Individual worker larvae were collected (before sealing) and homogenized in 0.2 ml of 0.25 M sucrose, with 3–5 mg of nicotinamide adenine dinucleotide (NAD) added to each homogenate. Homogenates were centrifuged at 1500 × g for 10 min, then 11 µl of supernatant was inoculated into preformed slots in gels. Electrophoresis was performed on 4.5% acrylamide (AM-9 grout, Cyanamid Australia) gel slabs, 20 cm × 11 cm × 0.3 cm, using a constant voltage gradient of 12.5 V/cm along the gel, for an average duration of 3.5 h. Gels were run at 4 ± 2 °C.

For general proteins, Poulik's discontinuous buffer[12] was used. Gels were stained in 0.5% amidoschwarz 10B in 7% acetic acid. Destaining was carried out by successive washes in 7% acetic acid. Homogenates for general protein staining had to be diluted 1:1 with distilled water before running on gels, to improve staining resolution.

Poulik's discontinuous buffer was also used for esterase. Gels were stained at room temperature in a solution containing 2 mg α-naphthyl acetate dissolved in 5 ml acetone; 35 ml 0.1 M Tris-HCl buffer, pH 7.0; 35 mg fast blue RR salt.

For malate dehydrogenase, the same sodium borate buffer, pH 8.2 (0.3 M boric acid) was used in the gel and in the electrode chambers, except that the buffer was diluted 1:5 in the gel. The gel buffer contained 0.3 mM NAD. Malate dehydrogenase was stained in the dark at 35 ± 3 °C in 40 ml of a solution made up in 0.1 M Tris-HCl buffer, pH 8.9, containing 100 mg D,L-malic acid; 15 mg NAD; 5 mg nitroblue tetrazolium and 2 mg phenazine methosulfate.

For alcohol dehydrogenase, a tris borate buffer, pH 8.1 (0.025 M Tris), containing 1.3 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 mM NAD was used in the gel, and a sodium borate buffer, pH 8.6 (0.13 M boric acid) was used in the electrode chambers. Gels were stained in the dark at 35 ± 3 °C in 40 ml of a solution made up in 0.1 M Tris-HCl buffer, pH 8.9, containing 0.35 ml of absolute ethanol; 15 mg NAD; 5 mg nitroblue tetrazolium and 2 mg phenazine methosulfate.

The table shows gene frequencies for each hive calculated from the phenotypes of 12 individual worker larvae. Phenotypes on gels and artificial insemination data (unpublished) are consistent with each of these enzymes being encoded by codominant alleles at single loci. Polymorphism could be detected at only 3 of the 4 enzyme and protein loci found to be variable in Brazilian honeybees. No genetically determined variation was detected in general proteins. This may be due to differences in technique, or to monomorphism in Australian stocks at all of the loci determining general proteins. In addition, only 2 alleles for alcohol dehydrogenase were segregating in Australian honeybees, compared with 3 alleles in the South American stocks. However, as in the South American honeybees, the esterase locus has a predominance of 'slow' alleles.

It is clear that while there may be differences in allelic frequencies between Australian stocks, only esterase appears to show a qualitative difference – in this case presence or absence of a rare allele. Hence, while these polymorphic enzyme loci may serve as quantitative genetic markers for population and commercial studies, they will not be absolutely diagnostic of different commercial stocks.

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## Ten-fold enhancement of 2,4-D effect on water hyacinth by addition of gibberellic acid

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*Summary.* Under greenhouse conditions the effective concentration of 2,4-D (amine salt) for killing water hyacinths could be decreased 10 times if 2,4-D was applied in combination with extremely low concentrations of gibberellic acid (6 g/ha or higher). This implies that in practice the risk of harming nearby vegetation is considerably reduced, and the cost of spraying programmes might be decreased.

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms., is generally considered the world's most troublesome aquatic weed[2,3]. The growth potential of this free floating plant is enormous and it frequently forms dense, impenetrable mats on the surface of water bodies in tropical and subtropical areas. If such vegetation blocks navigable rivers, irrigation canals, inlets of hydro-electric installations or other economically important stretches of water the consequences to local communities can be disastrous. Removal of vast water hyacinth masses by manual or mechanical means is often a Herculean task and as a consequence in many cases the application of herbicides cannot be avoided. In practice such chemical control programmes are carried out with only 1 herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), which is relatively cheap and effective against water hyacinths at concentrations of 2–5 kg/ha[3,4].

In 1976 it was reported that the addition of extremely low concentrations of gibberellic acid (GA) to the water in which water hyacinths grow (0.03 mg/l) caused an inhibition of vegetative growth and completely counteracted the formation of float leaves, i.e. leaves characterized by a bulbous swelling of the petiole which provides buoyancy[5]. Subsequently it was observed that an aqueous solution of GA brings about the same inhibiting effects on vegetative growth and float formation when sprayed upon the plants at 15 g/ha and higher concentrations[6]. It has been suggested that GA could perhaps be used to keep water hyacinths under control in certain areas and it was emphasized that

GA is a natural component of plants which does not endanger the environment[5]. In this context it should be noted that these extremely low concentrations are not likely to affect plants in adjacent areas and GA rapidly decomposes in water and soil.

Recent experiments in a greenhouse have shown that a 2,4-D concentration which has little or no effect on water hyacinths, i.e. 100 g/ha, kills the plants within 1 week when it is supplemented with extremely low concentrations of GA.

Water hyacinths were cultured during the summer season 1979 in a greenhouse in concrete reservoirs, 200 cm long, 100 cm wide and 50 cm deep. The reservoirs were filled up to the border with tap water and contained a 20-cm-thick layer of clay on the bottom. Each reservoir contained 10 water hyacinth plants which were sprayed by means of a small hand sprayer from a height of about 40 cm. The volume rate was 200 l/ha. The plants were first sprayed with 100 g/ha of 2,4-D (amine salt). Subsequently 0, 2, 4, 6 and 8 g/ha of GA (ICI product Berelex) was sprayed on plants in different reservoirs. The control plants were sprayed with either water, various concentrations of 2,4-D (50, 100, 200, 500 and 1000 g/ha) or a combination of water and 8 g/ha of GA. A 2nd and 3rd experiment were conducted with respectively 50 g/ha and 200 g/ha of 2,4-D. All experiments were repeated twice.

The results of the experiments are presented in the table. When the water hyacinths were sprayed with various concentrations of 2,4-D alone there was no effect at 50 g/ha and little or no effect at 100 g/ha. At 200 g/ha and 500 g/ha the plants were clearly affected (limp leaves) but most plants eventually survived the treatment. At 1000 g/ha the plants died within 1 week. A combination of 50 g/ha of 2,4-D with the various GA concentrations had a slight effect on the plants at the higher concentrations of GA. However, at 100 g/ha of 2,4-D the plants died within 1 week when the herbicide was applied in combination with 6 or 8 g/ha of GA. At 2 and 4 g/ha of GA there was a slight effect (limp leaves). In combination with 200 g/ha of 2,4-D the results were more pronounced but at 2 and 4 g/ha of GA most plants were able to survive. Applications of 8 g/ha of GA in the absence of 2,4-D slightly inhibited vegetative growth and float formation which is in accordance with the earlier report[5]. This effect became apparent after a period of 3 weeks. In controls which were sprayed with water only the plants remained healthy and grew profusely.

Effect of 2,4-D/GA spray combinations on water hyacinth

| Concentration 2,4-D (g/ha) | Concentration GA (g/ha) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| 0 | − | | | | −* |
| 50 | − | − | − | −+ | −+ |
| 100 | −+ | + | + | ++ | ++ |
| 200 | + | + | + | ++ | ++ |
| 500 | + | | | | |
| 1000 | ++ | | | | |

− No effect; −* slight effect on vegetative growth and float formation after a period of 3 weeks; −+ little or no effect; + slight effect (limp leaves) but most plants eventually survive; ++ plants die within 1 week, limp leaves after 2 days.

These greenhouse experiments clearly demonstrate that there is a synergistic effect of 2,4-D and GA with regard to water hyacinth. Consequently the usual field concentrations of 2,4-D could be decreased significantly if sprayed in combination with GA and this would considerably reduce the risk of harming nearby vegetation. Although GA products are relatively costly, smaller amounts of 2,4-D, approximately 10 times less than what is used at the moment, in combination with such extremely low concentrations of GA, might even lower the costs of spray programmes.

It is not clear by which mechanism GA influences the 2,4-D effect on water hyacinth. As in certain cases GA has been shown to enhance the translocation of auxins[7,8] it could be hypothesized that a similar system is involved.

Field experiments will be conducted in due course.

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## Occurrence of carotid labyrinth in the catfish group of teleost fishes

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*Summary.* A structure similar in topography and morphology to the carotid labyrinth of amphibians has been found in 4 catfishes, namely, *Clarias batrachus, Heteropneustes fossilis, Rita rita* and *Mystus seenghala.* The present finding of the presence of the carotid labyrinth in fishes is the first report, and the results are discussed in the light of the amphibious habit of these catfishes.

The carotid labyrinth - a spongy enlargement of the common carotid artery where it bifurcates into the external and internal carotid arteries - is confined to Amphibia, and most amphibians possess it[2]. The function of this structure is far from clear. It is generally believed that it is comparable to the carotid body of higher tetrapods, which possesses receptors[3] involved in circulatory and respiratory regulation by way of detection of fluid pressure and of tensions of oxygen and carbon dioxide in the blood. The presence of such receptors has not been confirmed in the carotid labyrinth[4-6] though it might be anticipated. However, so far, a piscine precursor or counterpart of the amphibian carotid labyrinth has not been reported. An 'amphibious' habit with bimodal respiration has evolved in a number of fishes, belonging to the Teleostei[7,8]. Since for fishes the general belief is that cardio-vascular events are