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EVALUATION OF DICARBOXIMIDE RESIDUES IN COLD-STORED KIWIFRUITS EXPOSED TO FIELD AND POST-HARVEST TREATMENTS

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The effectiveness of field and post-harvest treatments with dicarboximides (iprodione, procymidone and vinclozolin) against *B. fuckeliana* (*B. cinerea*) rot has been evaluated in cold-stored kiwifruits.

Although effective, field treatments did not allow the achievement of the desired level of control, whereas post-harvest applications permitted to maintain the absolute health of fruits even when these were artificially inoculated.

Dicarboximide residues were estimated after 4 months storage on fruits exposed to different treatments. Observations on the partitioning of fungicides between skin and pulp showed that the level of residues present in the edible part of the fruits was negligible. The maximum residue limits admitted in Italy are referred to unpeeled fruits. The validity of this criterion is briefly discussed.

KEY WORDS Dicarboximide fungicides, kiwifruit, effectiveness, fungicide residues, cold-storage.

INTRODUCTION

Kiwifruit (*Actinidia chinensis* Planchon) production is one of the newest and most rapidly developing agricultural activity in Italy. As with most new crops, the most suitable methods for handling, storage and transport of kiwifruits are largely unknown.

Kiwifruits are harvested unripe and are stored in the cold for periods up to 7 months before being marketed. Various fungi have been found associated with kiwifruit rot during cold-storage, but the most important pathogen is *Botryotinia fuckeliana* (de Bary) Whetz., teleomorph of *Botrytis cinerea* Pers.[1–6]

Although in some areas with a particularly humid climate, *B. fuckeliana* can infect kiwi plants already at the flowering stage,[7] generally the fungus has a very limited importance in the field, until fruits are nearly ripe.[6,8] Nevertheless, the climatic conditions occurring in the field exert a great influence on the severity of damages in stored fruits. Consequently, the incidence of B. *fuckeliana* rot varies

very much from season to season and with the location of the stand where fruits are produced. Although in most cases it is well below 1%, the disease is considered important, in the first because it is capable to damage up to 25% of fruits, and, secondly, because any infection is detrimental to the high-quality image that kiwifruit has on the market.[8]

In Italy, *B. fuckeliana* rot has been reported to occur on stored kiwifruits with a broad range of incidence, up to 50% of infected fruits, by several authors.[2,9–12]

The aims of the investigations reported in the present paper were: (a) to assess the importance of *B. fuckeliana* rot on kiwifruits produced in Southern Italy (Apulia); (b) to compare the effectiveness of field and post-harvest applications of the following dicarboximide fungicides: iprodione (3-(3, 5-dichlorophenyl)-N-isopropyl(2-4-dioxomidazolidine-1-carboxamide), procymidone (N-(3, 5-dichlorophenyl)-1, 2-dimethylcyclopropane-1, 2-dicarboximide), vinclozolin (3-(3, 5-dichlorophenyl)-5-ethenyl-5-methyl-2-4-oxazolidine-dione); (c) to evaluate the amount of dicarboximide residues present on fruits following different treatments.

MATERIALS AND METHODS

Field trials were carried out in a 5-year-old kiwifruit arbour of cv. Hayward located 10 miles far from Bari. Each experimental plot was formed by 34 plants.

The effectiveness of different dicarboximide fungicides was compared by spraying each of 4 plots on 30 September 1988 with one of the following: no fungicides (control); 1350 g Ha^{-1} vinclozolin (50%, Ronilan, Basf-Italia), 1350 g Ha^{-1} iprodione (50%, Rovral 50 WP, Rhône Poulenc), 1150 g Ha^{-1} procymidone (50%, Sumisclex, Shell-Italia). Treatments were made with a normal volume pump delivering 1500 l Ha^{-1} of fungicide suspensions.

Several control schedules involving a different number of treatments per season were tested. Each of 5 plots was sprayed with vinclozolin (50%, Ronilan, Basf-Italia) at rates as high as 750 g Ha^{-1} (a.i.). Treatment schedules were as it follows: plot a, 4 treatments (2 and 18 June, 24 August and 30 September, 1988); plot b, 3 treatments (18 June, 24 August and 30 September); plot c, 2 treatments (24 August and 30 September); plot d, 1 treatment (30 September); plot e, non treated control. Treatments were carried out with a normal volume pump delivering 1000 l Ha^{-1} of the fungicide suspension.

Harvesting was on 2 November 1988, 32 days after the last treatment. At that time, 160 fruits were randomly collected among the fruits harvested from each plot. These were subdivided in 4 groups of 40 fruits which were the experimental replicates in the storage.

Fruits that had never been exposed to field-treatments were harvested from a kiwifruit arbour (cv. Hayward) located 40 miles north of Bari. Part of the samples (70 fruits per each thesis) were dipped for 1 min in a suspension containing 1.0 g l^{-1} (a.i.) iprodione or vinclozolin, or 0.75 g l^{-1} (a.i.) procymidone. A flowable formulation of iprodione (25%, Kidan, Rhône Poulenc) was also used at the same concentration of active ingredient as the wettable powder. Control fruits were dipped in water. Unwounded fruits were allowed to air dry and then sprayed with

a suspension containing 2.5×10^6 *B. fuckeliana* conidia ml^{-1} water additioned of 0.01% Tween 20. A comparable set of fruits was inoculated but not treated, and another set was neither treated nor inoculated. Afterwards, all fruits were kept for 24 h at room temperature and high relative humidity before being boxed.

Fruits of each replicate were placed in a $30 \times 40 \times 18$ cm paraffined box and stored for 4 months in a cold room at 1 ± 1 °C and relative humidity higher than 95%.

Small bags containing potassium permanganate adsorbed on vermiculite were placed in the cold room next to the boxes, to capture the ethylene produced by fruits during ripening.[11]

Observations on the development of *B. fuckeliana* were carried out monthly 4 times during the storage by counting infected fruits. Data were transformed in Bliss angular values and statistically worked out by variance analysis and multiple range Duncan's test.

After each survey, 20 fruits (5 from each replicate) exposed to any given field treatment were individually analysed to evaluate the possible influence of fungicides applications on their organoleptic qualities. The parameters considered were those generally believed to be the best indicators of the physiological state of fruits.[11,13] Softness was assessed with a manual penetrometer having a 8-mm tip plunger, taking two measures on the opposite sides of the equatorial area of each fruit after localised (12–15 mm) peeling. Refractometer index was measured on juice drops from the opposite sides of single fruits with a manual refractometer. Total acidity was estimated by measuring the volume of N/10 NaOH needed to neutralise (phenolphthalein was used as a pH indicator) a 1:10 (w:v) solution containing 10 g of fruit juice in water.

At the end of storage, 20 fruits were randomly collected from each thesis (5 from each replicate) and peeled taking care to avoid any contact between pulp and skin. Pulp and peels from each sample were weighted and separately stored at −25 °C until analysis of fungicide residues.

Samples (200 g) of pulp or peel were blended at low speed. The samples of pulp had been formed pooling together pieces cut from different sides of 5 fruits. The fungicides were extracted on 3 replicate sub-samples (50 g) and partially purified by using a multiresidue method,[14,15] as previously described.[16] The reliability of the extraction procedure was estimated in 3 replicates on samples of both pulp and peel from untreated kiwifruits to which 2, 4, 8 and 16 $mg\,kg^{-1}$ of fungicides were added.

Gas-liquid chromatographic analysis was carried out with a Perkin-Elmer Sigma 115 chromatograph equipped with an electron capture detector, and a 2 m-long glass column (inside diameter 1.75 mm), acid washed, DMCS treated, packed with 1.5% OV 17 + 1.95% OV 210 on Gas-Chrom Q 100–120 mesh. The conditions of instrumental analysis were the following: oven temperature 230 °C (260 °C for iprodione); injector temperature 250 °C (280 °C for iprodione); detector temperature 300 °C; carrier gas nitrogen; column flow 50 ml/min^{-1}. Injection volume was 1 μl. Retention times were 1.02 min for vinclozolin, 1.91 min for procymidone, and 2.46 min for iprodione.

Fungicide concentrations in extracts were estimated by comparing pick areas

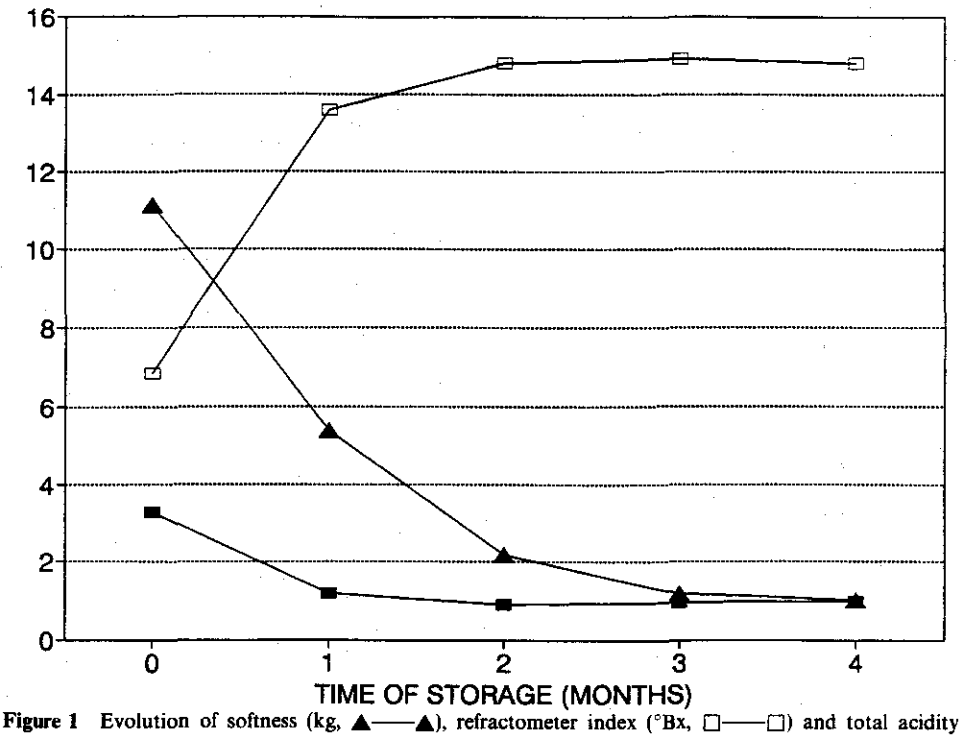

Figure 1 Evolution of softness (kg, ▲——▲), refractometer index (°Bx, □——□) and total acidity (meq NaOH per 10 g of juice, ■——■) during cold-storage of kiwifruits.

with the appropriate calibration curves. These were established by using standard solutions with concentrations ranging from 10^{-3} to $10\,mg\,l^{-1}$ of each fungicide.

RESULTS

The evolution of softness, refractometer index and total acidity of kiwifruits during storage showed that they ripened a little too early, being nearly-consumable already after 2 months (Figure 1).

Physical parameters of kiwifruits were significantly correlated among themselves and with the time of storage. Softness and, to a lesser extent, refractometer index were the parameters better correlated with the time course. The incidence of *B. fuckeliana* rot was related to softness and refractometer index, but not to total acidity (Table 1).

Fruits exposed to field treatments with different dicarboximide fungicides did not show any variation in the evolution of organolectic qualities in comparison to the control (data not shown).

Within 2 months storage, the incidence of *B. fuckeliana* rot was limited and differences among field treatments were not statistically significant. The influence of control schedules became more noticeable and acquired statistical significance after 3 months storage, while the percentage of rotting fruits was increasing in the

Table 1 Correlation matrix among time of storage, organolectic parameters and incidence of *B. fuckeliana* rot.[a]

	Time of storage	*Softness*	*Total acidity*	*Refractometer index*	*Infected fruits (%)*
Time of storage	1.00[b]	—	—	—	—
Softness	−0.92[b]	1.00[b]	—	—	—
Total acidity	−0.30[b]	0.52[b]	1.00[b]	—	—
Refractometer index	0.80[b]	−0.93[b]	−0.48[b]	1.00[b]	—
Infected fruits (%)	0.57[b]	−0.49[b]	−0.05	0.46[b]	1.00[b]

[a]The matrix was calculated on the ground of data referring to 160 fruits individually analysed.
[b]Correlation index with statistical significance at 0.01% level of confidence.

Table 2 Effectiveness of field exposition to several treatment schedules with dicarboximide fungicides against rotting of kiwifruits caused by *Botryotinia fuckeliana* during cold-storage.[a]

Fungicide	*Rate (a.i.)* $g\,Ha^{-1}$	*N. of treatments*[b]	*Time of storage (days)*			
			30	*60*	*90*	*120*
Control	—	—	3.7	5.6	8.7a	13.2a
Vinclozolin	750	1	3.7	3.7	5.6ab	10.8abc
Vinclozolin	750	2	1.9	2.5	3.7b	7.5ab
Vinclozolin	750	3	3.1	3.1	4.3b	10.8ab
Vinclozolin	750	4	1.2	3.1	4.3ab	7.9ab
Vinclozolin	1350	1	1.9	3.1	3.7b	9.1ab
Iprodione	1350	1	0.6	2.5	3.1bc	4.3bc
Procymidone	1150	1	0.6	0.6	0.6c	2.5c

[a]Figures represent average percentages of rotted fruits on 4 replicates. Values followed by different letters on the column differ significantly at $P = 0.05$ level according to the Duncan's multiple range test.
[b]See text for details.

control (Table 2). The number of field treatments with vinclozolin and its application rates did not exert obvious influence on the outcome of the storage. A single application with procymidone, and to lesser extent with iprodione, consistently showed higher efficacy than any control schedule based on vinclozolin (Table 2).

As to post-harvest treatments, non inoculated untreated fruits remained healthy till the end of storage (120 days). Artificial inoculations of fruits with conidia of *B. fuckeliana* proved very effective. Indeed, at the end of storage, as much as 28.6% of untreated inoculated fruits were rotted (Table 3). High levels of control were achieved with post-harvest applications. Vinclozolin showed the lowest activity, yielding as much as 20% of rotted fruits after 4 months storage, a value very similar to the untreated control (Table 4).

Fungicide recoveries at the extraction ranged between 95% and 98%.

Dicarboximide residues present on kiwi fruits after 4 months storage are reported in Table 4. Most of the chemical were confined to fruit peeling. On the average, the ratio between amounts of residues present in pulp and in peel was 7.1:1000. It should be considered that single fruits were made up for over 85% by

Table 3 Effectiveness of post-harvest applications of dicarboximides on kiwifruits artificially inoculated with *Botryotinia fuckeliana*.[a]

Fungicides[b]	*Concentration (a.i.) g/l*$^{-1}$	*Time of storage (days)*			
		30	*60*	*90*	*120*
Control I.F.	—	7.1a	12.8a	20.0a	28.6a
Vinclozolin	0.50	0.0b	0.0b	7.1b	20.0a
Iprodione WP	0.50	0.0b	0.0b	0.0b	0.0b
Iprodione FL	0.50	0.0b	0.0b	0.0b	0.0b
Procymidone	0.38	0.0b	0.0b	0.0b	0.4b
Control N.F.	—	0.0b	0.0b	0.0b	0.0b

[a]Figures represent average percentages of rotted fruits on 4 replicates. Values followed by different letters on the column differ significantly at $P=0.05$ level according to the Duncan's multiple range test.
[b]I.F. = fruits inoculated with *B. fuckeliana*; N.F. = non inoculated fruits: WP = wettable poweder; FL = flowable.

Table 4 Dicarboximide residues present on kiwifruits after 4 months of storage in cold rooms

Fungicide	*Rate*[a] *(a.i.)*	*Type and time of application*[b]	*Fungicide residue (mg kg*$^{-1}$*)* $\pm$ *S.D.*[c]			$\frac{Pulp}{peel} \times 1000$
			Whole fruit	*Pulp*	*Peel*	
Control	—	—	—	—	—	—
Vinclozolin	750	A	0.34 ± 0.01	$8 \cdot 10^{-3} \pm 10^{-3}$	1.6 ± 0.17	5.0
Vinclozolin	750	AB	0.35 ± 0.02	$16 \cdot 10^{-3} \pm 10^{-3}$	2.5 ± 0.12	6.4
Vinclozolin	750	ABC	0.31 ± 0.02	$15 \cdot 10^{-3} \pm 10^{-3}$	1.5 ± 0.12	10.0
Vinclozolin	750	ABCD	0.29 ± 0.03	$49 \cdot 10^{-3} \pm 10^{-3}$	1.4 ± 0.06	35.0
Vinclozolin	1350	D	0.50 ± 0.02	$6 \cdot 10^{-3} \pm 10^{-3}$	3.8 ± 0.17	1.6
Iprodione WP	1350	D	0.90 ± 0.02	$9 \cdot 10^{-3} \pm 10^{-3}$	6.0 ± 0.15	1.5
Procymidone	1150	D	0.46 ± 0.06	$10 \cdot 10^{-3} \pm 10^{-3}$	2.4 ± 0.32	4.2
Vinclozolin	0.50	P	2.6 ± 0.1	0.03 ± 0.06	11.7 ± 0.6	2.6
Iprodione WP	0.50	P	4.7 ± 0.8	0.04 ± 0.06	28.1 ± 4.4	1.4
Iprodione FL	0.50	P	7.9 ± 0.7	0.07 ± 0.06	42.4 ± 3.8	1.7
Procymidone	0.38	P	1.9 ± 0.5	0.10 ± 0.12	11.9 ± 2.9	8.4

[a]Rates are expressed in g Ha^{-1} for field treatments and in g l^{-1} (ml l^{-1} when flowable) for post-harvest applications.
[b]Field-treatments: A = 2 June, B = 18 June, C = 24 August, D = 30 September; P = post-harvest applications.
[c]Figures are average values of three replicate corrected for the proper average recovery.

pulp and for only 15% by peel. Field treatments left dicarboximide residues lower than 1 mg kg^{-1} of whole fruits. The number of field-treatments with vinclozolin did not affect markedly the level of residues present on the whole fruit and on its skin. However, control schedules involving a higher number of sprays left higher amounts of residues in the pulp, either in terms of absolute value (although in the range 10^{-3}–10^{-2} mg kg^{-1}) or in terms of proportion of total residues present on fruits.

When compared to field treatments, post-harvest applications with vinclozolin and iprodione brought about a dramatic increase of residues in the peel at which, however, did not correspond an equivalent increase in the pulp. The highest residue level in pulp (0.1 mg kg^{-1}) was given by post-harvest use of procymidone (Table 4).

The flowable formulation of iprodione left much more residue on fruits than the wettable formulation (Table 3).

DISCUSSION

In the present investigation, the experimental conditions of kiwifruit storage were not optimal because ethylene removal was not very effective and, intentionally, infected fruits were not removed after the periodical surveys. It is known that infected fruits produce ethylene which can trigger the ripening of healthy fruits.[11,13] This resulted in an early ripening of fruits. However, this was judged positively in view of the pursued aims of the trials, since the biological activity of fungicides was tested in the most favourable conditions to *B. fuckeliana* development.

The incidence of rotting was very limited. It is reasonable to ascribe this to the scanty inoculum of the pathogen present on the fruits as a result of the occurrence in the field of climatic conditions unfavourable to *B. fuckeliana*. Indeed, numerous fruits and flower residues were analysed during field trials for estimating the amount of *B. fuckeliana* inoculum through isolation on artificial media (water agar and potato dextrose agar) and incubation in moist chamber. The pathogen was found only sporadically even on untreated fruits (data not shown). In Northern Italy, a scanty presence of the pathogen in the field has been reported,[11,12] but in other occasions 20–30% of young fruits and flower residues were contamined by *B. fuckeliana* at the end of blossom.[2] This discrepancy can be considered as a clear evidence of the influence of environmental conditions on the development of the pathogen in the field.

In Northern Italy, two field-treatments, one soon after flowering, and one immediately before harvest, are recommended to prevent *B. fuckeliana* rot on stored fruits.[9,17]

In our conditions, the incidence of the disease was not influenced very much by treatments carried out long time before harvesting. It is reasonable to assume that these treatments act mainly by lowering the inoculum of the pathogen present in the field. If so, they have a limited importance in areas with a semi-arid climate, such as Southern Italy, where the level of *B. fuckeliana* inoculum is naturally very low. Likewise, the high effectiveness of late field-treatments can be related to a direct action of the fungicide still present on fruits after harvesting. It is known that fungicide residues decay very slowly on kiwifruit.[8,18,19] In the present investigation, the amounts of fungicides found on the skin of fruits after 4 months storage were still high enough to expect the establishment of infections be prevented.

Although effective, field treatments did not yield the desired level of control and seemed much less recommendable of post-harvest treatments. This observation is in agreement with the conclusions drawn by others and may be explained admitting that infection of fruits by *B. fuckeliana* occurs at times other than flowering, for instance during harvesting operations.[8,9,20]

How *B. fuckeliana* penetrates fruits is not yet clear. Wounding the skin was

essential to the establishment of infections during storage, which led to the conclusion that, even when a heavy inoculum is present, *B. fuckeliana* cannot enter unwounded fruits.[2] However, in other instances, it was found that stored fruits can be infected by the mycelium from neighbouring infected fruits.[6] In our experience, conidia suspended in water without any additive nutrients were able to infect unwounded fruits and, during storage, the passage of the fungus from infected to healthy fruits was observed with a certain frequency.

Artificial inoculation with *B. fuckeliana* was used in this study to simulate handling of fruits that carried a large inoculum and/or a high number of latent infections. In this case, fruit dipping with dicarboximides proved to be essential for a successful storage.

Dicarboximides are generally considered non-systemic fungicides, although a little systemic activity has been proved for procymidone in bean, cucumber and strawberry, and for iprodione in potato.[21–23] Our observations on the partitioning of fungicides between skin and pulp of kiwifruits showed these were confined on the skin. Similar conclusions were reached for phosmet and for captan, dichlofluanide, iprodione and vinclozolin.[8,19]

In Italy, post-harvest treatments of kiwifruit are not allowed by sanitary laws. Residues of iprodione and vinclozolin in unpeeled kiwifruits must not exceed $3\,mg\,kg^{-1}$. No maximum residue limits have been fixed for procymidone on kiwifruits; $1.5\,mg\,kg^{-1}$ is the maximum allowed on other crops (grapes, peach, tomato etc.). In the present trials, field treatments with iprodione, procymidone and vinclozolin left residues on unpeeled fruits which never reached $1\,mg\,kg^{-1}$. But, it must be considered that the time elapsed between the last treatment and the harvesting was 32 days, whereas this interval according to Italian legislation can be shorter, that is 15 days for iprodione and 10 days for vinclozolin.

With the exception of vinclozolin, post-harvest treatments with dicarboximides left in unpeeled fruits residues higher than the above cited limits. However, these were always lower than $0.1\,mg\,kg^{-1}$ on the edible part of the fruits. These data tallied with those previously reported.[8–11]

The observed partitioning of dicarboximide fungicides between pulp and skin of kiwifruits casts doubts on the feasibility of evaluating residues in unpeeled fruits. This may be right for fruits whose skin can occasionally be eaten or used as feed for animals, which is not the case of kiwifruits.

The high effectiveness of post-harvest treatments against *B. fuckeliana* rot of stored kiwifruit, the limited amount of fungicide residues in the edible part of fruits, and the advantages in environmental terms of a localised application of fungicides, seem to be a reasonable basis to prefer post-harvest to field treatments for controlling the pathogen.

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