and/or inhibiting GIH and is responsible for the ovarian enlargement.

The data clearly indicate that RSP can bring about an ovarian enlargement only in intact crabs (table, fig.). The ovarian index in the destalked crabs after RSP and 5-HT administration does not differ much from that in untreated destalked crabs. So, the changes obtained after RSP administration are mediated through the eyestalk, which is a source of moult inhibiting hormone (MIH) and GIH. It is possible that RSP is inhibiting the activity of GIH and thus upsetting the balance between GSH and GIH in the blood. The increased level of GSH in probably responsible for ovarian enlargement.

The problem as to why destalked crabs died after RSP administration can be explained with the help of our earlier studies on *S. serrata*[12]. We have shown that the eyestalk possesses a) hormone(s) which act against stress[12]. In the destalked crabs, as the hormone which acts against stress is lacking, the death of the crabs injected with RSP may be owing to its pharmacological action. It has already been demonstrated that RSP could produce toxic effects by its pharmacological actions[4], since the same dose of RSP is effective in the intact crabs and brings about changes in the ovary, and it has a toxic effect only in the destalked crabs. Perhaps some factors from the eyestalk may be preventing the toxic action of RSP.

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Nematicidal activity of dimethyl 1-dodecanephosphonate[1]

J. Feldmesser[2], J. Kochansky and W.E. Robbins

Nematology Laboratory and Insect Physiology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville (Maryland 20705, USA), July 20, 1982

Summary. Dimethyl 1-dodecanephosphonate has been shown in laboratory bioassays and greenhouse tests to be highly active against 2 species of nematodes. Other phosphonate esters showed little or no activity.

Plant parasitic nematodes cause damage in the United States estimated at about approximately 7.5% of crop value. Adjusted to current values these damages are estimated to total US$ 6×10^9/year[3].

Control of plant parasitic nematodes is difficult for several reasons. They are intrinsically resistant to chemical control because nematode cuticle withstands penetration by most pesticides. Many target nematodes live deep within the soil mass, or within roots in the soil. The soil itself serves as a formidable barrier, preventing many chemicals from penetrating target sites efficiently, or degrading or inactivating them before they can reach sites of action in concentrations sufficient to exert control. Consequently, nematicides must be applied at relatively high, costly, and frequently hazardous concentrations to be even minimally effective.

As a result, the number of currently registered nematicides (approximately 25) is small and inadequate. Many of these are specialty materials which cannot be considered for wide spectrum use. For example, phorate[1] is labeled only for use on easterlilies in the Pacific Northwest. Several are dangerous and may pose environmental risks and may consequently be removed from use by regulatory pressures.

Because of this dearth of relatively safe, effective nematicides, we have been engaged in evaluating various compounds found to be biologically active in other systems, for

Table 1. Range of concentrations (in ppm) of alkanephosphonate esters required to kill 95% of exposed *Panagrellus redivivus* populations in direct contact tests

Compound $RPO(OR')_2$	$R' = -CH_3$	$R' = -CH_2CH_3$	$R' = -(CH_2)_3CH_3$
$R = CH_3(CH_2)_3$	> 100		> 100
$CH_3(CH_2)_5$	> 100		
$CH_3(CH_2)_9$	20-40		> 100
$CH_3(CH_2)_{10}$	20-40	> 80	
$CH_3(CH_2)_{11}(I)$	0.5-1	> 80	
$CH_3(CH_2)_{12}$	20-40	> 80	
$CH_3(CH_2)_{13}$	80-100		> 100
$CH_3(CH_2)_{15}$	> 100		> 100

example the determination that a number of amines and amides are toxic to the nematodes *Panagrellus redivivus* and *Meloidogyne incognita,* in vitro[4-9]. The materials reported here have resulted from our research with compounds that disrupt hormonal and other processes in insects.

When dimethyl 1-dodecanephosphonate became available from research on Fire Ant toxicants, we tested it and found it extremely active against *Panagrellus redivivus* in our laboratory screen.

A search of the literature revealed no mention of nematicidal activity of long chain (> 2 carbons) alkanephosphonate esters. We then entered into a synthetic program to determine structure-activity parameters for this series of compounds.

The dibutyl esters were prepared and hydrolyzed to the acids by the methods of Kosolapoff[10]. Methyl esters were prepared by treating the acids with a slight excess of ethereal diazomethane. Ethyl esters were prepared from the phosphonyl chloride (from acid + thionyl chloride) and sodium ethoxide/ethanol. Products were all pure by GC and had NMR, IR, and, where run, mass spectra consistent with the expected structures.

The compounds were evaluated in a standard direct contact test[11]. *Panagrellus redivivus,* a saprophytic nematode and a sensitive indicator of nematicidal activity, was immersed for 48 h in mixtures containing water, quartz sand, and the toxicant in solubilized form. Each compound was tested at concentrations ranging from 100 ppm to 5 ppm, or lower when 5 ppm was lethal. The compounds were solubilized in a solvent-surfactant-water medium that is nontoxic to nematodes. This medium had the following composition: 1 part acetone and 1 part of an aqueous solution containing 5% Tween 20 (polyoxyethylated sorbitan monolaurate) and 5% Triton X-100 (polyoxyethylated octylphenol). Solvent-surfactant concentrations were 0.5% or less (vol/vol), of each formulated solution containing a candidate nematicide. Approximately 400 nematodes, in all developmental stages, were exposed in each test.

Effects were determined during the day immediately after exposure by microscopic examinations[12]. Normal unstressed *Panagrellus redivivus* are in continuous rapid motion, and the esophageal areas are hyaline. Exposure to nematicides results in reduced motility, immotility, and death, and when the nematodes are moribund or dead the esophageal structures disintegrate and darken. When reduction or cessation of motility appears to be the sole or major effect of exposure, nematodes are held for 24 h or longer after the end of the exposure period to determine recovery of motility, if any, and to determine mortality rates. Untreated checks were run along with all experimental compounds. Check mortality was < 10% (typically 3-4%).

P. redivivus, an ovoviviparous species, is also a sensitive indicator of penetrating effects of candidate nematicides. Gravid females contain embryonated eggs and second-stage larvae which hatch in utero. In circumstances where egg release to the environment is delayed, which may occur frequently in vitro, the larvae feed on maternal tissues (thus, *'endotokia matricida'*). As a result, these larvae may be shielded from direct contact with a candidate nematicide for the entire 48 h exposure period by random layers comprised of adult female cuticle, hypodermis, muscle layers, and internal tissues, and by egg shells and membranes. A candidate nematicide is considered effective in these tests only if it succeeds in killing these encased larvae. Under these test conditions, the LC_{95} for a standard commercial nematicide, DD (1:1 mixture of 1,2-dichloropropane and 1,3-dichloropropene and related C_3 chlorinated hydrocarbons), is 36 ppm and the lethal concentration (LC_{100}) is 40 ppm.

Under these conditions dimethyl 1-dodecanephosphonate ($CH_3(CH_2)_{11}PO(OCH_3)_2$, compound I in table 1) exhibited an LC_{95} of 0.5-1 ppm, and we observed 100% mortality as low as 1.25 ppm. This compares very favorably with lethal concentrations for the best commercial nematicides, aldicarb, carbofuran, phenamiphos, and phorate, all of which require 5 ppm in the *Panagrellus* assay and which are extremely toxic to mammals. This also compares well with the most active of the amines of Feldmesser et al.[4], which require 5-10 ppm.

The activity of the homologous dimethyl esters falls off rapidly on either side of the optimum chain length, as shown in table 1. Values are averages for 4 replications. Ethyl and butyl esters were essentially inactive (LC_{95} > 80 ppm). The differences in activity observed in this work may presumably be ascribed to variation in intrinsic toxicity, cuticular permeability, metabolic deactivation, and probably other factors. We have no way of assigning weights to these various factors, but it seems unlikely that a difference in activity of 20-80-fold between the dodecyl compound and the undecyl and tridecyl analogs could be ascribed solely to differences in permeability. We suspect that a combination of factors is involved.

Compound I was further tested against second stage (infective) larvae of *Meloidogyne incognita,* a widespread economically-important root parasite which attacks a large number of cultivated crops. Larvae were exposed in a vial test to a range of concentrations of the test compound for 48 h and then washed free of the candidate toxicant. Visual examinations showed darkened disintegrated structures in the esophageal areas of many of the exposed larvae. Since visual examination is not completely reliable for determining mortality, final viability determinations were made by the following bioassay procedure. Exposed larvae were used to inoculate small nematode-free tomato seedlings (*Lycopersicon esculentum,* var. Rugers), growing in nematode-free soil in small containers. 1000 exposed nematode larvae were placed in 3 or 4 small holes in the soil around the stem of each tomato seedling. The holes were then tamped shut, and the plants were watered lightly and thereafter maintained on a regular greenhouse schedule. Unexposed larvae were used to inoculate control plants.

Meloidogyne incognita causes root galls or 'root-knots' in the roots at and adjacent to nematode feeding sites. These galls become macroscopically visible, due to host plant reactions involving the proliferation of so-called 'giant cells'. Infections were evaluated on an arbitrary basis, the 'root-knot index', by assigning values of 0 = no infection, 1.0 = 1-25% of the roots galled, 2.0 = 26-50% galled, 3.0 = 51-75% galled, and 4.0 = 100% root infection.

The inoculated tomato seedlings were examined after 3 weeks to determine the viability of the nematode inocula expressed as root infections. Root-knot infections were indexed visually, and the roots were examined microscopically after differential staining to determine the absence or presence of nematodes in roots lacking visible knots. The results of inoculation with the exposed root-knot larvae are

Table 2. Effects of inoculating tomato seedlings with *Meloidogyne incognita* exposed to several concentrations of dimethyl dodecanephosphate for 48 h

Replicate	Root knot index[1] Concentration, ppm 5	10	20	40	80	Unexposed control
1	2.0	0.5-1.0	0.0	0.0	0.0	3.0-3.5
2	2.0	<0.25	0.0	0.0	0.0	3.0-3.5
3	2.0	<0.25	0.0	0.0	0.0	3.0-3.5

[1] Root knot index is defined in the text (see above).

shown in table 2. A concentration of 20 ppm was sufficient to prevent any root infection, and even 10 ppm reduced infection approximately 6-fold.

The mammalian toxicity of these materials is apparently low. None of three rabbits died or became visibly ill after single oral doses of 300 mg/kg of compound **I**.

This study showed, somewhat anticlimactically, that compound **I** (the first compound is this group to be tested) is the most active of the series. It is probably also the only one with sufficient activity to be potentially useful in the field.

The use of these compounds as nematicides has been patented[13].

1 This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the USDA nor does it imply registration under FIFRA, as amended. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture, and does not imply approval to the exclusion of other products that may also be suitable.

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Induction of trisomics by platinum diaminodinitrodichloride

J.S. Dhesi, S.S. Sandhu and M.D. Waters[1]

Department of Biology, North Carolina Central University, Durham (North Carolina 27707, USA), and Genetic Toxicology Division, United States Environmental Protection Agency, Research Triangle Park (North Carolina 27711, USA), March 5, 1982

Summary. Trisomics were produced in the pollen mother cells of *Pennisetum americanum* (L) K. Schum plants resulting from seeds treated with 10^{-6} M platinum diaminodinitrodichloride. On the basis of this preliminary study the relative potency of cis-PDD may be roughly equal to the well known plant clastogen, maleic hydrazide.

An interest in the study of trisomy in higher organisms is based on 2 facts. First, a number of congenital birth defects such as Down's Syndrome, Klinefelter's Syndrome and Turner's Syndrome are associated with the presence of an additional chromosome in the human genome. Second, primary trisomics have been useful in the past for establishing genetic linkage groups[2].

Trisomics occur spontaneously and also have been induced in crop plants through breeding techniques. Reports showing the induction of trisomics in higher plants through chemical treatment are rare.

An evaluation of the biological effects of platinum compounds has become of considerable interest as a result of their use in catalytic converters in the automotive industry. One of the platinum compounds, cis-platinum diaminodichloride (cis-PDD) shows potential in cancer chemotherapy[3]. However, cis-PDD has been reported to induce base-pair mutations in bacteria[4]. It has also been implicated as a mutagen on the basis of results in mammalian cells in culture[3,5-7], and in insects[8]. The results reported here show that another platinum compound, platinum diaminodinitrodichloride $[Pt(NH_3)_2(NO_2)_2Cl_2]$ induced trisomics in pearl millet, *Pennisetum americanum* (L) K. Schum.

The seeds of pearl millet inbred line Tift 23DB ($2n = 14$) were presoaked in distilled water for 24 h. Seeds were treated by soaking for 3 h with the freshly prepared solution of the test compound at the concentrations of

Germination percentage and seedling survival after PDDD and MH treatments

Test compound	Concentration	Germination (% control)	Survival 2 weeks after germination (% control)	Number of trisomic plants obtained
Control		100	100	0
MH	10^{-4} M	98	11	0
	10^{-5} M	105	86	2
PDDD	5×10^{-5} M	109	92	0
	5×10^{-6} M	103	80	0
	10^{-6} M	113	87	4
	10^{-7} M	97	88	0