FERTILE SEED PRODUCTION AND SELF-INCOMPATIBILITY OF *HYPERICUM CALYCINUM* IN ENGLAND

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ABSTRACT

Hypericum calycinum is shown to produce, in England, a small number of viable seeds which, in well-grown capsules, approximates to a constant proportion. Ovaries contain an average of some 800 or more ovules which, following self-pollination, exhibit a wide range of development. Well developed seeds yield a far higher percentage germination if sown when immature and it is suggested that self-incompatibility manifests itself not only through the first male gamete by degrees of failure in embryo development but also, through the second male gamete by inefficient endospermic nutrition during the attainment of the resting stage in this exalbuminous species. Germination, which extends over a period of months, is of the intermittent type, whether at normal or higher temperatures, and appears to be photoperiodically indifferent. Seedlings have not, however, been observed near colonies and possible reasons for this are discussed.

The Rose-of-Sharon (*Hypericum calycinum*) is found growing wild in south-eastern Europe and was introduced into this country in 1676 by Sir George Wheeler who had collected the plants from the neighbourhood of Constantinople (*Bot. Mag.* t. 146 (1796)). It has thus been with us not far short of three centuries and is more or less naturalised in many localities throughout Great Britain and Ireland, especially in the southern counties of England (Perring & Walters 1962, p. 57). More than sixty years ago, however, Hanbury & Marshall (1899) commented upon the fact that, although then well established in a few places in Kent, the Rose-of-Sharon apparently never spread in this country ' except by root extension '. So too, that excellent field botanist, James White, stated that ' this species is believed not to ripen seed anywhere in this country but to spread and maintain itself by root-extension only '(White 1912). Most gardeners and field botanists would probably subscribe to this generalisation although the phrase ' root-extension ' is liable to mislead, since the vegetative spread of *H. calycinum* is mainly, if not exclusively, by means of axillary underground stems. The distinction is significant since in other members of this genus, for example *H. perforatum* and *H. pulchrum* (see Salisbury 1942, Fig. 32), it is actually the roots which are responsible for the vegetative extension, since adventitious shoots arise from them. The production of such root-shoots can be greatly stimulated in *H. perforatum* by cutting down the erect stems but I have observed no such response in *H. calycinum*. Vegetative extension takes place slowly. The stoloniferous shoots (Fig. 3) grow obliquely upwards from the underground portion of the previous year's leafy shoots. The number arising from each is usually two to four, commonly paired and involving from one to three nodes. Each stolon bears some 8 to 15 pairs of small brown scale leaves below the soil and develops normal foliage leaves on reaching the light. The internodes do not usually attain a length of more than 1–2 cm so that the radial extension in a season rarely exceeds 15 to 20 cm. The very large areas, as along the cutting between Leatherhead and Dorking, that are often occupied exclusively by the Rose-of-Sharon are visual evidence both of the efficacy of its stoloniferous spread and its stability.

A similar generalisation concerning the absence of reproduction by seed in Britain has been made with respect to the two Periwinkles (*Vinca major* and *Vinca minor*) likewise characterised by conspicuous vegetative vigour. Both these species I recorded as having fruited successfully in this country in 1961 (see Salisbury 1961). In the warm late autumn of 1961, examination of capsules of *H. calycinum* revealed a small number of, apparently, fully developed seeds and this discovery, as also my experience with the species

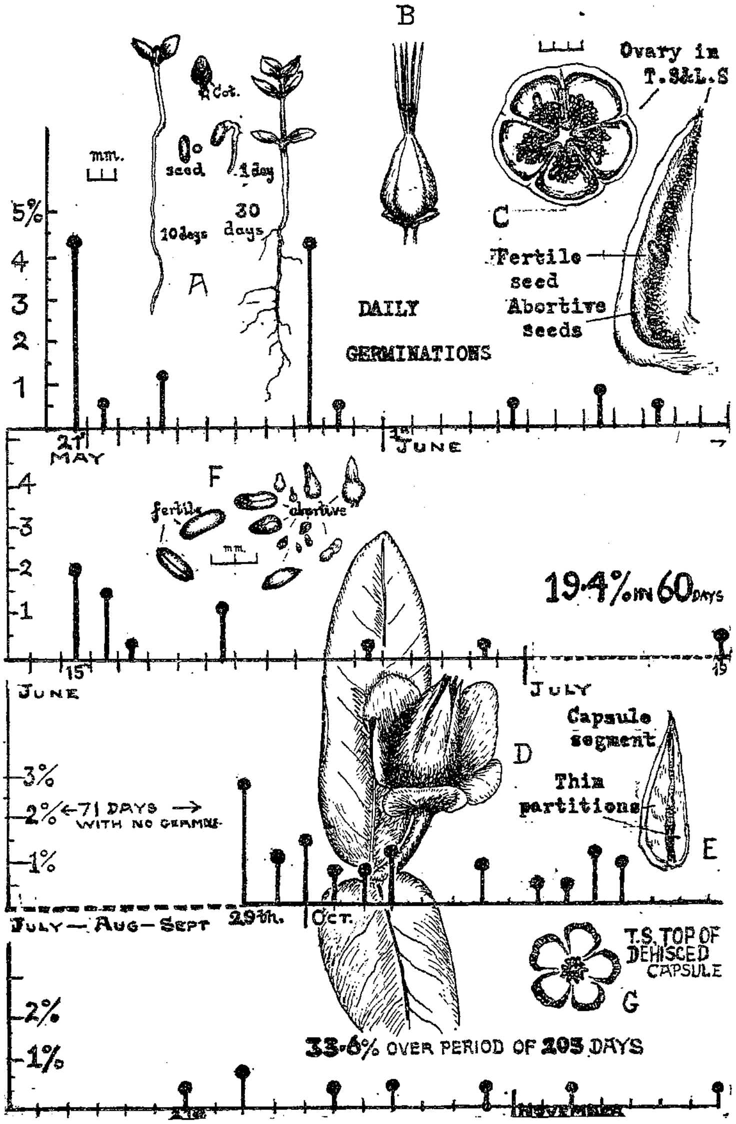

Fig. 1. Intermittent germination of 300 apparently fertile seeds of *H. calycinum*, with (inset) details of the fruit, seeds and seedlings. Magnifications as shown by millimetre scales.

of *Vinca*, led me to look carefully at diverse colonies of *H. calycinum*, both in 1961 and subsequently, from which it would appear that some seed production, though very sparse, is not uncommon, at least in southern England.

Normally the Rose-of-Sharon is probably self-incompatible and, again as with the Periwinkles, this would tend to be accentuated by the large colonies formed by the vegetative spread of a single individual, since the attentions of pollinating insects might be confined to the flowers on only one plant, even if another individual were in the neighbourhood. Moreover it is quite likely that the entire population of *Hypericum calycinum* in this country may have been derived from a single stock. However that may be, the five styles that surmount the ovary are relatively long (see Fig. 1 *B*) so that perhaps only exceptionally favourable weather conditions when pollination occurs, especially with regard to temperature, can enable the pollen-tubes from the plant's own pollen to traverse this distance and grow sufficiently rapidly to effect fertilisation. Eight large capsules obtained in October 1961, and eight more in November 1962, were dissected carefully and the numbers of apparently fertile seeds, abortive seeds and ovules alike counted with the following results.

Fully-developed apparently 'fertile' seeds	*Percentage 'fertile' seeds*	*Obviously abortive seeds*	*Total number of seeds in capsule*
11	1·8	581	592
18	2·9	597	615
22	3·4	629	651
7	1·0	647	654
13	2·0	647	660
37	5·1	678	715
36	4·7	721	757
24	3·2	767	791
18	2·1	828	846
14	1·6	840	854
15	1·5	966	981
27	2·7	964	991
19	1·8	1040	1059
11	1·0	1071	1082
35	3·2	1058	1093
12	1·0	1155	1167
Average number of fertile seeds 20 [s. d. 9·6: s.e.m. = 2·4]	Average % 2·4	Average number of abortive seeds 824	Average total number of seeds in capsule 844 [s.d. 261: s.e.m. = 65]

Those sixteen capsules were deliberately selected because of their exceptional size and might therefore be expected to contain the highest proportion of fertile seeds and thus provide an indication of maximum fertility. In these the observed range was from seven to thirty-seven with an average of twenty. A random sample, of a number of other 1961 capsules, was also examined, in which the apparently fertile seeds were alone counted, and these provided an average of only 6·2 per capsule.

The apparently fertile seeds were 1·5 to 2 mm in length and from 0·7 to 0·9 mm in width. Almost cylindrical in shape, with rounded ends and a slight ridge along one side, the symmetry of the seeds, as well as their size, usually distinguishes the viable from the abortive ones. The colour is dark brown and the average weight of a seed is 0·00058 gm (the largest from 0·0006 to 0·0007 gm). This is about five or six times the weight of the seeds of the herbaceous species (see Salisbury 1942); they are, nevertheless, sufficiently small and light for gusts of wind to disperse them readily (Fig. 1, C & F).

Subsequently, three hundred of the 'apparently fertile' seeds, harvested in late autumn, were sown on 1 May following. The outcome is presented in the accompanying histogram (Fig. 1) from which it will be seen that germination began within three weeks and was then intermittent through May and June, with a total of only 19% in the first forty days followed by a pause of seventy-one days and then a further surge of germinations bringing the total to 33% in about 5½ months with only two more (0·6%) on 15 December, after 205 days. It should be emphasised that, in order to obtain a sufficiency for a representative test the seeds were collected from various plants at different times. More seeds were collected in 1962. Of these some were harvested from a number of capsules, produced by a single colony and all obtained and sown on the same day, 4 November. There was a total of 183 apparently good seeds but whereas 106 were deep-brown and presumably mature, the remaining 77 ranged in colour from cream to pale-brown and were clearly immature. Both batches were sown side-by-side in the same seed-pan and placed where the temperatures during the daytime attained about 20° C and the minimum night temperatures were about 5° C.

It was anticipated that the immature seeds might yield a low germination but, in fact, their percentage was the highest observed for the species, namely 92%, and the much lower percentage germination of the riper seeds from the same capsules, namely 62·2%, would strongly suggest inadequate nutrition by the endosperm during the later stages of maturation, a point to which I shall revert. The pattern of germination, in both batches, was again intermittent but, as might be expected from the temperatures being appreciably higher than those in which the 1961 seeds were germinated, it was far less protracted (Fig. 2) occupying about ninety-nine days instead of two-hundred and five. A point that may be of significance is that although the number of viable seeds was almost the same in both the 1962 groups, germinations of the immature seeds took place on only twenty-three separate days compared with germinations on thirty-three days for the mature seeds.

When considering self-incompatibility attention has, hitherto, been almost exclusively focussed upon the normal embryo which, in endospermic seeds, has achieved its resting stage *before* the transfer of food from the endosperm. In an exalbuminous seed such as that of *H. calycinum*, the resting stage is not attained till *after* the endospermic nutrition has been transferred.

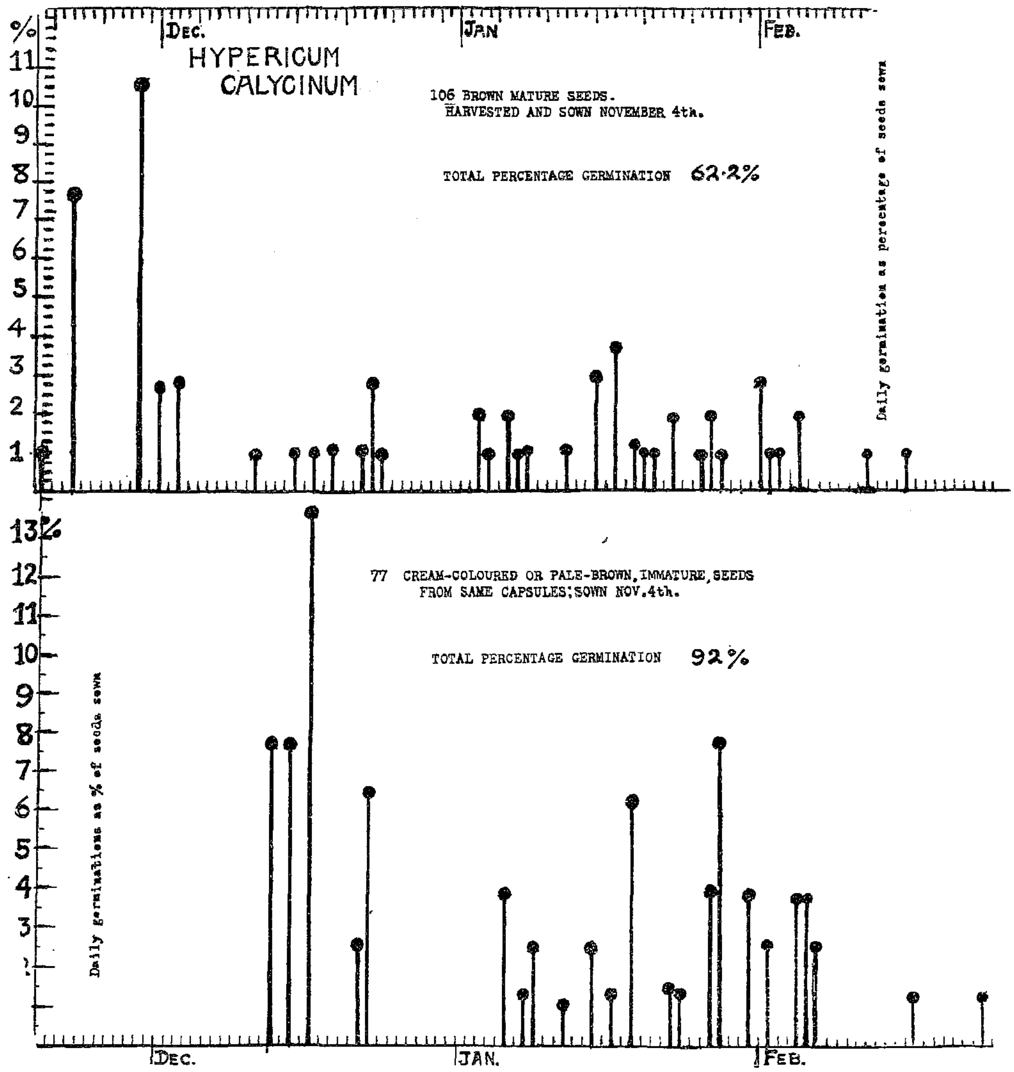

Fig. 2. Germination of fully formed mature and immature seeds from the same capsules sown immediately after harvesting, showing much higher percentage germination of latter.

Having regard to the fact that partially developed embryos were found in some of the seeds regarded as abortive and which a green coloration suggested were present in an appreciable proportion, 1,800 of the better-developed of these were sown (at the same time as the 'good' seeds and under like conditions), to check the validity of one's assessment of their abortive character.

Of these seeds which, from their size and shape, were deemed to be abortive, two germinated after 68 days and ten more between the 83rd and 116th day after sowing. The error, in judging the abortive seeds visually, would then appear to be almost negligible and, since those tested consisted of the most promising only, the error of assessment was certainly much less than 6 in 1000. It is, moreover, not unlikely that this small fraction proved viable only because they were sown immediately and should be considered in the

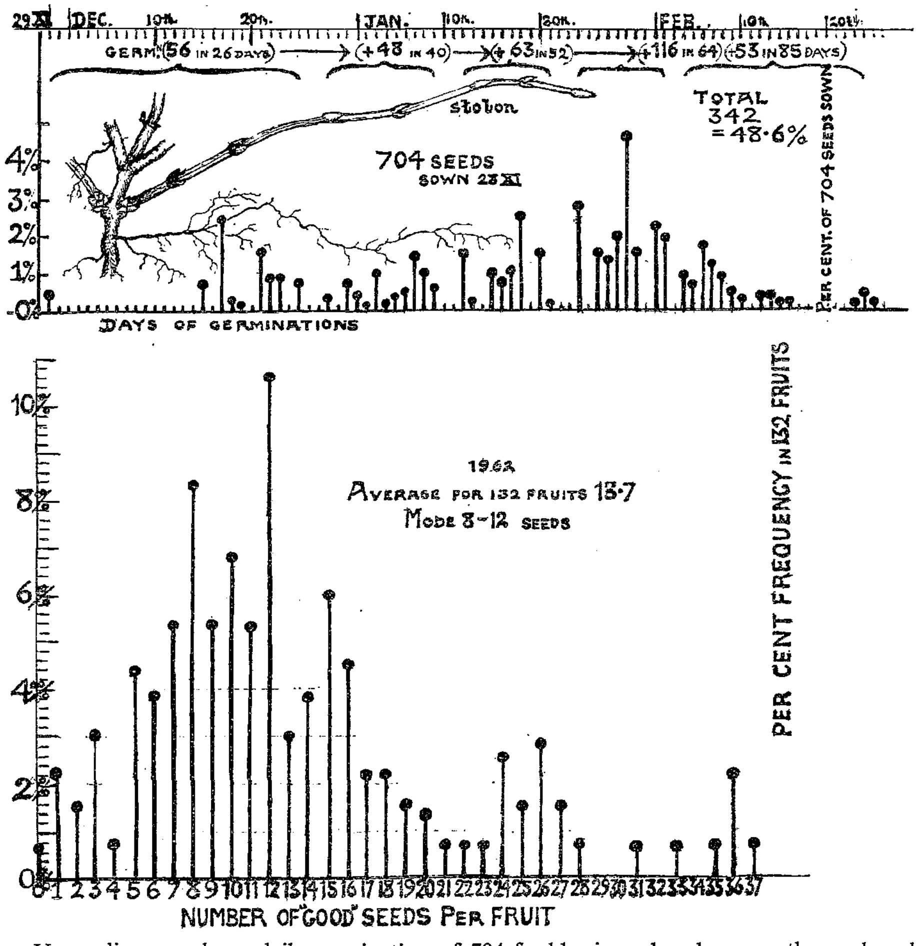

Fig. 3. Upper diagram, shows daily germination of 704 freshly-ripened and apparently good seeds over period of 85 days. Inset, drawing of stolon half natural size. Lower diagram shows number and frequency of 'good' seeds in 132 fruits.

light of the enhanced percentage germination of immature seeds. However, the noteworthy feature that these germination tests collectively reveal is that there is obviously no abrupt transition from the viable to the non-viable but the distinction between them is one of degree rather than of kind. Most of the fruits examined were from Sussex plants growing at approximately 49° 48′ N. Fruit collected near Wendover (c. 51° 45′ N) contained some apparently fertile seeds and it would be of interest to know how far northwards the ability to produce fertile seeds can extend[1]. It should be noted that there is no obvious negative correlation between seed production and the formation of stolons (cf. Fig. 3), and that the emphasis here on vegetative spread, rather than seed development, is not, as in so many species, a concomitant of polyploidy since the Rose-of-Sharon is a diploid species with twenty chromosomes (Darlington & Wylie 1955 p.114).

From various localities 132 fruits were collected in October 1962, before they were quite mature, but beginning to change colour. As the seeds at this stage are not yet fully pigmented, one is able not merely to distinguish between the possibly viable seeds and the apparently abortive ones but also in some degree to assess, in many of the latter, the amount of development before abortion. Even the collapsed and withered ovules were still distinguishable, somewhat resembling microscopic autumn leaves. The well-developed symmetrical seeds, that germination tests indicate as including almost all the only viable ones, ranged in number from one to thirty-seven in a capsule and the frequencies of occurrence are represented in the accompanying graph (Fig. 4). The arithmetic mean was 13·8, compared with 6·2 in 1961, but it will be seen that the mode is between 8 and 12 (the average for the capsules represented in Fig. 4 was 18 but these were not a random sample, having been selected for range of size).

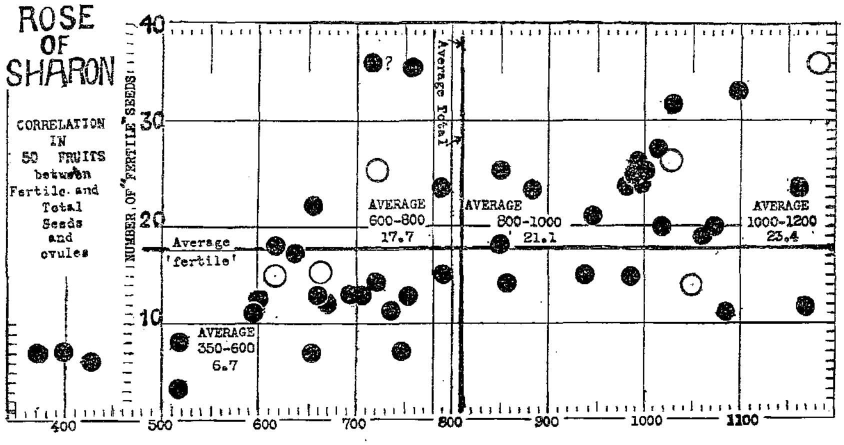

Fig. 4. Each spot represents one capsule; its position in the vertical direction corresponds to the number of apparently fertile seeds which it contained and its horizontal position to the total content of ovules whether fertilised or abortive. O = Capsules from 'Highdown'

It may, I think, be assumed that the usual percentage of those that would germinate is unlikely to exceed the average for the 1187 actually tested, namely 48 %. If so, and if the average total of ovules in the fruit be taken as about 800, this would imply an effective development of not more than about eight per thousand of the ovules present. This may represent the normal incidence of compatibility and effective fertilisation in this species when self-pollinated.

One can thus envisage that, in a normally self-incompatible species, the triple fusion which produces the endosperm and involves the second male nucleus may similarly exhibit partial or complete failure with correspondingly variable effect upon the development of the embryo. Precocious planting of seeds with ineffective endosperms, might however provide substitutional nutrition and so account for the enhanced percentage germination of the immature seeds of a capsule.

[1]From information provided by Professor Pichi Sermolli, it would seem that in Northen Italy, at Genoa, seed production of this species is as sparse and unreliable as with us.

Another collection of 704 mature seeds was obtained, about three weeks later in 1962, from several widely separated colonies, growing in various situations and soils. These also were sown at once, under the same conditions as those just considered. This composite sample yielded only 48·4% seedlings, a reduction perhaps associated with the seeds having ripened in the less favourable temperatures of mid-November. These 704 seeds, having been culled from capsules produced in distinctive environments, the intermittence, as might have been anticipated, is obscured but is nevertheless manifest as repeated surges in the germinations (cf. Fig. 3). Elsewhere evidence has been furnished (Salisbury 1962 p. 394 et seq.) to indicate that the potentiality for intermittence is genetically determined but that its manifestation is probably dependent upon developmental conditions. If this be accepted we should not expect the intermittent germination of seeds from distinctive individuals and habitats to exhibit contemporaneity.

Since the 1961 seeds were sown in May and all the 1962 seeds late in autumn, it would appear that the difference in photoperiodic environment (short nights for the one: long nights for the others) had little, if any, effect. No replication was possible since all available seeds have been utilised for the experiments here described.

The lower percentage germination of the 300 seeds of 1961 (a decrease of 15%, as compared with that of the 704 seeds of 1962) is to be attributed not merely to seasonal differences but to the the former having been kept for several months whereas the latter were sown immediately. The decrease of germination with increasing age is a familiar feature accompanying the passage of years, or even months, but here, as we have seen, this decline appears to go back to the period within the capsule and a gradual diminution in the proportion of viable seeds would seem to be indicated *from the time of their formation onwards*.

Although the number of ovules to be fertilised in an ovary is so large and, as mentioned later, may be over 1400, it seems unlikely that inadequate pollination plays any part in bringing about the poor seed production, since there is a multitude of stamens (350 or more in a flower) each of which perhaps produces some 900, or more, pollen-grains; a ratio of pollen-grains to ovules of the order of 300 to 1! Moreover the greater the number of ovules present in an ovary the more would appear to be fertilised (cf. Fig. 4), a relationship that could scarcely obtain were quantity, and not quality, the limiting condition.

Investigators, using a diversity of types, have provided evidence that the incompatibility between individuals of a species depends on a series of alternative genetic characteristics. These determine the biochemical relations between the germinating pollen-grain and the tissues of the pollinated plant, a subtle disparity between them being necessary for effective growth and fertilisation. External conditions, as Professor D. Lewis demonstrated with respect to temperature (Lewis 1942), can modify their interaction. It is scarcely surprising therefore that some pollen tubes do, in fact, reach the egg cell and effect fertilisation. Even for the markedly self-incompatible sweet cherries, Crane & Lawrence (1938) obtained about 0·1% fertility when selfed. Thus, the occurrence of a small proportion of viable seeds in self-pollinated *H. calycinum* is not unexpected. Why, in the race against time to the ovules, some pollen-tubes achieve success may be an outcome of the shuffling of the genes that takes place during the formation of the sexual cells. Theoretical considerations lead us to recognise that the nuclei of both pollen-grains and egg cells, though derived from the same parent, may yet have individual differences that could influence their effective union. So we may postulate that successful self-fertilisation could depend upon the chance mating of such male and female nuclei as exhibit compatible constitutions. If this hypothesis be correct then with an increased number of matings the number of successes should augment although the *proportion* of these might be expected to remain roughly constant. Both these conditions are, in fact, apparently fulfilled.

Owing to the fortunate circumstances that the successes and failures in the fruit can alike be determined with a fair degree of accuracy, it is possible to check these features although such determinations, owing to their laborious and time-consuming character, could only be made upon a limited number of fruits, actually fifty-one, selected for the range of size they presented. The data for fifty of these are displayed in Fig. 4. By grouping these capsules, according to increments of about 200 in their ovular content, the rise in the average numbers of ' fertile seeds ', accompanying the total increase, is made evident.

The examination included some large capsules from a colony, established for more than half-a-century, growing on an exceptionally sheltered, south-aspect slope in Sir Frederick Stern's garden at ' Highdown '. One of these (not included in Fig. 4) was quite outstanding, with no less than 58 apparently fertile seeds out of a total content of 1458; a proportion still barely achieving 4%, of which not more than a third would probably germinate. If all the fifty-one fruits in which the contents were determined be grouped, we find that the *proportion* of apparently fertile seeds is strikingly similar, in all but the lowest category of contents, namely 370 to 600, 1·4%; 600 to 800, 2·6%; 800 to 1,000, 2·2% and 1000, to 1,458, 2·3%. The facts then would appear to correspond with our hypothetical presentation, the more so that the discrepancy in the class-interval representing the smallest capsules could be due to nutritional stresses inoperative for the larger fruits.

A very noteworthy feature of the incompatibility in this species is that various stages of abortion appear to have occurred subsequent to nuclear fusion. The green colour of many of the abortive seeds is indicative of the presence of embryos arrested in their development at different stages. The pollen tubes of incompatibles may often grow in the style to different degrees and any which reach the egg-cell may effect fertilisation. The genetic barriers thus act by affecting the temporal aspects of growth and perhaps this extends further, beyond the fertilisation of the egg, to affect also mating of the second male nucleus with the fused polar nuclei to provide the endospermic nourishment for the developing embryo. If in *H. calycinum* this second fusion is similarly retarded it might explain the arrest of the embryo at various stages. The much higher % germination of immature seeds is in harmony with such an interpretation.

It would not appear likely that nutrition materially influences the number of ' good ' seeds produced since, although the shoots of the Rose-of-Sharon are normally single-flowered, I have found paired fruits not infrequently and very rarely a shoot bearing three fruits. On three-fruited and two-fruited shoots alike, the capsules did not exhibit any diminution below the average in numbers of ' good ' seeds.

Although flowering may begin in July, I have rarely found ripe fruit before the end of October and frequently not till late November. This retarded maturation may be the result of the different climatic conditions that prevail here, compared with those of S.E. Europe. Dehiscence takes place from the apex towards the base into five segments (Fig. 1, D & G) surrounding a central, conical column formed by the placentas. Frequently, however, the capsules do not appear to split open sufficiently widely for the larger seeds to escape readily. Though sometimes erect, the fruits are usually inclined so that, when the segments spread at the apex, the contained seeds should be shaken out by the gusts of wind. Two circumstances appear to militate against the successful operation of this ' censer ' device. Firstly, owing to the high proportion of seeds which abort, the capsule is largely empty and as it dries and contracts may do so without creating the tensions that, with a well-filled capsule, would more readily ensure the effective rupture and gaping apart of the segments towards the top of the fruit. To test this aspect, ten capsules were selected which had pronounced dehiscence slits and these were each in turn held by the stalk and violently shaken and jarred, to simulate wind movement in an exaggerated form. The result was that 54% of the total of fully-developed seeds escaped. However the 46% that were still retained included, not unnaturally, the larger ones, and if only 22% of the total were actually viable might well account for all of these.

The second circumstance is that since the fruits ripen so late in the year the chance of a thorough sun-baking in these latitudes is meagre. Capsules, which had dehisced but in which the slits were too narrow to permit the escape of any but the abortive seeds, were placed in a warm room to simulate sun-drying. Only after several days was it that the segments separated sufficiently to permit the ready escape of the fully-developed seeds. This would seem to indicate that lack of adequate insolation probably impairs seed-dispersal under the climatic conditions that normally prevail here in late autumn. Obviously, with decay of the capsules, all seeds would eventually be released but, during the intervening period, they would have been subjected to great fluctuations of temperature and probably also to recurrent changes from dryness to saturation, conditions known to be the reverse of those conducive to the retention of viability.

Towards the end of February a random collection of 62 fruits was obtained from an exposed habitat and their contents examined. All the capsules were dehisced and had been subjected to severe winds that had attained gale force on several occasions. Nevertheless although just under one third contained no seeds, 43 of the capsules contained apparently good seeds the number of which ranged from 1 to 32, the average being 4·2 (s.d. = 6), so that even under these extreme conditions, so favourable to dispersal, an average of only about eight 'good' seeds would be liberated from a capsule and this would imply an average seedling potential for the escaping seeds of not more than four. In an actual test of 210 of these retained 'good' seeds germination attained 37·6% and was completed in 13 days, which suggests the leaching out or inactivation of an inhibitor. Ineffective dispersal would then seem to be an important contributing factor, but not a complete explanation, for the apparent absence of seedlings.

From the foregoing considerations it is not unlikely that an appreciable proportion of the potentially viable seeds may not reach the ground until after their germinative capacity has become impaired. Further, the scanty seeds and even scantier seedlings might perhaps be largely, or even entirely, accounted for by predators. It will be obvious from the data presented in Figs. 1 and 3 that germination takes place in a discontinuous manner which implies that the number of seedlings at any one time could, at best, only be small and so might readily be overlooked. However, I have in fact searched the fringes of colonies, in various localities and on various soils, for seedlings, so far without success. Furthermore, there does not seem to be that degree of discontinuity in occurrence, near the borders of colonies, that might be expected if the species had spread from seedlings.

Although we now know, with certainty, that some viable seeds are produced by the species in southern England, it still remains to be ascertained whether, in the open, seedlings do actually develop and, if so, when. Are conditions normally adverse to germination or, if not, what are the circumstances responsible for the non-survival of seedlings? Only further observations can resolve these questions.

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