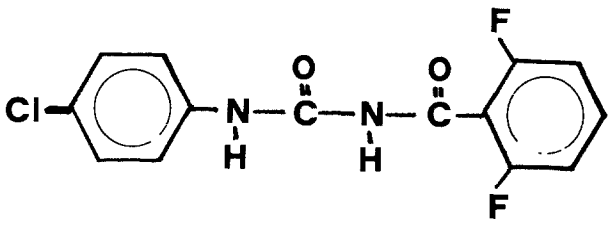
Thin Layer Chromatographic Detection of Diflubenzuron in Biological Samples

Paolo Maini[1] and Katalin V. Deseö[2]

[1]*Pesticide Residue Laboratory—Centro Esperienze Ricerche SIAPA SpA, 40015 GALLIERA (BO)*
[2]*Centro di Fitofarmacia dell'Università di Bologna Italy*

INTRODUCTION

Diflubenzuron is the proposed common name of a new experimental insecticide with a particular mode of action. It is known also under the trade name Dimilin(R), or PH 60-40, or TH-6040. Chemically, it is 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea or, according to the Chemical Abstracts usage: N-[[(4-chlorophenyl)amino] carbonyl]-2,6-difluorobenzamide.



Diflubenzuron inhibits larval development in many coleopterous, lepidopterous, and dipterous species (PHILIPS DUPHAR; VAN DAALEN et al., 1972; WELLINGA et al., 1973,a,b). The larvicide properties of this compound are based on the inhibition of the chitin biosynthesis (POST and VINCENT, 1973; MULDER and GIJSWIJT, 1973; MULDER and SWENNEN, 1973; POST and MULDER, 1974), therefore, it causes severe lesions in the endocuticular tissues and interferes with the moulting process. The product acts only if ingested and does not

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Bulletin of Environmental Contamination & Toxicology, Vol. 16, No. 6 © 1976 by Springer-Verlag New York Inc.

show systemic activity in plants (PHILIPS-DUPHAR; POST and VINCENT, 1973; BERNARDI and MASSASSO, 1975).

Diflubenzuron was tested for biological efficacy also by SCHAEFER et al. (1975), MILLER et al. (1975), GRANETT and DUNBAR (1975), GAMBERINI and PEPE (1975), LINSER and FABIANI (1975), PASSARINI and LODI (1975). It may show sometimes also an ovicidal effect (ASCHER and NEMNY, 1974). MOORE and TAFT (1975) found also some chemiosterilizing activity.

Our observations with diflubenzuron were carried out in an apple orchard in Galliera (Bologna, Italy). In a part of the orchard diflubenzuron alone was used as an insecticide against harmful insects, mostly the leaf miner Tortricides (PASSARINI and LODI, 1975). The orchard showed also a heavy infestation (43%) of Zeuzera pyrina Zell. (Lepidoptera: Cossidae). The larvae of this species live in galleries in the twigs and trunks. The presence of the larvae is easily noticeable, since huge amounts of excrement are pushed out from the holes at the entrances to the galleries.

A superficial, visual comparison between the excrements produced by the larvae of the treated and that of the untreated trees, showed already organoleptic differences. However, an explanation of this difference was difficult, as on one hand the older Z. pyrina larvae are generally known to live only in their galleries, and on the other hand diflubenzuron is not a systemic compound. So, the question arose whether diflubenzuron really did cause the observed difference, and if so, whether the larvae did come into contact with the compound coming out and re-entering, through new holes bored in the treated bark. Therefore, the first step was to show the presence of diflubenzuron in the metabolic products of the larvae.

It seemed reasonable to analyze the excrements for diflubenzuron residues and with this aim a chemical method was developed.

EXPERIMENTAL

Field treatments and sample collection

Samples of the excrements were collected from treated and untreated trees by the application of small boxes over the holes of the inhabited galleries. The size of the excrements showed the age of the larvae and only

the larvae terminating their development in summer and autumn were observed. Furthermore, samples from the bark and from the living wood immediately below it, were collected separately for chemical analysis.

Treatment rates were of 50 g active ingredient (200 g of Dimilin 25% w.p.)/100 l of water spraying 12 l/tree to run-off, by means of a motor pump. The data of the treatments were: april, 15th; may, 16th; june, 17th; july, 17th; and august, 4th; and that of sample collection: june, 27th; july, 3rd, 8th, and 11th; august, 14th; and september, 8th. Sample amounts ranged 1-20 g.

Chemical analysis

A simple and quick procedure was developed for the qualitative detection of diflubenzuron residues in the above mentioned samples by thin layer chromatography.

Extraction and Clean-up

Samples of 1-10 g were mixed for 5 min. in a 1 pint glass jar with a Omni-Mixer homogenizer (Ivan Sorvall Inc., Norwalk, Conn.), after addition of a spoonful of Filter Cel and 100 ml of ethyl acetate and 50 ml acetone. The slurry was filtered under suction on a G-4 synthe rized glass filter, directly into a 300 ml round bottomed flask and the filter cake was twice washed with diethyl ether. The filtered extract was evaporated in a Rotavapor apparatus (Büchi, Switzerland) till dry and taken up in 10 ml of methylene chloride.

Clean-up was made, following MILLER et al. (1975), by elution of the methylene chloride solution on a small column of 5 g silica gel (Silica gel RS, Ø 0,05-0,20 mm, C. Erba, Milan, Italy) and continuing the elution with an additional 100 ml of CH_2Cl_2. Then the column eluant was concentrated till dry and taken up with acetone in a graduated small test tube to a suitable measured volume for analysis by thin layer chromatography.

An alternative clean-up procedure, using a florisil column eluted first with 50 ml of petroleum ether to be wasted and then with 100 ml of the mixture of petroleum ether/ethyl acetate, 60:40 (w/v), gave less satisfactory results.

Thin layer chromatography

Silica Gel G precoated TLC plates, 0,25 mm thick (Merck, Darmstadt, G.F.R.) were used as purchased, without any activation treatment.

The standard solution used was 0,25% diflubenzuron in ethyl acetate, prepared from the technical product Dimilin(R), as 25% wettable powder. Suitable amounts (5-40 ul) of the standard and of the sample solutions under examination were applied on the layers, by means of Beckman polyethylene micropipettes (Beckman, Spinco Division, Palo Alto, Calif.). The plates were developed for a distance of 10 cm, at room temperature, in a pre-saturated chamber, its walls having been covered with filter paper, with the developing mixture chloroform:ethyl acetate:methanol, 100:2:1. After careful air drying of the plates, the detection of diflubenzuron was carried out spraying with a fresh $AgNO_3$ reagent, prepared according to the "ANALYTICAL METHODS FOR PESTICIDE RESIDUES IN FOOD" (1973), and placing the plates under uv lamp light for 2-3 minutes. Diflubenzuron was detected as a white spot on a light brown background and with a Rf value = 0,34 (Figure 1). The chromatograms remained unchanged for 4-5 h, then the background began to turn darker and diflubenzuron spots tended to disappear. Small variations in the mixture composition can lead to great changes in the Rf value of diflubenzuron (0,25-0,65); therefore , a comparison with standard product should always be made.

RESULTS AND CONCLUSIONS

The minimum amount of diflubenzuron detected was 1 ug. This method is not very sensitive, but none of the other spray reagents tested to detect diflubenzuron, gave comparable results.

The extraction and clean-up procedure used gave good and clear chromatograms: some other white spots due to an unknown compound appeared near the solvent front.

A dark brown-black spot with Rf = 0,55 appeared in the chromatograms of all the bark samples and of all excrements collected from both treated and untreated trees (Figure 1). As this spot behaved as an organochlorine compound and as the whole apple orchard had been previously treated with a Captan based fungicide, a comparison with Captan standard was made and its presence was confirmed both in the bark and in the excrements from the whole orchard.

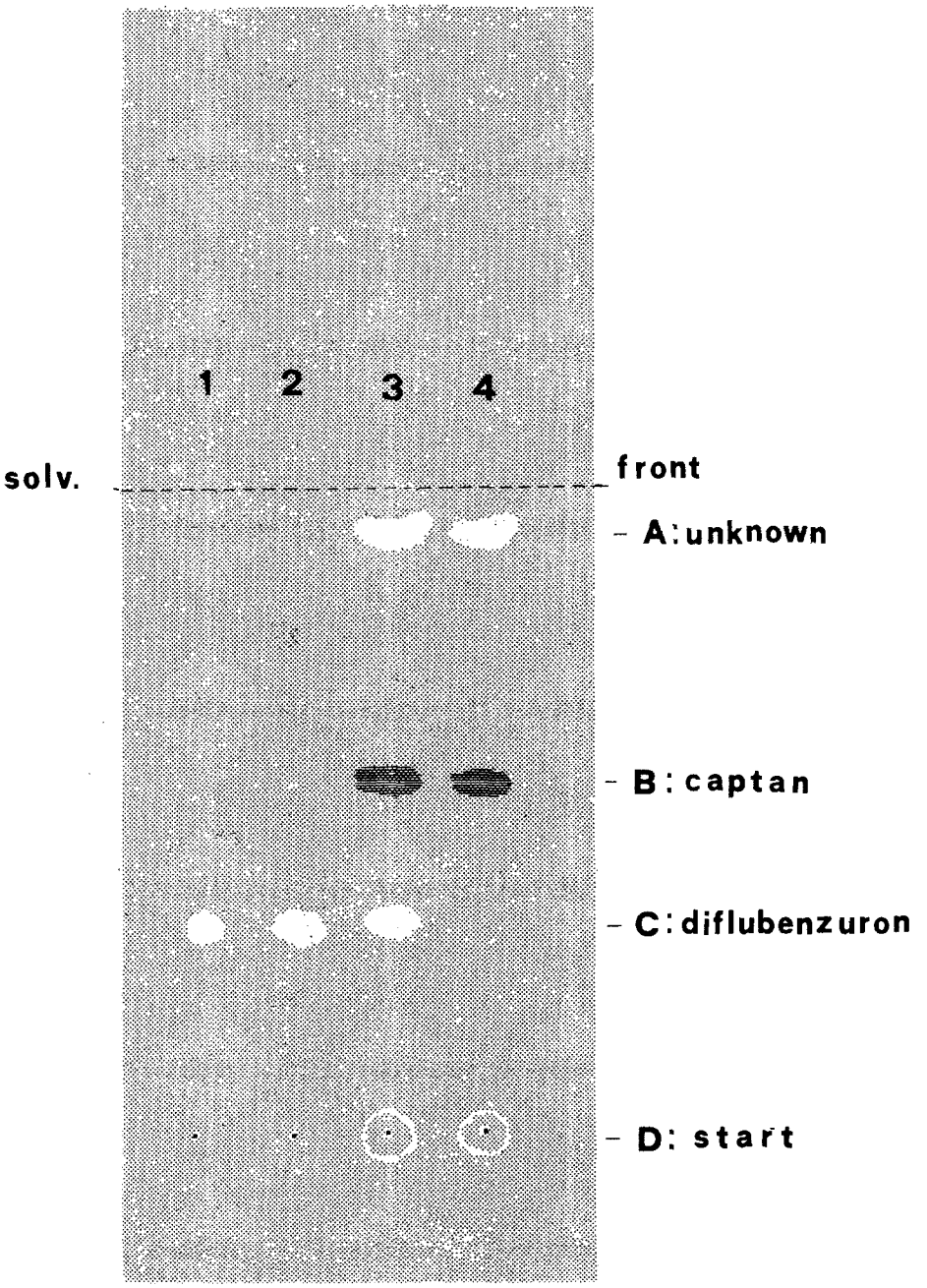

Fig. 1. 1 and 2 = 1.25 and 5.0 µg, resp., of pure diflubenzuron; 3 and 4 = 40 µl (≡ 200 mg) ext. of excrements from diflubenzuron treated and untreated trees, resp.

Diflubenzuron, in various concentrations, was present in all the excrements from the treated trees, except the last collected samples (August 14th, and September 8th) and was also present in greater amounts in all the bark samples from the treated trees.

All the wood samples, on the contrary, were completely devoid of diflubenzuron residues; therefore, the systemicity of the product was excluded.

Therefore, the hypothesized (and from 10th june until 10th july also observed) behavior of Z. pyrina larvae, getting out from their bores and re-entering by boring new holes in the trunk, with an unavoidable ingestion of small amounts of bark, found a partial confirmation.

However, the diflubenzuron amounts ingested by Z. pyrina caterpillars did not reach toxic levels, as no significant difference was observed between the number of deformed or dead larvae and pupae in treated and untreated trees.

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