

# A Review of the Place of *in vitro* Cell Culture Systems in Studies of Action, Metabolism and Resistance of Biocides Affecting Photosynthesis

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The homogeneity, density and axenic nature of cell culture systems have made them an amenable tool for studying many aspects of biocide research; screening, metabolism, mode of action and resistance. Cell cultures are “multi-homogeneous” and each homogeneous state may be analogous to different plant parts. Cultures can be homogeneously green, homogeneously white in either a homogeneously exponential or stationary phase of growth. Examples are presented showing why no single homogeneous state should be envisaged to be analogous to a whole plant in biocide studies.

Because of the possibility of more uniform herbicide application, culture systems have a use in isolating resistant crop strains, at higher selection pressures and much higher “plant” densities than is possible in the field, with recent successes. Protoplast fusion techniques may allow the transfer of genetic resistance between related but genetically incompatible crop species.

## Introduction

Cell culture systems offer quite obvious advantages for studying biocide effects in plants, *e. g.*, cells in suspension facilitate rapid kinetics; the sterility is important for metabolic studies; the immense number of potentially totipotent “plants” in small volumes of suspensions should represent hectares of fields in attempts to select for herbicide resistant phenotypes (*cf.* [1]). They have not received as much attention in studies with pesticides affecting photosynthesis as they have for other herbicides. This has occurred for a variety of reasons; not all herbicides affecting photosynthesis, affected cell cultures in one early report [2]. This was most probably due to the historically ill conceived notion that just because cells can ultimately be regenerated into plants they should always have all systems of the plant operative. We can measure photosynthesis in whole plants or we can measure it in isolated plastids. Why then should we need the middle ground of the isolated cell, especially as algae can also be considered to be isolated cells? For the study of photosynthesis, isolated plastids are excellent in many cases; why then do we need cells? Isolated plastids are of no help with inhibitors that work at the level of plastid

development, *e. g.* those that prevent carotene synthesis. Examples will be shown below that *cells* fulfil a gap in research needs if it is the *herbicide* (and not just *photosynthesis*) that is of interest. Cells will allow us to ascertain whether photosynthesis is the sole function affected by a herbicide. The cells may help us in deciding how a compound inhibiting at a known site actually brings about the death of the plants; something that may not be evident from plastid studies. Would not then algae or leaf discs be better than cells? Not always as they too have their limitations. Often one wishes to find out if compounds have potential herbicidal effects, especially for structure-activity correlations. Such correlations are not just a function of reactivity at the site of action; penetration is a major factor. The leaf and the leaf discs bear cuticles which must be traversed. Without permeability barriers one can construct structure-activity relationships more closely related to the site of action. Algae have permeability barriers, probably because they evolved in “liquid media” which often contain hostile compounds. Algae and plants can react quite differently to some herbicides (*e. g.* [3]). If plants are of interest, it might be better to use plants or their cells.

**Abbreviations:** The International Standards Organization, British Standards Institute and Weed Science Society of America common names of biocides are used throughout.

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## Problems: do cell cultures mirror plants?

On the one hand, cell cultures offer us a comparatively homogeneous, axenic, cuticle free system with almost all cells metabolically active and little



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need for intercellular transport. On the other, a plant is a cuticle bearing, heterogeneous system with most of its cells being either highly differentiated or dead. The last are unaffected by biocides but we must know how well cell cultures mirror heterogeneously differentiated plants. Steady state growing cultures are selected for rapid division and a lack of organ and tissue differentiation. Most callus and suspension cultures are white (colorless); *i. e.* they lack chlorophyll. In recent years only a few chlorophyllous cell cultures have been isolated; of these fewer yet are photoautotrophic [4]. The philosophical assumption (possibly subconscious) that all plant cells should have all biochemical pathways operative did not deter researchers from treating calli or cell suspensions with herbicides thought or known to affect photosynthesis, with mixed results (*e. g.* [2]).

A degree of rationalization of using cell cultures for herbicide research involved using green and white cultures [5–7] and cultures at different stages of development [8, 9]. It was possible to show that cell cultures reflect the whole plant but in a manner more complicated than had been previously envisaged. Cell cultures are not completely undifferentiated and the target of many herbicides is in cells in a differentiated state. Using green and white callus cultures of different species, ranked correlations could be made between the effects of various herbicides on the cultures and on plants [5]. Some of the herbicides which seemed “out of rank” in either green or white cultures were later tested in green and white callus cultures of the same species [6, 7]. For example, napropamide, a herbicide which is not toxic to tomato shoots but is toxic to tomato roots, at certain concentrations was lethal to white calli without affecting green tomato calli [6]. On the other hand, herbicides which affect photosynthesis are much more toxic to green than to white cultures. Concentrations of simazine that were 85% inhibitory to green *Rumex obtusifolius* calli were without affect on white calli of the same species [7]. Similar results to the calli can be obtained with suspension cultures of two closely related *Solanaceae*; tomato (green cultures) and *Solanum nigrum* (achlorophyllous cultures). Suspension cultures have the advantage over calli in that metabolic parameters, which are easier to measure, can be used to estimate effect instead of growth. Some of the findings with such cultures are summarized in Table I. These results follow and expand the

Table I. Effects of herbicides with various modes of action on vitality of green and white cell suspensions cultures.

Herbicide M	White <i>Solanum nigrum</i> cultures	Green <i>Lycopersicon esculentum</i> cultures
	<sup>14</sup> C amino acid incorporation (% of untreated control)	
Atrazine		
3 × 10 <sup>-10</sup>	138	55
10 <sup>-3</sup>	106	28
Metribuzin		
10 <sup>-3</sup>	107	92
3 × 10 <sup>-3</sup>	106	61
Diallate		
3 × 10 <sup>-5</sup>	99	14
10 <sup>-4</sup>	117	11
3 × 10 <sup>-4</sup>	110	9
10 <sup>-3</sup>	64	7
Napropamide		
3 × 10 <sup>-4</sup>	62	99
10 <sup>-3</sup>	2	87

The cultures used were essentially those described in [10] except that in the past year the tomato cultures have greened under high light intensities although they are still obligate heterotrophs, (with a measured ability to photosynthesize). The herbicides were placed in 10% packed volume cultures, incubated for 45 h and <sup>14</sup>C amino acid incorporation was then measured for one hour as a measure of viability and growth of the culture essentially as outlined in ref. [11]. These data are previously unpublished.

results with calli [6, 7]. Napropamide affected only white cultures; atrazine affected only green cultures (previously unpubl.) and diuron affected green cultures more than white cultures. Atrazine and diuron are supposed to affect the same binding site on plastids (*e. g.* [12]) thus one might wonder why no concentration of diuron could be found which gives a total differential toxicity between the green and white calli [7]. A highly plausible explanation is that diuron can inhibit at more than one site and the *K<sub>i</sub>*'s of the different sites are not that dissimilar. Such findings which could say much about the modes of action of diuron, are not obtainable with plastids. The results have strong implications about the rate at which diuron resistant weeds would appear in the field, as will be discussed in a later section. Metribuzin also discriminated strongly between green and white cells hurting the green cells (Table I). This may help in explaining the data of Ellis [13] who found that the differential tolerance of different tomato strains to metribuzin did not carry over to cell cultures. His cell structures were not green, and the tolerance could well be at

the level of the differentiated plastid. Detoxification of metribuzin does not require green cultures; suspension cultures of a soybean variety which is more tolerant to metribuzin in the field detoxify it more rapidly than suspension cultures of susceptible varieties [14].

The concentrations used in experiments with cell suspensions (*e.g.* Table I) may seem exceedingly high to those used to seeing results with algae. The critical factor seems not to be the concentration per unit solvent but the absolute amount of herbicide per cell volume (*cf.* Fig. 8 in ref. [15]). Thus a 1000 times more herbicide is needed with a 10% packed cell volume of plant cells than with a 0.01% packed cell volume of algal cells.

The heterotrophic nature of the green calli [7] and green suspension cultures (Table I) add something to our knowledge on how certain herbicides kill the plant. That triazines stop photosynthesis is obvious from the plethora of literature on the subject but that may not be the direct cause of death. Plants could die from the free radicals formed in the presence of triazines or from starvation: White cells lack thylakoids containing photosystem II and cannot form free radicals, and are not killed by atrazine. The green cells used have functional photosynthesis which contributes very little to growth. Still the cells will not live without an exogenous sucrose supply [7]. Thus, death as a result of atrazine treatment cannot be from starvation in a heterotrophic photosynthetic culture; the free radicals formed were probably the toxic factors. It would be impossible to reach such conclusions with plastids, and complicated to do so with isolated leaves or whole plants.

The stage of development of cells may affect their reactivity to different growth regulators. This was analyzed in some depth with dikegulac, a growth regulator which affects growing apices in minute doses but does not affect the leaves receiving spray treatments (*cf.* [8]). At certain concentrations this compound had no effect on stationary cultures while being completely toxic to exponentially dividing cultures, when both were diluted to the same packed cell volumes [8]. Such results occurred irrespective of whether the cultures were green or white [8].

From the examples of differential reactivities of green and white cells and stationary and exponential cultures we came to the conclusion that it is best to consider cell cultures as being somewhat dif-

Table II. The "multihomogeneous" nature of plant cell culture systems.

Culture	Possible analogy in plant
White exponential	root (and shoot?) meristems
Green exponential	developing leaves (?)
White stationary	mature root tissue
Green stationary	mature leaf tissue

ferentiated and having "multihomogeneous" states, with different states being somewhat analogous to different tissue types in the plant (Table II). It is not clear what analogies a green exponentially dividing culture has in the plant; the shoot apex does not have mature chloroplasts and not that many divisions occur in the developing leaf.

### The uses of cultures in biocide screening

From the above we see that cultures, especially those on liquid media, have a potential as a pre-screen for herbicides. Neither chlorophyllous nor achlorophyllous cultures could be used alone as a universal screen because of the multihomogeneous nature of the cultures (Table I). If both are used we may better determine whether a compound is a potential herbicide. A system was developed using green and white tissue which requires <3 mg of the compound being screened in a "multimetabolic" test [16]. By using distantly related species, selectivity could be canceled out. Thus, 29 of the 29 commercial herbicides tested at 1 mM (roughly equivalent to a field concentration of 400 mg/m<sup>2</sup>) were toxic to one or the other cell type. Only 19 were toxic to both [16]. Of five insecticides and acaricides tested only carbaryl was toxic. Six fungicides were tested and all were toxic [16], justifying the botanical classification of fungi as plants. If it were not for the cuticle or other barriers, pesticides affecting pathways common to plant and fungi will be expected to kill plants. Thus the pre-screen predicts toxicity, and there will be a certain number of "false toxics", *i.e.* compounds which will not be toxic in a standard greenhouse screen. In most industrial primary screens with seedlings, more than 80% of the compounds tested are inactive and a cell culture prescreen could eliminate the majority of them. It could also find compounds which have potential toxicity but have penetration problems; the test would suggest which compounds should have changed formulation, or modifications

in molecular structure which might render them active.

Because of the minuscule requirement for compounds and because the cultures lack cuticles, they are ideally suited for structure-activity relationship studies. Many of the modern rapid methods for organic syntheses can be used only for the synthesis of a few mg of material. Whole plant testing requires much more material than cell culture microtests and isolated plastids are only suitable for compounds interfering with photosynthesis and not those interfering with plastid development. This leaves a vast potential use of cell culture systems.

### Cell cultures for studying biocide metabolism

The obvious advantages of cell culture for studying biocide metabolism have brought about their use in a large array of studies, such that a recent review of the subject contains >100 references [17]. The aims of these studies were manifold; the group of Sandermann has studied pesticide detoxification by plants (*cf.* [17]). They studied the metabolism of DDT and kelthane [18] in parsley cultures. Many of the metabolites that they found have not been previously reported in other (non-plant) systems. The problem of green plant *vs.* white culture again may enter in. Feung [19] has found different paths of DDT metabolism in green soybean plants and white soybean calli; the plants seem to metabolize more towards volatiles and the calli more to TDE. The selective detoxification of 2,4-D has also been studied in calli and suspension cultures (*cf.* [20]). Many metabolites and conjugation products were reported and models for herbicide detoxification presented. Despite the excellent chemistry, the conclusions of these type studies are hard to evaluate; 2,4-D was presented at hormonal levels (0.5–2 mg/l) [20] and not at herbicidal levels, which would seem more logical. Monocot cells should be resistant to a few orders of magnitude greater 2,4-D-concentrations than were used. It has not been excluded that the 2,4-D (auxin) and 2,4-D (herbicide) degradation pathways have vastly different  $K_m$  values for 2,4-D.

It is now well known by those studying secondary metabolite production that many of the secondary pathways become "inoperative" in cell cultures. This may have been borne out also in a study of cisanilide metabolism in leaves and cultures of carrot and cot-

ton [21]. The cell cultures had a reduced capacity to form secondary glycoside conjugates, forming more methanol-insoluble residues than the leaves [21]. Still, part of the difference may well be due to the fact that the authors compared achlorophyllous suspensions to green leaves, especially as it has been reported that the herbicide inhibits the Hill reaction in isolated chloroplasts (*cf.* [21]).

The degradation of other groups of herbicides has been studied in cell suspensions, often with results parallel to those found whole plants; *e.g.*, varietal differences in detoxification of metribuzin carried over to soybean suspension cultures [14].

There is a wealth of yet unpublished work, especially from the groups in Fargo, N.D., USA, on the metabolism of various pesticides, describing conjugations with glutathione, cysteine and malonyl cysteine, hydroxylations, demethylations, and other cleavages of diclofop-methyl, diphenamid, fluoro-difen, fluometuron, linuron, monuron and PCNB in cultures of carrot, soybean, wheat, hibiscus and peanut [22].

### Other uses of cultures to localize sites and modes of action of biocides

Some of the uses of cultures to localize biocide effects were reviewed in the previous section. The "multi-homogeneity" of the cultures allowed the pinpointing of effects to the same tissues as were pinpointed in whole plant experiments; to the roots and white cultures (napropamide), to green tissues only (triazines), to both green and white tissues with some differential between them (diuron) and to dividing tissues (dikegulac). With cultures, large amounts of amenable tissue are available in each of these "multihomogeneous states" for studying each particular steady state. Other *in vitro* systems are available but (at least in theory) the steady state is perturbed; stationary leaf cells are no longer stationary when enzymatically prepared into protoplasts or suspensions; storage root slices, leaf discs and tuber explants are also not the same "*in vitro*" as they were (before removal) *in situ*. Steady state growing tissues are preferred for metabolic, kinetic and mode and site of action studies.

There are many cases where the precise mode of action(s) of a herbicide is hard to ascertain. There is hardly a herbicide that has not been reported to affect protein or nucleic acid syntheses. Surprisingly,



researchers seem to forget that the transcriptional and translational capacities of plants are severely impeded by death resulting from other causes. To perform the necessary experiments to ascertain primary modes of action, kinetics are needed to show which biochemical reactions are hindered first. Kinetic experiments are problematic in whole plants or even tissue pieces; results are confused by diffusion or transport times to the different depths of tissue. Cell suspension cultures again may be the answer.

An interesting recent attempt at this was made by Ashton *et al.* [23]. They isolated leaf cells and measured the time course of inhibition of 5 different major processes at various herbicide concentrations. The most sensitive sites for herbicides such as atrazine, bromacil, monuron and paraquat seemed to be photosynthesis whereas other herbicides seemed to inhibit other sites first [23]. It is unfortunate that this group took their first time point only 15 minutes after herbicide treatment. Many of the herbicides affected more than one site by that time [23]. There is no problem of getting adequate control activity before that time. Their tissue was far removed from being in steady state; the cells had just passed through the plasmolysis and enzyme treatments necessary to prepare single isolated leaf cells. Thus, they used ex-stationary cells in a dynamic period of regeneration, lag phase and possible preparation for division.

More rapid kinetics were achieved with steady state cell suspensions [9]. It was possible to show that dikegulac inhibited amino acid uptake with the kinetics extrapolation back to time zero. This effect preceded suppression of incorporation into proteins by 10 minutes suggesting that the effect of this compound may well be on cell membranes. To obtain even more rapid kinetics, a flow device was constructed and dye leakage from cells was measured. In this manner it was also possible to show the immediate effect of dikegulac [9]. The previously reported greater tolerance of stationary cells to this compound [8] carried through to the kinetics of dye leakage; the rate of leakage was greater for exponential phase cells at any given dikegulac and cell concentration [9]. Despite the drawbacks of recently isolated cells and protoplasts described above, protoplasts are more amenable than cells for microscopic assessment of membrane damage. We were able to show with protoplasts that cell division is

affected at lower concentrations than those affecting membranes, and that the tonoplast is affected quite a bit after the effects on the plasmalemma [24].

In summary, the first very useful attempts have been made in using cell cultures to study metabolism and localize effects of herbicides within plants and cells. The multi-homogeneous nature of cell cultures can be useful in these studies and must always be considered.

### **Selection for herbicide resistance\* using cell cultures**

The number of selective herbicides available is both finite and small, especially when compared to the number of crops. All too often there is not a good match between a crop, the weeds attacking it, with an economic selective herbicide. The cost in time, resources and money of developing new herbicides seems almost prohibitively great but the problems of obtaining permission to use a known herbicide used on other crops ("new use registration") are not nearly as large. For these reasons alone it would be useful to select for herbicide resistant strains in many crops. Both photosynthesis research and herbicide research have profited from the recently found strains of weeds resistant to S-triazines (*cf.* [25, 26]).

A problem which should be addressed first is "why have weeds developed genetic resistance only to S-triazines"? Does this mean that despite all the years of herbicide use we should only expect to find resistance to the triazines alone? This problem has been discussed at length mathematically [27] and empirically [27, 28]. Basically it can be said that resistance to other herbicides will yet appear but to most at a much lower rate. In weeds, resistance is delayed by a *compounded* dampening effect brought about by low selection pressure and low persistence of most herbicides, and the staggered germination of weed seed and the lower competitive fitness of resistant biotypes that do appear [27, 28]. When high selection pressure, highly persistent S-triazines were used repeatedly, resistance and differential tolerance appeared (*cf.* [25, 26]). If we were to try

\* Resistance is herein defined as being completely tolerant to a concentration of herbicide that kills the "wild" type. Lesser degrees of tolerance are noted as tolerance or differential tolerance. This definition conforms to that of the FAO Working Party on Pesticide Resistance.

to select herbicide resistant crop strains in the field, the task would be formidable and very time consuming. This is because the selection pressure which we can impose in the field can rarely be made to exceed 90–95% kill; the vast majority of plants remaining are “escapees”. Increasing the dose does not increase kill of susceptible plants but may be a lethal to the few resistant plants among the escapees, because of interactions with lower affinity sites of action of the herbicide. The time involved (measured in generations) in obtaining resistance in field conditions would seem prohibitive (Fig. 1). One would have to treat very large numbers of plants, planted in very large areas for 5–10 years without knowing whether (or how much) enrichment for herbicide resistance has occurred. The likelihood of picking up resistance inherited by more than single gene alleles are minimal considering the limitations in numbers and space. If resistance to the herbicide of choice is multigenic, what then? A huge loss in time, effort and space.

The case with differential tolerances may be different as there may well be greater initial frequencies of more tolerant individuals. There is but one report of a successful field selection for herbicide tolerance

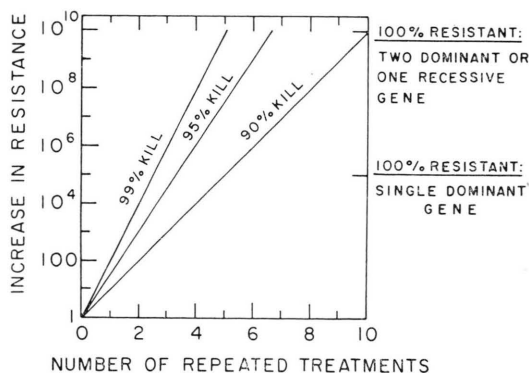


Fig. 1. Rate of increase in resistance strains. The rate of enrichment for resistance for crop strains was calculated using the equations in [27] assuming that germination is uniform and immediate ( $\bar{n} = 1$ ), and that no fitness differential is allowed to be expressed between resistant and wild type strains. The number of repeated treatments represents the number of generations treated. In the field it would equal seasons and in cell cultures would equal divisions in the presence of the herbicide. On the right axis, the estimated increase needed to attain 100% resistant with inheritance at different allelic situations is shown. The point for one recessive gene may be much closer to that of a single dominant gene if the data from lower plants [39] can be extrapolated. Note that one or two treatments before 100% resistance is achieved that the frequency of truly resistant biotypes will still be undiscernable ( $< 1\%$ ).

in a crop leading to the release of a new variety [29]. The pasture grass *Lolium perenne* is somewhat tolerant to treatments with paraquat. Faulkner took a population that already undergone repeated paraquat treatments over several years and then performed recurrent selection. He achieved sufficient resistance such that rates of paraquat can now be used to severely inhibit unwanted species while allowing the *Lolium* to expand [29]. This could be done despite the multigenic heritability of the paraquat tolerance (*cf.* [29]). The differences in susceptibility seem not to be at the level of uptake, translocation, distribution or metabolism of paraquat but to activities of enzymes which may detoxify the toxic products of paraquat action [30].

The projections from Fig. 1 suggest that it should be far more feasible to select for resistance using cell cultures. In theory, one can have a million totipotent cells representing a million plants in a milliliter of suspension with a generation time of a few days, not the 6–12 months in the field. Thus enrichment for resistance should be fast and easy. Additionally, the possibility of greater uniformity of application to cells in culture should vastly reduce the number of escapees, allowing higher effective selection pressures without affecting secondary lower affinity sites of action. In cell cultures we have a different problem; often when the number of viable cells decreases beyond a certain point, no colonies appear on plating. Assuming we use feeder cultures or other techniques we can somewhat raise the plating efficiencies. The problem of decreased fitness of most mutants must be considered. All of the triazine resistant weed strains are less fit when they are in competition with the wild type in non-selective situations [31]. This may well be due to the modified plastid properties of these strains (*cf.* [26]); the level of field photosynthesis (“productivity”) of these strains has not yet been measured. With crops, the problem of “fitness” may be marginal; crop species have been so heavily selected for, for so long that they already are quite naturally “unfit” except in the highly artificial “cultural” environments where they are grown. Anyway, they are not usually grown in mixed intraspecific combinations. Thus, herbicide resistance should hardly decrease this lack of fitness in a crop species, in the crop environment, although denser planting may be necessary. It must be clear that not all herbicide resistant mutants will be useful; with herbicides

affecting photosynthesis they can be detrimental. The diquat and paraquat resistant *Lolium* [29] is surely useful but the diquat resistant maize is blocked on the oxidizing side of photosystem II [33] and would hardly be agriculturally productive. When we have a pure cell culture of a resistant strain, fitness does not pose a problem (unless there are back mutations). In mixed resistant-susceptible cultures, such as one might encounter during a step-wise enrichment for resistance, it might be expected that the wild type cells will be twice as fit as the resistant cells (*cf.* [31]). Fitness differentials under selective and non-selective conditions have been found, as would be predicted, in mixed biotype suspension cultures [32]. The fitness problem can be alleviated by keeping cultures under selective conditions at all times, *i.e.* by cultivating continuously in the presence of the herbicide.

The use of plant cell culture systems for crop improvement has been reviewed extensively [34] but the section on the possibilities and results with herbicides is of necessity rather short. The first report of a selection for true resistance to a herbicide affecting photosynthesis was by Zenk [35]. He obtained soybean cultures with complete resistance to atrazine. Unfortunately soybean is the one species which has not been "willing to cooperate" and demonstrate its totipotential to regenerate plants.

Barg and Umiel have been able to select lines in green tobacco calli which had a differential tolerance to amitrole. The two best lines showed 50–62% of the growth of controls at  $10^{-4}$  M (which essentially stopped growth of the parent line) [36]. More recently they have shown that one of these lines could be classified as "resistant" at  $7.5 \times 10^{-5}$  M amitrole [37]. Data are not yet available on the resistance of plants regenerated from these lines, although they have been shown to be totipotent [37].

The first report of resistant plants being regenerated from selected resistance in cell cultures appeared at the end of last year. Chaleff and Parsons selected for picloram resistance in tobacco and isolated a (monogene dominant) resistant strain [38]. Their work is of especial interest (despite their use of a herbicide that does not act through photosynthesis) because they were able to select without mutagenesis or haploidization. As appealing as haploids may be for the purpose of crop selection, they impose certain problems in breeding. If we start out with a "name" variety, we wish to return to that variety

(with the added herbicide resistance). The likelihood of getting back the name variety after haploidizing and rediploidizing will probably be nil even in the most homozygous of varieties. Standard backcross breeding will be required before the new variety can be released. It still remains to be shown that it is that much harder to select for single gene recessive mutants in diploid plants. It has already been demonstrated that recessive mutant phenotypes appear at a much higher frequencies than expected in yeast and slime molds. A recessive slime mold mutant classically expected at a diploid frequency of  $1.4 \times 10^{-13}$  appeared a frequency of  $9 \times 10^{-8}$  [39]. The usefulness of mutagens to increase the frequency of resistant mutations can also be questioned. There is a strong possibility that resulting resistants will have received  $>1$  "hit" and will then have other undesirable features.

The first "full cycle" selection for resistance to herbicides affecting photosynthesis has also just appeared. Radin and Carlson [40] selected for tobacco resistant to phenmedipham and bentazon. They used a mixed whole plant/tissue culture technique as they recognized the problems of selection with non photosynthetic tissue. They treated mutagenized haploid plantlets (derived from anther cell cultures) and later sprayed the partially expanded leaves. Leaves of tobacco are basically constructed of clones of cells, as there are divisions of the cells of the primordial leaf during growth. After spraying, all the "wild type" clones in the leaves yellowed, leaving a few green resistant clonal spots. These green spots were transferred to tissue culture medium for multiplication and regeneration. Some of the plants were truly resistant with the resistance to both bentazon and phenmedipham being inherited as recessive alleles [40]. Their lines should be intriguing for use in mode of action studies as they are inherited on nuclear genes yet compete with triazines for the same binding site [12]. The triazine resistance is cytoplasmically inherited [41].

Just because tobacco has been used for most of the studies on herbicide (as well as other) resistances (*cf.* [34]) does not mean that this is the crop of choice. Though not all crops have been regenerated, many have [34], and some of these could use new herbicides.

There may be ways to use the triazine resistant biotypes that have appeared in the last few years (*cf.* [25]). Some of these weeds are related to crops

and it would be of interest to try to transfer the genetic information. At least in *Brassica* [41] and probably in *Amaranthus* and *Chenopodium* [42] triazine resistance is cytoplasmically inherited, presumably on the chloroplast genome. Attempts are already being made to transfer the triazine resistance from the weed *Brassica campestris* to the related *Brassica* crops, (especially the seed rapes) using classical means [42]. This breeding program is confounded by the different chromosome numbers of the different species. It would be appealing to transfer this type of resistance using protoplast fusion techniques (*cf.* [43]). This type of inter-specific transfer of a cytoplasmic gene has recently been elegantly performed by Zelcer *et al.* [44]. They inactivated the nucleus of the species donating the cytoplasm by X-irradiation such that only the cytochromes could be expressed in the fusion product [44]. This type procedure allows (in theory) a rapid transfer of plastid gene information without the necessity of classical cross and backcross techniques. Thus, there is much untapped potential in this direction.

Care must be taken in choosing the species and the correct "multihomogeneous" cell type for selection of resistants. At the cell level, the use of white

calli for isolating resistance to a herbicide known to act primarily (*i. e.* at its lowest inhibitory concentration) on photosynthesis would seem misconceived, although a detoxifying mutant might appear. Equally misconceived would be to try to use cell cultures to isolate mutants to herbicides whose known mechanisms of selectivity are based on morphological barriers. Considering the possibilities for genetic drift occurring in cultures, it might be wise to use recently isolated cells if we wish to return to our original variety. Still, there is quite a future in using cell cultures for isolating genetically resistant varieties, at far less effort and cost than selecting for resistance in the field.

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