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# CHEMICAL GAMETOCIDES FOR WHEAT AND BARLEY IN RELATION TO F1 HYBRID BREEDING PROGRAMMES

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Summary Research programmes in the USA have demonstrated enhanced yield and other advantages of hybrid wheat over conventional, pure-line varieties and seed of the first, commercial hybrid varieties is now available. Seed production systems for hybrid wheat presently rely on male sterile cytoplasms, which are both difficult and costly to handle, to control pollination. Use of a chemical gametocide would offer significant advantages over this system. Chemicals with known gametocidal activity are listed and the activity of two compounds - RH531 and DPX3778 is discussed. A specification for the 'ideal gametocide' is advanced.

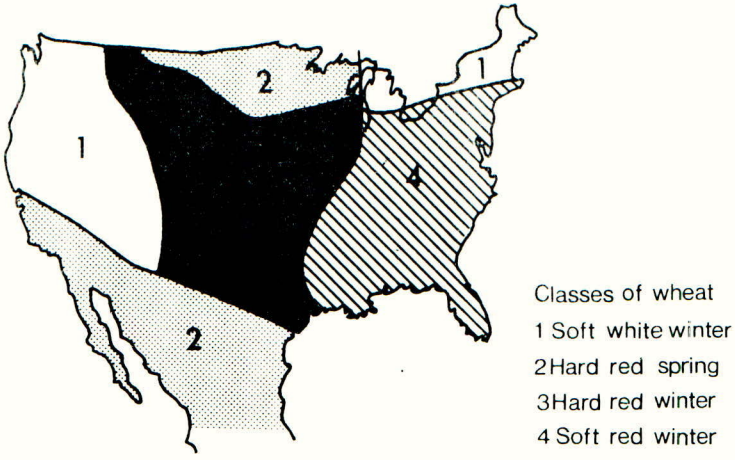
## THE IMPORTANCE OF $F_1$ VARIETIES IN CEREALS

Ever since the successful commercial exploitation of hybrid vigour in maize, of which approximately 80-90% of the North American acreage is now planted with hybrid varieties (19), the possibility of developing hybrid varieties of other crops, such as wheat and barley, has been considered. Research into hybrid cereals has been carried out in W. Europe and USA. In W. Europe, where the research has been devoted mainly to wheat, with some work in barley and rye, there has been only limited success. No commercial hybrid varieties are currently available, nor are they likely to be in the near future. A possible exception is rye, where hybrids may be released in the next 2-3 years. The reasons for this lack of success have been the poor levels of heterosis which have been achieved and which are insufficient to pay for the costs of production of hybrid seed. In addition strong conventional breeding programmes have maintained a steady increase in yield with pure line varieties.

In the USA however, the position is somewhat different. Research into hybrid wheat started in the 1950s and after initial setbacks the first hybrid wheat varieties are now just beginning to be released for commercial production. The distribution of the major types of wheat grown in the U.S. is shown in Figure 1. Winter wheats account for the largest proportion - more than 52M acres or approximately 70% of the U.S. wheat acreage. Hard red winter wheats (HRW) form the single most important class - 40M acres or approximately 55% of the total wheat acreage. Not unnaturally, hybrid research has concentrated on HRW types. The first hybrid variety was released by the De Kalb Co. in 1971, and now HRW hybrids are grown on approximately 1M acres. Progress with the other classes of wheat has been less rapid. Soft wheat hybrids are perhaps 3-5 years from commercial release. Spring wheats are further behind, largely due to problems with fertility restoration in the adverse environmental conditions under which the spring wheats are typically grown.

The success of hybrid breeding programmes in the USA, may be attributed partly to the American 'belief' in the concept of hybrid wheat which has been backed by large investments in long term research programmes, and partly to the lower level of

FIGURE 1 Distribution of major wheat types in the U.S.A.




effort in conventional, pure line breeding programmes. The following advantages over conventional varieties are claimed for the new hybrid wheats (28):

Heterosis: although it was uncertain whether wheat hybrids would exhibit heterosis, research has shown that yield increases may be obtained. Increases of 15% to 30% compared with conventional pure line types are generally quoted, although this figure is obtained by comparison with commercially-available varieties adapted to the area in which the hybrid is grown rather than with the pure line parents of the hybrid. Critics claim this to be an artificial comparison and comment that the success of hybrids owes much to the poor pure-line varieties with which they are compared.

Improved pasture yield: there is a large heterotic effect on vegetative growth and this is important in many areas where winter wheat is grazed for forage and then grown on to yield.

Increased stem strength: prevents lodging.

Increased seedling vigour and tillering: allows reductions in seeding rate of about 25%.

Increased protein content of grain.

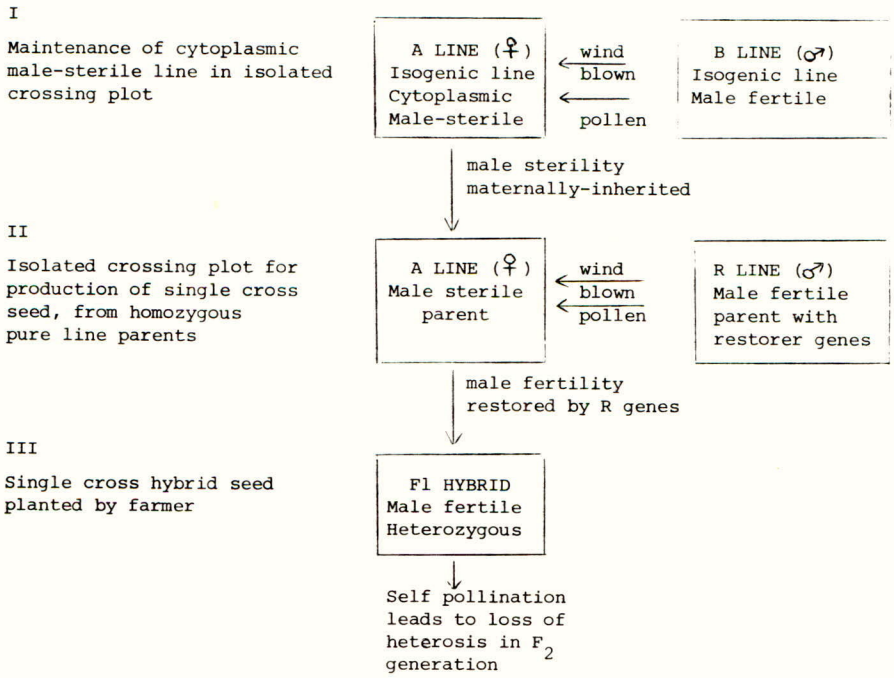
Control of seed supply by seed companies: hybrid seed must be produced annually from the parental foundation stock by a specialised operation, allowing seed companies to control supply and ensure economic returns.

Hybrids currently occupy only a small proportion of the total US wheat acreage. The expansion of the hybrid acreage is hindered by the practical problems of seed production - whether hybrid seed can be produced on a field scale and whether the benefits of hybrid wheat will pay for the increased costs incurred in its production.

PRODUCTION OF HYBRID WHEAT VARIETIES

The production of an F1 hybrid cereal variety is illustrated in Figure 2.

Figure 2. Method of producing seed of hybrid cereals




An F1 hybrid variety is produced by the controlled hybridisation of two pure line, homozygous parents (Figure 2;II); it is hezerozygous and displays heterosis (Figure 2;III). However, this hybrid variety segregates in F2 and succeeding generations and is not stable. It must be recreated annually from the two pure line parents.

Since cereals such as wheat are normally self-pollinating, the production of an hybrid requires the control of pollination, by making one parent male-sterile. Cross pollination is thus assured. In small scale glasshouse studies this is achieved by hand emasculation or by heat treatments. On a field scale cytoplasmic or genetically-controlled male sterility is used. The cytoplasmic system is best known and is the system presently used in the production of hybrid seed. A cytoplasmically-inherited factor, isolated from the primitive wheat Triticum timopheevi and incorporated in the female parent, suppresses pollen development giving rise to a male sterile A Line. This is crossed with the male fertile parent or R line, which has nuclear, restorer genes. Provided these lines are isolated from other pollen sources, all seed set by the A line will be the F1 hybrid. The restorer genes, conferred by the R line, suppress the expression of the maternally-inherited male sterile cytoplasm and restore fertility to the hybrid.

Parental lines, or foundation stock, must be maintained continually for the production of F1 hybrids. The R line is fully fertile and is maintained by self pollination. The A line however is male-sterile and is maintained with difficulty

through an isogenic line - the B line or Maintainer line - without the cytoplasmic male sterile factor (Figure 2;I). The A and B lines are crossed and seed collected from the A line. Because A and B are isogenic, the genotype remains unchanged. However, because cytoplasmic factors are maternally inherited, this seed is male sterile.

The complexity of this system is one of the main difficulties associated with hybrid seed production. In addition, few male sterile cytoplasms are known, and of these only that isolated from T. timopheevi has proved suitable for commercial exploitation. There is a danger that if a single cytoplasm were to be incorporated in large areas of hybrid varieties, pathogen epiphytotics could occur. This has already happened with maize hybrids, where the use of Texas male sterile (T) cytoplasms led to the rapid spread of Southern Leaf Blight through much of the commercial crop (11).

The use of a chemical gametocide would offer certain advantages over cytoplasmic male sterility: i) male sterility could be produced as required, eliminating the need to introduce sterility factors into prospective parents by lengthy backcrossing programmes; ii) the need for restorer genes would be avoided; iii) the complex procedure using B lines to maintain female parent would not be necessary; iv) the reliance on a single cytoplasm, with its consequent dangers, would be avoided. It is these potential benefits which have triggered research for a suitable chemical gametocide.

## CHEMICALS WITH KNOWN GAMETOCIDAL ACTIVITY

Some of the more important chemical types which have been shown to have gametocidal activity are listed in Table 1. It is not intended to review their chemical activity in this paper, other than to note the wide range of species in which they are active and to draw attention to the fact that none has proved satisfactory for the commercial production of $F_1$ hybrid seed.

In the following section the activity of two of these compounds - RH531 and DPX3778 - is discussed in more detail.

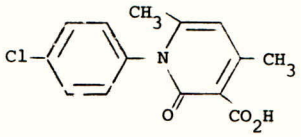
Table 1. Chemicals with known gametocidal activity

| CHEMICAL NAME(S) | CROP SPECIES |
|---|---|
| Ethrel (Ethephon) (2-chloroethylphosphonic acid) | wheat (44,45,49,2,23), barley (30,22), triticale (47), sugar beet (20,21), cucurbits (31,42,32) |
| RH531 | wheat (50,24), barley (22,56), triticale(50,47) |
| RH 532 | wheat (25) |
| DPX 3778 | maize (51,29), wheat (26) |
| MH30 (maleic hydrazide) | maize (34,38), wheat (8,40), tomato (41), capsicum (7), grape (1) |
| UNI D513 | triticale (47) |
| FW450 (Mendok) | cotton (9,12,16), sugar beet (10,20), soya (46), tomato (35,36), alfalfa (33), wheat (40), rye (37), ryegrass (15) |

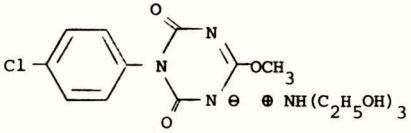
| CHEMICAL NAME(S) | CROP SPECIES |
|---|---|
| Gibberellic acid | maize (19,39,57), wheat (40), sunflower (48), lettuce (13,55) |
| TIBA | wheat (40), grape (1) |
| Morphactins | cucurbits (6,27) |
| Oestrone | wheat (14), sugar beet (20) |
| methano pyrrolidines (17)<br>alkyl pyridones (52)<br>benzyl pyridones (53)<br>cinchoninic acids (54) | recent patents |

## EFFECTS OF SOME IMPORTANT CHEMICAL GAMETOCIDES

The chemical structure and name are shown below for RH531 and DPX3778



RH531: Sodium 1-(p-chlorophenyl)-1,2-dihydro-4,6-dimethyl-2-oxonicotinate.



DPX3778: 3-(p-chlorophenyl)-6-methoxy-s-triazine-2,4-(1H,3H)-dione; triethanolamine salt.

The experiments described below were carried out during the summer of 1975 using barley (Hordeum vulgare L. cv Mari) as the test species. Plants were grown 4 per 10 cm pot in a sand/peat compost, and were maintained in a glasshouse under an 18h daylength in which natural daylight was extended with high pressure mercury fluorescent lamps (type MBFR).

The experiments were designed to look at the effects of rate and time of application of the chemical gametocides. There were 8 rates (0,125,250,500,1000, 2000,4000,8000 ppm ai) and 4 times of application (to coincide with premeiotic, meiotic and early and late post-meiotic stages in pollen development). Treatments were a factorial combination of the levels of these two factors. There were four replicate pots for each treatment and 20 ml of solution was sprayed to run-off on these four pots. The active ingredient was formulated in 4 ml Acetone + 2 ml 1% Lissapol NX.

At each time of application, plants were sampled to determine the stage of the development of the main shoots according to the numbers of fully expanded leaves, the length of the developing ear, the stage of ear development and the stage of pollen development. The stage of pollen development was assessed by microscopic examination of anthers from the most advanced florets situated in the middle of the ear. Details of the sequence of ear and pollen development may be found elsewhere (3,4,5).

At ear emergence the main shoot ears of the plants were bagged to prevent cross-pollination. Thus any grain set by these ears would be the result of self-pollination only; a low grain set is indicative of gametocidal activity. The measure of grain setting used in the data which follows is Floret Fertility, which is defined as

$$\frac{\text{Number of grains per ear}}{\text{Number of florets per ear}} \times 100\%$$

The first experiment investigated the effects of RH531. The stages of development of the plants at each of the times of spraying are shown in Table 2.

Table 2. The stage of development of the main shoot at each of the times of spraying. Data are based on 4 replicate pots each containing 4 plants

| CHARACTER | STAGE OF SPRAYING | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Age (days) | 23 | 25 | 28 | 31 |
| Number of fully-expanded leaves | 5 | 6 | 7 | 7 |
| Ear length (mm) | 8.1 | 29.4 | 56.4 | 57.8 |
| Stage of ear development | awn initials | awns longer than spike | spike elongation | awn emergence |
| Stage of pollen development: (1) | | | | |
| premeiotic mitosis | 5 | - | - | - |
| premeiotic interphase | 11 | - | - | - |
| meiosis | - | 7 | 1 | - |
| early post-meiotic | - | 9 | 4 | - |
| late post-meiotic | - | - | 11 | 16 |

(1) Data for pollen development are the totals amongst 16 plants sampled at each time of spraying.

The effects of the RH531 treatments on grain setting in the bagged main shoot ears are given in Table 3. These show that:

i) Effects varied with the stage of development at which the chemical was applied. Applications made 23 or 25 days after sowing led to large reductions in floret fertility, applications made 28 days after sowing had very small effects on fertility, whereas applications made 31 days after sowing were without effect on floret fertility.

ii) The 'sensitive stage' corresponds with stages in pollen development around meiosis. Thus at 23 days pollen cells were premeiotic and at 25 days pollen was at meiosis or in the early young pollen stages (Table 2).

iii) There was no evidence of a dose response with applications made at the sensitive stage; rates of as little as 125 ppm inhibited grain setting.

iv) The controls showed no effects on floret fertility confirming that neither the formulation blank nor the bagging process affected grain setting.

v) The RH531 treatments did, however, lead to abnormal ear development. Ears were stunted with shortened rachis internodes giving a characteristic compacted appearance.

Table 3. Effects of the RH531 treatments on grain setting in bagged main shoot ears Data for floret fertility are presented as percentages (%) and also arcsin transformed (trans) LSDs' for floret fertility are for arcsin transformed data only

| Rate (ppm ai) | Stage of Application | Florets | Grains | Fertility (%) | Fertility (trans) |
|---|---|---|---|---|---|
| 0 | | 15.1 | 11.6 | 76.5 | 61.0 |
| 125 | | 15.6 | 1.4 | 2.6 | 9.3 |
| 250 | | 18.9 | 2.5 | 4.9 | 12.8 |
| 500 | | 16.1 | 0.0 | 0.0 | 0.0 |
| 1000 | I | 18.3 | 0.6 | 0.9 | 5.4 |
| 2000 | | 18.0 | 0.4 | 0.8 | 5.1 |
| 4000 | | 16.6 | 0.3 | 0.4 | 3.6 |
| 8000 | | 17.9 | 0.0 | 0.0 | 0.0 |
| 0 | | 17.0 | 12.0 | 70.6 | 57.2 |
| 125 | | 17.5 | 0.3 | 0.3 | 3.4 |
| 250 | | 18.0 | 0.3 | 0.4 | 3.4 |
| 500 | II | 17.6 | 0.6 | 2.2 | 8.5 |
| 1000 | | 15.9 | 0.3 | 0.2 | 2.5 |
| 2000 | | 15.9 | 0.3 | 0.4 | 3.7 |
| 4000 | | 16.9 | 0.6 | 1.4 | 6.7 |
| 8000 | | 16.9 | 0.3 | 0.4 | 3.6 |
| 0 | | 15.1 | 11.5 | 76.2 | 60.8 |
| 125 | | 16.1 | 10.4 | 62.5 | 52.2 |
| 250 | | 16.0 | 12.4 | 77.1 | 61.4 |
| 500 | | 16.8 | 13.0 | 78.7 | 62.5 |
| 1000 | III | 15.8 | 10.1 | 63.6 | 52.9 |
| 2000 | | 14.6 | 6.4 | 43.7 | 41.4 |
| 4000 | | 16.1 | 9.4 | 54.0 | 47.3 |
| 8000 | | 15.6 | 9.9 | 56.8 | 48.9 |
| 0 | | 16.0 | 13.4 | 84.8 | 67.1 |
| 125 | | 16.9 | 12.1 | 73.2 | 58.8 |
| 250 | | 16.3 | 14.0 | 88.2 | 69.9 |
| 500 | IV | 17.0 | 13.0 | 78.0 | 62.0 |
| 1000 | | 16.0 | 12.6 | 80.4 | 63.7 |
| 2000 | | 16.0 | 13.0 | 82.3 | 65.1 |
| 4000 | | 15.9 | 12.0 | 76.3 | 60.9 |
| 8000 | | 16.6 | 13.8 | 83.3 | 65.9 |
| Untreated control | | 16.3 | 12.6 | 79.0 | 62.7 |
| LSD (5%) | | | | | |
| treated v untreated | | 1.8 | 3.7 | - | 17.4 |
| treated v control | | 1.6 | 3.3 | - | 15.0 |

Table 4. The stage of development of the main shoot at each of the times of spraying with DPX3778. Data are based on 4 replicate pots each containing 4 plants

| CHARACTER | STAGE OF SPRAYING | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Age (days) | 22 | 26 | 28 | 30 |
| Number of fully expanded leaves | 5 | 6 | 7 | 7 |
| Ear length (mm) | 5.0 | 15.5 | 39.3 | 68.1 |
| Stage of ear development | awn initials | awns longer than spike | spike elongation | awn emergence |
| Stage in pollen development (1) | | | | |
| premeiotic mitosis | 16 | 1 | - | - |
| premeiotic interphase | - | 1 | - | - |
| meiosis | - | 14 | 2 | - |
| Early post meiotic | - | - | 14 | - |
| late post meiotic | - | - | - | 16 |

(1) Data for pollen development are the totals amongst the 16 plants sampled at each time of spraying.

The second experiment investigated the effects of DPX3778. The stages of development of the main shoots at each time of spraying are shown in Table 4 and the effects of the treatments on grain setting in the bagged main shoot ears are given in Table 5. These show that:

i) The effects of DPX3778 were dependent on the stage of development at the time of treatment. The stage which was most sensitive occurred 26 days after sowing when all rates led to almost complete failure of grain setting. These treatments coincided with meiotic stages in pollen development (Table 4)

ii) The plants were also sensitive at stage I (22 days) which coincided with the premeiotic pollen stages (Table 4). However, the plants were less sensitive and only 4000 and 8000 ppm rates reduced fertility.

iii) Applications made at stage III (28 days), coinciding with the early postmeiotic stages (Table 4) were similarly less sensitive to the chemical; the 1000, 2000, 4000 and 8000 ppm rates gave slight reductions in fertility.

iv) Applications made at stage IV, at awn emergence, had no effect on fertility.

The experiments demonstrate that it is possible to inhibit grain setting completely with chemical gametocides. In these studies no observations of ovule development were made and no cross-pollinations were performed. It is thus possible that both male and female development could have been affected and that the chemicals induced total sterility. However, in other work both RH531 and DPX3778 have been found to selectively inhibit pollen development (29,56). The effectiveness of the chemical gametocides depended on the stage at which they were applied. The critical stage for both RH531 and DPX3778 occurred at or just before meiosis, and this agrees with similar observations made with ethephon as a gametocide (2,43). However, the plants were also sensitive to the RH531 and DPX3778 treatments immediately before and after this critical stage, and there were differences between the two chemicals. At a given rate RH531 was active over a wider range of stages than DPX3778, which would allow a greater flexibility of timing of application to induce complete male sterility. Although it is thus technically-feasible to control pollination by means of chemical gametocides, there are problems still to be solved if chemical gametocides are to be used in the commercial production of hybrid seed.

Table 5. Effects of the DPX3778 treatments on grain setting in bagged main shoot ears Figures in brackets were not included in analyses of variance; LSD's are not applicable to these estimates

| Rate (ppm ai) | stage of application | Florets | Grains | Fertility (%) | Fertility (trans) |
|---|---|---|---|---|---|
| 0 | | 16.3 | 11.5 | 71.0 | 57.4 |
| 125 | | 17.0 | 13.3 | 79.0 | 62.7 |
| 250 | | 16.9 | 14.9 | 88.5 | 70.2 |
| 500 | | 18.8 | 15.4 | 82.8 | 65.5 |
| 1000 | I | 18.0 | 16.0 | 89.1 | 70.8 |
| 2000 | | 17.8 | 14.4 | 83.8 | 66.2 |
| 4000 | | 18.3 | 11.1 | 61.3 | 51.5 |
| 8000 | | 17.0 | 3.3 | 10.9 | 19.3 |
| 0 | | 18.1 | 14.5 | 81.8 | 64.8 |
| 125 | | 17.5 | (0) | (0) | (0.0) |
| 250 | | 18.8 | (0) | (0) | (0.0) |
| 500 | | 18.4 | (0) | (0) | (0.0) |
| 1000 | II | 17.8 | (0.3) | (1.3) | (6.5) |
| 2000 | | 18.3 | (0.3) | (1.4) | (6.8) |
| 4000 | | 17.5 | (0.3) | (1.4) | (6.8) |
| 8000 | | 18.5 | (0) | (0) | (0.0) |
| 0 | | 17.6 | 15.1 | 86.4 | 68.4 |
| 125 | | 18.8 | 14.8 | 80.6 | 63.8 |
| 250 | III | 17.5 | 15.8 | 90.4 | 71.9 |
| 500 | | 17.3 | 14.9 | 87.3 | 69.1 |
| 1000 | | 15.5 | 10.3 | 65.8 | 54.2 |
| 2000 | | 17.8 | 11.3 | 65.2 | 53.8 |
| 4000 | | 17.8 | 9.6 | 53.2 | 46.8 |
| 8000 | | 16.3 | 7.3 | 44.0 | 41.6 |
| 0 | | 17.0 | 13.5 | 79.8 | 63.3 |
| 125 | | 19.1 | 14.3 | 76.3 | 60.9 |
| 250 | | 17.8 | 11.5 | 59.7 | 50.6 |
| 500 | | 17.6 | 13.3 | 75.9 | 60.6 |
| 1000 | IV | 17.1 | 12.5 | 74.4 | 59.6 |
| 2000 | | 17.0 | 10.3 | 61.2 | 51.5 |
| 4000 | | 16.8 | 13.3 | 79.8 | 63.3 |
| 8000 | | 17.5 | 8.0 | 45.0 | 42.1 |
| Untreated control | | 18.6 | 16.1 | 87.3 | 69.1 |
| LSD (5%) | | | | | |
| treated v treated | | 1.8 | 4.1 | - | 15.6 |
| treated v control | | 1.6 | 3.5 | - | 13.4 |

## SPECIFICATION OF THE IDEAL CHEMICAL GAMETOCIDE

The 'ideal' chemical gametocide, for use in the production of hybrid seed on a field scale, should possess the following properties:

1) Induction of male but not female sterility: any reduction in female sterility would reduce hybrid seed yields and raise seed price.

2) Complete inhibition of pollen development: partial male sterility would lead to

selfing. Since the parental lines are naturally homozygous and show no inbreeding depression, this would not lead to large reductions in yield in the hybrid crop, but rather to a reduction in the proportion of heterosis. Confidentiality of breeding lines would also be difficult to maintain.

3) Independence from environmental conditions: a gametocide must produce reliable effects in a range of environments. It is probably the major reason for the failure of experimental compounds and is perhaps the single most important criterion.

4) Independence from genotypic differences: a gametocide must be effective in the range of parental genotypes emerging from breeding programmes. It is likely that the commercial life of hybrid varieties will be short; in maize hybrid varieties are replaced on average every 5-6 years.

5) Wide flexibility of rate and stage of application: chemicals which are too dependent on rate or growth stage would be impractical where large acreages required spraying or adverse weather conditions prevented spraying. Chemicals applied as a seed dressing would be ideal.

6) Absence of phytotoxicity or other adverse effects.

7) Environmental safety.

8) Cost effectiveness.

A chemical matching the above criteria has yet to be discovered.

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## References

1. ARUMAGAM, R. and RAO, V.N.M. (1973). Indian Journal of Plant Physiology 16, 1-4.
2. BENNETT, M.D. and HUGHES, W.G. (1972). Nature (London) 240, 566-8.
3. BENNETT, M.D., RAO, M.K., SMITH, J.B. and BAYLISS, M.W. (1973). Philosophical Transactions of the Royal Society, Series B, 266, 39-81.
4. BONNETT, O.T. (1933). Journal of Agricultural Research 51, 451-7.
5. BONNETT, O.T. (1966). Bulletin of the Illinois Agriculture Experimental Station 721, 1-105.
6. CANTLIFFE, D.J. (1974). Canadian Journal of Plant Science 54, 771-6.
7. CHAUHAN, S.V.S. and SINGH, S.P. (1972). Indian Journal of Plant Physiology 15, 138-47.
8. CHOPRA, V.L., JAIN, S.K. and SWAMINANTHAN, M.S. (1960). Indian Journal of Genetics and Plant Breeding 20, 188-99
9. CHOUDHURRY, A.R. (1966). Pakistan Journal of Science 18, 175-88.
10. DUDLEY, J.W. (1960). Journal of the American Society of Sugar Beet Technology 11, 25-36.
11. DUVICK, D.N. (1975). Proceedings of the 27th Annual Conference on Corn and Sorghum Research, 192-201.
12. EATON, F.W. (1957). Science (New York) 126, 1175.
13. EENINK, A.H. and LOUPIAS, S. (1976). Zaadbelangen 10, 300-2.
14. FENDAL, R.K. (1967). Washington State University, Research Progress 474.
15. FOSTER, C.A. (1969). Annals of Botany (London) 33, 947-50.
16. GAGNEJA, M.R. and SINGH, T.P. (1973). Indian Journal of Genetics and Plant Breeding 33, 340.

17. GERMAN PATENT NO. DT 2641295, 16 September 1975.
18. GRILICHES, Z. (1960). Science 132, 275-80.
19. HANSEN, D.J., BELLMAN, S.K. and SACHER, R.M. (1975). Plant Physiology 56, (2, Supplement), 44.
20. HECKER, R.J. BILGEN, T., BHATNAGAR, P.S. and SMITH, G.A. (1972). Canadian Journal of Plant Science 52, 937-40.
21. HECKER, R.J. and SMITH, G.A. (1975). Canadian Journal of Plant Science 55, 655-6.
22. HOCKETT, E.A. (1972). Barley Newsletter 15, 95-8.
23. HUGHES, W.G., BENNETT, M.D., BODDEN, J.J. and GALANOPOULOU, S. (1974). Annals of Applied Botany 76, 243-52.
24. JAN, C.C., QUALSET, C.O. and VOGT, H.E. (1974). Euphytica 23, 78-85.
25. JAN, C.C., QUALSET, C.O. and VOGT, H.E. (1976). Euphytica 25, 375.
26. JOHNSON, R.R. and BROWN, C.M. (1976). Crop Science 16, 584-7.
27. KAUSHIK, M.P. and SHARMA, J.K. (1974). Indian Journal of Experimental Biology 12, 599-600.
28. KINNEAR, H. (1975). The American Farmer, September 1975 16.
29. LAIBLE, C.A. (1974). Proceedings of the 29th Annual Corn and Sorghum Research Conference, 174-84.
30. LAW, J. and STOSKOPF, N.C. (1973). Canadian Journal of Plant Science 53, 765-6.
31. LOWER, R.L. and MILLER, C.H. (1969). Nature (London) 222, 1072-3.
32. McMURRAY, A.L. and MILLER, C.H. (1969). Journal of the American Society of Horticultural Science 94, 400-2.
33. MILLER, D.A. and HITTLE, C.N. (1963). Crop Science 3, 397-400.
34. MOORE, R.H. (1950). Science 112, 52.
35. MOORE, J.F. (1959). Science 129, 1738-40.
36. MOORE, J.F. (1964). Proceedings of the American Society of Horticultural Science 84, 474-9.
37. NATROVA, Z and HLAVIC, M. (1975). Biologia Plantarum 17, 251-5.
38. NAYLOR, and DAVIS (1950). Botanical Gazette 112, 112.
39. NELSON, P.M. and ROSSMAN, E.C. (1958). Science 127, 1500-01.
40. PORTER, K.B. and WIESE, A.F. (1961). Crop Science 1, 381-2.
41. REHM, S. (1952). Nature (London) 170, 38.
42. ROBINSON, R.W., WHITACKER, T.W. and BAHN, G.W. (1970). Euphytica 19, 180-3.
43. ROWELL, P.L. (1972). Ph.D.Thesis, Washington State University, Pullman, Washington.
44. ROWELL, P.L. and MILLER, D.G. (1971). Crop Science 11, 629-31.
45. ROWELL,P.L. and MILLER, D.G. (1974). Crop Science 14, 31-3.
46. de SACKS, R.L. and ROSAS, G.S. (1966). Plant Breeding Abstracts 33, 1654.
47. SAPRA, V.T., SHARMA, G.C. and HUGHES, J.L. (1974). Euphytica 23, 685-90.
48. SEETHARA, A. and KUMARI, P.K. (1975). Indian Journal of Genetics and Plant Breeding 35, 136.
49. STOSKOPF, N.C. and LAW, J. (1972). Canadian Journal of Plant Science 52, 680-3.
50. TRUPP, C.R. (1972). American Society of Agronomy Abstracts, 29.
51. U.S.A. PATENT APPLICATION NO. 325359, 22 January 1973.
52. U.S.A. PATENT NO. 4038065, 11 October 1974.
53. U.S.A. PATENT NO. 4028084, 11 October 1974.
54. U.S.A. PATENT NO. 4009020, 6 May 1975.
55. VAN DER MEER, Q.P. and VAN BENNEKOM, J.L. (1973). Euphytica 22, 239-43.
56. WANG, R.C. and LUND, S. (1975). Crop Science 15, 550-3.
57. WITTWER, S.H. and BUKOVAC, M.J. (1958). Economic Botany 12, 213-55.
58. YIH, R.Y., McNULTY, P.J., SEIDEL, M.C. and VISTE, K.L. (1971). Horticultural Science 6, 460-1.