Invited Trends Article:

Paraquat Resistance of Weeds – the Case of *Conyza canadensis (L.) Cronq*

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The paper gives an overview of literature on paraquat resistance of weeds and the proposed mechanism of resistance. New results we achieved on horseweed (*Conyza canadensis /L./, Cronq.*) are discussed in detail.

It was demonstrated that there is no significant constitutive difference related to the paraquat resistance between untreated susceptible and paraquat-resistant horseweed plants. The lower sensitivity of flowering resistant plants may be due to the fact that paraquat content in treated leaves of flowering resistant plants was only 25% as compared to those measured at rosette stage. Our results confirm that paraquat resistance is not based on elevated level and activity of antioxidant enzyme system. The hypothesized role of polyamines in the resistance mechanisms can be excluded. The higher putrescine and total polyamine content of paraquat treated resistant leaves can rather be regarded as a general stress response, than as a symptom of paraquat resistance. A paraquat-inducible protein is supposed to play a role in the resistance, which presumably functions by binding paraquat to an inactivating site and/or by carrying paraquat to metabolically inactive cell compartment (vacuole, cell wall). From model experiments it is concluded that paraquat and diquat preferentially form hydrophylic interactions with proteins containing a higher amount of lysine and glutamic acid. Consequently, the reason for paraquat resistance in horseweed is probably a hydrophylic interaction of paraquat with a protein, leading to inactivation of paraquat through forming a conjugate and/or sequestration into the vacuole or the cell wall.

Bipyridyls and their mode of action

The two best-known bipyridyl compounds, paraquat (1,1′-dimethyl-4,4′-bipyridinium) and diquat (9,10-dihydro-8a,10a-diazoniaphenanthrene), are used, generally in the form of haloid salts, as the active ingredients of post-emergent, non-selective, contact herbicides. These compounds are applied in vineyards, orchards and forests for total weed control, in horticulture for the weed control of seedbeds, and in the field for defoliation and desiccation of crops. These compounds are inactivated in the soil, as they bind irreversibly to clay minerals, so they have no residual effects. The compounds are extremely toxic to vertebrates, and thus to man: the LD_{50} value characterising the oral toxicity in rats is 157 mg/kg body weight for paraquat. The minimum lethal dose by oral ingestion in human beings is about 35 mg/kg body weight. Because of the high toxicity the use of herbicides containing paraquat is severely restricted or banned in many European countries. Nevertheless, investigations on its effect and resistance mechanisms continue with scarcely diminished intensity.

The bipyridyls exert their phytotoxic effect by diverting electrons on the reducing side of PS I from their normal physiological pathway at the FeS_x component(s) of the electron transport chain (Hiyama *et al.*, 1993), thus forming cation radicals and also preventing the formation of NADPH. These radicals are extremely reactive and generate superoxide anion radicals in the chloroplasts by interacting with molecular oxygen present due to photosynthetic oxygen evolution. In this process the bipyridyl radicals themselves revert to cations. The bipyridyl cation radicals and the superoxide anion radicals generate hydroxyl radicals in subse-

quent reactions. The active oxygen species, especially the hydroxyl ($OH^•$) and superoxide anion ($O^{•2-}$) radicals rapidly peroxidize the fatty acid side chains of membrane lipids, leading to a loss of membrane integrity.

Occurrence of paraquat resistance in weeds

The first resistant weed biotypes were detected 12–15 years after herbicides containing bipyridyl were introduced in the sixties. In each case paraquat containing Gramoxone was applied 5–10 times a year for 5–11 years. These plants also exhibited a lower extent of resistance to herbicides containing diquat. The resistant weeds include both monocots (e. g. *Hordeum glaucum, H. leporinum, Poa annua, etc*) and dicots (e. g. *Amaranthus lividus, Solanum nigrum, S. americanum, Vulpia bromoides, etc*) and were found over a wide range of geographical locations (for further details see Preston, 1994). The resistance was independent of paraquat pretreatment in some plants e.g. *Rehmannia glutinosa (Gaertn.) Libosch* (Chun *et al.*, 1997). Paraquat tolerance was also demonstrated in *Chlamydomonas reinhardtii* mutants (Kitayama and Togasaki, 1992; Vartak and Bhargava, 1999), *Chenopodium rubrum* cell cultures (Ranade and Feierabend, 1991), *Nicotiana tabacum* calli (Furusawa *et al.*, 1984), transgenic tobacco (Aono *et al.*, 1995) and *Arabidopsis thaliana* (Kurepa *et al.*, 1997). Among the cultivated plants, paraquat tolerance was recorded for, among others, pea (*Pisum sativum* L., Donahue *et al.*, 1997) and a soybean variety (Kim and Hatzios, 1993).

Hypotheses on the mechanism of paraquat resistance

The resistance of weeds to herbicides and the tolerance of crops is generally based on their ability to detoxify the herbicides, or on the site of action becoming insensitive to the applied active agent.

On the basis of experiments carried out on paraquat-resistant (PqR) biotypes of weed species originating from various regions and developing under differing climatic and herbicide application conditions, numerous hypotheses have been evolved on possible reasons for resistance. The idea that the redox potential relations at the entry site of paraquat in the photosynthetic electron transport chain changed so that the paraquat was incapable of accepting electrons was rejected at an early stage. No changes could be observed at the active site in any of the experiments carried out on *Lolium perenne* (Harvey *et al.*, 1978), *Conyza bonariensis* (Fuerst *et al.*, 1985) and *Hordeum glaucum* (Powles and Cornic, 1987). In a resistant biotype of *Conyza canadensis* it was found that the active site was just as accessible to paraquat as in susceptible ones, i.e. the redox potential on the acceptor side of PS I did not change (Lehoczki, personal communication).

The hypothesis that paraquat becomes metabolised in the plant was also rejected, since no paraquat metabolites had been detected in plants, and the same proved to be true of two paraquat-resistant weeds (*Lolium perenne*, Harvey *et al.*, 1987; *Conyza bonariensis*, Norman *et al.*, 1993). Data in the literature indicate that only certain soil-born bacteria (Funderburk and Bozarth, 1967) and fungi (Carr *et al.*, 1985) are capable of metabolising paraquat, while there is one single indirect experimental evidence of its metabolisation in *Rehmannia glutinosa* (Chun *et al.*, 1997). Convincing proof of plant metabolisation is difficult to obtain due to the fact that paraquat undergoes spontaneous decomposition on plant leaves when exposed to UV light. The question of the metabolisation of paraquat in plants is thus still open, mainly due to methodological problems.

During investigations on the limited mobility of paraquat in R plants it was demonstrated with four different resistant species that paraquat is bound to cellwall components. This adsorption proved to be paralleled by the recovery of functional activity, characterised by carbon dioxide fixation and chlorophyll-a fluorescence, after transitory inhibition (Fuerst and Vaughn, 1990).

A more viable hypothesis is that resistance is associated with the enhanced activity of antioxidative, protective enzymes (Shaaltiel and Gressel, 1986). Plants are known to possess an antioxidant enzyme system capable of eliminating the active oxygen forms arising in the chloroplasts under physiological conditions. The superoxide anion radical is transformed by the superoxide dismutase (SOD) enzyme into hydrogen peroxide and molecular oxygen, and subsequently the hydrogen peroxide is eliminated from the chloroplasts by the ascorbate-glutathione cycle, consisting of the

ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase enzymes (Foyer and Halliwell, 1976). During oxidative stress the activity of these enzymes is generally enhanced. In experimentally juvenilised tobacco a certain degree of paraquat tolerance and resistance to other oxidative stresses could be achieved by increasing the quantity of antioxidant agents and enzymes (Barna et al., 1993). However, the enhanced activity of the enzymes in this cycle could not be detected in most of the paraquat-resistant plants (Powles and Cornic, 1987; Carroll et al., 1988). In some of these plants, although the enzymes exhibited 1.3–1.6 times higher activity (Shaaltiel and Gressel, 1986; Shaaltiel et al., 1988), this was not sufficient to explain the resistance factor of 10 observed. Further investigations on paraquat-resistant *Conyza bonariensis* 24 hours after sublethal treatment with paraquat showed a similar increase in activity for numerous antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione peroxidase (Ye and Gressel, 2000). It remains doubtful whether this mechanism has any decisive role in paraquat resistance, since the paraquat radical reacting with molecular oxygen reverts to a cation, and is thus able to continuously generate superoxide radicals. The protecting enzyme cycle, which utilises the NADPH pool of the chloroplasts is not capable to cope with this situation indefinitely. The opinion prevails that the antioxidant enzyme cycle only provides a temporary protection, until some other type of mechanism(s) ensuring long-term survival starts to operate. Experiments carried out on plants belonging to five different R biotypes of *Conyza canadensis* L. growing at various sites in Hungary proved convincingly that despite the very different resistance factors there was neither a difference in SOD activity (Turcsányi et al., 1994) nor in ascorbate peroxidase, glutathione reductase and catalase before or after spraying with paraquat (Turcsányi et al., 1998).

According to another possible explanation paraquat is prevented from reaching its site of action in the chloroplasts of R plants. Experiments carried out using ^{14}C-paraquat indicated that in certain cases, e.g. in *Erigeron philadelphicus* (Tanaka et al., 1986), *Hordeum glaucum* (Bishop et al., 1987) and *Conyza bonariensis* (Fuerst et al., 1985)

the resistance may be caused by the strong inhibition of translocation. This could contribute to the induction of an inducible protective mechanism, since less paraquat would reach the cells, putting a lighter burden on the protective system. Paraquat enters the cell with the aid of a transporter molecule localized in the plasmalemma. This became clear when it was demonstrated that the dynamics of the concentration-dependent uptake of paraquat could be divided into a linear component dependent on the permeability of the cell wall and a saturable component indicating a transporter molecule (Hart et al., 1992b). Putrescine competitively inhibited uptake, which could indicate that a polyamine transporter is responsible for the uptake of paraquat. The most important feature of paraquat from the point of view of transport is the charge distribution within the molecule which is similar to that of putrescine (Hart et al., 1992a). Since polyamines can be transported into the vacuoles, it is probable that a polyamine transporter assists the paraquat to enter the vacuoles (Pistocchi et al., 1988). In some of the R biotypes the binding of paraquat to the cell wall (Fuerst et al., 1985; Vaughn et al., 1989) or its sequestration in the vacuoles (Hart and DiTomaso, 1994; Lasat et al., 1997) could be observed. Certain polyamines also have a putative role in exclusion mechanisms (Preston et al., 1992), based on the fact that putrescine competitively inhibited the uptake of paraquat in the cell membranes of maize root protoplasts (Hart et al., 1992a).

Data in the literature on the inheritance of paraquat resistance mention a number of possibilities for various species, ranging from recessive inheritance (*Ceratopteris richardii*, Hickok and Schwarz, 1989) through dominant (*Conyza bonariensis, Erigeron philadelphicus,* Itoh and Miyahara, 1984) and semidominant (*Arctotheca calendula, Hordeum glaucum,* Islam and Powles, 1988; Purba et al., 1993) to polygenic inheritance (*Lolium perenne, Poa annua,* Fuerst and Vaughn, 1990). Genetic studies also indicated that plants with low resistance factors (below 10), such as *Poa annua* and *Lolium perenne,* showed polygenic inheritance, while weeds with higher resistance factors, such as *Hordeum leporinum, H. glaucum* and *Arctotheca calendula,* had semidominant inheritance associated with a single gene (Islam and Powles, 1988; Purba et al., 1993).

Characteristics of paraquat-resistant biotypes of *Conyza canadensis (L.) Cronq.*

Due to unclear nomenclature in the scientific literature with respect to horseweed we have to emphasize that *Conyza canadensis (L.) Cronq.* as the correct scientific name has been used since 1976 (Cronquist, 1976). The first paraquat-resistant *Conyza canadensis* plants found in Hungary developed in an atrazine-resistant population (Pölös *et al.*, 1987). Later, horseweed populations resistant only to paraquat were also found. For quantitative characterisation of the resistance the paraquat resistance factor (RF) is determined as the concentration ratio resulting in 50% inhibition in R and S plants (I_{50R}/I_{50S}). In the rosette stage the RF value of plants resistant only to paraquat was 180 expressed as the change in F_v/F_m, characteristic of the quantum efficiency of PS II. In paraquat-atrazine coresistant (PqAR) plants, RF values varied between 210 and 650, depending on the prehistory of the plant (Szigeti *et al.*, 1988, 1994). In the flowering stage RF values as high as 1000 were reported (Turcsányi *et al.*, 1998). When treated with diquat, the RF value of the PqR biotype, resistant only to paraquat, was 47, while that of the PqAR biotypes found at various sites in Hungary exhibited resistance factors of between 50 and 110 (Szigeti *et al.*, 1994). These data indicate that the simultaneous resistance to atrazine and paraquat always results in a higher resistance factor with respect to bipyridyls.

It was found that in the rosette stage of development, RF depended greatly on the parameters used for the RF determination [CO_2 fixation, F_v/F_m, ratio of fluorescence decrease: $Rfd = (F_m-F_s)/F_s$], on the treatment conditions, on previous treatments and on the site of origin of the plants. The effect recorded is also substantially influenced by light intensity used during the bipyridyl treatment (Lehoczki *et al.*, 1992). Light plays an important role in inducing the resistance mechanism, and is also a criterion for the initial uptake of paraquat (Váradi *et al.*, 2000). Light conditions applied in the experiments must thus be taken into consideration when comparing our data or data from the literature. Also the stage of development should not be ignored when determining the RF or comparing biotypes, since the resistance factor is generally higher in the flowering stage, as observed in the case of *Conyza bonariensis* (Amsellem *et al.*, 1993). This rise in the RF value could be due to a higher sensitivity of S plants during flowering. In the case of susceptible pea plants analysis of several functions indicated that the damage caused by paraquat in older leaves was twice as great as that observed in younger ones (Donahue *et al.*, 1997). Our latest studies show that in *Conyza canadensis* the difference in sensitivity as a function of developmental stage may be due to the fact that paraquat content in leaves of flowering R plants was only 25% as compared to that measured during the rosette stage (Fig. 1.).
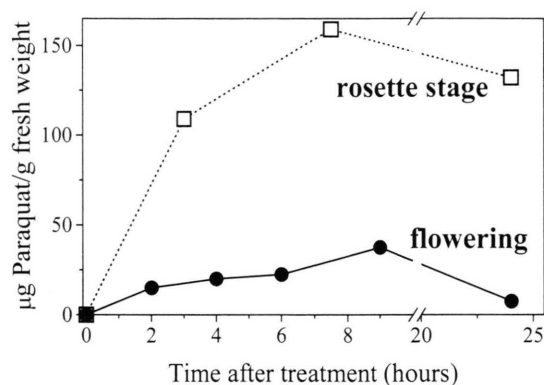

Fig. 1. Paraquat content of paraquat-resistant (PqR) *Conyza canadensis* leaves of different developmental (rosette and flowering) stage after spraying with 1.5 mm paraquat and washing of the leaves.

Effect of paraquat treatment on the chloroplast structure, photosynthetic functions and the activity of certain antioxidant enzymes in R plants

Under laboratory conditions the effect of paraquat depended to a great extent on whether the intact plants were sprayed or detached leaves were floated. Therefore, it is always important to emphasise the mode of treatment employed. Certain phenomena associated with resistance, such as the strong inhibition of physiological functions as the result of paraquat treatment and their subsequent recovery, can only be observed in sprayed plants, not on leaves treated by floating. In the latter, on the other hand, a pronounced stimulation of carbon dioxide fixation was observed (Pölös *et al.*, 1988). The technique used for treatment must therefore be taken into consideration when evaluating the results.

The reduction in the chlorophyll content, as a secondary effect of bipyridyls, exhibited strong time- and concentration dependence in S leaves treated by floating on paraquat solution (Pölös *et al.*, 1988). The reduction in chlorophyll content is due to oxidative pigment decomposition induced by the superoxide anion radicals generated by paraquat and by the hydroxyl radicals formed. The capacity of natural protective mechanisms is not sufficient to eliminate the superoxide continuously generated by paraquat. Nevertheless, the chlorophyll content of R plants did not decrease significantly after a 24-h treatment up to a concentration of 0.1 mM paraquat. In R plants the mechanism ensuring resistance hinders chlorophyll destruction by preventing the formation of active oxygen species. In S plants treated by spraying, chlorophyll destruction could not be measured because the leaves lost their turgor and withered in the light at room temperature even before a decrease in chlorophyll content. Under similar conditions the chlorophyll content of R plants did not change (Pölös *et al.*, 1988).

The ultrastructure of the chloroplasts in untreated S and R plants did not show any significant difference (Pölös *et al.*, 1988). In S leaves treated by floating in 10 μM paraquat or diquat for 24 h the grana membranes dilated, the envelope membranes became disorganised and osmiophilic globuli could be observed in its place. The envelope membranes of the chloroplasts in PqR leaves treated with bipyridyls remained intact, although the intrathylakoidal space was enlarged. In the thylakoid membranes of S plants treated with paraquat the quantity of oligomer LHCP II decreased and this reduction probably led to a change in the association between LHCP II and the PS II reaction centre complex. This is confirmed by the lowering of the Mg^{2+}-induced changes of the short wavelength fluorescence intensity and increase in the relative quantum requirement values. The decrease in LHCP II also correlated well with the reduction in the Δ^3-transhexadecanoic acid (16:1) content (Szigeti *et al.*, 1992). This observation is in agreement with the view that Δ^3-transhexadecanoic acid is an important factor in the stabilisation of LHCP II (Rémy *et al.*, 1984; Krol *et al.*, 1989). The chloroplast thylakoid membranes of PqR plants contained relatively less CP1 and CP1a (the chlorophyll-protein complexes of PS I) and less 70 kD apoprotein, and exhibited less intense fluorescence than the S plants at 730 nm. All these results suggest damage to the chloroplast membranes of S plants after treatment and the relative intactness of the plastid membranes of R plants.

After spraying with paraquat the PqR plants exhibited transitory inhibition of their photosynthetic functions (*in vivo* CO_2 fixation, O_2 production) amounting up to 60%. Within two hours of treatment the inhibition no longer increased and thereafter gradually decreased, although the level of functional activity of the untreated control was not reached after 72 hours (Lehoczki *et al.*, 1992).

The difference between plants treated by floating or spraying could perhaps be attributed to the fact that constant contact with the solution ensures continual supplies of paraquat even after the protective mechanism has been activated. In the course of the treatment by floating the S leaves also exhibited different behaviour, since the destruction of the leaf was much slower than after spraying, taking 18–24 h, in contrast to 4 h. This can be explained by the fact that the leaves did not suffer water loss, which may be an important factor in the case of sprayed intact plants.

The *in vivo* CO_2 fixation characterising the functioning of the whole photosynthetic apparatus was far more sensitive to paraquat treatment in both S and R biotypes than the F_v/F_m parameter. This means that the superoxide anion radicals generated by paraquat not only damage certain proteins in the electron transport chain, but probably also affect proteins participating in carbon dioxide fixation, or having some influence on it.

The CO_2 fixation of R leaves treated with paraquat by floating fluctuated around the initial value for the first three hours after treatment, then it was gradually stimulated, by as much as 80% depending on the paraquat concentration (Pölös *et al.*, 1988). Similar observations were published on other plants, irrespective of paraquat resistance. Some authors explain this phenomenon as a stimulation of the ribulose-1,5-bisphosphate-carboxylase activity through changes in the transthylakoidal ΔpH due to the acceleration of the photosynthetic electron transport chain by paraquat (Salvucci *et al.*, 1987).

The CO_2 fixation of intact R plants treated by spraying showed recovery after a transitory inhibi-

tion, and approached the initial level again after approx. 72 h. As the result of spraying with 50 μM diquat this same transitory inhibition and subsequent recovery could be observed in the R biotype, although the recovery was slower than in the case of paraquat. The initial quenching of F_v after spraying and the intense inhibition of CO_2 fixation indicate that even in R plants paraquat and diquat are rapidly able to enter the chloroplasts and exert their electron-diverting effect, which is then gradually eliminated by a mechanism activated after the treatment. The induction of the eliminating mechanism is reflected in the transitory nature of the inhibition. Two hours after the treatment the decrease in the photosynthetic function was found to stop, and a gradual recovery was observed (Szigeti *et al.*, 1994). This indicates that resistance is not due to a constitutive exclusion of the paraquat from its site of action, but to the functioning of an inducible eliminating mechanism. The functional recovery of paraquat-R plants after spraying could also be detected through changes in the oxidation of P700 (Szigeti and Lehoczki, 1992).

The possibility of an increase in SOD activity as part of the resistance mechanism was excluded by the simultaneous application of the Cu-chelator N,N-diethyldithiocarbamate (DDC) and paraquat (Darkó *et al.*, 1994; Turcsányi *et al.*, 1998). DDC is the *in vivo* inhibitor of Cu/ZnSOD (Heikkila *et al.*, 1976). However, even after combined spraying with these two compounds, the R plants functionally recovered, which was not expected in the presence of the chelator if Cu/ZnSOD played a decisive role in the resistance mechanism. In the presence of 0.5 mM menadione the functional activity of PqR plants, characterised by F_v/F_m, decreased to the same extent as that of S plants (Rácz *et al.*, unpublished results). These results indicate that in our paraquat-resistant horseweed biotypes the antioxidant enzyme system is not involved in the resistance mechanism.

Depending on their site of origin, the RF value of PqAR plants with respect to diquat ranged from 50 to 120, while it was 47 for PqR plants. Consequently, an increase in the activity of the antioxidant enzyme system can not be responsible for resistance. If this were true, resistance to diquat should be similar in extent to the paraquat resistance, since both herbicides are equally capable of generating superoxide anion radicals.

So what type of substance is synthesised in R plants sprayed with paraquat, which is capable to stop inhibition, excluding or metabolising(?) the active agent, and thus restoring photosynthetic functions?

Darkó *et al.* (1994) found that even R horseweed plants were destroyed if they were simultaneously treated with cycloheximide and paraquat. In the concentration applied, cycloheximide alone was not lethal to the plants. The *in vivo* effect of cycloheximide, which inhibits protein synthesis of the eukaryotic type, is based on the fact that by binding to the 80S ribosome it inhibits the translocation of the new protein. It can thus be concluded that the recovery of the functions in PqR plants might be caused by a nuclear-coded protein. The existence, the characteristics and the role of the hypothesized protein is still open, since it is not clear from the results achieved to date how the protein participates in the probable exclusion of paraquat; as a transporter, as a binding protein, or possibly as a metabolising enzyme. The first results of attempts to detect and identify the protein indicated that a 32-kD protein appeared in the cytosol 6 h after paraquat treatment in the leaves of the PqR biotype and could still be detected 30 days later. Whether this protein arose due to *de novo* synthesis or as the degradation product of another protein is still unclear.

Binding of paraquat and diquat to various biomolecules in model systems

Considering the results achieved with other plants (Fuerst *et al.*, 1985; Vaughn *et al.*, 1989), it seemed reasonable to determine whether resistance was due to inactivation of paraquat or diquat, to their transport to another cell compartment, or to their possible binding to certain substances.

The binding of both compounds to cellulose was reduced by K^+ and Na^+ ions; the binding strength decreased logarithmically with the increase in the ion concentration. Ca^{2+} and Mg^{2+} ions had a similar effect, but this was ten times stronger than in the case of monovalent cations (Cserháti and Szigeti, 1991). According to the literature the cell wall bound 70–80% of the paraquat in the cell,

regardless of the degree of resistance (Hart *et al.*, 1992b). The role of cellulose, as the main cell wall component, in resistance is thus unlikely, despite its ability to bind bipyridyls.

As the phase transition parameters of various phospholipids containing polar groups of varying polarity (dimyristoyl phosphatidylcholine, DMPC; dipalmitoyl phosphatidylethanol-amine, DPPE; dipalmitoyl phosphatidic acid, DPPA), as determined by differential scanning calorimetry, were not influenced by either paraquat or diquat, the interaction of bipyridyls with phospholipids can be excluded in both the action mechanism of the bipyridyls and in the resistance mechanism (Szőgyi *et al.*, 1989). The interaction observed between paraquat and phospholipids has been confirmed by other authors (Toreggiani *et al.*, 1997).

Both paraquat and diquat are capable of binding to cationic and anionic adsorptive centres. In a reversed phase thin layer chromatography system it was demonstrated that they bind preferentially to the polar (basic or acidic) side chains of amino acid residues. The configuration of the amino acids (L or D) has no role in this hydrophilic interaction (Cserháti *et al.*, 1988). The two substances have a tendency to interact with proteins, as confirmed by differential scanning calorimetry. The phase transition temperature of the model proteins, lysozyme and papain, was significantly increased by both compounds even in a 1:100 molar ratio (Szőgyi *et al.*, 1989). It thus appears probable that if a protein with these properties is present in R plants, it must be capable of binding paraquat and diquat, and/or of transporting them into a metabolically inactive compartment, or possibly inactivating them enzymatically. If a protein interacts with paraquat in R plants, the dibasic or dicarboxylic amino acid residues of this protein, e.g. glutamic acid or lysine, will actually take part in the process (Cserháti *et al.*, 1988).

The interaction of paraquat and diquat with proteins is considerably influenced by the presence and concentration of divalent ions if the ions have to compete with the bipyridyls for adsorptive binding sites in the given chromatographic system. They must behave in the same way *in vivo*, and this is associated with the fact that the two bipyridyls behave as divalent cations (Cserháti and Szigeti, 1991).

Possible role of polyamines in resistance

On the basis of data in the literature it seemed likely that certain polyamines played a role in paraquat resistance mechanisms based on exclusion (Preston *et al.*, 1992). Since the exclusion mechanism in the PqR biotypes of *Conyza canadensis* was not originally present, but proved to be of an inducible nature, it was important to determine the polyamine content.

These determinations proved that the putrescine and total polyamine contents of untreated leaves of plants of the PqR and PqAR biotypes was 3–4 times higher than that of S plants (Szigeti *et al.*, 1996b). Other authors made similar observations on paraquat-resistant *Conyza bonariensis* (Ye *et al.*, 1997).

In the early nineties it was found that a mutant of *Escherichia coli* with decreased polyamine synthesis had increased sensitivity to paraquat treatment (Minton *et al.*, 1990). This increased sensitivity disappeared after treatment with exogenously added putrescine and spermidine. The present authors made an analogous observation, when they found that 100 µM exogenous putrescine substantially reduced the strong inhibitory effect of paraquat in detached S leaves ($F_v = 0.12 \rightarrow 0.31$) (Rácz *et al.*, 2000), which could be based on the radical-scavenging effect of polyamines (Drolet *et al.*, 1986). The protective effect of polyamines against paraquat toxicity was demonstrated on sunflower leaf discs by other authors, who attributed this effect to the antioxidant nature of polyamines (Benavides *et al.*, 2000). The variable fluorescence of PqAR and PqR leaves showed hardly any change (0.83–0.81) even at higher concentrations of paraquat (1 mM), and exogenous putrescine had no effect on these values. This observation can be explained by the higher polyamine contents of R biotypes.

Investigations on changes in the level of polyamines after combined spraying with paraquat and cycloheximide indicated that in the presence of cycloheximide paraquat caused irreversible damage despite the fact that the contents of putrescine and total polyamine rose to three times the initial value (2100 nmol/g fresh weight) (Szigeti *et al.*, 1996a).

No direct correlation was found, however, between the higher polyamine content induced in

maize leaves by a long period (30 days) of chilling treatment (8 °C) and the level of paraquat resistance or the characteristic symptoms observed following spraying (the recovery of physiological functions) (Szigeti *et al.*, 1996a). Although preliminary treatment with abscisic acid increased the polyamine content in the model plant, it had no influence on paraquat sensitivity (unpublished data). The rise in putrescine and total polyamine levels in R *Conyza* plants treated with paraquat appears to be a general stress response, rather than a specific reason for or symptom of resistance. At the same time, the higher polyamine content may be involved in the induction of protein synthesis, the specific cause of which is, however, paraquat itself (Rácz *et al.*, 2000).

Examinations on the distribution of free polyamines (including the most characteristic – putrescine) between the cell fractions indicated that in untreated S and PqR plants the bulk of polyamines was localised in the cytosol fraction (nearly 80%). Four h after paraquat treatment the putrescine content increased in each fraction without preferential enhancement in any on of them, suggesting that the intracellular compartmentation of the polyamines is unaffected by the treatment, i.e. polyamines do not take a direct part in determining the direction of paraquat transport (Rácz *et al.*, 2000).

Acknowledgements

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