

Short communication

Effects of fungicide and insecticide mixtures on apple tree canopy photosynthesis, dark respiration and carbon economy[☆]

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Abstract

Fungicide/insecticide mixtures were applied at times and doses commonly used in commercial orchard practice. Their effects on photosynthesis and dark respiration were evaluated in two seasons with respect to the potential stress they impose on an apple tree using cv. ‘Elstar’. The mixtures included the fungicides mancozeb, flusilazol and dithianon, and the insecticides oxydemeton-methyl or pirimicarb.

A new technology was employed to continuously examine photosynthesis, dark respiration and carbon balance of apple trees based on six canopy chambers, which enclosed apple trees under natural conditions in the field, with on-line measurements and continuous analysis of CO₂ exchange and automated data acquisition.

The fungicides mancozeb and flusilazol combined with the insecticide oxydemeton-methyl reduced whole tree canopy CO₂ assimilation mostly at midday and, using hourly means, by an averaged 7.4% on the day of its application. This reduction in whole canopy photosynthesis declined with time, restoring most of the original photosynthetic potential within 3–5% in 3 days, hence, indicating acceptable phytotoxicity. This fungicide/insecticide mixture overproportionally, in relation to the changes in photosynthesis, *increased* dark respiration by up to 72% in the night after application, thereby drastically affecting the tree’s carbon balance in an adverse way.

In contrast, the fungicide dithianon combined with the insecticide pirimicarb *decreased* dark respiration by 15–21% with reductions in canopy photosynthesis in the order of 6–9%. Because the decrease in dark respiration exceeded that in photosynthesis, the apple tree overall gained carbon in a balance.

Overall, effects on photosynthesis were smaller than on dark respiration. The effects of the pesticide combinations on photosynthesis are attributed to the CO₂-independent Hill reaction in photosynthesis and to uncoupling the photosynthetic electron flow from phosphorylation, thereby inhibiting energy, viz. ATP formation or its transfer, rendering dissociation of ATP into ADP and P_i.

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1. Introduction

Interdisciplinary studies reporting plant responses to pesticides traditionally rely upon photosynthesis measurements taken either on individual leaf disks or sections. Reliance on individual leaf measurements is mostly attributed to technological constraints, because the standard equipment for gas exchange measurements is not large enough to allow measurements to be taken on an entire leaf, plant or tree. A literature review by

Evans (1993) concluded that investigations relying on individual leaf measurements do not accurately represent whole plant responses. Poor correlations (Evans, 1993) can be attributed to (1) measurements taken from a section of a leaf, which is not representative of an entire plant or tree, (2) selection of a leaf that does not reflect the gas exchange of the canopy and (3) inability to account for diurnal changes in photosynthesis and dark respiration (Klingemann et al., 2000). To exclude the danger of leaf section responses with excessively large values, whole tree canopy chambers have been built for field-sized fruit trees since 1994, when continuous measurements with on-line data recording started. This enabled combined diurnal photosynthesis and dark respiration measurements, the latter a valuable index for plant stress (Groschowska and Lubinska,

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1973; Wibbe and Blanke, 1996, 1997; Untiedt and Blanke, 2001).

The use of fungicides and insecticides in integrated fruit production (IFP) is restricted in terms of choice of compounds, concentration, frequency and time of application. The fungicides mancozeb, flusilazol and dithianon, as well as the insecticides oxydemeton-methyl and pirimicarb, are registered for use in integrated fruit production (IFP). It is common practice to mix these fungicides and insecticides for field applications, which may impose abiotic stress on the fruit tree in addition to existing biotic stress (Klingemann et al., 2000; Untiedt and Blanke, 2001). Two cases of phytotoxicity were reported, where mancozeb and dithianon, two contact scab fungicides, induced leaf spots/lesions and leaf drop in cv. 'Golden Delicious' apple (Bremer and Bünemann, 1982). Information on pesticide effects on plant physiology, viz., photosynthesis and dark respiration of whole trees, aids understanding the underlying regulatory mechanisms as a precondition to judge the phytotoxicity of a compound. To our knowledge, neither whole tree/plant canopy measurements nor dark respiration data exist on these commonly used compounds employed in this study. Hence, the aim of this study was to investigate the effects of these three fungicides and two insecticides, all approved in IFP, at times and doses commercially used, on photosynthesis, dark respiration and carbon balance to describe the stress they impose. Apple cv. 'Elstar' was used in this new approach of whole tree canopy gas exchange measurements.

2. Materials and methods

2.1. Trees

Three-year-old apple (*Malus domestica* Borkh.) cv. 'Elstar' trees on M9 rootstock were trained to slender spindles in the orchard of Institut für Obstbau und Gemüsebau in Bonn. They were cultivated in lysimeters in coarse sand of 1.2 mm diameter in 801 pots and supplied with complete Long Ashton nutrient solution (Blanke, 1997). For the present experiment, 21 apple trees were selected for uniformity in 1994 and 1995, of which six served as control trees.

2.2. Tree canopy chamber

Six tree canopy cuvettes of 0.1 mm transparent polyethylene film sacks each housed a single apple tree in their centre (Wibbe and Blanke, 1996). Film sacks were 2.50 m high and conical in shape with a diameter of 1.50 m, 1.75 m at the top or base, respectively. Each canopy cuvette contained a volume of 7 m³ with the film sacks of 1.6 × 1.8 × 2.5 m³ suspended from a stainless-steel frame.

The film sacks were sealed gas tight against the base board using water barriers (Wibbe and Blanke, 1997).

2.3. Air flow and leaf area

Outside air was sampled from a 0.6 m wide chimney 6 m above ground to serve as CO₂ reference air and pumped through the six canopy chambers. The flow rate of the outside air pumped into the chamber at its base could be adjusted to give large differential readings for the infrared gas analyser (IRGA) irrespective of weather conditions. The air left each canopy cuvette through a 1.5 m long pipe of 15 cm diameter at the top where air was sampled for CO₂ analysis. Flow rates were measured exactly in the range of 0.2–20 m s⁻¹, equivalent to a volume flow of 4.8–475 m³ h⁻¹ with an anemometer (Höntzsch Co, Waiblingen, Germany). Volume flows of 6–100 m³ h⁻¹ were employed, catering for the different environments throughout the year and their effects on the magnitude of CO₂ exchange to achieve cuvette air volume turnovers of 1–17 times h⁻¹. A fan circulated the air within the tree canopy chamber to avoid formation of boundary layers. The CO₂ reference and analysis air from the six chambers were passed to a differential CO₂ infrared gas analyser via a sequential sampling unit type WA 161 (ADC) and cold trap. The six channels were identified by the binary code of the WA 161. Net photosynthesis and dark respiration were calculated based on differential CO₂, volume flow and leaf area using the formulae cited in von Willert et al. (1995). Leaf areas were measured in situ and non-destructively at weekly intervals using a portable leaf area meter type CI 201 (CID, Moscow, USA).

2.4. Spray application

Sprays 1 and 2, consisting of the fungicides and insecticides listed in Table 1, were applied until run-off using a manual knapsack sprayer at 09:00. Apple trees were sprayed five times with each spray during 1994 and 1995 at concentrations commonly used in horticultural practices for pest control as listed in Table 1. The six control trees were sprayed at the same time with water containing 2 ppm 'Citowett' as surfactant to monitor possible adjuvant effects in the commercial formulations used.

3. Results

3.1. Effects of mancozeb, flusilazol and oxydemeton-methyl

The fungicides mancozeb (Dithane Ultra) and flusilazol (Benocap) combined with the insecticide oxydemeton-methyl (Metasystox) were applied five times in

Table 1
Doses and times of application of employed fungicides and insecticides

Common or trade name	Active ingredient (mode of action)	Purposes in fruit production	Concentrations employed (per l)	Date of application
Spray 1				1994:
Dithane Ultra	Mancozeb (contact)	Scab control	5 g/l	26/6, 30/8
Benocap	Flusilazol (systemic)	Scab control	3.33 ml/l	1995:
Metasystox	Oxydemeton-methyl (systemic fungicide)	Insect control	4 g/l	18/7, 16/8, 9/9
Spray 2				7/6/94
Delan	Dithianon (contact)	Scab control	1.6 ml/l	1995
Pirimor	Pirimicarb (systemic)	Aphid control	1 g/l	7/6, 30/6, 4/7
Control	Water	n/a	2 ppm 'Citowett'	Dates as above

Table 2

Percentage changes in apple tree canopy CO₂ assimilation during the daytime and CO₂ efflux (dark respiration) during the night after application of the combined fungicides mancozeb (Dithane; 5 g/l) and flusilazol (Benocap; 4 g/l) with the insecticide oxydemeton-methyl (Metasystox; (3.3 ml/l). Control trees were sprayed with water (+ 2 ppm Citowett as surfactant)

Date		Photosynthesis (CO ₂ assimilation)					Dark respiration (CO ₂ efflux)				
		1	2	3	4	5	1	2	3	4	5
Day 1	Day	-1.4	-3.3	-0.2	-7.4	+2.3					
	Night						+25.6	+38.1	+72.2	+7.4	+58.9
Day 2	Day	-2.5	-0.1	+1.1	-2.2	-6.1					
	Night								+65.0		
Day 3	Day			+3.2							
	Night								+56.5		

(1) 26/6/94; (2) 30/8/94; (3) 18/7/95; (4) 16/8/95; (5) 9/9/95.

Tree canopy photosynthesis or dark respiration was measured simultaneously of treated and control trees. Negative values designate reduced photosynthesis, while positive values indicate increased dark respiration—data from two seasons.

1994 and 1995 “on new apple trees” in commonly used doses, while the control trees received water plus 2 ppm ‘Citowett’ as a surfactant to mimic all-purpose adjuvants in commercial pesticide formulations. Diurnal courses of whole apple tree canopy CO₂ exchange, i.e. photosynthesis and dark respiration, were recorded simultaneously for treated and control trees. Photosynthesis and dark respiration of the same apple tree on the day prior to the application were designated as another control in addition to the water (+ surfactant) treatment. Percentage calculations in Tables 2–4 are based on hourly means which served as 100% control to allow for environmental changes throughout the measured period.

The control trees, treated with water and surfactant, showed comparable canopy photosynthesis during the previous day relative to trees selected for subsequent treatments (data not shown). The fungicide and insecticide mixture reduced apple tree canopy

Table 3

Percentage changes in apple tree canopy CO₂ assimilation during the daytime and CO₂ efflux (dark respiration) during the night after application combining the fungicides dithianon (Delan; 1.6 ml/l) and the insecticide Pirimicarb (Pirimor; 1 g/l) on 7 June 1995. Control trees were sprayed with water (+ 2 ppm Citowett as surfactant)

Date	Period	Photosynthesis (CO ₂ assimilation)	Dark respiration (CO ₂ efflux)
7 June	Day	-6.63	
	Night		-21.2
8 June	Day	-6.24	
	Night		-20.7
9 June	Day	-2.04	
	Night		-18.3
10 June	Day	-5.51	

Tree canopy photosynthesis or dark respiration was measured simultaneously of treated and control trees. Negative values designate reduced photosynthesis or reduced dark respiration.

photosynthesis immediately after spray application at 09:00 (Fig. 1). The typical steep rise in photosynthesis in the morning and the midday peak were both reduced and lasted all day (Fig. 1).

To show the effects over time, data were calculated based on hourly means (Table 2). During the next three consecutive days, spray 1 altered apple tree canopy photosynthesis by +2.3% to -7.4% relative to the control trees treated with water and surfactant (Table 2).

The relatively small average daytime changes, resulting from averaging hourly means, in tree canopy photosynthesis with a maximum daytime of -7.4% were associated with more drastic increases in dark respiration. These great increases in dark respiration due to the fungicide/insecticide mixture of up to +72% of the apple trees in the subsequent night after the morning (09:00) spray application declined over three nights from

72% to 57% (Table 2), reflecting the decline in metabolic changes associated with the detoxification and metabolism of the pesticides.

3.2. Effects of dithianon (Delan) plus pirimor (Pirimicarb)

The fungicide dithianon (Delan) combined with the insecticide pirimor (Pirimicarb) were applied four times during 1994 and 1995. Like spray 1, they reduced whole apple tree photosynthesis after at least one day with the reduction being either small (Table 3) or retarded up to one day (Table 4). However, this mixture did not increase dark respiration like spray 1, but unexpectedly decreased dark respiration after treatment on 7 June by up to 21% (Table 3) and on 4 August 1995 by up to 15% (Table 4). Both decreases in dark respiration were consistent, but differed in their slope/response time. The instant reduction in dark respiration (Table 3) declined consistently with time from 21% to 18% over 3 days in the June treatment. The reverse applied to the August application where dark respiration still decreased with time over 3 days (Table 4). While the reductions in canopy photosynthesis were smaller for spray 2 than with spray 1, the reduced dark respiration was in contrast to spray 1, which enhanced dark respiration (Table 2).

Table 4

Percentage changes in apple tree canopy CO₂ assimilation during the daytime and CO₂ efflux (dark respiration) during the night after application combining the fungicides Delan (1.6 ml/l) and the insecticide Pirimor (1 g/l) on 2 August 1995. Control trees were sprayed with water (+2 ppm Citowett as surfactant)

Date	Period	Photosynthesis (CO ₂ assimilation)	Dark respiration (CO ₂ efflux)
2 August	Day	-4.1	
	Night		-0.9
3 August	Day	-9.3	
	Night		-7.6
4 August	Day	-8.5	
	Night		-15.5
5 August	Day	-0.5	

Tree canopy photosynthesis or dark respiration was measured simultaneously of treated and control trees. Negative values designate reduced photosynthesis or reduced respiration.

4. Discussion

4.1. Effects of combined fungicides and insecticides on apple canopy photosynthesis

To our knowledge, these appear to be the first data available on the effects of pesticides on CO₂ assimilation in fruit trees using tree canopy chambers and field conditions.

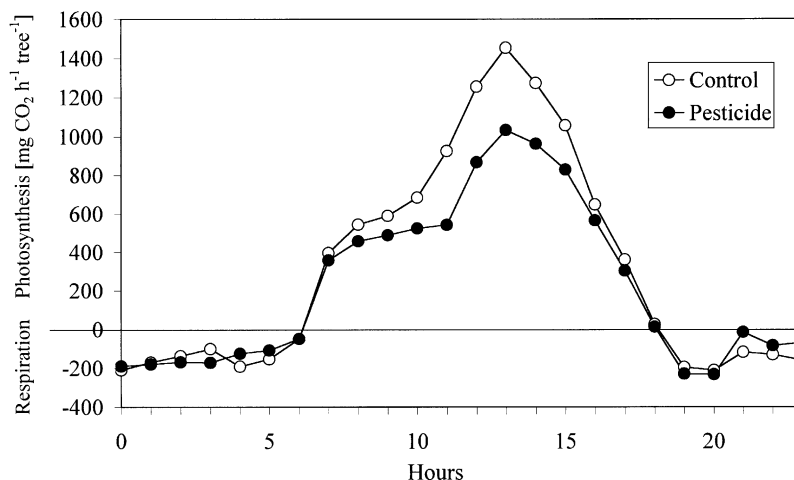


Fig. 1. Diurnal course of CO₂ exchange (photosynthesis during the day, dark respiration during the night) of the whole apple tree canopy before and after applying the fungicide/insecticide mixture (Table 1).

The present results of reductions in the diurnal course were particularly pronounced in the morning and at midday (Fig. 1) of whole apple tree canopy photosynthesis on the day of application. This diurnal course of CO₂ exchange was not investigated before. Percentage reductions were ca. 10% (Tables 2–4) when expressed on a hourly average confirm previous findings of reductions of 10% by guthion, and lower than the 20–30% by benomyl in apple leaf disks (Kristevea and Kristev, 1971) and 30% by diazinon obtained also with single apple leaves (Heinicke and Foott, 1966) and reductions in single leaf photosynthesis in strawberry by methomyl, carbaryl and permethrin which declined from 9% to 7.5% from day 1 to 13 (Trumble et al., 1988).

The observed reductions in apple canopy photosynthesis of less than 10% (Tables 2–4) confirm results of less than a 7% reduction in photosynthesis obtained also with cv. 'Elstar' in the same whole tree chambers after the application of 15 ppm 1-naphthyl acetic acid, used for fruit thinning, where photosynthesis was restored to its original level (within 0.6%) 24 h after application (Untiedt and Blanke, 2001).

4.2. Delayed effects on canopy photosynthesis

The phenomenon of a 24 h retarded trough in canopy photosynthesis after application of spray 2 (Table 4) was shorter than that found with the fungicide, methomyl, or the insecticide, methyl parathion. These compounds were reported to decrease photosynthesis of lettuce by 8% or 10%, respectively, after one day and further by 20% or 18% after 7 days (Murthy, 1983).

4.3. Effect of multiple pesticide applications

To our knowledge, combined sprays, as common in orchard practice, have not been investigated with respect to their effects on photosynthesis. However, multiple applications of up to five times of the same compound have been used previously. The results obtained with combinations of either of the two insecticides, oxydemeton-methyl (Fig. 1, Table 2) or pirimicarb (and diazinon) (Tables 3–5), confirm those of Ferree and Hall (1978), who found no effect of single applications of the insecticides diazinon, methomyl or oxamyl sprays on photosynthesis of greenhouse-grown potted apple cv. 'Golden Delicious' trees. This also confirms the previous classification of diazinon as a compound without significant effect on photosynthesis or dark respiration (Ayers and Barden, 1975). Multiple sprays of the same compound at the recommended rate were necessary to decrease photosynthesis by 20–30% (3 sprays), using leaf disks in a Warburg apparatus over 60 min (Kristevea and Kristev, 1971), or by 10–47% (5 sprays), using measurements of an exposed peripheral single leaf in a phytotron (Ferree and Hall, 1978). Similarly, a

single application of nine insecticides and four fungicides reduced photosynthesis of pecan, reductions which increased with multiple applications from 10% to 30% (Wood and Payne, 1984).

4.4. Effects of combined fungicides and insecticides on dark respiration of the apple canopy

Previous studies concentrated on spot measurements of single leaves in which estimates of dark respiration are difficult. To our knowledge, only one investigation (Ayers and Barden, 1975) examined dark respiration. The present work has shown that the major and, possibly more interesting, effects of the pesticides were on dark respiration. Two opposing effects were observed, an increase (Table 2) and a decrease in dark respiration (Tables 3 and 4). The first, the increase in dark respiration due to spray I (Table 2) resembles the results of Ayers and Barden (1975), who found an increase in dark respiration after insecticide application in apple initially by 23% which declined to 5% after day 11, and by Untiedt and Blanke (2001) who examined the effect of 100 ppm NAA + Ethrel, used for fruit thinning which dramatically increased dark respiration by 106%. The second effect, the decrease in dark respiration, has not been observed before with insecticides, but resembles the effects of the plant growth regulator 1-naphthyl acetic acid (Amid-thin and Rhodofix) which decreased dark respiration by 46% in the first week after application (Untiedt and Blanke, 2001). The two opposing effects in terms of dark respiration may indicate a valuable and more sensitive stress indicator than photosynthesis.

4.5. Mode of action

The observed reduction in apple canopy photosynthesis by some pesticides was attributed to the Hill reaction (Kristevea and Kristev, 1971), the CO₂-independent reduction or water splitting process of photosynthesis in the chloroplast, and uncoupling the photosynthetic electron flow from phosphorylation, thereby inhibiting ATP formation (Murthy, 1983) or to inhibition of energy (ATP) transfer, rendering dissociation of ATP into ADP and Pi (Younis and Mohancy, 1980), the latter using herbicides. The increase in dark respiration may be associated with ethylene release as a stress reaction which enhances respiration (Solomos and Laties, 1976).

5. Conclusion

The fungicide/insecticide mixtures used in the present study decreased whole tree canopy photosynthesis, particularly at midday. Overall averaged reductions of

less than 10% (Tables 2–4) declined to 3–5% within 4 days indicating their acceptable phytotoxicity for apple. However, the largest, opposing and most interesting effects were observed with dark respiration. This increased up to 72% on the day after application of spray I (mancozeb, flusilazol and oxydemeton-methyl), but decreased up to 21% by spray 2 (dithianon [Delan] plus pirimor [Pirimicarb]). The increase in dark respiration can be explained by (a) additional energy requirement, (b) metabolic breakdown of the compound and (c) activation of the alternative, cyanide-insensitive, respiration.

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