Fast plants for finer science—an introduction to the biology of rapid-cycling *Brassica campestris* (*rapa*) L.

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Introduction

It is not uncommon to find that a new teaching approach or some new material with which to teach leads our pupils to a much improved understanding of biology. The introduction to British schools of 'fast plants', which are novel rapid-cycling cultivars of one brassica species, may mark such an innovation. This article explains what fast plants are, how they relate to other brassicas, how they may be obtained for classroom use, and how they may be grown. From this initial account their potential for teaching should become apparent.

Fast plants are an exciting new teaching material with potential to enliven the driest areas of botany. There are many basic concepts in plant biology, such as plant growth, tropisms, floral reproduction, pollination, embryonic development, and plant genetics that are unexcitingly taught. To some extent this must be because plants are not easily produced to order at particular life-cycle stages, nor are they able to be grown sufficiently quickly or predictably to fit into a set period of teaching time.

Rapid-cycling brassicas are very easily grown to a predictable and reliable time schedule of a few weeks regardless of season (figure 1). They are ideally suited to modular teaching. The plants are attractive and small (less than 30 cm high on a 2.5 cm diameter base), so that sufficient can be kept in a laboratory for each student to have his or her own individual plant. These readily become personal to the younger pupil. If a secondary student can work with several plants then extensive experimentation can be done. The plants are therefore ideally suited to the problem-solving, hypothesis-making approach inherent in good teaching. Investigations with these plants into embryonic growth or the effects of environmental toxins are free of the many limitations associated with animal experimentation. Furthermore, the plants are phenotypically varied, with many single-allele variant forms: for genetics teaching they promise to rival *Drosophila*. They have a life cycle short enough to fit a Mendelian experiment, from parent to F_2 generation, inside the duration of a 12-week term. Last but not least, rapid-cycling brassicas are at the frontiers of contemporary science: they are new (they did not exist a quarter of a century ago); they are employed in current research and have uncharted possibilities for developments in both teaching and plant breeding. This plant allows students tangible experience of modern plant technology and provides a window into

Abstract

Rapid-cycling brassicas, originally developed for plant breeding research, promise to be of value in school and college teaching. Within the family Cruciferae, they are members of the same genus and species as the crop brassicas of great economic importance. The crop brassicas are briefly reviewed as they set the rapid-cycling plant cultivars in their context. The way in which the cultivars, with their five-week seed-to-seed cycle, were developed is described and an account of their developmental morphology given. The standardized methods for growing these fast plants on capillary matting under light banks is explained. Their insect pollination and a method of artificial pollination with beesticks are described and methods for circumventing the flower's self-incompatibility reviewed. Practical physiology and genetics exercises are listed to illustrate the plant's potential value for teaching.

Nomenclature

There is unfortunately some confusion over the species (but not the cultivar) nomenclature. *Brassica campestris* is synonymous with *Brassica rapa*, a specific name which it is equally legitimate to use but which courts confusion with winter oil-seed rape, *Brassica napus*. *B. campestris*, as a species, embraces our native wild and domestic turnips, stubble-turnips, a form of annual rape, and many oriental (Chinese) cabbages. Although the name *B. rapa* is used currently in the *Flora of the British Isles* (Clapham, Tutin, and Moore, 1987) and by the Institute of Horticultural Research, Wellesbourne, *Brassica campestris* (Linnaeus) is the name preferred in the bulk of contemporary research literature and by Science and Plants for Schools, the organization that is promoting this material in education.
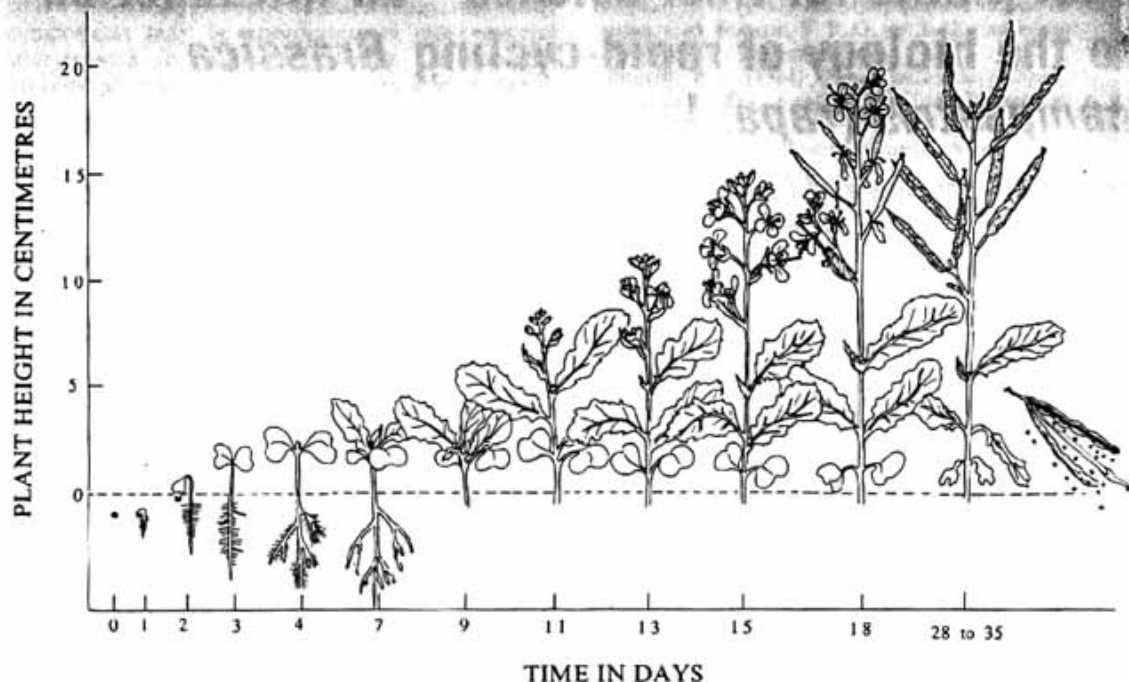
Figure 1 The life cycle of rapid-cycling *Brassica campestris* (*rapa*) L.

the research worlds of crop plant evolution, selective breeding tissue culture and even plant transformation (Williams, 1990).

The brassicas

When teaching with rapid-cycling brassicas, it is valuable to place them in the biological context of the genus *Brassica* and the family Cruciferae. (For a good recent account of the *Brassica* crops, see Simmonds, 1976).

The brassicas belong to a family of plants carrying four petals in a cross shape—the Cruciferae. In the genus *Brassica* the flowers are yellow and commonly borne in an apical or axillary raceme. With their attractive yellow flowers, fast plants remind one, at first sight, not of a typical cabbage but of a small charlock, rape, or mustard.

The brassicas are principally leaf or flower-vegetables and oil-seed crops. Many are household names: cabbage, cauliflower, sprouts, spring-greens, broccoli, radishes, white and black mustards, turnips, swedes, pak-choi, and Chinese leaves, just to name some that have found their way into many homes in Britain. Many people are also familiar with kale and forage root-crops for livestock: these animal-feeds are but one stage further removed from us in our food chain. Who also can have failed to notice the midsummer fields of lemon-gold when the oil-seed rape is in flower?

Brassicas are among the world's economically most important plants. Rape seed ranks fourth in the world as an oil crop. Fodder crops have considerable importance as overwintering feed for temperate zone livestock, whilst 'greens' of diverse kinds provide a substantial dietary component for over half the world's population.

The plants in the crucifer family have rich nutritious leaves, many with bitter-tasting chemicals in them, such as the mustard oils and glucosinolates. The chemistry of the Cruciferae has been reviewed by Vaughan, MacLeod, and Jones (1976). Glucosinolates may have evolved as a protection to the plants against their predators, but oddly they may make them attractively flavoured as well. The brassicas are often rich in vitamins A and C; recently bred non-bitter Brussels sprouts have twice as much vitamin C as oranges. Also important nutritionally are the leaf starch and protein found in spring greens, kale, and Chinese leaves (petsai). A million tonnes of the latter are consumed per week by the people of China. However, many of the selected varieties of the major species have been chosen because their winter food reserves are laid down compactly in just one part of the plant. Head cabbages and sprouts have food stores in their dormant apical and axillary buds. Cauliflowers and broccoli have theirs, respectively, in the form of abortive and potential flower buds. Others have their reserves in the leaf bases (pak-choi), the stem above ground (kohlrabi), the hypocotyl stem tuber (swede), or the tap root below ground (turnip) (see figure 2). Many of the brassicas are oil-seed
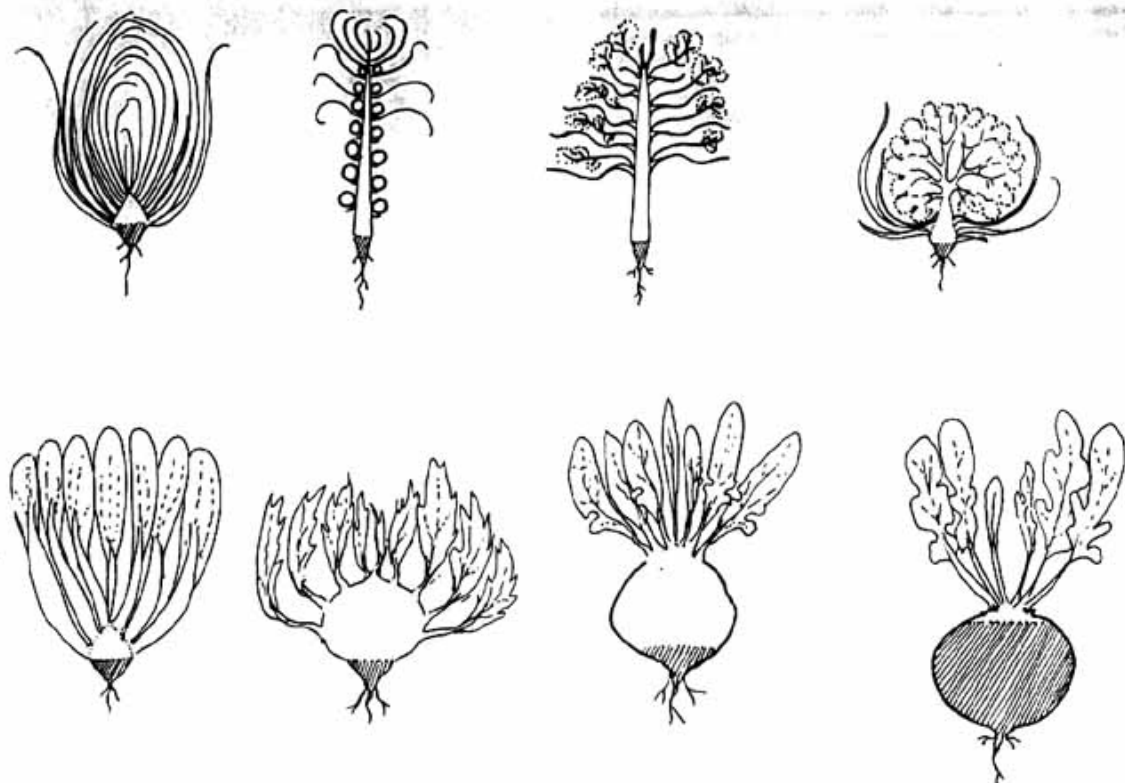
Figure 2 The brassica vegetable morphotypes, illustrating the anatomically varied regions in which selected brassicas, used as vegetables, invest their food reserves. (Drawing not to scale. Roots are shaded.)
Top row, from left: apical leaves and bud (head cabbage and Chinese cabbage); axillary buds (Brussels sprouts); axillary flower buds (sprouting broccoli); apical flower buds (cauliflower).
Bottom row, from left: petiole of leaf (pak-choi); stem (kohlrabi); hypocotyl (swede); tap root (turnip).

crops. Mustard seed is 25 per cent oil, and rich in aromatic glucosides. Rape has a seed in which much of the plant's energy and protein reserves are concentrated in a most readily harvested form.

The cultivated brassicas are represented by six interrelated species. Three are diploids and three are amphidiploid (allotetraploid) derivatives. The original crop plants are centred on the Mediterranean area of the Old World. Wild turnips (*B. campestris* [*rapa*]) and black mustard (*B. nigra*) are native European plants, both occurring as weed species as well as crops in Britain, whilst wild cabbages (*Brassica oleracea*) grow on the cliffs of Europe by the sea. These first three are given the letters a, b, and c to describe their respectively different genomes. As each is diploid, that is they have two sets of chromosomes, they are genomically described as aa, bb, and cc respectively (table 1).

These three diploid species are strongly out-breeding and possess self-incompatibility, controlled by a multiple allelic series at the S locus. *Brassica campestris* (synonymous with *B. rapa*) has numerous cultivars, including not only Chinese leaves and turnips but also an annual rape. *B. nigra* is the black (French)

mustard and the proverbial 'mustard seed' of Biblical parable. *B. oleracea* has at least 14 races of which perhaps the most extraordinary is the Jersey kale, *B. o. palmifolia*, otherwise known as the long-jack cabbage, that will grow to six metres.

At some point in recent evolutionary history, perhaps between or after the most recent of the Ice Ages, three new species of *Brassica* arose by hybridization. As with other crop polyploids this may well have happened under human cultivation, for rape and swedes have no wild relatives. Such interspecies hybrids are infertile unless chromosome doubling occurs to make accurate meiotic pairing a possibility. These

Table 1 The diploid brassicas

Species	Genome	Chromosomes	Crop names
Brassica campestris	(aa)	$2n = 20$	Turnips, Chinese leaves
Brassica nigra	(bb)	$2n = 16$	Black mustard
Brassica oleracea	(cc)	$2n = 18$	Cabbage, broccoli, sprouts, cauliflower, kohlrabi, kale

Table 2 The amphidiploid brassicas

Species	Genome	Chromosomes	Crop names
Brassica juncea	(aabb)	$2n = 20 + 16 = 36$	Brown mustard
Brassica carinata	(bbcc)	$2n = 16 + 18 = 34$	Ethiopian mustard
Brassica napus	(ccaa)	$2n = 18 + 20 = 38$	Oil-seed rape, swede

are then diploid for both chromosome sets and are therefore referred to as amphidiploids (table 2).

These three amphidiploid species are predominantly self-pollinating (75 per cent of plants in oil-seed rape) and are therefore not so dependent as the diploids on insect vectors of pollen. Two are less well known. *Brassica juncea* (brown mustard) has many mustard crop and spring-greens cultivars whilst the similar *B. carinata* is an Ethiopian leaf vegetable with a higher protein content than spinach. *Brassica napus* exists in two familiar forms, as oil-seed rape and the swede. The former is the most important economically. Rape seed is 40 per cent oil and often contains erucic acid, a 22-carbon unsaturated fatty acid, which is toxic to mammals. Varieties of rape seed oil that are low in erucic acid are used in margarine for cooking, whilst the cake is a useful animal feed; oil from those varieties high in erucic acid is used as a jet engine lubricant and in polymer resins. Swedes, once a staple root vegetable and now rather less popular, may well have arisen as turnip/kale hybrids in mediaeval times; they were first scientifically noted in Europe in 1650 and came to Britain from Sweden in 1775.

The principal brassicas are thus closely related to each other, their relationships being exemplified by the 'brassica triangle' (figure 3). Knowing about their genomic relationships makes the transfer of genetic material within the whole group readily intelligible and this knowledge has been exploited by breeders. For ezample, an economically important problem with brassicas is clubroot disease, caused by the fungus *Plasmidiophora brassicae* (see Austin, 1986). Genetic resistance to clubroot disease is found in Dutch stubble-turnips (*Brassica campestris*). A domi-
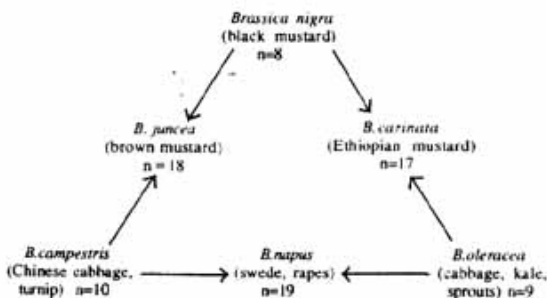
nant allele has been introduced to the oil-seed rape crop from the stubble-turnips by hybridization of the *B. campestris* (aa) with *B. oleracea* (cc) to form a *B. napus* (aacc). Such transfers may be speedily done with the new rapid-cycling material. Breeders may want to improve crop size, nutrient content, disease resistance, growth rate, harvesting ease, or just market features such as colour. Well over a thousand scientists in 45 countries are working to improve just these brassica crops alone.

Many taxonomists today might put *Raphanus* in the same genus as the brassicas. The first inter-generic cross ever produced (Karpechenko, 1929) was between a cabbage (*B. oleracea*) and a radish (*Raphanus sativus*) to produce the new hybrid crop genus *Raphanobrassica*. This plant has a very high dry weight biomass productivity and has been used as a breeding bridge, bringing disease-resistant alleles from the radish group into the brassica group (McNaughton and Ross, 1978).

The rapid-cycling cultivars

The rapid-cycling brassicas are not pure-bred lines, but exist as populations that have been selected for their rapid completion of a seed-to-seed life cycle. The fastest of these 'fast plants' grows from seed to flowering in 14 days and completes the maturation of its seed in a further 22 days. Under optimal conditions it can therefore pass through ten such 36-day cycles in one year.

Most brassica species are biennials or annuals. Their normal seed-to-seed cycle times are too long for the convenience of plant breeders interested in rapid gene transfer. It must be remembered that commercial plant breeding is competitive, costly, and time consuming. It was this time and money constraint that led to the fast plant idea.

The earliest rapid-cycling plants were developed in the 1960s from the parent stock of a Polish annual spring rape *Brassica campestris* (Friend and Helson, 1966). Rapid-cycling plants promised not only to speed up gene transfer but also to provide a rapid sexual means of quickly bulking up the breeding population upon which selection could then be set to work. The Canadian Department of Agriculture was the first to employ a rapid-cycling variety, 'Arlo', in their annual-rape breeding enterprises. This first one had a seed-to-seed cycle of 50 days. Friend had observed that flower buds were produced by plants within ten days of planting if the plants received a single 'long day' of more than 20 hours continuous light from the fourth day onwards. It is to the credit of Postlethwaite and Enochs (1967) that they immediately recognized the potential educational merits of these 'tachyplants' (from the Greek *tachos* meaning speedy) in a paper published the following year.

In 1970, one of the authors (Paul Williams) began a programme of reviewing the germplasm collections of brassicas from around the world (Williams and Hill,

Figure 3 The *Brassica* triangle, showing the origin of the three amphidiploid species from the three pairs of contributing diploid genomes. (After Hemingway, J. S., in Simmonds, 1976.)

1986). The United States Department of Agriculture had 2000 wild or cultivated brassicas in its seed bank collection. It was noted that in each species there were a few forms that would rapidly come to flower after a long-day treatment of continuous light. These were selected to produce the rapid-cycling cultivars of the principal *Brassica* species. The aim was set out to grow small, rapid-flowering, highly fertile forms under optimum and standardized laboratory growth conditions. The resultant cultivars of each species were then inter-pollinated. Populations were then multiplied up separately and then subjected to recurrent selection for the following criteria:

1. Petite plant habit.
2. Minimum time from seed to flowering.
3. Uniformity of flower maturation.
4. High female fertility.
5. Rapid seed maturation.
6. Absence of seed dormancy.

Plants were grown in mini-pots, at densities of 880 per square metre, in a moss peat and vermiculite mix. The plants were fed water and dissolved mineral nutrients through their roots and kept under the continuous irradiance of white fluorescent strip-lighting at a temperature of 25 °C. A 10 per cent selection was exercised in each generation for early flowering. Selection only ceased when 50 per cent of the population first flowered within a two-day period. The population was then stabilized by bulking up again to a base population. Table 3 shows the growth characteristics of the resulting rapid-cycling populations of the six principal *Brassica* species.

The clear winner in this 'tachyplant' contest was the *Brassica campestris* population. It is these that have become the most celebrated as materials for teaching and research.

The use of *B. campestris* for the teaching of genetics was first practised by one of the authors (P. Williams) at the University of Wisconsin–Madison. Interest in the plants developed. Seed was supplied by Williams to Hawk and Crowder at Cornell University (see Hawk and Crowder, 1978a and b, and also Crowder *et al.*, 1980). Much of the plant's potential for teaching both genetics and other aspects of plant biology has been realized through the establishment of the Crucifer Genetics Cooperative, at Madison–Wisconsin, which has supplied seed to members, and, through the *CrGC Resources Book*, supplied copious

information (Williams, 1985). The 'fast plants' programme in the USA has been advanced by the funding of educational programmes of research and teacher support (Hafner, 1987).

Besides their potential for use in education the present germplasm collection of rapid-cycling base populations (RCBPs) is immensely important for research. Their biotechnological potential is hinted at by the scattered pieces of an incomplete puzzle. For example, the control mechanisms of fatty acid and protein production in a rape crop may be analysed from studying the genetics of rapid-cycling *Brassica napus*. This leads us to ask whether highly specific fatty acids or seed-cake proteins could be made to order by oil-seed rape. To do this we need to understand the genetic control of the developmental system and introduce the right genes. Much investigation of genetic control systems has already been done in *Arabidopsis thaliana*, a related crucifer 'tachyplant'. We know that brassicas may be transformed by *Agrobacterium tumefaciens* which has been modified by molecular biologists to insert genes of choice into the *Brassica* genome. Once created, a novelty can be mass produced. Brassicas are easily tissue cultured. For example, haploids may be obtained from anther microspores. 'Double haploids' can be induced to form from plants with a haploid microspore origin— literally growing embryos out of anthers. Homozygous plants, so derived, have no hidden recessive characters and could form a remarkably uniform crop (see Williams and Hill, 1986, and Dickinson, 1989). Some of these new techniques are now routine in the lab and could be developed in the classroom. Fast plants could bring contemporary research practice into ordinary teaching.

The morphology of rapid-cycling *Brassica campestris*

Figure 1 illustrates the size, proportions and many of the morphological stages of development of fast plants, through the life cycle. Observation of a population of these plants reveals an intriguing degree of variability between them. From this base population various genetic forms may be isolated or discovered. The typical features of fast plants and some of the variations observed by one of the authors (Tomkins), on first growing them, are described below.

Table 3 The growth characteristics of rapid-cycling *Brassica* populations

Brassica species	Genome	Mean days to first flowering	Height at first flowering/cm	Seeds/plant	Days/cycle	Cycles/year
B. campestris (rapa)	A	14	13	78	36	10
B. nigra	B	20	27	69	40	9
B. oleracea	C	30	23	18	60	6
B. juncea	AB	19	30	107	39	9
B. carinata	BC	26	42	67	56	6
B. napus	CA	25	35	76	55	6

Seeds, about 1 mm in diameter, are typically dark brown, but yellow and black ones occur. Seedlings, have twin heart-shaped cotyledons, grow rapidly, and are a dark green in colour. The first true leaves are barely present at germination, but after one week are well grown and at this stage even flower bud initials are visible. The cotyledons thicken up to make fleshy and long-lived leaves that only wither towards the end of the plant's life. The plant population varies in anthocyanin expression. Most plants show a degree of purple colour on the stem, particularly the hypocotyl, and in such places as the flower buds, anther tips, and testa.

Generally the plants are a slightly glaucous grass-green in colour, with a bloom of wax on the leaves, though some more shiny bloomless morphs occur. They are only slightly hispid (bearing long hairs) with, typically, relatively few spiky hairs on both leaf surfaces and on the stem; again there is variation. The most hairy plants tend to adhere to each other. There are no short (pubescent) hairs. The main basal leaves have a very short petiole, are entire or somewhat pinnatifid (divided almost to the midrib) and lyrate (having the largest lobe terminally). There is clear variation in leaf shape between plants. Higher leaves are smaller and more oblong-lanceolate, with a cordate (heart-shaped) leaf base that clasps the stem.

Most flowers are in a terminal raceme, though axillary flower buds commonly form later. There is certainly variation in the inflorescence form, perhaps reflecting the strength of apical dominance. Flower buds formed in the apical whorl open at about eight-hour intervals. Each flower is offset in the whorl from the next by approximately 135° on the spiral axis. The flowers are relatively large and bright yellow (rarely cream yellow). There are four sepals, and four entire clawed petals (angled outwards into a cross shape). There is some variation in petal margins and curvature. Flowers (see figure 8) generally have six stamens, four of which elongate around the pistil as the flower opens. Four small nectaries are found between the filament bases of the inner four anther filaments. The stigma has a single head but is sometimes two-lobed.

The fruit, correctly described as a siliqua, is perhaps best called a 'pod' for classroom purposes. It contains two longitudinal chambers of seeds, divided by a membrane. The pods vary in their shape, length, and the acuteness of the angle at which they are carried to the stem, as well as in their degree of inflation and constriction around seeds and in their translucence. Each pod has a terminal beak, derived from the style. The pods are relatively indehiscent and if dried off at day 35 will store well without loss of seed.

Growing rapid-cycling brassicas

This section of the paper sets out the key factors in the successful practice of fast plant culture. It must be emphasized that the optimum growing conditions for rapid cycling Brassica campestris differ from the conditions that teachers are most familiar with in growing other plants for the lab and classroom. Science and Plants for Schools (SAPS) has tested growing methods and produced full instructions for fast plant growing with the kits now commercially available (the MacIntyre Fast Plants Basic Kit, see details and address at the end of this paper).

The 'long day' of continuous bright fluorescent light is essential for early flower bud initiation. For optimal growth rates the leaves should be between 2 and 7 cms from the fluorescent tubes. Plants were originally selected to grow under 'white' argon-filled phosphor-lined tubes. The contemporary krypton-filled tubes, that have now largely replaced them, are equally effective and more economical. Light-banks of six tubes side by side are recommended. For most purposes three twin-tube fluorescent fittings of 120 cm length, assembled side by side, under a supporting structure have been found to be most effective. Each krypton tube is about 36 watts. This light bank costs about 30 to 35 pence per day to run. A thick plywood board mounting for the tubes (60 cm × 120 cm) is recommended, with a suspension system from wall, rack, or trolley. SAPS has produced notes on the construction and operation of light banks, with the construction and running costs for a school clearly set out (these are available free from SAPS, see addresses at the end of this article). The most adaptable system in use may prove to be the light bank trolley (figure 4). This has the advantage of being mobile, coming in for classroom use whilst being put away, with the lights continuously on, in a closed cupboard at other times. Up to 1000 plants may be grown simultaneously under one such light
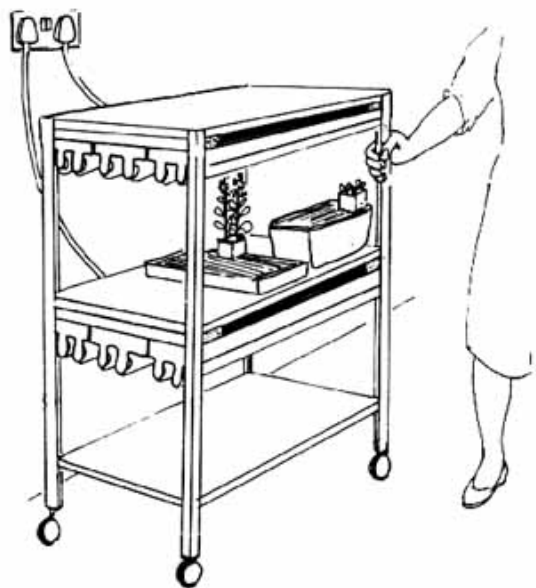

Figure 4   The trolley light bank.

bank, though handling them is much easier at half this density. Those interested in lighting systems for plants would be well advised to consult the excellent Electricity Council publication on lighting for horticultural production (Electricity Council, 1987).

Each plant requires only 10 cm³ of peat-vermiculite rooting medium. A 50:50 mix of Sphagnum moss peat and fine grade vermiculite is best. It has been found that this relatively nutrient-free medium produces the most consistent growth conditions when used in conjunction with slow release NPK pellets. Those retailed by MacIntyre, with the kits, are designed to release the minerals over a 70-day period at 15 °C. At the higher temperatures of indoor growing (22–27 °C) this produces a full release of the plant's requirement over the 35-day growth period. Plants with higher mineral nutrient supplies are more leafy and can produce more seed, but an element of root volume constriction and slight nutrient deficiency undoubtedly keeps plants more petite in their growth form.

Employing the SAPS/MacIntyre system, seeds are sown in a polystyrene 4-pot (5 cm × 5 cm × 5 cm). Each of the four mini-pots is fitted with a wick and packed gently with between 7 and 10 cm³ of peat-vermiculite. About four NPK pellets and the seeds are sown just beneath the upper surface (see figure 5). An alternative system is to use single 10 cm³ test-tube caps as plant pots. These are autoclavable and may be recycled many more times than a polystyrene pot. In all cases water is supplied to the wick by capillary matting beneath. The MacIntyre kit utilizes a large sandwich box as the reservoir for water, the plants being supported by the lid (figure 6). This will bear a class set of fifteen 4-pots containing 60 plants in all.
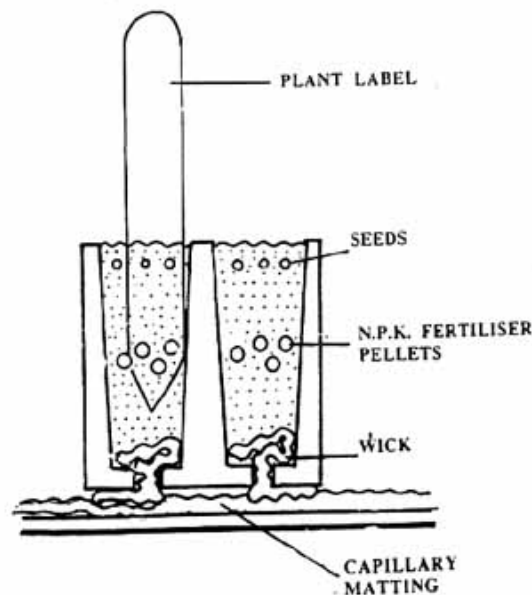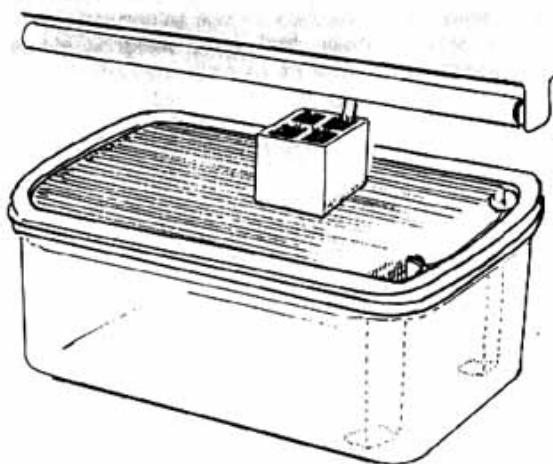

Figure 6 The MacIntyre fast plants growing system.

At least eight of these class sets will go under a standard light bank. On a larger scale it is better to use standard laboratory equipment trays as water reservoirs, each fitted with a Perspex sheet lid and capillary matting. Whatever system is employed, the light-to-plant distance will need to be adjusted to the optimum. Either the reservoirs can be raised and lowered to the lights, or the lights raised and lowered to the plants.

The water reservoir should have the spare capacity to supply water over a long weekend. The MacIntyre system will supply water for at least five days without refilling. Copper sulphate should initially be added to the reservoir water to discourage algal growth in the capillary matting and to discourage fungal attacks to the seedlings. This dose may be administered, in solution, at a strength of 0.12 g dm⁻³ of the blue hydrated copper sulphate crytals. This is equivalent to 30 ppm of copper ion and is well tolerated by plants up to twice this level. Capillary matting should be bleach (sodium hypochlorite) treated, between generations of plants, because fine rootlets go from the 4-pots into the matting and without sterilization may cause subsequent infection of healthy plants.

The sowing rates recommended for classroom practice are three seeds per mini-pot. However, germination is well over 95 per cent and it is quite unusual to have no seedling in a mini-pot at this sowing. Seedlings should be thinned to a single specimen per mini-pot on the fifth day (see figure 7).

Plants grow rapidly after the first week, putting on several centimetres per day once they are 10 to 15 days old. It is at this age that they need most watching with respect to the plant-to-light distance. After pollination (discussed in the next section) there are two more practices that will help speed up the life cycle. Once 15 or so flowers have been pollinated, the apical buds and any axillary flower buds may be pinched out. This is referred to as terminalization. This con-

Figure 5 Section through a 4-pot.

Labels in figure: PLANT LABEL, SEEDS, N.P.K. FERTILISER PELLETS, WICK, CAPILLARY MATTING

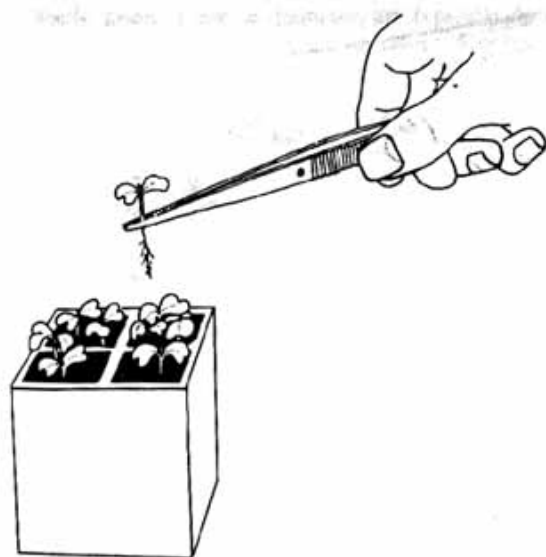Figure 8   *Brassica campestris* flower at anthesis.

Figure 7   Thinning seedlings at five days.

centrates the plant's photosynthetic production into the fruits. If ten pods per plant are left to ripen, good seed production will be obrained from each of them (mean of 175 seeds per plant). Pods begin to yellow when mature (about day 35). If the plants are then removed from the capillary matting and allowed to dry for a few days, seed is readily harvested. Removal is important, for plants left on a source of water after 40 days may be so damp as to cause germination of the seed in the pod. There is no seed dormancy.

The reproductive biology of fast plants

Plants grown under light banks at 22 to 27 °C may come into first flower as early as 13 days after the sowing of seed; many flowers will be open by the 15th day. With this degree of predictability it is possible to time pollination and to timetable student practical work on floral reproduction with extraordinary precision.

All the brassicas have bee-pollinated flowers. Their bright yellow colour, UV reflectance pattern, and scent undoubtedly serve to attract insects to the copious supplies of nectar and pollen. There is some exciting teaching possible in this area. What is a bee to a flower? What is a flower to a bee? Each has changed and adapted to the other over the millions of years since the Cretaceous era. The co-evolution of bees and flowers is well exemplified by the floral anatomy and floral behaviour of fast plants and there is much sense in teaching these in conjunction with the honey-bee's pollination behaviour and functional anatomy. In this respect, the pollination biology of fast plants challenges us to a more holistic approach to our teaching. Dead bees, if imaginatively

employed, can be used to teach this topic in a novel and exciting way.

The opening of flower buds (anthesis) takes place in a regular progression, with one bud opening about every eight hours. The pistil begins a rapid elongation ahead of the stamens so that the stigma emerges first from the bud. Stigmas are receptive (but protected) for up to three days before anthesis and for the first two to three days afterwards (when unprotected). Pollination should therefore be performed daily to ensure maximum seed set. Anthers dehisce by the second day of opening after the stamen filaments have rotated outwards and away from the stigma.

Although these behaviour sequences may reduce self-pollination, it is prevented more surely by the self-incompatibility mechanism of the stigma. This is a well-studied phenomenon (Richards and Thurling, 1973; Saaren and Kakar, 1975). On the stigmatic surface there are pollen grain recognition systems under genetic control of multiple alleles at the S locus. For example, a heterozygous parent plant S^1S^2 would freely give access to S^3 pollen grains, but an S^1S^3 parent would not. There are many alleles possible at the S locus. If either of the alleles is the same as that of the pollen then the pollen grains will not germinate and hence not effect a subsequent fertilization.

The self-incompatibility mechanism comes into operation just before anthesis but develops after stigmatic receptivity (see above). If, therefore, buds are opened by dextrous dissection two days before they would open normally, the mechanism is not yet fully in place and bud-pollination of self-types is possible (note that buds at minus-two days may be identified by counting six buds up the whorl from the most recent anthesis) (see figure 9). Bypassing the self-incompatibility system has obvious significance in genetic procedures. Avoiding the self-incompatibility mechanism is also possible with at least three other trialled methods. Firstly, slicing off the stigmatic surface with a razor blade removes the inhibition, and
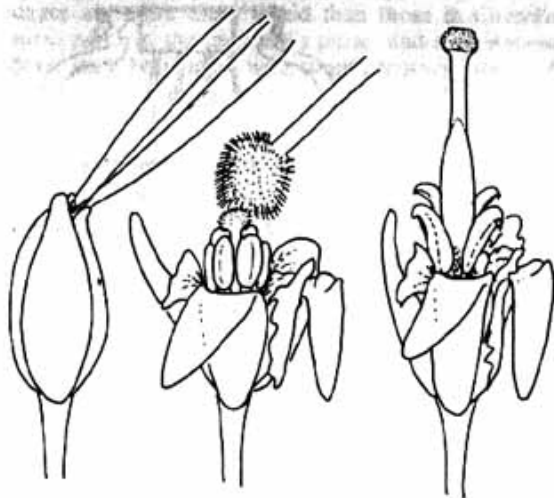
Figure 9 A successful bud pollination.

pollen applied to the cut stylar suface may germinate, especially if applied with a drop of pollen germination medium. Secondly, exposure of flowers to 3–5 per cent carbon dioxide before pollination is also effective in narcotising the mechanism (Nakinishi and Hinaka, 1973). Thirdly, the application of a micropipetted drop of common salt solution at 15 g NaCl dm^{-3} on to the stigma 10 to 15 minutes before pollination yields about eight seeds per pod (Williams, 1986). The last is perhaps the easiest practical method.

The honey bee *Apis mellifera* landing on a brassica flower grips the petals and thrusts its proboscis down to the base of the stamens in pursuit of nectar. Pollen is shed readily on to the short feathery hairs (plumose setae) of the bee's body (figure 10). The pollen grains are well secured as they adhere between and in the forks of the hairs. The bee periodically combs itself free of pollen with the pollen brushes on its fore-legs and forms the pollen load into the baskets on its hind-legs.

Observation of bees at work and observation of the highly adapted, co-evolved, plumose setae on the bee's body gave birth to an important technological step in fast plant science. In seeking a better brush for pollination and a means of storing pollen, one of the authors (Williams) developed the beestick method (Williams, 1980). It has been found, of course, that these are extremely effective as pollen vectors and vehicles for pollen storage. To prepare bees and make beesticks follow the procedure below (see figure 11).

Dead bees may be obtained from hives, when the floor is swept in the spring. Alternatively, live bees may be collected from a hive entrance with a large pooter. Bees are most humanely killed by chilling and then deep freezing. Bees are most hairy as young workers. Dead bees should be air-dried at 60 °C for 24 hours, and kept cool in storage with a desiccant (blue silica gel). The heat treatment effectively kills any pollen they may already be carrying.

When held by the thorax, the head, legs, and abdomen of the bee are readily removed with forceps or a mounted needle. A round double-pointed cocktail stick, with one end dipped in some quick-setting adhesive, may then be used to mount the thorax on the end of the stick (see figure 11). Once the glue has dried and the toxic vapour evaporated, the beestick may be employed as a pollinating agent. Very gently and slowly rotate the thorax brush in the open flower (figure 12). It is tempting to twirl it, but this showers a lot of pollen away from the flower and breaks off the bee hairs! Transfer pollen from the flower of one plant to the stigma of the next. Cross-pollination must be across plants—not just across flowers on the same plant. When pollinating a group of fast plants with a fresh beestick it is best to collect pollen from the more mature flowers of the first, visit all the flowers of each plant in turn, and then return to the youngest flowers of the first few again. One such beestick will last for several hundred pollinations.

Pollen is not only easily transferred by this method but may also be stored on the beestick. This is best

Figure 10 Fast plant pollen trapped by the plumose setae of the honey bee (SEMs).
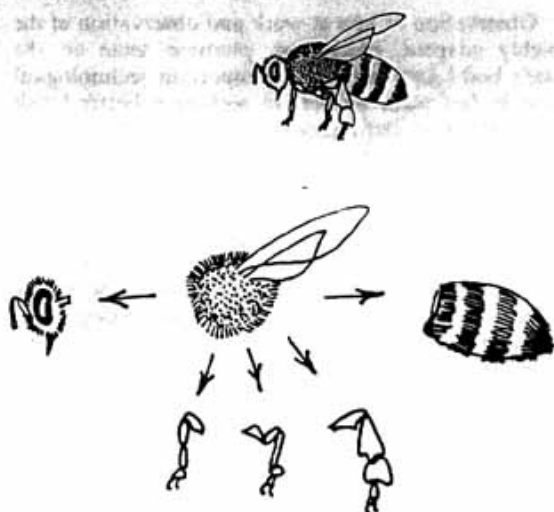
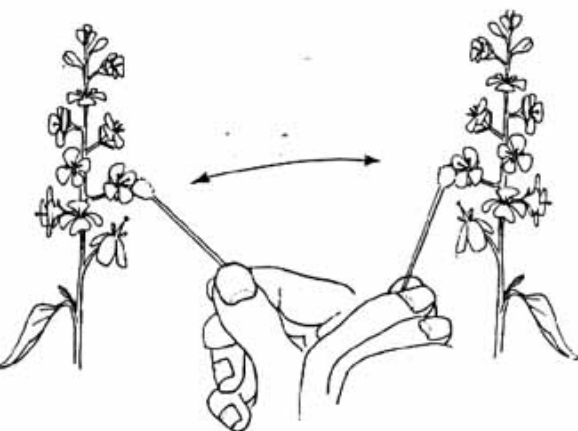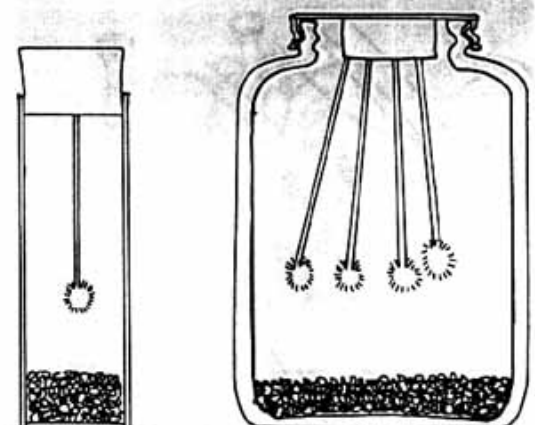**Figure 11** The manufacture of beesticks (see text for description).

Figure 13 Storage of beesticks (see text for description).

done in a screw-top bottle with silica gel in the bottom (figure 13). A polystyrene pad inside the lid will hold many beesticks. Pollen is viable for four days at normal temperatures and humidities. Viability may be extended by storing the pollen in a dry environment at colder temperatures. If dry and in a deep freeze it will be viable for many months. When hybridizing or performing genetic crosses (or even sending genes through the post) beesticks have proved to be very valuable short-lived stores of male germplasm.

All ovaries elongate in the few days following anthesis but only those successfully pollinated continue to elongate beyond the initial extension and to swell. One week after anthesis (days 20 to 24) it will be clear how many fruits will be formed. Fruits of this age are ideally suited to studies of embryogenesis. Dissected ovules reveal the developing green embryos in the clear endosperm of the embryo sac. The embryos start as a small globular initial on a chain of suspensor cells. This then extends to form a heart-shaped embryo as the cotyledons are formed, and then becomes a more elongated torpedo as the radicle is developed downwards. Finally, as the embryo is reflexed over, prior to 'curling up' in its seed-embryo form, a walking-stick shape is developed (figure 14). These developmental

100 µm
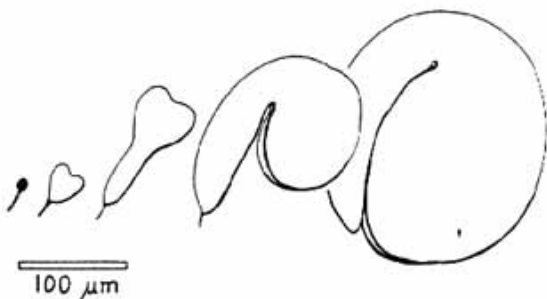
Figure 14 Embryonic stages of *Brassica. From the left:* a globular embryo, heart shape, torpedo shape, walking stick stage, and immature seed embryo. All stages drawn to scale.

Figure 12 Pollination with beesticks.

stages are more easily found than those in *Capsella bursa-pastoris*, the shepherd's purse, and their age, in days, may be gauged with some accuracy from the known date of pollination.

Practical work opportunities

Fast plants provide the material for an infinite variety of projects and practical work in biology teaching. This article has aimed to set out the basic biology of the rapid-cycling plants, and it is very much to be hoped that teachers interested in their potential for teaching will start growing them. In this respect the *MacIntyre Fast Plants Basic Kit*, on the market since May 1990, serves as a useful introduction to the plants. The experimental topics for investigation that are outlined in the basic kit include germination, growth, fertilizer trials, photomorphogenesis, phototropism, geotropism, floral structure, pollination, pollen germination, embryogenesis, natural variation, hybridization, physiological studies, pests and pathogens, root-zone interactions, and bioassays.

Further information

Science and Plants for Schools (SAPS) was set up to promote exciting plant science teaching. It has an affiliate and a sponsored schools scheme and sends a newsletter to schools. In addition, SAPS seconds teachers and funds research and in-service workshops. It does not supply fast plant seed (see MacIntyre below). Teachers in the United Kingdom wishing to know more about Science and Plants for Schools should write to:
The Programme Coordinator
Science and Plants for Schools
Homerton College
Cambridge
CB2 2PH (tel: 0223 411141, ext 233).
   MacIntyre Fast Plants Basic Kits are marketed by:
MacIntyre (FAST PLANTS KITS)
Mottingham Garden Centre
Mottingham Lane
London SE12 9AW (tel: 081 857 2425).

Acknowledgements

References

Austin, R. B. *et al.* ed. (1986) *Molecular biology and crop improvement.* Cambridge: Cambridge University Press.

Clapham, A. R., Tutin, T. G., and Moore, D. M. (1987) *The flora of the British Isles.* 3rd edn. Cambridge: Cambridge University Press.

Crowder, L. V., Hawk, J. A., Gradziel, T. M., and Fast, S. E. (1980) In *Tested studies for laboratory teaching.* Dubuque, IA: Kendall Hunt. pp. 155–169.

Dickinson, H. ed. (1989) *Core biology of plant breeding.* A Review published for the Commission of the European Communities Biotechnology Division.

Electricity Council (1987) *Lighting for horticultural production.* The Farm Electric Centre, Kenilworth, Stoneleigh, Warwickshire CV8 2LS.

Friend, D. J. C. and Helson, V. A. (1966) Floral induction by one long day in *Brassica campestris* L. *Science,* **153,** 1115.

Hafner, R. S. (1987) *Brassica rapa*—a resource book for secondary biology teachers. Unpublished master's thesis, University of Wisconsin–Madison.

Hafner, R. S. (1990) Fast plants—rapid-cycling brassicas. *The American Biology Teacher,* **52**(1), 40–46.

Hawk, J. A. and Crowder, L. V. (1978a) *Brassica campestris* L. a higher plant with the potential for teaching genetics. *Journal of Heredity,* **69,** 121–124.

Hawk, J. A. and Crowder, L. V. (1978b) The inheritance of four mutants in early flowering *Brassica campestris. Journal of Heredity,* **69,** 125–127.

Karpechenko, G. D. (1929) *Raphanobrassica. Journal of Genetics,* **14,** 375.

McNaughton, I. H. and Ross, C. J. (1978) Inter-specific and inter-generic hybridization in the Brassicae with special emphasis on the improvement of forage crops. *SPBS Report (1977–78).*

Nakinishi, T. and Hinaka, K. (1973). An effective time of CO_2 gas treatment in overcoming self-incompatibility in *Brassica. Plant and Cell Physiology,* **14,** 873–879.

Postlethwaite, S. N. and Enochs, N. J. (1967) Tachyplants—suited to instruction and research. *Plant Sciences Bulletin, Botanical Society of America,* 13, 2.

Richards, R. A. and Thurling, N. (1973) The genetics of self incompatibility in *Brassica campestris.* L. *Genetica,* **44,** 428–438.

Saaren, P. K. and Kakar, S. N. (1975) The genetics of

incompatibility in brown sarson, *Brassica campestris* L. *Pflanzenzucht*, **74**, 291–297.

Simmonds, N. W. (1976) *Evolution of crop plants*. London: Longman.

Vaughan, J. G., MacLeod, A. J., and Jones, B. M. G. (1976) *The biology and chemistry of the Cruciferae*. London: Academic Press.

Williams, P. H. (1980) Beesticks, an aid to pollinating the Cruciferae. *Horticultural Science*, **15**(6), 802–803.

Williams, P. H. (1985) *CrGC Resources Book*. Wisconsin–Madison, WI: Department of Plant Pathology, University of Wisconsin–Madison.

Williams, P. H. and Hill, C. B. (1986) Rapid cycling populations of *Brassica. Science*, **232**, 1385–1389.

Williams, P. H. (1990) Rapid-cycling brassicas: a context for plant biotechnology. *Biotechnology Education*, **1**(3), 111–114. (Published by the National Centre for School Biotechnology, Reading.)

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