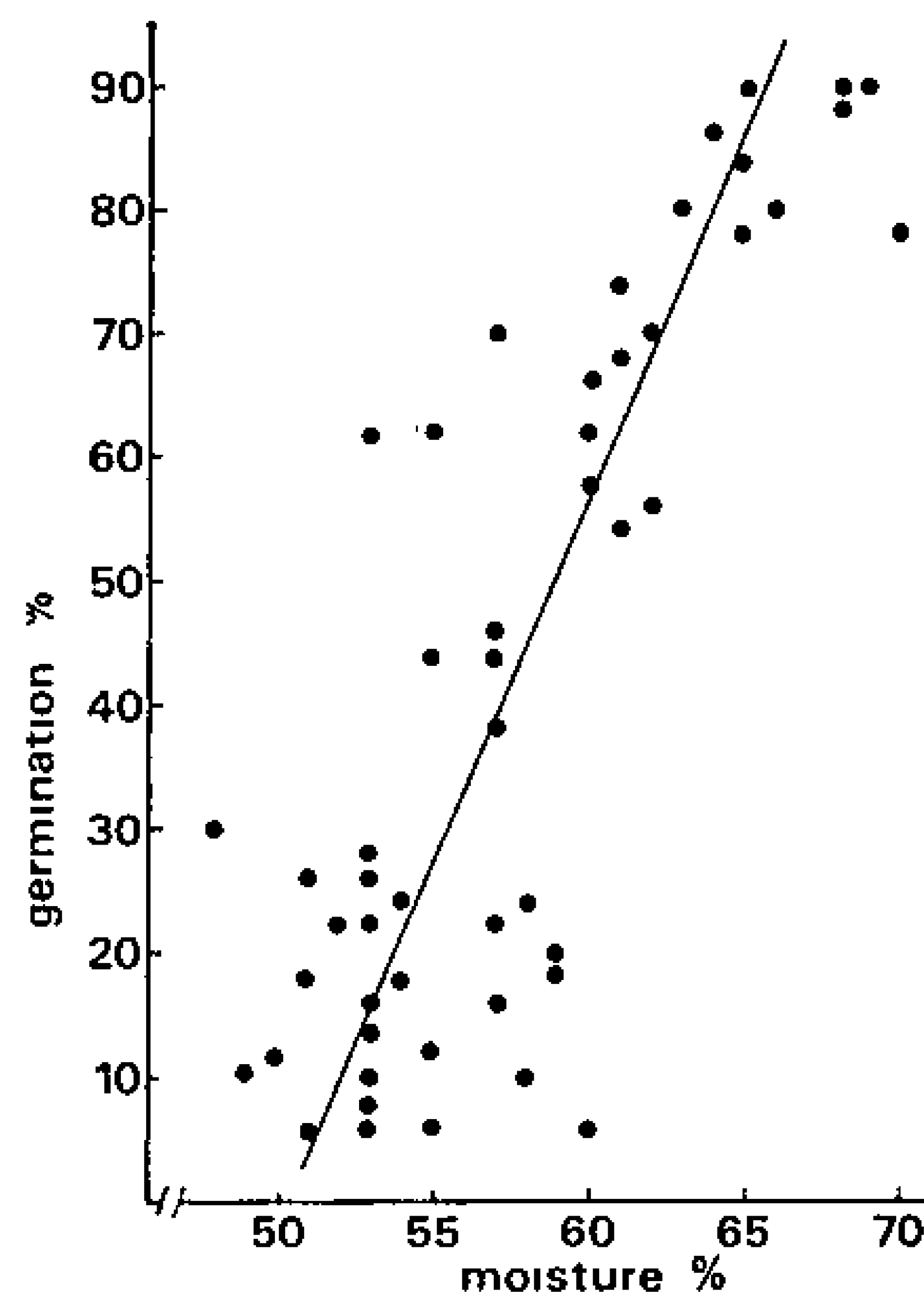

Figure 5. Correlation between germination and seed moisture content. Line fitted by regression analysis ($x=0.17y + 50\ 4$)

DISCUSSION

Before we began this work, we felt from experience that the length of the warm period might be critical, and that it might be possible for seeds to be too long in the warm period. Our results do not support this hypothesis: certainly 8 weeks warm seems to be a practical minimum, but there is no evidence that longer warm is detrimental; indeed 12 or even 16 weeks warm are beneficial if subsequent cold is sub-optimal.

Clear evidence has been presented that dormant embryos produce a diffusible lipase inhibitor, presumably acting on

lipases in the endosperm. Production of this inhibitor appears to cease only when embryos have received a suitable combination of treatments to enable them to start growth. Since the contamination which enabled us to observe this phenomenon was fortuitous, it occurred on only some plates and even then was very variable, future work would require more standardised conditions.

The anomalies in our endosperm/radish seed test need further study. Treatment 8,4,2,16 achieved 84% germination despite the apparent presence of the inhibitor; this can be explained if we hypothesise that inhibitor from a single endosperm is sufficient to produce the effect on radish. Our tests were conducted with the combined extract of 10 endosperms; if inhibitor from just one is sufficient, this could explain why we have found the test so definite in almost all cases. If none of the endosperms contain an inhibitor, the radish seeds germinate, if one or more contain an inhibitor, the radish seeds do not germinate. Our results do not provide sufficient evidence to test this hypothesis.

To explain the result with 8,4,8,4 where there was no germination despite embryo growth and apparent absence of endosperm inhibitor, requires that we postulate yet another source of inhibition, and we have two candidates to propose: the testa and the integument. The testa has been shown by colleagues at Plymouth Polytechnic, using the radish-test (S.D. Lane, personal communication), to contain one or more potential inhibitors, but we have not attempted to consider it in this study. It is often remarked that the very hard testa is perhaps a barrier to imbibition; there is however a relatively soft micropylar region and we have shown in other studies that whole seeds, cracked seeds, and seeds from which the testa has been completely removed, have identical rates of imbibition.

The integument is a membrane immediately beneath, but not attached to the testa. It is intimately connected to the endosperm and often appears to be very tough. It is quite possible that, under certain conditions, this membrane constitutes an effective barrier to gaseous exchange. Furthermore, we have often observed, outside this particular experiment, germinated seedlings which emerge with the cotyledons seemingly trapped within the integument; whether this is due to the integument's strength or to developmental incapacity on the part of the epicotyl, we do not know.

CONCLUSION

Our results have been presented largely in qualitative form because the treatments were unreplicated and most of

the information is not amenable to statistical analysis. Had we replicated, the already large number of operations would have become utterly unmanageable. Nevertheless, we have provided a practical basis on which it should be possible to achieve high germination regularly; and we have highlighted several areas, which will be of great interest to academic researchers, where further work could be done.

PROPAGATION AT BRIDGEMERE NURSERIES

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I have worked at Bridgemere Nurseries in Cheshire for the last 14 years. I achieved the position of head of propagation some 9 years ago, due to a knowledge based purely on practical experience and advice from fellow propagators.

I have a minimal involvement in field propagation and this is limited to fruit trees and the easy evergreens, such as laurel and *Vinca* spp. The latter are propagated under low polythene tunnels on a sheltered, well-drained section of the field.

The breakdown of the 800,000 cuttings which are rooted by my department each year, is as follows: 30% shrubs, 25% heathers, and 25% conifers, the balance being split among everything from Exbury azaleas, *Pieris* spp., *Mahonia* spp., with about 5% of this balance being climbers.

The second type of propagation under my control is the division of bareroot herbaceous plants, a crop which is increasingly being home nursery produced. The reason for this is customer demand which is creating a demand for almost limitless cultivars of plants of all kinds. This means that I am constantly having to add new plants to my propagation lists, the present range covering some 750 different ones. Sadly, once the newer cultivars become popular, we have to axe some of the more traditional lines in order to keep a careful balance.

One of the few types of hardy plants which we do not propagate as yet are the alpines, but we may one day add them to our range.

Propagation Techniques. In the main, our cuttings are rooted in Macpenny mist units, which provide mist between March and October.