northern bluegill and the black crappie indicate the possible presence of a minor component of greater mobility than the major components. The extremely characteristic specificity of the electrophoretic patterns for each of the species included in this study also corroborates the observation of Connell (*1*) that the electrophoretic method of analyzing the muscle protein of fish may be a means of "fingerprinting" any species. We have noted some evidence (not included in this report) that differentiation of a taxonomic category of subspecies may be possible.

In addition, the eletrophoretic method of analysis can be used in fish hybridization studies, in studies of phylogeny, and—to cite a very practical application—in connection with game enforcement procedures to prove illegal possession of fish fillets.

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3. We thank George W. Bennett of the Illinois Natural History Survey for the white crappie used in this study and Rene Evard and Parvin Medhat for technical assistance.
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Synergism of Malathion against Resistant Insects

Abstract. Several tris-substituted derivatives of phosphoric acid synergized the toxicity of malathion to resistant houseflies and mosquitoes. In some tests it was possible to overcome acquired resistance completely. The synergists had much less effect on the toxicity of malathion to susceptible strains of the same species.

Studies with mammals have shown that the toxicity of malathion (*1*), one of the safest of insecticides, can be increased by simultaneous administration of, or prior treatment with, a number of organic phosphates. Thus, the potent insecticide EPN (*2*) synergizes malathion against mammals (*3*), as does the noninsecticide tri-*o*-cresyl phosphate (*4*). With both compounds it is known that the effect of their synergizing action is to block the degradation of malathion through carboethoxy ester hydrolysis (*5*).

The effect of a series of phenyl phosphorus materials on the toxicity of malathion to mice has been investigated, and a correlation between the ability to synergize malathion and the ability to inhibit ali-esterase activity has been demonstrated (*6*). Recent work has shown that resistance to organophosphates in insects is associated with a decline in ali-esterase activity and a change in the nature of the ali-esterase from an enzyme (or enzymes) inhibited by organophosphates to an enzyme (or enzymes) capable of degrading them (*7*). These findings prompted us to investigate the possibility that acquired resistance to malathion in insects might be overcome through use of ali-esterase inhibitors. Experiments were conducted with several tris-substituted aromatic and aliphatic derivatives of phosphoric acid which are known to be ali-esterase inhibitors.

For these experiments 2- to 4-day-old adult female houseflies, *Musca domestica* L., of a malathion-susceptible (Orlando Regular) colony and a malathion-resistant (Grothe) colony were used. The mosquitoes used were fourth-instar larvae of a susceptible and a malathion-resistant strain of *Culex tarsalis* Coq. In the tests with flies, groups of 20 adult females were exposed to films of the insecticide with or without synergist in 1-pint glass jars. Mosquito larvae were tested by placing groups of 20 in 250 ml of water in glass jars containing the toxicants. In all tests mortality determinations were made 24 hours after initial exposure to the toxicant.

Technical-grade malathion of more than 90 percent purity was used in these studies. The synergists were samples of commercially available materials (*8*). The materials were tested on a weight-to-weight basis.

Table 1. The effect of some synergists on the toxicity of malathion to susceptible and resistant houseflies and mosquitoes.

Ratio of insecticide to synergist	Toxicity (LC_{50} in μg per jar)			
	Houseflies		Mosquitoes	
	Resistant	Susceptible	Resistant	Susceptible
	Malathion only			
	1800	17	2.4	0.025
	Malathion plus triphenyl phosphate			
1:1	80	17	0.024	.016
1:10	50	25	.025	.022
	Malathion plus tributyl phosphorotrithioate			
1:1	25	9	.030	.014
1:10	20	10	.014	.010
	Malathion plus tributyl phosphorotrithioite			
1:1	40	12	.025	.014
1:10	18	13	.015	.018

The results of experiments with several of the most effective synergists are presented in Table 1. As shown by the data, the synergists reduced the resistance to malathion of flies of the Grothe colony from about 100-fold to less than 5-fold. None of the synergists tested increased the toxicity of malathion to flies of the susceptible colony by as much as a factor of 2. In tests with mosquito larvae the same synergists completely overcame the 100-fold resistance. As with the flies, the synergists failed to produce striking increases in toxicity to larvae of the susceptible colony.

At present the effect of the synergists is not fully understood. All the synergists are known to be inhibitors of ali-esterase activity in flies and mosquitoes under both in vivo and in vitro conditions. They also synergize the toxicity of malathion to mammals (*9*).

Preliminary studies have indicated that the synergists inhibit the degradation of malathion by mosquito larvae. The most logical explanation is that the synergists inhibit the ability of the insects to degrade malathion by cleavage of the carboethoxy ester linkages. Both houseflies and mosquitoes are known to degrade malathion partially through hydrolysis of these bonds, and with *Culex tarsalis,* increased ability to degrade through carboethoxy ester hydrolysis is known to be a factor in resistance (*10*).

The results provide evidence that, at least in certain cases, acquired resistance to organophosphate insecticides can be overcome through selective inhibition of degradation mechanisms with noninsecticidal compounds.

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References and Notes

1. Malathion is the common name for *S*-[1,2-bis (ethoxycarbonyl) ethyl] O,O-dimethyl phosphorodithioate.
2. EPN is the trade name for *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate.
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Flower Initiation in Excised Stem Disks of Wedgwood Iris

Abstract. The presence of substances promoting flower induction was demonstrated in both scales and buds of Wedgwood iris. The flower-inducing extracts of buds appear to contain gibberellin-like compounds.

In the study of flower-initiation phenomena, more and more use is being made of the technique of aseptic culturing both of whole plantlets (*1*) and of isolated buds (*2*). However, until now only the physiology of flowering of dicotyledons, and in particular of those sensitive to the length of the day, has been studied in this way. For a variety of reasons a day-neutral, monocotyledonous plant—the iris cultivar Wedgwood—was chosen as the test material in the study reported here.

The bulbs of Wedgwood iris are lifted at the end of August. They arrive at the laboratory 4 to 6 days after lifting and are immediately stored at 25.5°C. At this temperature they remain vegetative for more than 10 months. In the bulbs, flowers are initiated during dry storage at 13°C. The transition of the shoot apex from the vegetative to the reproductive stage occurs about 4½ weeks after the beginning of the low-temperature treatment. Shorter exposure to temperature of 13°C and also prolonged storage at 25.5°C result in a threshold condition in which the bulbs are slightly activated. The state of activation is shown by the rate of initiation and development of the flower primordium in the excised shoot apex growing on an agar medium.

The shoot apex was in the vegetative state at the time of excision. The threshold condition was the starting point of all our experiments. The threshold was the result of a combination of a period of storage at 25.5°C and a so-called "pretreatment" at 13°C for various lengths of time. The bulb of Wedgwood iris consists of a short axis, called a stem disk, which carries typical scale leaves, young foliage leaves, and, in the center, the shoot apex. After removal of the scales, the remaining part of the bulb was sterilized for 30 minutes with hypochlorite and rinsed in sterile water. Then, part of the stem disk with the two or three youngest leaf primordia and the shoot apex was excised under aseptic conditions and placed on a nutrient medium. The medium contained macronutrients according to the formula of Knop, micronutrients according to the formula of Heller (*3*), sucrose, and agar. Culturing was done in tubes or petri dishes at 13°C in the dark. The average duration of the experiments was 7 weeks. At the end of an experiment the stage of development of the shoot apex was determined under a dissecting microscope.

Isolated stem disks carrying only the shoot apex and the two or three youngest leaf primordia, and excised from bulbs that had been stored at 25.5°C without any prior treatment at 13°, did not become reproductive when kept on the medium at 13° for a period of 7 weeks. On the other hand, explants carrying either all the young foliage leaves present at the time of excision or a piece of scale, when exposed to temperature of 13° for a similar period of time develop flower primordia. It therefore appears that the presence of young leaves and scales promotes flower initiation. Since the medium contained sugar, it does not seem likely that the effect of leaves and scales is merely that of supplying simple organic nutrients.

The promotive influence of scales on flower formation could also be demonstrated in the absence of any connection of living tissue with the shoot apex. This was done in two ways.

1) An excised stem disk and a piece of scale were placed together on the nutrient medium, with their cut surfaces on the agar. The piece of scale used in these experiments, as in those mentioned above in which the scale was attached to the shoot apex, corresponded to one-fourth of the total mass of the scale material and weighed about 4 g. The weight of the stem disk was about 500 mg. The results are shown in Table 1, as experiments 1 through 4. Although there was no union of tissue between the epidermis of the scale and the cut surface of the stem disk, the presence of the scale fragment induced flower initiation in 60 to 100 percent of the explants.

In comparing the results of these experiments, it should be borne in mind that they were carried out at various times—that is, after various periods of storage at 25.5°C. Experiment 2 was carried out in January, experiment 1 in April. So the bulbs of experiment 1 had been exposed to a temperature of 25.5°C almost twice as long as those of experiment 2 and were more acti-

Table 1. Influence of scales and bud extracts on flower induction in excised stem disks of Wedgwood iris. Treatment A: bulbs exposed at 13°C prior to, respectively, incubation of scales and extraction of flower-inducing substances. Treatment B: bulbs exposed at 13°C prior to excision of test stem disks. N_t, total number of stem disks; N_r, number of stem disks that became reproductive. In experiments 1 through 4, scales and apexes were incubated at the same time; in experiments 5 and 6, scales were incubated for 1 week prior to the incubation of apexes.

Experiment	Experimental group				Control group	
	Treatment at 13° (wk)		N_t	N_r	N_t	N_r
	A	B				
			Influence of scales			
1			10	6	12	0*
2			9	2	13	0
3	1		11	11	19	13
4	2		23	14	15	2*
5	1		13	5	32	0*
6	3		13	3	32	0*
			Influence of bud extract			
7	3	1	24	7	30	2†
8	4	1 4/7	23	10	30	2*

*$P < .01$. †$P < .05$.