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Research on Dithianon to Evaluate Suspects of Reduced Sensitivity of *Venturia inaequalis* with Different Methodologies in Italian Apple Areas

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Abstract

Dithianon is a multi-site fungicide and has never been object of suspects and reports of reduced sensitivity and activity. Italian IFP technicians had the suspect of reductions of activity by this fungicide on *Venturia inaequalis.* Methodologies, *in vitro* and *in vivo* were carried out to verify this suspect. Populations poorly controlled with suspects on dithianon and sensible ones were utilized. The tests *in vitro* permitted to evidence light and non-significant reductions of sensitivity of poorly controlled populations with respect to sensible ones. *In vivo tests* on seedlings were non-reliable for a general low activity of dithianon. On the contrary, the original *in vivo* methodology on grafted apple plants showed several reductions of activity, with moderate levels and a spot distribution in orchards. The cause was probably due to the increased treatments with dithianon caused by problems on other groups of fungicides and by a high infective pressure in some years. It is discussed if this reduction can be considered a resistance phenomenon or a temporary modification of the interactions of plant-fungus-fungicide.

Keywords

Venturia inaequalis, Sensitivity, Dithianon, Apple Scab Control, Reduced Control

1. Introduction

Dithianon is a fungicide commercialized since 1963 with a broad spectrum of activity on many phytopathogen fungi belonging to Ascomycetes or Deuteromycetes [1] that infect crops all over the world [2] and is registered on many diseases, comprehending also on *Venturia* (*inaequalis* and *pirina*).

It is a multi-site fungicide and its mode of action, not well known, is based on

activity against thiol groups in proteins and peptides, especially glutathione [3] [4]. It is substantially a surface product with preventive applications.

In the control of *V. inaequalis*, dithianon has often acquired an important role and it is active mostly in preventive applications, 3 - 5 day before infection [5] [6], but also in timely post-infection sprays, better 12 hours after the beginning of the infection [7] [8]. It is utilized during a long vegetative period (from "mouse ears" to "fruit set"), generally with a rather high number of treatments. The importance of this fungicide is increased by the fact that regulations of European Community [9] revoked or limited many pesticides, reducing particularly those with multiside action and favoring the news, more toxicologically sure but with a much higher risk of resistance.

In bibliography, there are not reports about loss of sensitivity and activity caused by reduced sensitivity, also because dithianon is generally used against diseases that do not require a high number of treatments or it is alternate with other fungicides.

In some Italian apple areas the number of treatments with dithianon increased, over 10 or 15, caused by the occurrence of resistance to other fungicides, as QoI's [10], AP's [11] and DMI's [12].

Subsequently to this context, the technics of some Italian apple areas had the suspect that the dithianon could present a reduced activity.

Therefore, we set up researches to assay different methodologies to verify if these reductions could be possible.

2. Methodology

Three types of *Venturia inaequalis* populations with different origin and scab management (and with presumably different sensitivity) were collected from apple areas and assayed with different methodologies.

The different types were collected from:

- Trees in uncultivated and untreated areas (sensible-wild-types).
- Orchards often treated with dithianon where the control of *V.inaequalis* was good. There was also a population "mixed" constituted by a mix of conidia from many "well (good) controlled" populations of several Italian apple areas (sensible, treated).
- Orchards often treated with dithianon, with control complains and suspects of reduced activity by this fungicide ("poorly controlled" and suspects on dithianon).

The fundamental comparison was between well and the poorly controlled population with suspects on dithianon. The methodologies assayed were:

2.1. *In Vitro*

The dithianon was tested as antigerminative activity on conidia of *V. inaequalis* populations in Petri plates amended with the fungicide [2] utilized as active material (PESTANAL® Sigma-Aldrich International). Every sample was tested with

two or three replicates. In each one, a part of inoculum (subpopulation) constituted by about 0.014 g of scabbed leaves was shacked in sterile water, filtered and adjusted to a conidia concentration of 1 - 2^5/ml. Drops of 20 µl from this dispersion were put in agar with the following concentrations of dithianon: 0, 0.01, 0.1, 0.3, 1, 3, 10, 30 mg/L. Before dilutions, streptomycin sulphate (200 mg/L) was added. The percentage of germination was assessed 24 hours later and data transformed in probits to obtain EC_{50} values (the concentration reducing of 50% the spore germination respect the test). The data were transformed to obtain RF (Resistance Factor: mean EC_{50} each treated population/mean EC_{50} sensible ones).

The same germination data were used also for assessment of M.I.C. (Minimal Inhibiting Concentration able to completely inhibit germination of spores) and concentrations used were: 0.3, 1.0, 3.0, 10 and 30 mg/L. Another used parameter is the RF (Resistance Factor) as the ratio: EC_{50} examined population/EC_{50} reference sensible population.

The data were collected and examined in 2008 and 2010 year, and compared with sensible ones.

Statistical Analysis

The EC_{50} values were analyzed with a model of variance with years and populations as effects (Statgraphics 12.0, $P \leq 0.05$).

2.2. *In Vivo*

These tests were realized with different methodologies on different types of apple plants:

2.2.1. Apple Seedlings

Apple seedlings cv. Golden with 5 - 6 leaves were preventively or curatively sprayed with dithianon. Different concentrations: 0.25, 0.50, 1 (field dose) and 2 g/L as commercial formulate of Delan (70 WG, 70% a.m., BASF) were used in preventive treatments. In curative ones only the field dose (1 g/L) was used

In preventive ones, 24 hours after applications, the plants were inoculated with a suspension of conidia by populations of *V. inaequalis* with a concentration of 1 - 2 × 10^5 conidia. After 48 hours of incubation with continuous wetness and 20˚C as mean temperature, the leaves of seedlings dried up and the curative application was realized. Subsequently all plants were incubated for 20 - 21 days at about the same temperature. In the end, the assessment of infection was carried out as mean percentage of scabbed leaf area and relative efficacy (%)*. The control, in field and *in vivo* trials, is classified as "good" when efficiency % is superior to 90% and "poor" when it is inferior. Statistical elaboration on grafted apples will give a more precise value of "good or poor control" in these trials.

$$\text{Relative efficacy \%} = \frac{\text{degree of infection of test} - \text{degree of infection of treated sample} \times 100}{\text{degree of infection of test}}$$

2.2.2. Grafted Apple Plants

After the two previous assays, a new type of *in vivo* trials (for dithianon) was by me pointed out and realized on potted grafted apples cv. Imperatore Dallago (very sensitive to scab, comparable to cv Mc Intosh), two or three years old, with 4 - 6 branches.

The two groups of populations were compared: sensible (well controlled ones) confronted with the poorly controlled ones with suspects on dithanon, while wild-types were not utilized, considering the high amount of work and being less essential for the comparison respect the last two types. The sensible ones were represented by a population (n.608) well controlled in Ravenna province and another sensible defined "mixed" constituted by conidia of several populations (already declared "*In Vitro* tests").

In comparison, populations from Emilia-Romagna Region and Trentino Province with suspects of reduced control by dithianon were tested (poorly controlled).

From two to four replications were carried out for every population. In each test and population, four plants were inoculated: two as control ad two treated with dithianon. The application was realized 16 hours before the inoculation and every plant was treated with about 35 ml of dithianon as commercial product Delan 70 WG (70% active material), BASF using 1 g/L. The last formed leaf of every bud was detected with a plastic bind and application was realized with a handle mechanic sprayer, until the drip limit.

Inoculations were realized utilizing, for each plant, about 0.007 g of scabbed leaves and 14 ml of distilled water. The amount of scabbed leaves and water was soaked, filtered and the conidia concentrated 0.8 - 1.3^5 /ml.

They were maintained in glasshouse with a temperature about 20˚C and relative humidity 60% - 98%, while the assessment of infection was realized 21 - 24 days after inoculation on 30 - 60 leaves/plant, evaluating the percentage of scabbed leaf area. The data were transformed in relative efficacy %*.

2.2.3. Statistic Analysis

The relative efficacy of dithianon was calculated starting from the mean percentage of scabbed area on leaves of the group of sensible and well-controlled populations, compared with that of suspected ones. Activity data, expressed as efficacy (%), were subjected to Bliss transformation (arcsen√efficacy %) and elaborated as "incomplete and unbalanced randomized blocks" design (SAS 9.0). This program was used for the different number of tests for every sample and, The C.I. (Confidence Interval) on efficacy (%) of sensible and well controlled populations was used to identify a suspected population, as statistically different (Table 4).

3. Results

3.1. *In Vitro* Tests

Each population tested in 2008 and 2010 is presented in Table 1 with type of

scab management, location, treatments, EC_{50} and MIC.

The statistic elaboration of EC_{50} values (Table 2), expressed as mean in each type of population sensitivity, shows that wild-type populations present sensitivity data like that of well-controlled ones but are at the limit of significance with referring to the poorly controlled ones. On the contrary the well-controlled populations present no significant differences with respect to the poorly controlled ones.

3.1.1. *In Vivo* Tests on Seedlings

Table 3 reports the results referred to the degree of infection and to the efficacy (%) of dithianon applied on several populations inoculated both preventively (with different doses) and curatively (only field dose).

The data show low or moderate levels of activity by dithianon, not only in reduced doses (until 1/4 of field dose), but also at field dose on both the types of tested populations (well or poorly controlled). Increasing the dose (from 1/4 to 2x), the degree of infection generally does not show reductions and consequently the efficacy % does not increase. The same tests replicated on population n. 99 (another well controlled population) show very uneven results, irrespective of each concentration. Likewise curative applications offer moderate or low levels of activity on well or poor controlled populations.

Table 1. Results *in vitro* sensitivity to dithianon (EC_{50} & CMI) by populations from wild types, well-controlled and poorly controlled with suspects on dithianon in 2008 and 2010 survey.

2008 Survey						
Samples					Results	
N°	Year	Type of population	Location and (Province) of apple trees	Mean n° treatments/ year with dithianon***	Mean DE_{50}* (mg/L)	MIC** (mg/L)
12-02	2008	Wild-types	Bologna	0	0.053	n.d.****
158	"		Suviana lake (BO)	0	0.030	n.d.
62	"		Po river foodplain (MN)	0	0.034	n.d.
313	"		Bagnacavallo (RA)	9	0.055	n.d.
222	"	Well-controlled	Formignana (FE)	3	0.060	n.d.
402	"		Ferrara	6	0.065	n.d.
99	"		Cona (FE)	3	0.092	n.d.
98	"		Cona (FE)	3	0.095	n.d.
225	"		Fosso Ghiaia (RA)	6	0.105	n.d.
202	"		Pordenone (PN)	5	0.11	n.d.

Continued

303	"	Reduced control and suspects on dithianon	Cona (FE)	7	0.10	n.d.
212	"		Cona (FE)	7	0.13	n.d.
211	"		Viconovo (FE)	7	0.25	n.d.
304	"		Cona (FE)	6	0.19	n.d.
428	"		Lavezzola (RA)	12	0.18	n.d.
403	"		Chiesuol del Fosso FE	5	0.25	n.d.
409	"		Ostellato (FE)	13	0.27	n.d.
408	"		Borgo Faina (RA)	7	0.29	n.d.
406	"		Bagnacavallo (RA)	9	0.43	n.d.
319	"		Fosso Ghiaia (RA)	6	0.015	n.d.
413	"		Altedo (BO) sperim.field	8	0.14	n.d.
416	"		Altedo (BO) sperim.field	8	0.48	n.d.
417	"		Altedo (BO) sperim.field	8	0.47	n.d.
			2010 survey			
36	2010	Wild-types	BASF	0	0.016	0.3
62	"		Po river foodplain (MN)	0	0.034	1
12	"		Bologna	0	0.052	1
97	"	Well controlled	Bagnacavallo (RA)	4	0.038	1
98	"		Cona (FE)	3	0.14	3
202	"		Pordenone (PN)	5	0.26	10
608	"		Faenza (RA)	14	0.27	10
Mixed	"		Localities from several Regions and Provinces	2 - 7	0.20	10
501	"	Reduced control and suspects on dithianon	Lavezzola (RA)	9	0.46	10
611	"		Consandolo (FE)	10	0.09	3
609	"		Bagnacavallo (RA)	11	0.13	3
605	"		Granarolo (RA)	12	0.18	3
616	"		Cona (FE)	7	0.18	1
615	"		Gaibana (FE)	14	0.22	3
Ba-3	"		S.Michele (TN)	10	0.26	3
600	"		Lavezzola (RA)	8	0.30	10
602	"		Fosso Ghiaia (RA)	7	0.34	10
704	"		S.Antonio (RA)	3	0.52	10
705	"		S.Antonio (RA)	3	0.45	10

* Concentration that reduced germination by 50% respect test; ** Concentration Minimum Inhibiting; *** Referred to the last two or three years; **** Not Detected.

Table 2. Mean sensitivity *In vitro* tests (EC_{50} values) of the three types of populations and results of statistic elaboration.

Type of population	Mean EC_{50} (mg/l)	RF ***	Comparison	Standard Error	DF	T-value	P-value	Significance (P: ≤0.05)
Wild types	0.03	__	Poorly controlled	0.055	2	−3.93	0.049	**
Well controlled	0.15	5.0	Wild types-well controlled	0.057	2	−2.07	0.17	*
Poorly-controlled	0.25	8.3	Well-controlled-poorly controlled	0.039	2	−2.47	0.13	*

* Values statistically non-significant; ** Values statistically significant; *** Resistance Factor respect wild-types.

Table 3. Results of the degree of infection and the efficacy (%) of dithianon on well and poor controlled populations of *V. inaequalis* inoculated on seedlings with curative and preventive applications.

Population n°	Field-control	Test *	Preventive treatments								Curative treatments	
			1/4 field dose		1/2 field dose		Field dose***		2x field dose		Field dose	
			*	**	*	**	*	**	*	**	*	**
99	Good****	44.4	44.5	0.0	38.8	12.6	41.6	6.3	16.6	62.0	27.7	32.0
		33.3	11.1	66.6	nr	nr	14.8	55.5	40.0	0.0	27.4	17.7
203	Good	29.6	40.7	0.0	21.2	28.3	19.4	34.2	33.0	0.0	50.0	0.0
608	Good						17.7	63.0				
602	Poor****	48.1	48.1	0.0	29.0	39.4	55.0	0.0	36.1	25.0	36.9	24.9

* Mean percentage of scabbed area on apple leaves; ** Efficacy %; *** Field dose dithianon: 1 g/L; **** "Good control or well controlled" when efficacy % is superior to 90%; "poor control" when it is inferior and with suspects on dithianon.

3.1.2. *In Vivo Tests* on Grafted Apple Plants

Table 4 presents each type population, populations, their location and results (relative efficiency %) obtained in each subpopulation. It is visible that there are moderate differences among efficacy % of all populations, both sensible and "suspected of reduced control".

Table 5 presents statistic elaboration where C are sensible and well controlled populations, while N are those "Suspected of reduced control" and RC all the populations. General statistic diversity presents $P > 0.019$, so among all populations there are not statistical differences.

We chose the C as referring group, calculating mean percentage. The results expressed as relative efficacy show that the highest levels in assays are presented by good-controlled populations (n. 608 and "mixed") with values from 100 to 93.3%, with a mean as 96.9% and a confidence interval of 10.6. So subpopulations inferior to an efficacy of 86.6% are considered statistically different.

Suspected samples (Table 4) from Emilia-Romagna region show that five populations (n.704, 603, Ba3, 2915, 601) present non significant values of dithianon efficacy (%) in all subpopulations.

The other four populations (n.501, 602, 615 and 3015) show a part of trials where subpopulations evidence significant reductions of activity by this fungicide, with values inferior to the I.C. (Confidence Interval). In Trentino Province (Table 4) only populations n. 16, 21, 23, 24 present one assay with significantly inferior values. The others, n. 17, 19 and 25 present no significant values of all subpopulations

Table 4. Efficiency (%) of dithianon *in vivo* (grafted plants) on two types of *V. inaequalis* populations: Well controlled and suspected of reduced control by the fungicide in two apple areas.

Populations		Subpopulations tested*						
Type	N° or denom. sample	n.1	n.2	n.3	n.4	n.5	n.6	Mean
Well-controlled	Mixed	93.3	98.2	99.3	96.9	96.4	94.6	96.48
	608	94.9	98.4	100				97.76
		Emilia-Romagna Region						
	501	90.3	87.0	73.8*				83.7
	602	95.6	92.3	85.2*				91.0
	704	94.0	92.0	94.2				93.4
	603	89.9	98.6					94.3
	Ba3	90.7	92.9					91.8
	615	94.0	62.0*					78.0
	601	93.2						93.2
Poorly controlled with suspects on dithianon	2915	89.3	89.2					89.2
	3015	91.0	86.1*					88.5
		Trentino Province						
	16	91.4	82.6*					87.0
	17	92.1						92.1
	19	97.9	89.5					93.7
	21	81.4*	94.2					87.8
	23	85.0*	97.6					91.3
	24	81.7*	92.4					87.0
	25	94.3	87.7					91.0

* Reduction of efficacy (%) out of the Limits of Confidence, so statistically significant.

Table 5. Statistic analysis on results of efficacy % of dithianon in test on grafted apple trees.

Comparison of C and N populations (C: well controlled; N: suspected reduced control) after Bliss transformation				
C-RC	Num DF = 8	Den DF = 6	F = 6.17	P(F): >0.019
Statistic discrimination of suspected subpopulation respect sensible utilizing C.I. "Confidence Interval"				
N	Standard error of mean	Mean of well controlled populations	Confidence interval (C.I. - 95%)	Lower Limit of C.I.
9	0.058	96.9%	10.6	86.3 (efficacy %)

These reductions are generally light (efficacy percentage superior to 80%) and only two subpopulations (one from sample n. 501 and one from n. 615) present

efficacy values inferior to 80%.

With referring to samples from Trentino Province, three populations (n.17, 19 and 25) respect seven total, show light reductions of activity, that are inside "confidence interval". On the contrary four populations (n. 16, 21, 23, 24) present, in one of the two realized tests, a subpopulation with efficiency values inferior to C.I. of well controlled ones. Nevertheless, the reduction of activity percentage is moderate, with relative efficiency always superior to 80%.

4. Discussion and Conclusions

The research on eventual reductions of sensitivity and activity of *V. inaequalis* populations to dithianon has been carried out with different methodologies: *in vitro* as antigerminative activity of dithianon on conidia; *in vivo,* verifying preventive and curative activity of this fungicide on scab presence of different kinds of plant: seedlings or grafted plants.

In vitro tests, the three types of populations examined (wild type, well-controlled and poorly-controlled with suspects on dithianon) were compared as EC_{50} and MIC values. The results showed that there are no statistical differences among the three types of populations, especially when we compare well and poorly controlled ones in commercial orchards. The statistical analysis confirmed what was observed by Stammler [2] with several samples from Germany.

The second methodology, based on preventive and curative applications of dithianon on apple seedlings, was realized with only a few samples of compared populations (well controlled and poorly ones, dithianon suspected), because it was clear that this methodology is not apt to show sensitivity and activity differences, as informed by Stammler (*pers. comm.*).

It is not clear why the activity of dithianon on apple seedlings was so low, considering that similar tests with seedlings on other fungicides as AP's [12] and DMI's [13] permit high correlation between this type of test and different sensitivities with reliable results, as shown by me. It was also advised by FRAC [14].

The last methodology was carried out with populations inoculated on apple leaves, not on seedling but on grafted apple trees and tested as comparison between sensible well-controlled populations and suspected of reduced control. All populations present no significant differences. With referring to subpopulations there are inferior activities in some populations both in Emilia-Romagna and Trentino.

I can detect that a reduction of activity is possible, also for fungicides with multi-site action, as dithianon, but now we examine and compare characteristics.

It must be pointed out that the detected reductions of dithianon in activity appear light and highly inferior to the decreases of sensitivity/activity ascertained by me with other fungicides, as QoI's [9] and DMI's [13] in presence of resistance by *V. inaequalis.*

This decline of dithianon is caused by new resistant genotypes, or it is more

adequate to impute this to temporary modifications activity and sensitivity by apple scab (*V. inaequalis*).

In this regard I contacted technics of Emilia-Romagna region and Trentino province, and learned that in the following years these reductions of activity were almost absent, while continuing to use dithianon. This leads to belief that the moderate reductions of sensitivity and activity in surveyed years were determined by temporary modifications in the target of fungicide with biochemical and physiological interactions. They were probably caused by very high infective pressures (meteorological conditions) with difficulties to intervene in time. In addition must be considered the diffused presence of apple cultivars with medium or high susceptibility (initial or acquired) to scab [15]. In very case, an annual number of dithianon treatments not superior to 9 are advised.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Rubenson, J. (1999) Handbook of Pest Management. CRC Press, Boca Raton, 348. https://doi.org/10.1201/9781482273281

[2] Stammler, G. (2014) Sensitivity of *Venturia inaequalis* to Dithianon. *Modern Fungicides and Antifungal Compounds*, **7**, 271-272.

[3] Sturdik, E., Drobnica, L., Sladkova, V. and Datelinka, I. (1984) Fungicide 2,3-Dinitrilo-1,4-dithia-9,10-anthraquinone (Dithianon) Is a Thiol-Combining Agent. *Biologia*, **39**, 1179-1190.

[4] Brown, J. and Ogle, H. (1997) Plant Pathogens and Plant Diseases. Australasian Plant Pathology Society, Toowoomba, 382-383.

[5] Cesari, A., Fiaccadori, R. and Galassi, T. (1980) Studio sull'attività residua svolta da fungicidi polivalenti applicati nella difesa antiticchiolatura del melo. *Atti Giornate Fitopatologiche*, **2**, 385-390.

[6] Schwabe, W.F.S., Jones, A. and Jonker, J. (1984) Greenhouse Evaluation of the Curative and Protective Action of Sterol-Inhibiting Fungicides against Apple Scab. *Phytopathology*, **74**, 249-252. https://doi.org/10.1094/Phyto-74-249

[7] Schwabe, W.F.S. (1980) Curative Activity of Fungicides against Apple Leaf Infection by *Venturia inaequalis*. *Phytophylactica*, **12**, 199-207.

[8] Battistini, G., Finestrelli, A., Brunelli, A. and Fiaccadori, R. (2016) Valutazione dell'attività curativa di vecchi e recenti fungicidi su *Venturia inaequalis*. *Atti Giornate Fitopatologiche*, **2**, 345-352.

[9] EU n.540/2011. Commission Implementing Regulation (UE) n.540/2011 25 May 2011. http://www.eurlex.europa.eu/legalcontent/IT/TXT/PDF/?uri=CELEX:02011R0540-20190530&from=DE

[10] Fiaccadori, R., Cicognani, E., Abbatecola, M., Collina, M. and Brunelli, A. (2005) Sensitivity of *Venturia inaequalis* to Strobilurin Fungicides in Italy. *Communications in Agricultural and Applied Biological Sciences*, **70**, 73-78.

[11] Fiaccadori, R., Collina, M. and Brunelli, A. (2013) Reduced Sensitivity of *Venturia inaequalis* to Strobilurins and Anilinopyrimidines in Italy. 8*th International Conference on Integrated Fruit Production*, Kusadasi, 7-12 October 2012, *IOBC-WPRS Bulletin*, **91**, 345-350.

[12] Fiaccadori, R. (2018) *In Vitro*, *In Vivo* and in Field Sensitivity of *Venturia inaequalis* to Anilinopyrimidine Fungicides with Different Types of Scab Management and Degree of Control. *America Journal Plant Sciences*, **8**, 2056-2068.

[13] Fiaccadori, R. (2017) Researches on Methodologies to Verify Reduced Sensitivities of *Venturia inaequalis* in Field to Difenoconazole and First Indications of a Survey in Italy. *American Journal of Plant Sciences*, **8**, 2056-2068. https://doi.org/10.4236/ajps.2017.89138

[14] Fungicide Resistance Action Committee (2006) Monitoring Methodologies. www.frac.info/docs/default-source/monitoring-methods/approved-methods/ventin-monitoring-method-syngenta2006-v1

[15] Sierotzki, H., Eggenschwiler, D., Boilat, O., McDermot, J. and Gessler, C. (1994) Detection of Variation in Virulence toward Susceptible Apple Cultivars in Natural Populations of *Venturia inaequalis*. *Phytopathology*, **84**, 1005-1009. https://doi.org/10.1094/Phyto-84-1005