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Disruption of mating of the persimmon fruit moth, *Stathmopoda masinissa*, by a synthetic sex pheromone

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ABSTRACT

Synthetic pheromone components from the persimmon moth *S. masinissa* were found to effectively attract male moths in a persimmon orchard. Polyethylene dispensers containing one or more pheromone components disorientated males and inhibited mating of tethered virgin females in the field which resulted in reduced damage to the fruits by *S. masinissa* larvae.

INTRODUCTION

The moth, *Stathmopoda masinissa* is a major pest of persimmon fruit. The species is distributed throughout Honshu, Shikoku and Kyushu Islands in Japan and has two generations a year in the central Honshu Island (Naka *et al.*, 2002). The adults appear in late spring and summer and the 5th instar larvae over winter. Eggs are laid on succulent sprout shoots and hatch about one week later. The early instar larvae feed inside the succulent sprout shoots of the persimmon tree. The third to last (fifth) instar larvae bore into the core of the persimmon fruit to feed from the calyx of the fruits and subsequently the damaged fruits rot and drop to the ground. Protection from damage caused by this species has been mainly achieved by spraying chemical pesticides and the most effective application is carried out in a very limited period when the larvae move from the unfolding buds to the fruits to feed on the core. Monitoring the population density of this species is laborious because they are not easily attracted to light traps and it is necessary to count the moths on the persimmon leaves in the day time.

Using a gas chromatographic-electroantennographic detection (GC-EAD) technique (Strubble and Arn, 1984), three distinct electroantennogram (EAG)-active materials (components I-III) were found in a crude extract of the pheromone glands of *S. masinissa* females. (Naka *et al.*, 2003). Their mass spectra, chromatographic behaviors, and chemical properties indicated that components I, II, and III were hexadecadienal, hexadecadienyl acetate, and hexadecadien-1-ol with a conjugated diene system, respectively. Further analyses and comparison with synthesized geometrical isomers of each 4,6-hexadecadienyl compound (alcohol, acetate, and aldehyde; Nishida *et al.*, 2003) led to the conclusion that the *S. masinissa* pheromone was composed of three components; E4,Z6-16:Ald (component I), E4,Z6-16:OAc (component II), and E4,Z6-16:OH (component III) (Naka *et al.*, 2003). This paper reports the use of synthetic pheromone components of *S. masinissa* as a monitoring tool and their development as a disruptant of mating communication for control of this pest on persimmon fruit.

MATERIALS AND METHODS

Testing chemical components of the *S. masinissa* pheromone in traps

Lures and traps for capturing *S. masinissa* males were prepared by applying 100 µl of each of the three synthesized components of the sex pheromone in hexane either alone or in combination to individual rubber septa (8 mm diameter, white rubber) and tested using sticky traps (30 x 27 cm bottom plate with a roof, Takeda Chemical Ind., Ltd., Osaka, Japan) separated by at least 10 m at 1.5 m above ground. For a virgin female trap, a round screen cage (5 cm diameter) with two 1 to 2 d old virgin females was placed at the center. In the cage, a few drops of 5% sugar water were placed as a food source. The traps were inspected each day after setting.

Disruption of mating using a polyethylene dispenser with pheromone components

In 2003, (4*E*,6*Z*)-4,6-hexadecadienyl acetate (E4,Z6-16:OAc) was formulated using stabilizers (Sumisorb 300 and Sumilizer BHT, Sumitomo Chemical Co., Ltd., Osaka, Japan, 3%) and dispensed into polyethylene tubes (*ca.* 50 mg/tube) placed in a persimmon orchard from May to September. Three experimental sites were set up in persimmon orchards of the Gifu Prefecture; a low-density field (A, 0.1 ha with 30 tubes, 300 tubes/ha), a high-density field (B, 0.1 ha with 180 tubes, 1800 tubes/ha), and a non-treated field (C, 0.3 ha). To test whether *S. masinissa* males could be disorientated from the females by the polyethylene tubes, traps baited with the synthetic pheromone (10:1 mixture of E4,Z6-16:OAc and E4,Z6-16:Ald) or two virgin females were set up in these areas and the number of males captured in the traps recorded from July 15 to August 19. To test whether mating could be disrupted, tethered *S. masinissa* females were placed in these test areas on the evenings of July 26, July 30, August 6, and August 13. The next morning, they were dissected to examine the presence of a spermatophore indicating that mating had occurred, and the percentage that mated with males was recorded. Finally, the percentage of persimmon fruits infected with *S. masinissa* larvae was also assessed in each field area on September 4. In 2004 and 2005, a mixture of E4,Z6-16:OAc and E4,Z6-16:Ald (20:1) was also formulated (*ca.* 80 mg/tube) and evaluated in the same way as in 2003.

RESULTS AND DISCUSSION

Testing chemical components of the *S. masinissa* pheromone in traps

Single-component lures baited with E4,Z6-16:Ald or E4,Z6-16:OH attracted no male moths but the rubber septum with only E4,Z6-16:OAc (0.6 mg) effectively captured *S. masinis* males, indicating that this acetate was essential for attraction (Table 1). The activity of E4,Z6-16:OAc, which was greater than that of its geometrical isomers, was enhanced by mixing with E4,Z6-16:Ald. A 10:1 mixture was most effective at attracting males to the lures. The effect of E4,Z6-16:OH was unclear, and higher contents of this and E4,Z6-16:Ald decreased the number of captured males. The optimum dose for pheromone components applied to the rubber septum was 0.66 mg (0.6 mg of E4,Z6-16:OAc and 0.06 mg of E4,Z6-16:Ald), although lures baited with concentrations 100 times less could still attract the males.

Table 1. Field attraction of *S. masinissa* males by synthetic pheromone component lures and virgin females in 2003 (from May 9 to June 10)

Lure (mg/septum) E4,Z6-16:OAc	E4,Z6-16:Ald	E4,Z6-16:OH	Total males captured by two traps
0.6	0	0	3
0.6	0.06	0	83
0.6	0.3	0	34
0.6	0.06	0.06	73
0.6	0.3	0.3	11
0.1	0.01	0.01	69
0.06	0.006	0.006	40
0	0	0	0
Virgin females			92

Disruption of mating using a polyethylene dispenser with pheromone components

Field tests in 2003 showed that the main pheromone component E4,Z6-16:OAc was successful at disorientating *S. masinissa* males from the females. The number of captured males in the virgin female traps or the pheromone traps in the fields permeated with the pheromone (A and B) was much lower than that of the no pheromone control (C) (Table 2). A similar result was obtained in the experiment with tethered females (Table 3). 30-67 % of the females placed in the no pheromone control area (C) mated until the next morning, but females in the high-density pheromone treated field (B) never mated. Mating only occurred on one occasion in the low-density field. There was a reduction in the number of fruits infected with *S. masinissa* larvae in the pheromone treated areas compared to the control field. While 28-34% fruits were damaged in the control (331 from 1,052 fruits examined), infestation was reduced to 17-18% in field A (325 from 1,899 fruits examined) and 12-15% in field B (268 from 1,958 fruits examined). Field tests in 2004 and 2005 with a 20:1 mixture of E4,Z6-16:OAc and E4,Z6-16:Ald in the polyethylene dispensers gave even an better reduction in fruit damage. Typical pest damage levels were 50% in the control (no pheromone), 21 % in a field permeated with E4,Z6-16:OAc, and 6 % in a field permeated with the 20:1 mixture.

Table 2. Attraction of *S. masinissa* males by monitoring traps in fields permeated with synthetic E4,Z6-16:OAc

	Total males captured from July 15 to August 19, 2003		
	Test field		
Lure	A; 300 tubes/ha	B; 1800 tubes/ha	C; 0 tubes/ha
Synthetic pheromone traps	0	0	48
Virgin females	1	1	109

Table 3. Percentage of tethered *S. masinissa* females mating in fields permeated with synthetic E4,Z6-16:OAc

	Mating percentage		
	Test field		
Date, in 2003	A; 300 tubes/ha	B; 1800 tubes/ha	C; 0 tubes/ha
July 26	0 %	0 %	67 %
July 30	31 %	0 %	67 %
August 6	0 %	0 %	50 %
August 13	0 %	0 %	30 %

The use of a pheromone as a disruption method is highly selective and also ensures the survival of natural enemies (Cardé and Minks, 1995). The sex pheromone, which shows no toxicity to mammals, is therefore an ideal insect-behavior regulator (IBR). Since the registrations of "Nomate PBW," consisting of Z7,Z11-16:OAc and Z7,E11-16:OAc, for the control of the pink bollworm moth, *Pectinophora gossypiella,* in the USA (1976) and "Hamaki-con," containing Z11-14:OAc, for the simultaneous control of some leafroller species in Japan (1983), several synthetic pheromones have now been utilized as mating disruptants for more than 20 lepidopteran species (Ando *et al.*, 2004). The results presented here indicate that synthetic pheromone components are potentially useful as a control measure for the persimmon fruit moth and provide an alternative to spraying with conventional insecticides.

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A particle film crop protectant provides a bio-rational approach for control of pear psylla and other important insect pests

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ABSTRACT

A crop protectant based on particle film technology has been co-developed by the USDA and Engelhard Corporation, USA and is already used for protection against economically important physiological and environmental stresses; particularly the damaging manifestations of heat stress and sunburn. This formulated kaolin particle film (FKPF) containing 95% active kaolin, has been commercialised in wettable powder form in the USA and several other countries and is used against a number of insects. Field trials conducted in Europe over the last four years have shown that FKPF resulted in equal or superior performance, compared with pyrethroid or organo-phosphate (OP) standard insecticides, against pear psylla, vine leafhoppers and the olive fruit fly. Application dose rates ranged from 25 to 50 kg/ha and spray frequencies ranged from one to four per season. In all trials, no crop phytotoxicity was observed. In vines, no adverse effects were found from FKPF applications on fermentation processes, wine properties or taste. Ongoing trials in Europe and elsewhere are now providing encouraging results against other key insect pests. This novel technology therefore offers a reduced risk alternative for insect control and resistance management in addition to its current uses on crops for environmental stress protection.

INTRODUCTION

A formulated kaolin particle film (FKPF) product commercialised by Engelhard Corporation as 'Surround' WP crop protectant was developed by the United States Department of Agriculture's - Agricultural Research Service (USDA-ARS) and Engelhard Corporation as part of a programme designed to find reduced risk alternatives to chemical pesticides. This current particle film technology is based on a specially engineered form of the mineral kaolin. Although widely used for many years as an inert carrier of pesticides, recent advances in kaolin processing and formulating have created new opportunities for this technology in agriculture. Kaolin used for these applications typically has a particle size <2 μm and is formulated to spread and create a uniform porous film on the plant surface that is removable after harvest. The film does not interfere with photosynthesis, yet blocks a significant amount of harmful ultraviolet and infrared radiation and alters insect pest behaviour (Glenn and Puterka, 2005).

The main mode of action against insects is considered to be repellency, although other mechanisms can also be important, such as reduced survival when eggs hatch on a particle film treated surface, reduced mating success, impeded movement, host camouflage, and impeding an insect's ability to hold on to plant surfaces. Particle films may also alter the taste or smell of the host plant. Unlike most chemical insecticides, kaolin has no specific site(s) of action and therefore resistance is unlikely to develop.

Over the last few years, field trials testing formulated kaolin particle films have been conducted around the world to assess performance against a wide range of insect pests and to quantify the physiological benefits to crops from the reduction of heat stress and sunburn. In Europe, work to register a FKPF for use in the management of a number of economically important insect species is well advanced. The main target insect species include *Cacopsylla pyri, Empoasca vitis, Cydia pomonella, Bactrocera oleae, Prays oleae, Ceratitis capitata, Thrips spp*, and various Curculionidae.

Kaolin is a naturally occurring inert mineral, and is an integral part of most soils and aquatic habitats. Kaolin is approved for use as a food additive (by EU, US & CODEX) and as an ingredient in pharmaceuticals, cosmetics, paper, paint and thousands of other everyday goods. Residues on crops are indistinguishable from other natural clay deposits, and if present at harvest, can be removed by using appropriate washing methods or left on the crop if the crop's skin or husk is to be removed. The commercial formulated kaolin particle film product, containing 95% active kaolin is currently used in many countries including the United States, Canada, Chile, Argentina, Mexico, Costa Rica, South Africa, Australia, New Zealand, Belgium, Spain, Italy, Greece and Turkey. The main crops are apples, pears, grapes, olives, citrus, tomatoes, cherries, melons, and walnuts. FKPF is also commercially used in pineapples, onions, peppers, and bananas.

MATERIAL AND METHODS

Field trials to evaluate the performance of FKPF against pear psylla, vine cicadellids and olive fruit fly were conducted according to European Plant Protection Organisation (EPPO) guidelines and mini-vinification and taint tests were conducted following Commission des essais biologiques (CEB) guideline N°143. All field trials were carried out by Good Experimental Practice (GEP) accredited organisations.

Pear / *Cacopsylla pyri* trials

Thirteen trials were conducted in Europe between 2001 and 2004 to evaluate FKPG against *C. pyri* (pear psylla). Applications were targeted against the over-wintering generation of *C. pyri*. Generally, the first application of FKPF was made just before or at the beginning of egg-laying. Two to four sprays, depending upon rainfall, were generally applied between BBCH 51 (inflorescence bud swelling) and BBCH 55 (flower buds visible but still closed). The standard reference treatment in most trials was deltamethrin, applied either stand-alone or in tank mixture with a mineral oil; it was usually applied only once, at the same time as the first application of FKPF. Spray volumes used were generally between 500 to 1000 litres/ha. Trial plot sizes ranged from 40 to 3760 m² with three or four replications except in some large plot trials. Data were collected on adults, eggs and nymphal stages at different times before and after treatment in all trials. However for clarity, only data on nymphs are presented, as they are the most damaging and difficult stage of *C. pyri* to control. Nymphs were counted on 10 to 40 shoots/plot or 10 to 100 flower clusters/plot.

Vines / *Empoasca vitis* trials

Four trials were conducted in South East and South West France, from 2002 to 2004 to evaluate FKPG against *E. vitis* (vine leafhopper). FKPF was applied at 25 and 50 kg/ha in three trials and lambdacyhalothrin was applied as a standard reference treatment at 12.5 g a.i. /ha. Applications were targeted at the second generation of *E. vitis*, generally during July (crop stage BBCH 77 to 79) when egg hatching was at its highest. FKPF was applied only once in 2002 and 2003 but one and two applications were compared in 2004. With two applications, the first was applied at beginning of hatching and the second one week later at the normal timing. Plot size varied from 23 to 540 m² and treatments were replicated four times. Spray volume ranged from 150 to 400 litres/ha. Leafhoppers were counted on 50 leaves / plot.

Vines / Mini-vinification trials

Two trials were conducted on white grapes (Riesling and Sauvignon) and two on red (Merlot and Syrah) in four different wine growing areas of France (Alsace, Loire Valley, Bordeaux and Rhone Valley). Three replicated plots of three rows with a length sufficient to produce 70 kg of grapes/plot were used. In order to cover future targeted uses of FKPF against both *E. vitis* and grape berry moths, three applications were made at 25 kg/ha, in July, with a one to two week spray interval. Lambdacyhalothrin was applied twice as a standard reference product, at 12.5 g a.i./ha (*E. vitis*) and 17.5 g a.i./ha (grape berry moths). All fermentation and taint testing procedures were conducted following established methods in GEP accredited organisations.

Olive tree / *Bactrocera oleae* trials

Four trials were conducted in southern Spain to evaluate FKPF against *B. oleae* (olive fruit fly). Generally two applications were made when adult flight was intense; the first during the second part of June and the second during September, when high summer temperatures were declining. FKPF was tested at 3 kg/100 litres and 5 kg/100 litres, tank mixed with 0.1 litre/100 litres of a non-ionic adjuvant. The reference product was dimethoate 400 g/ litre EC sprayed at 150 ml/100 litres in June and then only locally applied during September in a mixture (at 0.65%) with a bait, based on hydrolysed proteins. Tree density at the trial locations varied from 70 to 100 / ha; individual plots contained 20 to 25 trees and each treatment was replicated 3 times. Spray volumes ranged from 800 to 1000 litres/ha. Evaluations of efficacy were carried out every two weeks, by assessing 20 or 30 fruits on each of 10 centrally located trees per plot (total = 200 or 300 fruits/plot).

Statistics

Data for all trials presented were analysed using ANOVA, followed by Newmann & Keuls multiple range test with the exception of the trials against *B. oleae* in olive. In tables, mean values of treatments followed by different letters are significantly different ($P<0.05$).

RESULTS

Pear / *Cacopsylla pyri* control

No adverse crop effects of FKPF were evident. The only difference, compared to the untreated plots, was a delay at flowering of 2 days, in one trial conducted in 2003, in Southern France. In this trial however, there was no delay in fruit maturity at harvest.

A key assessment in pears is the infestation level of *C. pyri* at flowering time, because sprays to control the second generation are generally triggered when 20% of the shoots are infested by eggs or nymphs. Flowering in pears is between the end of March (Southern France and Italy) to mid April (Belgium and Central/Northern France). Since the timing of the first application of FKPF is at the beginning of egg-laying of over-wintering females, there is therefore 1.5 to 2 months between first sprays and the assessment of nymphs at flowering (BBCH 65).

In all the field trials, FKPF protected the pear crop from *C. pyri* better than deltamethrin, with and without oil (Table 1). The high level of control of the first generation resulted in important advantages due to synchronised egg hatching which facilitated optimised timing of the spring applied insecticides, delayed application timing of insecticides against the second generation and in some situations a reduced number of spring applications.

Table 1. Percentage control of pear psylla nymphs with FKPF at flowering

Year	Country	FKPF kg/ha x sprays	Reference g a.i./ha* or untreated	FKPF % Control	Reference % Control
2001	France	30kg x 3	Decis/17.5	100 a	87 ab
2001	Italy	90kg x 2	Mineral oil/40.5L	99 a	89 b
2002	Italy	90kg x 2	Mineral oil/40.5L	100 a	57 b
2002	Belgium	30kg x 4	Decis/17.5	98 a	92.5 b
2002	Belgium	30kg x 4	Untreated 0.8 nymphs / cluster	99	-
2003	Belgium	30kg x 3	Untreated 1.2 nymphs / shoot	98	-
2003	France	30kg x 3	Untreated 2.0 nymphs / shoot	89	-
2003	France	30kg x 4	Decis+oil/17.5+20L	95 a	70 b
2003	France	30kg x 4	Decis+oil/17.5+20L	83 a	17 b
2004	France	50/25/25 kg	Decis/17.5	93 a	19 b
2004	France	50/25/25/25 kg	Decis/17.5	100 a	76 b
2004	France	50/30/30 kg	Decis+oil/17.5+20L	99 a	60 b
2005	France	50/30 kg	Untreated 48% cluster infested	96	-
Mean control over 13 trials				96	-
Mean control over 9 trials (compared with reference)				96	63

Infestation level is given for untreated plots when no reference treatment was included.
Mean values of treatments followed by different letters are significantly different ($P<0.05$).
*Dose rate of Decis expressed by g a.i./ha; dose of oil expressed in formulated/ha.

Vines / *Empoasca vitis*

No adverse crop effects from FKPF were observed in any of the trials.

The efficacy of FKPF against *E. vitis* two weeks after either a single or a second application is shown in Table 2. This timing was selected as the population of *E. vitis* often decreased in the untreated control in later assessments. Results showed that a single application of FKPF at 25 kg/ha, at the same time as the pyrethroid standard, provided similar control of *E. vitis* while two applications of FKPF at 25 kg/ha or one at 50 kg/ha resulted in only slightly superior performance.

Table 2. Mean percentage control of *Empoasca vitis* nymphs on vines with FKPF (France)

Year		2002	2003	2003	2004	
No. of sprays		1	1	1	2	
Spray interval		14 d	12 d	14 d	23/14 d	
Treatments	Dose (kg/ha)					Mean % Control
Untreated (nb/50 leaves)	-	(95.8) a	(48.3) a	(39.8) a	(63) a	n/a
FKPF	25	55 b	89 b	87 c	81 b	78
FKPF	2 x 25	-	-	-	87 b	87
FKPF	50	82 cd	92 b	95 d	-	90
Lambdacyhalothrin	12.5*	62 bc	-	68 b	89 b	73

Pest infestation level in the untreated control is shown in parentheses.
Mean values of treatments followed by different letters are significantly different ($P<0.05$).
*Dose rate of Lambdacyhalothrin given in g a.i./ha due to use of different formulations.

Vines / Minivinification

Analysis of key parameters for must and wine, e.g., pH, sugar, alcohol content, total acidity, free and total SO_2, optical density, and fermentation curves for density and temperature were all normal and similar to the reference product. Taint testing following the triangular method (AFNOR V 09-013) could not distinguish between wines produced from FKPF or lambdacyhalothrin treated vines.

Olives / *Bactrocera oleae* control

No phytotoxic symptoms related to FKPF were reported in any of the trials.

The highest level of fruit damage for both stings and stings plus larvae were observed between mid-October and mid-November. Assessments made during this period were therefore used to calculate the percentage fruit attacked and to assess statistical differences between treatments using ANOVAR and Tukey's test. All treatments gave a significant reduction in the percentage of olives attacked (Table 3). However, although FKPF resulted in less fruit damage than dimethoate in all four trials, the differences were not statistically significant. There was no difference between dose rates of 3 and 5 kg/100 litres of FKPF.

Table 3. Mean percentage of olives attacked (stings and stings plus larvae) by *B. oleae* (Spain)

Trial		MOL 01-02	MOL 02-02	MOL 03-02	MOL 04-02
Volume (litres/ha)		1000	800	830	1000
T1		19/06	24/06	05/09	24/06
T2		07/09	30/09	-	30/09
Assessment date		14/11	20/11	18/10	20/11
Treatments	Dose kg/100 litres				
Untreated	-	27.2 a	24.7 a	9.0 a	21.4 a
FKPF	3	4.7 b	4.5 b	2.1 b	5.3 b
FKPF	5	4.0 b	4.9 b	2.1 b	4.6 b
Dimethoate	*	9.0 b	7.4 b	2.9 b	6.9 b

Mean values of treatments followed by different letters are significantly different ($P<0.05$).
*Dimethoate 150 ml/100 litres applied in all standard plots at T1, localised at T2 with bait.

DISCUSSION

In European field trials, a commercial formulated kaolin particle film (FKPF) that contains 95% active kaolin gave effective control of the over-wintering generation of pear psylla, leafhopper nymphs in vines and olive flies. In all trials, FKPF provided protection that was equal or superior to the standard reference products (deltamethrin, lambdacyhalothrin, and dimethoate) with no significant phytotoxicity or adverse effects on grape fermentation processes, wine properties or taste. Other efficacy trials currently in progress are showing positive results against grape berry moth in vines, codling moth and aphids in pome fruit, thrips in table grapes and tomato, and *Heliothis spp.* in tomato. The FKPF product has already been successfully commercialised for protection against physiological and environmental stresses such as sunburn and heat stress in Spain, Italy, USA, Latin America, South Africa, New Zealand, and Australia. FKPF therefore offers a novel crop protectant approach to reducing insect damage in a range of crops, in addition to providing known benefits such as the reduction of heat stress and sunburn.

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Biological control activity of *Gliocladium virens* and *Trichoderma* spp. against soil-borne plant pathogens

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ABSTRACT

Gliocladium virens and two *Trichoderma* spp. were found to be antagonists of the soilborne pathogens *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) and *Rhizoctonia solani* in dual-cultures. In tests with tomato plants, all three fungal isolates reduced Fusarium wilt caused by *Fol* and increased plant fresh weight. An isolate of *T. harzianum* increased both the number and weight of tomato fruits whereas isolates of *T. viride* and *G. virens* increased fruit weight only. *G. virens* also significantly increased tomato plant fresh weight compared to the untreated control. These fungi therefore have potential as biological control agents.

INTRODUCTION

Direct application of fungal antagonists such as *Trichoderma* spp. and *Gliocladium* spp. to the soil is a common method for biological control of soilborne plant pathogens (Beagle-Ristaino & Papavizas, 1985; Lewis & Papavizas, 1995) and mycelial preparations of both *G. virens* and *Trichoderma harzianum* grown in sand-bran and corn-meal sand medium, have been shown to reduce Fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) (Vagelas *et al.*, 2000, 2004). In this present study, the biological control agents (BCAs) *G. virens, T. harzianum* and *T. viride* were initially tested against *Fol* and *Rhizoctonia solani* (*Rs*) on agar. Conidial suspensions of the BCAs were then tested for activity against *Fol* on tomato plants in the glasshouse to determine if this resulted in effective disease control as observed previously with mycelial preparations (Vagelas *et al.*, 2000, 2004). Such tests aim to assess the potential use of the BCAs as an alternative means of controlling Fusarium wilt on tomato.

MATERIALS AND METHODS

Cultures

Fusarium oxysporum f.sp. *lycopersici* (IMI 194417) and *Rhizoctonia solani* (University of Reading culture collection) were used in this study. The BCAs *Gliocladium virens* and *Trichoderma viride* were derived from the culture collection of the Department of Plant Production, T.E.I. of Larissa, Greece, whereas the *Trichoderma harzianum* isolate was from the CAB collection (IMI 110150). *Fol* conidial suspensions were prepared by incubating Czapek-Dox broth cultures inoculated with six *Fol* agar plugs for 7 d at 25°C. Conidial suspensions of BCAs were prepared by washing the surface of PDA colony plates in sterile distilled water.

Antagonism on agar plates

Potato dextrose agar (PDA) and a laboratory prepared V8 juice based agar (V8A), were used for antagonism tests. Plugs of mycelium (4 mm diameter) taken from PDA plates of *F. oxysporum* f.sp. *lycopersici* (*Fol*) and *R. solani* (*Rs*) were placed 4 cm away from plugs of the BCA fungi, also taken from PDA plates, on PDA or V8A. The interactions were recorded after 144 h incubation of the agar plates at 25°C as: 1, no inhibition; 2, inhibition of *Fol* or *Rs* at a distance from the edge of BCA colonies; 3, inhibition of *Fol* or *Rs* colonies as soon as they contacted BCA colonies; 4, overgrowth of the pathogens by the BCAs mycelia (Table 2). Colony diameters of *Fol* and *Rs* were measured in culture without BCAs (control) and in the dual cultures with the BCAs. The percentage of *Fol* or *Rs* colony growth in dual cultures compared to the colony growth single control cultures of *Fol* or *Rs* was calculated (Royse and Ries, 1987).

Glasshouse experiment

The effect of the BCAs on *Fol* was studied in 2 litre pots containing a commercial mixture of peat, loam and sand (3:1:1, v:v; Roffey Brothers Ltd U.K.). The soil mixture was proved pathogen free in a preliminary test. A *Fol* conidial suspension (20 ml) was added to each pot and pots were kept in a glasshouse for a month until *Fol* chlamydospores were formed. After this period, the concentration of chlamydospores was 10^3-10^4 chlamydospores per g of soil as determined by series dilution of 1 g air dried soil samples on Komada medium (De Cal *et al.*, 1997). Conidial suspensions of all BCAs were prepared and adjusted to 10^7 spores ml^{-1} and 7 ml added to each pot. There were ten replicate pots per treatment. Inoculated control (*Fol* only) and uninoculated control (no *Fol*, no BCA) treatments were also included. After 30 d, plant fresh weight (not including roots) and tomato fruit number were recorded. Fusarium wilt was assessed by visual inspection of the plants for wilt symptoms and recorded as the percentage of chlorotic or wilting leaves.

Data analysis

All data were analysed in a factorial analysis of variance (ANOVA), using the statistical package *GenStat5*. When the *F* test was significant ($P = 0.05$), the treatment means were compared using Tukey's multiple range test.

RESULTS AND DISCUSSION

Antagonism on agar plates

G. virens proved a significantly better inhibitor of *Fol* on V8A plates compared to *T. viride* and *T. harzianum*. In PDA plates there was no significant difference between BCAs as inhibitors of *Fol* (Table 1). *G. virens* and *T. viride* were significantly better inhibitors of *Rs* than *T. harzianum*, on V8A plates than on PDA plates (Table 1). *T. harzianum* rapidly grew over *Rs* colonies. Interaction scores are shown in Table 2. Under the microscope, *T. harzianum* hyphae were observed to grow parallel with the *Fol* hyphae, and coil around them (Figure 1a). *T. viride* rapidly grew towards *Rs* and *Fol* mycelia and a clear contact line was seen between them, followed by a profuse production of green conidia, the appearance of which was initially observed across the contact line (Figure 1b). *G. virens* produced visible inhibition zones in dual cultures with both *Fol* and *Rs*. The *Rs* hyphae were also parasitised by hyphae of *G. virens* as described by Howell (1982). In all cases an unidentified visible light green substance was also observed diffused in the PDA.

Table 1. Mean percentage inhibition of radial extension growth of *F. oxysporum* (*Fol*) and *R. solani* (*Rs*) after 144 h by three potential BCAs in dual cultures at 25°C

BCAs	Mean % inhibition on PDA for *Fol*	Mean % inhibition on V8A for *Fol*	Mean % inhibition on PDA for *Rs*	Mean % inhibition on V8A for *Rs*
G. virens	85.3 ± 2.35 a	94.0 ± 1.17 c	74.1 ± 3.38 a	88.7 ± 3.05 b
T. viride	70.6 ± 6.69 a	83.7 ± 2.36 b	70.2 ± 5.89 a	88.5 ± 2.37 b
T. harzianum	85.5 ± 3.76 a	58.9 ± 1.62 a	99.1 ± 0.61 b	70.2 ± 3.9 a
$LSD_{0.05}$	NS 13.45	5.18	11.42	9.2

Values are means ± SEM (Standard error of the mean) based on 10 replicates per treatment. Values within a column followed by the same later do not differ significantly according to Tukey's multiple comparisons test (P=0.05). NS=not significant.

(a) (b)

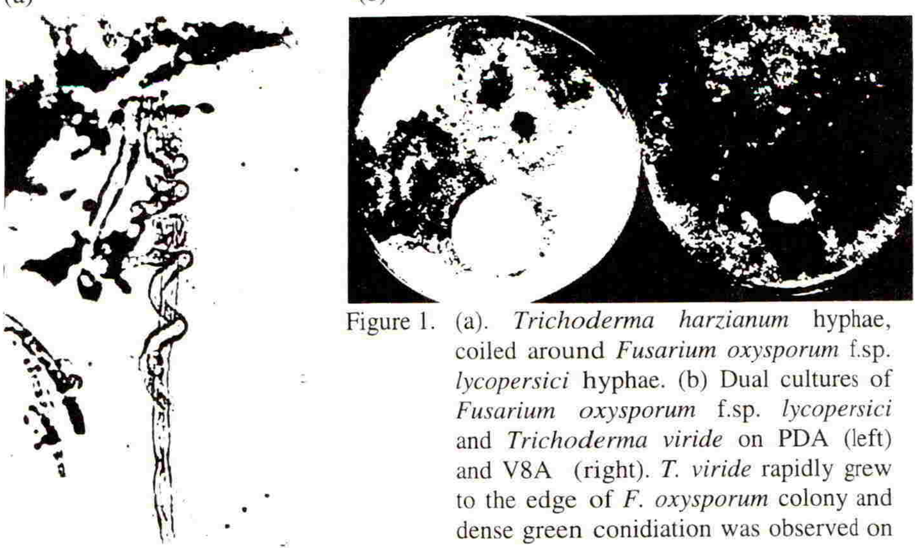


Figure 1. (a). *Trichoderma harzianum* hyphae, coiled around *Fusarium oxysporum* f.sp. *lycopersici* hyphae. (b) Dual cultures of *Fusarium oxysporum* f.sp. *lycopersici* and *Trichoderma viride* on PDA (left) and V8A (right). *T. viride* rapidly grew to the edge of *F. oxysporum* colony and dense green conidiation was observed on PDA medium.

Table 2. Interaction scores of three BCAs against *F. oxysporum* f.sp. *lycopersici* and *R. solani* mycelia in dual cultures on PDA

BCA	Interaction score	
	Fol mycelia	*R*s mycelia
G. virens	2	2
T. viride	3	3
T. harzianum	3	4

Values are based on 10 observations per treatment.

Glasshouse experiment

All BCAs reduced the number of leaves with *Fol* symptoms compared with inoculated control plants (Table 3). In addition, all BCAs tested significantly increased tomato plant fresh weight ($P < 0.001$) compared with the inoculated (*Fol* only) control treatment (Figure 2) with *G. virens* showing the greatest effect. There was no significant difference however between the inoculated (*Fol* only) and uninoculated (no *Fol*, no BCA) control treatments (Figure 2). In this study *G. virens* also resulted in statistically significantly greater tomato plant fresh weight compared to the untreated control (no *Fol*, Figure 2). This suggests that *G. virens* promotes tomato plant growth, as found by Vagelas *et al.* (2000, 2004).

Table 3. Effect of BCAs on percentage of chlorotic and wilted tomato plant leaves caused by *F. oxysporum* f.sp. *lycopersici* (*Fol*)

Treatment	Percentage of chlorotic and wilt leaves
Control (untreated plants)	0a
F. oxysporum f.sp. *lycopersici* (*Fol* only)	51c
Fol + T. viride	16b
Fol + T. harzianum	21b
Fol + G. virens	12b

Values within a column followed by the same letter do not differ significantly according to Tukey's multiple comparisons test (P=0.05).

Of the three BCAs only *T. harzianum* resulted in a significantly higher number and weight of tomato fruits compared to the *Fol* inoculated control (Figure 3). Although *G. virens* and *T. viride* treatments had no effect on the number of the tomato fruits, the weight of the tomato fruits was significantly greater than the inoculated control and comparable with *T. harzianum*. None of the BCAs had any effect compared to the uninoculated control in respect of the number and weight of tomato fruit (Figure 3).

In conclusion, the results of this study suggest that the BCAs have the potential to control *Fol*, but further experimentation is required to confirm this in the field.

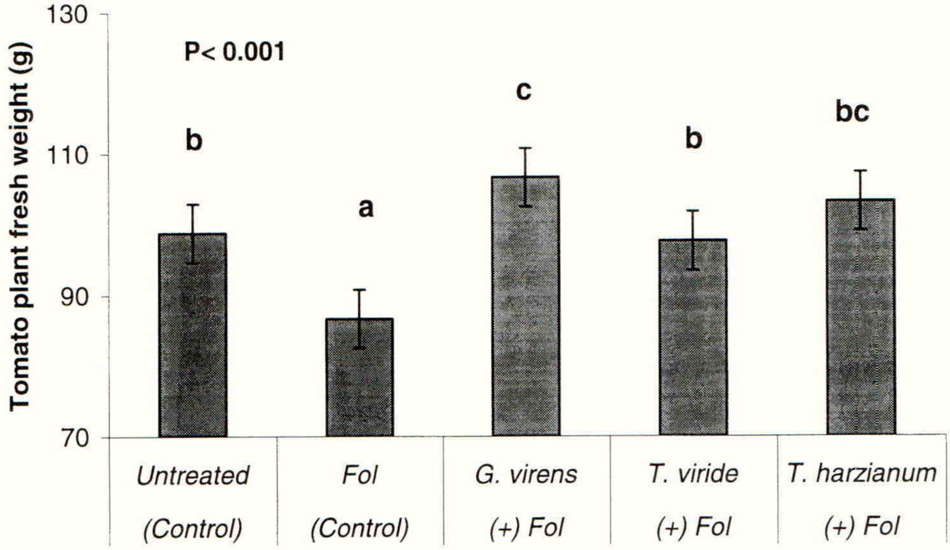
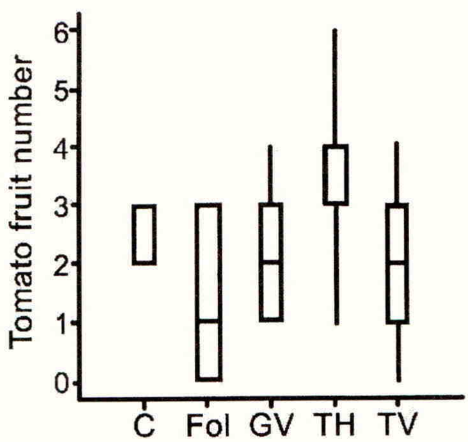


Figure 2. Effect of BCAs on tomato plant fresh weight in soil mix with *F. oxysporum* f.sp *lycopersici* (Stem fresh weigh were used as a model). Bars with the same letter do not differ significantly according to Tukey's tests (P=0.05). Values are based on 10 replicates per treatment

(a)



(b)

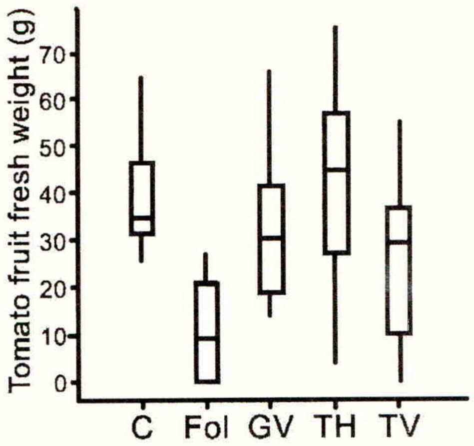


Figure 3. Box plot comparing the number (a) and fresh weight (b) of tomato fruits for BCA treatments (TH = *T. harzianum*, GV = *G. virens*, TV = *T. viride*) and inoculated (*Fol* only) and uninoculated controls (C). Values are based on 10 replicates per treatment

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A new bio-rational fungicide formulation of potassium bicarbonate for horticultural crops, vines and ornamentals

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ABSTRACT

Potassium bicarbonate has been recognised as having fungicidal properties for many years. However recently, a more effective formulation has been developed that offers both good efficacy and crop selectivity. This product which has been registered and sold in the USA is now under development in Europe against a range of important crop diseases. Results from 13 vine trials heavily infected with powdery mildew confirmed that 4250 g a.i./ha (Austria and Switzerland) or 5100 g a.i./ha (France and Spain) of this formulation gave only slightly less control than a high rate of sulphur (10 kg a.i./ha) or the synthetic reference fungicides, spiroxamine and penconazol. Two trials in apples have also demonstrated that the product can give similar control of scab on fruit as either captan or kresoxim-methyl and only slightly reduced control of scab on leaves. Similarly, data from 10 trials in strawberries showed that when applied preventively, potassium bicarbonate at 2125 to 4250 g a.i./ha provided effective powdery mildew control. Potassium bicarbonate therefore offers a low risk, bio-rational alternative for disease control in some important horticultural crops.

INTRODUCTION

The fungicidal activity of bicarbonates against various fungal diseases has been known for more than 70 years (Marloth, 1931) and many researchers have described the effects of both potassium and sodium bicarbonate on a range of diseases including *Sphaerotheca* (Horst *et al.*, 1992), *Sclerotium* (Punja *et al.*, 1982), and *Botrytis* (Palmer *et al.*, 1997). Articles on web sites (e.g. http://www.attra.org/attra-pub/bakingsoda.html) also confirm the activity of bicarbonates against most important species of powdery mildews. Although not fully confirmed, the mode of action of bicarbonates is thought to be related to osmotic and pH effects on both fungal hyphae and spores. As this is a non site specific mode of action, the development of resistance is unlikely. Bicarbonates therefore represent potentially new resistance management tools that can be used in rotation with classical synthetic fungicides (e.g. triazoles and strobilurins) that are more prone to resistance issues. In general, potassium bicarbonate is preferred over sodium bicarbonate because it can be more effective, less phytotoxic, does not accumulate in soil and may also have plant nutrient benefits.

In simple aqueous solutions, potassium bicarbonate can give limited control due to poor spreading on the foliage and poor penetration of the fungus. For this reason, much effort has gone into screening a wide range of adjuvants to find the optimum combination that offers good efficacy, crop selectivity and consumer and environmental safety. The result of this research by workers at Cornell University and Church & Dwight Co Ltd has been the development of a product containing 85% FCC grade potassium bicarbonate, which is now registered in the USA and commercialised under the brand names of Agricure® and Armicarb® 100. The same product, known as Armicarb 85SP®, is now being developed in Europe to control a range of diseases of vines, tree fruits, strawberries, vegetables and ornamental plants.

In addition to offering effective control of some important agricultural and storage diseases, potassium bicarbonate is a naturally occurring inorganic salt found in soil, water, sediments, plant and animal tissues. It is registered by both CODEX and the EU as a food additive and is classified as GRAS (Generally Regarded As Safe) in the USA. When applied to crops it is indistinguishable from natural potassium and bicarbonate and is therefore exempt from residue tolerances (MRLs), thus allowing for minimal (e.g. <1 day) pre-harvest intervals in fresh fruits and vegetables.

This paper presents some of the results obtained in Europe against three crop/disease targets; vine powdery mildew (*Uncinula necator*), apple scab (*Venturia inaequalis*) and strawberry powdery mildew (*Sphaerotheca macularis*). Other crop disease targets in development in Europe, include powdery mildews in vegetables and fruit, *Botrytis* in vines and strawberries, *Monilinia* in stone fruits and several diseases in ornamental plants.

MATERIAL AND METHODS

Vine Powdery Mildew trials

During the 2003 and 2004 seasons, potassium bicarbonate was evaluated in 13 field trials, against powdery mildew in vines. The trials were conducted in several countries, Austria (2), France (5), Spain (1) and Switzerland (5). All 13 trials were not sufficiently infected to be evaluated on both on leaves and bunches and therefore only 11 were assessed on bunches and 11 on leaves. Plot sizes varied between 10 and 50 m² and were replicated 3 to 4 times.

Potassium bicarbonate was applied at 4250 g a.i./ha in Austria and Switzerland and at 5100 g a.i/ha in France and Spain. Spray volume varied between 200 and 1000 litres/ha with the lowest volumes used in France. Standard reference products, either sulphur at 10 kg a.i./ha, spiroxamine at 250 g a.i./ha or penconazol at 25 g a.i./ha were included for comparison. First sprays were usually applied either preventatively or from first appearance of the disease, which was generally during the first two weeks of May. However, some French trial sprays were initiated when the disease was already developing. In total, three to ten applications were made at 8 to 14 day intervals over all the trials.

Visual assessments of powdery mildew incidence and severity were made for both leaves (100/plot) and bunches (50/plot) before the first treatment and at different intervals during the trial. Final assessments, between mid-July and mid-September were related to disease progress.

Apple Scab trials

The activity of potassium bicarbonate against apple scab was evaluated in two trials, in 2002 in Belgium and in 2004 in Austria. Plot sizes were 6 or 24 trees respectively and each treatment was replicated four times in Austria and only twice in Belgium. Apple varieties were Jonagold (Belgium) and Golden (Austria).

The first applications of potassium bicarbonate were made preventatively, during early April and a total of 10 or 11 sprays were applied at 7 to 12 day intervals. The last applications were made during the second half of July. In the Austrian trial, potassium bicarbonate was applied at 4250 g a.i./ha and the reference was captan at 1000 g a.i./ha. In Belgium, potassium bicarbonate was applied at 3825 g a.i./ha and compared with kresoxim-methyl at 100 g a.i./ha. Spray volumes varied from 200 and 1000 litres/ha, depending upon tree size and stage.

Strawberry powdery mildew trials

From 2002 to 2004, the new formulation of potassium bicarbonate was evaluated against powdery mildew (*Sphaerotheca macularis*) on strawberries, grown under plastic. Ten trials were conducted: seven large (200 m^2) un-replicated demonstration trials in France; two replicated small plot trials in Belgium (7.5 m^2); and one in Spain (11 m^2). Dose rates in the trials were 2125 g a.i./ha in France, with spray volumes between 150 and 1200 litres/ha. The number of applications varied between two and eight, and the interval between sprays was generally one week. The first application was usually applied preventatively at or before first appearance of the disease. Varieties tested were: Chiflon (Spain), Elsanta (Belgium), Darselect, Fraise des bois, Garriguette and Mara des bois (France). Assessments for efficacy were based on the frequency (% incidence) of powdery mildew symptoms on 50 to 100 leaves per plot.

Statistics

Data for all trials presented were analysed using ANOVA, followed by Newmann & Keuls multiple range test. In tables, mean values of treatments followed by different letters are significantly different ($P<0.05$).

RESULTS

Vine powdery mildew control

Crop selectivity was generally good but some moderate phytotoxicity was observed in two trials. The main symptoms observed were small black necrotic spots on some of the grapes. Phytotoxicity was not variety-dependent as the cultivars affected, Chasselas and Gamay, showed no symptoms in other trials carried out under similar conditions, with the same number of applications. A thorough evaluation of available information showed that these trials were both sprayed twice within two days during the fruit development period and this may therefore have been responsible for causing the observed phytotoxic effects.

Disease pressure in the trials was generally high. Potassium bicarbonate applied at 4250 g a.i./ha in Austria and Switzerland and at 5100 g a.i./ha in France and in Spain, provided good control, although this was generally slightly lower than the standard but not statistically different. (Tables 1 and 2). French trial results confirmed that potassium bicarbonate performs best when applied preventatively. However, spray volumes may also have played an important role, as they were only about 200 litres/ha throughout the season in the French trials, compared with between 600 to 1000 litres/ha (depending upon crop development stage) in the other countries.

Preventative applications and sufficient spray volume, to correctly protect leaves and bunches, are key parameters to successfully using potassium bicarbonate against vine powdery mildew.

Table 1. Mean percentage control and range of control (in parentheses) of vine powdery mildew on bunches

Country Number of trials	Austria 2	Switzerland 4	France 4	Spain 1
Untreated control (% infected area)	(30%)	(34.3%)	(62.9%)	(3.4%)
Potassium bicarbonate 4250 g a.i./ha	86 ab (75-97)	89.8 ab (74-100)	-	-
Potassium bicarbonate 5100 g a.i./ha	-	-	68 ab (41-94)	98 a
Sulphur 10000 g a.i./ha	-	-	77.7 a (50-100)	80 ab
Penconazol 25 g a.i./ha	96.5 a (94-99)	-	-	-
Spiroxamine 250 g a.i./ha	-	100 a	-	-

Mean values of treatments followed by different letters are significantly different ($P<0.05$).

Table 2. Mean percentage control and range of control (in parentheses) of vine powdery mildew on leaves

Country Number of trials	Switzerland 5	France 5	Spain 1
Untreated control (% infected area)	(51%)	(30%)	(11%)
Potassium bicarbonate 4250 g a.i./ha	89 a (76-95)	-	-
Potassium bicarbonate 5100 g a.i./ha	-	77.4 a (46-99)	96 a
Sulphur 10000 g a.i./ha	-	87.4 a (65-100)	91 a
Spiroxamine 250 g a.i./ha	96 a (91-100)	-	-

Mean values of treatments followed by different letters are significantly different ($P<0.05$).

Apple scab control

Selectivity of potassium bicarbonate was good, with no fruit russeting or foliar damage observed. The trial in Austria was located in a fruit growing area characterized by high scab pressure and from the beginning of April to mid June 15 infection possibilities were noted. Potassium bicarbonate applied at 4250 g a.i./ha gave similar control of scab on fruits as the standard reference captan, which was applied at 1000 g/ha (Table 3). However, potassium bicarbonate resulted in slightly inferior control of scab on leaves but this was not significant. Assessments in the trial carried out in Belgium were based only on the incidence on leaves or fruits attacked by scab, although the severity of infection in the untreated control in both leaves and fruits was very high (99-100%, Table 4). Under these conditions, potassium bicarbonate applied at 3825 g a.i./ha demonstrated good scab control and was similar to the standard kresoxim-methyl on fruits but inferior on leaves. This trial was not statistically analysed as there were only two replicates.

Table 3. Mean percentage control of apple scab in Austria

Type of assessment	Leaves Incidence	Leaves Severity	Fruits Incidence	Fruits Severity
Untreated (% infected)	(100%) a		(100%) a	
Untreated (% infected area)		(6%) a		(6%) a
Potassium bicarbonate 4250 g a.i./ha	64 b	75 b	65 b	79 b
Captan 1000 g a.i./ha	76 b	83 b	64 b	76 b

Mean values of treatments followed by different letters are significantly different (P<0.05).

Table 4. Mean percentage control of apple scab in Belgium

Type of assessment	Leaf Incidence	Fruit Incidence
Untreated (% leaf or fruit attacked)	(100%)	(99%)
Potassium bicarbonate 3825g a.i./ha	70	94
Kresoxim-methyl 100g a.i./ha	85	97

Mean values of treatments followed by different letters are significantly different (P<0.05).

Strawberry powdery mildew control

Crop selectivity was good in all ten trials, even in hot temperatures under plastic. In the seven large plot demonstration trials conducted in France, potassium bicarbonate was not compared with either a standard reference or an untreated control as the growers wanted to avoid powdery mildew infestations in all situations. In strawberries grown in natural soil substrates, potassium bicarbonate gave excellent powdery mildew control, even on Mara des Bois, a very susceptible cultivar. In fact it only failed to give satisfactory control in one trial which was conducted on an artificial substrate, where powdery mildew is known to be more difficult to control. In this trial, the first application was also made when flowers were already open, which was perhaps too late for a preventative treatment. The results obtained in the Belgian and Spanish small plot replicated trials also confirmed that potassium bicarbonate gave good control of powdery mildew in strawberries that was equal or superior to the standard reference products (Table 5).

Table 5. Mean percentage control of powdery mildew in strawberries

Country	Dose Rate	Belgium Trial 1	Belgium Trial 2	Spain
Untreated (% incidence)	-	(62.5)	(82)	(17)
		% control of powdery mildew		
Potassium bicarbonate	3788 g a.i./ha	-	-	88
Potassium bicarbonate	4250 g a.i./ha	72.5 b	60.5 a	-
Sulphur	10000 g a.i./ha	-	-	56
Myclobutanil	60 g a.i./ha	47.8 b	54.7 a	-
Kresoxim-methyl	150 g a.i./ha	94.9 a	74.4 a	-

Mean values of treatments followed by different letters are significantly different ($P<0.05$).

DISCUSSION

Although the fungicidal properties of bicarbonates have been known for many years, the recent formulation of a product containing potassium bicarbonate has now provided commercially acceptable control of some key crop diseases. This product has been registered and commercialised in the USA and is under development in Europe; where it is formulated as a soluble powder containing 85% potassium bicarbonate. Since potassium bicarbonate is a naturally occurring inorganic salt, there are no expected adverse effects on man and the environment from the proposed use on crops. In addition, an exemption for crop residue tolerances has been granted in the USA and is being requested in Europe. This will provide the opportunity to use a fungicide close to harvest when residues from conventional fungicides are often problematic. Field trials in Europe focussing on vines, apples and strawberry have shown promising results. In vines, potassium bicarbonate applied at 4250 - 5100 g a.i./ha gave similar or only slightly reduced control of powdery mildew as either a high dose rate of sulphur or the registered rates of spiroxamine and penconazol. In apples, control of scab at 3825 - 4250 g a.i./ha was similar in fruits as with captan or kresoxim-methyl and only slightly inferior on leaves. In strawberries, potassium bicarbonate applied preventively at 2125 - 4250 g a.i./ha provided high powdery mildew protection. Other trials ongoing in Europe are demonstrating efficacy with potassium bicarbonate against powdery mildews in apples, ornamentals and several vegetable crops, as well as *Botrytis* in vines. In conclusion, potassium bicarbonate correctly formulated, offers a new bio-rational alternative for controlling some important crop diseases of horticultural crops.

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The use of a Risk Assessment Index to reduce fungicide applications and control powdery mildew in California vineyards

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ABSTRACT

Different fungicide treatments and combinations were applied according to a Risk Assessment Index (RAI) for control of powdery mildew (*Erysiphe necator*) in a mature vineyard and compared with a typical grower's standard treatment which followed a calendar schedule. Results showed that by using the RAI, the total number of fungicide applications could be reduced without loss of effective disease control.

INTRODUCTION

Grape production in California (estimated value, 2.5 Billion USD) contributes significantly to the state's economy. Grapes cover greater than 300,000 ha throughout California's unique environmental and climatic conditions. Regardless of the grape product or market, powdery mildew caused by *Erysiphe necator* var *necator* (formerly *Uncinula necator*), continues to be the most important pathogen. Grape cultivars grown for raisin, fresh market, juice or wine have varying susceptibilities. Left untreated, highly susceptible *Vitis vinifera* cvs Fiesta, Crimson Seedless, Carignane, and Chardonnay, show yield reductions and reduced quality. The reduction in fresh fruit quality can also affect the end product in the form of sub-standard raisins, infections by secondary pathogens of stored fresh market grapes and off flavours in wine. Consumer demand for high quality fresh market fruit requires growers to produce fruit that is free of disease and blemishes. In order to meet this demand, California's San Joaquin Valley grape growers apply fungicides on a calendar schedule regardless of environmental factors that may negate their necessity as a result of no or poor fungal growth. It is these very conditions where grape growers can take advantage of novel technologies such as disease forecasting to better manage grapevine powdery mildew.

Prior to the early 1980's when triadimefon was introduced, there were few chemicals registered for powdery mildew control although elemental sulfur used primarily as a dust has been used for over 150 years in California. Shortly after its introduction, triadimefon resistance became apparent in California's coastal vineyards and hence growers statewide reverted to sulfur as the primary fungicide applied in commercial vineyards. The low cost, ease of application, multi-site inhibition, and exclusive use in organic programs, now makes sulfur the most widely used fungicide in grape production systems despite that fact that there are approximately 20 non-sulfur based fungicides registered in California to control grapevine powdery mildew. Although sulfur is the most widely used fungicide, its use can result in eye and throat irritation, drift to non-target sites, and contributions to poor air quality. Sulfur drift incidences have caught the attention of state regulators, whose aim is to impose stronger regulation and potentially eliminate it as a tool for grapevine powdery mildew management. The potential loss of sulfur means that understanding the characteristics of and utilization of multiple products with different modes-of-action in conjunction with use of the RAI must be implemented.

The development of the UC Davis Powdery Mildew Risk Assessment Index (RAI) has helped growers determine optimal times to apply elemental sulfur dust or other fungicides registered for *E. necator* management. Developed by Thomas *et al.* (1994), the RAI is a phenology model based on optimal growth temperatures for grapevine powdery mildew. The RAI is based on a point system with a range of 0-100, which is calculated by identifying six continuous hours with grapevine canopy temperatures ranging between 21-30°C. The model begins to accumulate points when two events take place: 1) budburst must happen and 2) temperatures must be in the range as described previously for three contiguous days. A more detailed account of the RAI parameters can be found at http://ucipm.ucdavis.edu/. The advantage of the RAI is that spray intervals can potentially be lengthened, thereby eliminating unnecessary sprays. This is possible during periods of low mildew pressure when temperatures are not optimal for fungal growth. Growers can access daily RAI values and weather data from loggers with temperature sensors in the grape canopy, via the UC IPM website. Weather stations are distributed throughout Fresno (7 stations) and Madera (2 stations) counties, representing greater than one third of the state's grape production.

The purpose of this research was to demonstrate that implementing the RAI in a grapevine powdery mildew management program could reduce disease effectively while minimizing the use of fungicides. Eliminating fungicide sprays benefits growers by saving them time and money. Additionally, exposure to people, non-target sites, and the surrounding environment are reduced.

MATERIALS AND METHODS

Fungicide treatments using the grower's standard on a calendar schedule and programs using multiple products either on a calendar schedule or in combination with the RAI were compared. The experiment was conducted in a mature, flood irrigated vineyard in Madera, California in 2004. Own rooted *Vitis vinifera* cv Carignane (2.1 x 3.7 m spacing) was planted in 1974 on a uniformly deep (>4m) Hanford fine sandy loam with rows in the east-west direction using a California sprawl two-wire vertical trellis system. There were four replicated blocks of five vine plots that included six fungicide treatments and an untreated control. Fungicide treatments were applied in combinations that would be recommended by chemical representatives or pest control advisers (Table 1). All treatments were applied using a backpack mist blower calibrated to deliver 935 litres/ha (April 6-28) and 1,402 litres/ha (May 5 through veraison). The spray volume was increased for better coverage as the grapevine canopy grew. Micronized sulfur was used in lieu of elemental dusting sulfur for ease of application on a small scale. Disease severity ratings were taken on June 30, 2005. Disease severity data was transformed utilizing the Arcsine transformation, subjected to ANOVA and means separated by the LSD method at P = 0.05. The means were back-transformed for reporting purposes. RAI values were calculated from 2004 weather data using the weather station in Ripperdan, CA.

RESULTS AND DISCUSSION

Grapevine powdery mildew was reduced for all fungicide treatments when compared with the untreated control (Table 1). Fungicide applications applied according to the RAI resulted in a reduction of between four and six applications of sulfur compared to a program based on a calendar schedule. Disease severity levels indicated that all of the fungicide programs were effective in controlling powdery mildew, but some may be more applicable to certain grape production systems. For example, although the severity of mildew in the grower's standard treatment is not a problem for raisin production, it would not be adequate for fresh market grapes. Treatments 6 and 7 which had the lowest disease severity and to a lesser extent treatment 5 would be more appropriate for producing fresh market grapes. The RAI accumulated 100 points prior to budburst and was declining when budburst occurred (Figure 1). Consequently, the RAI was recalculated in relation to budburst for the cultivar 'Carignane' and not 'Thompson Seedless', which occurred March 9. Fungicide treatments were initiated 14 days after budburst in anticipation of favorable temperature for mildew growth and then followed a calendar schedule according to the respective labels with the exception of the treatment 2 and 6. Treatment 2 was applied weekly and the interval for treatment 6 was extended 7 days starting June 9, when the RAI indicated low powdery mildew pressure. Treatment 7, which was similar to treatment 6, followed a calendar schedule according to product labels after the initial application date. Understanding *E. necator* biology and integrating the RAI into a powdery mildew management program can save a grower time and money and also reduce the air particulate matter load by eliminating sprays early in the season, while maintaining good disease control.

Table 1. Effect of fungicide treatments on 'Carignane' fruit applied at different timing using the grower's standard and the powdery mildew RAI.

Treatment		Timing[abc]	Disease severity[d]		Total applications[e]
1	Untreated control	-	90.61	a	-
2	copper + micronized sulfur	GS/CS[a]	8.04	b	12c + ms
3	micronized sulfur, fenarimol, and kresoxim-methyl	RAI/CS[b]	5.65	b	7 = 3ms + 1r +1s+1r + 1s
4	micronized sulfur, fenarimol	RAI/CS	4.22	b	8 = 3ms + 4f + 1ms
5	micronized sulfur, quinoxyfen	RAI/CS	2.53	bc	6 = 3ms + 3q
6	micronized sulfur, pyraclostrobin & boscalid + Latron B1956-Cal	RAI[c]	0.07	cd	6 = 3ms + 3p&b
7	micronized sulfur, pyraclostrobin & boscalid + Latron B1956-RAI	RAI/CS	0.03	d	6 = 3ms + 3p&b

[a] GS = grower standard. CS = calendar schedule. Fungicide program initiated when shoots were 12-15 cm long and then every 7 days.

[b] Fungicide program initiated after budburst when shoots were 12-15 cm and followed the calendar schedule.

[c] Treatment 6 was initiated using the RAI parameters but followed the calendar schedule until June 9, 2005.

[d] Percent cluster area infected with *E. necator*.

[e] Represents the number of applications and order of fungicides for each application.

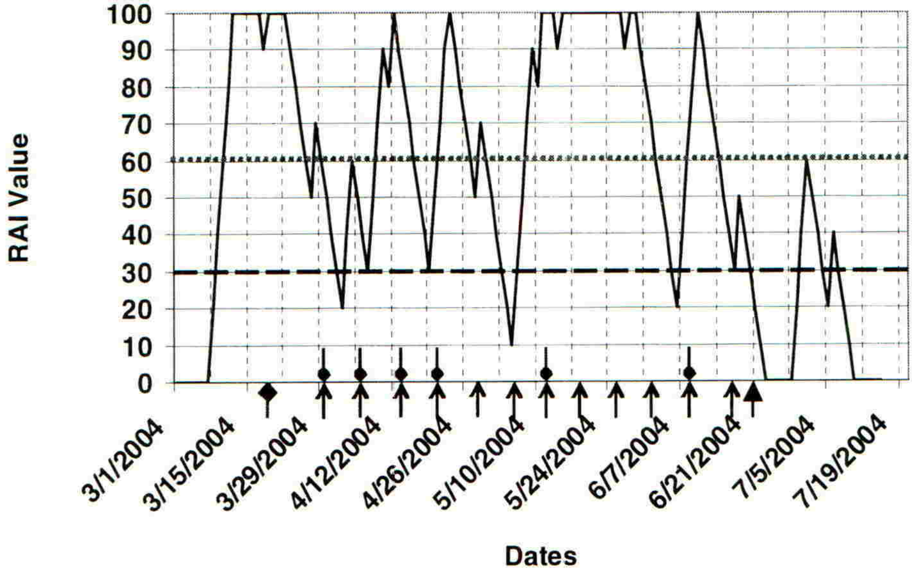

Figure 1. RAI calculated from 2004 weather data network station in Ripperdan, CA. Short dash line represents the high threshold (60) and long dash line represents the intermediate threshold (30) for *E. necator* growth. Box arrow represents budburst (March 23, 2004). Upward open-end arrows represent application dates at initiation through veraison (April 6—June 23, 2004) for treatment 2. Downward circle arrows represent application dates at initiation through veraison (April 6-June 23, 2004) for treatment 6. Upward closed-end arrow represents evaluation of fruit for disease severity.

It has been demonstrated that using the RAI and a program of fungicides with different modes-of-action, a grape grower has the potential to reduce applications of sulfur without loss of disease control. This is important given the pressure being placed on the grape industry to clean up its act with respect to the problems with using sulfur dust and shows that there are alternative fungicides effective against powdery mildew should sulfur be eventually withdrawn.

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Vegetable crop sensitivity to mesotrione soil residues

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ABSTRACT

The objective of this research was to identify the potential for injury and yield reductions to vegetable crops grown in rotation with corn, following mesotrione application to corn. Mesotrione was applied pre-emergence to corn after planting in the spring of 2002 at 175 g a.i./ha and 350 g a.i./ha, and post-emergence at the 5-leaf stage of corn at 100 g a.i./ha and 200 g a.i./ha (Site 1). In the spring of 2003, tomato, potato and green beans were planted into the same site followed by peas, black beans and green beans in the spring of 2004. Mesotrione was also applied pre-emergence to corn in the spring of 2003 at 175 g/ha and 350 g/ha (Site 2). In the spring of 2004, broccoli, onion, carrot, pepper and cucumber were planted into this site. All experiments were arranged in a randomized complete block design, with a control plot and four replications. Injury ratings and final yield of each crop were recorded. Only green beans showed significant injury in 2003 at Site 1 at 350 g/ha, and this resulted in a significant yield reduction but no injury or yield reductions were observed in 2004, two years after mesotrione application. At Site 2 in 2004, one year after mesotrione application, onion, carrot and cucumber showed significant injury at 350 g/ha, but only carrot showed significant yield reductions. Broccoli and pepper were not affected.

INTRODUCTION

Mesotrione is a triketone herbicide for pre-emergence and post-emergence broadleaf weed control in corn. The triketone herbicides are derivatives from the plant-produced phytotoxin leptospermone (Duke *et al.*, 2000). Mesotrione belongs to the callistemone class of chemistry and disrupts pigment biosynthesis by inhibition of p-hydroxy-phenylpyruvate dioxygenase (HPPD) enzyme in susceptible plants (Wichert *et al.*, 1999). Mesotrione has many desirable features for use in integrated weed management programs in corn providing broad spectrum control of all the major broadleaf weeds and some grasses as well as residual control of later germinating weeds common in corn production, including triazine resistant weeds such as red root pigweed (*Amaranthus retroflexus*) and common lambsquarters (*Chenopodium album*). Corn is naturally tolerant to mesotrione because of its reduced uptake and ability to rapidly metabolize the herbicide, compared to sensitive weeds. Mesotrione has a favorable environmental and toxicological profile. Weeds are expected to have low potential to develop resistance to this new, novel, mode of action since it is a competitive inhibitor and acts on an unexploited herbicide target site (Duke *et al.*, 2000).

Vegetable crops in southern Ontario are commonly grown in rotation with agronomic crops. Herbicide persistence is an important consideration in crop production since residue levels of some herbicides can persist to the next growing season and may injure sensitive crops in the rotation (O'Sullivan *et al.*, 1998). Herbicide characteristics (method of degradation, water solubility and rate of application) and site characteristics (soil type, rainfall and temperature) influence persistence (Dyson *et al.*, 2002). Drought conditions in the year prior to planting rotational crops results in higher levels of herbicide carryover and consequently more injury to the crops grown in the rotation and many vegetable crops are susceptible to herbicide residues. Managing herbicide applications to prevent problems with herbicide residues in the rotation is important. Crop response to herbicide residue depends on various factors, including species and variety, soil type and environmental conditions after planting. Damage to high value susceptible crops can result in a considerable economic loss and herbicide carryover from persistent herbicides has been a particular problem for growers of fruit and vegetable crops (O'Sullivan *et al.*, 1998).

Mesotrione was recently introduced for annual weed control in corn, including sweet corn and seed corn. Mesotrione has both soil and foliar activity and is being rapidly accepted as a primary tool for weed management in corn crops. Mesotrione provides excellent residual control and breaks down readily in the soil provided there is sufficient moisture, but there is concern about its persistence in the soil and carryover to sensitive crops grown in rotation (Maeghe *et al.*, 2004; Torma *et al.*, 2004). The rate of application, timing of application and soil characteristics are key influences on the rate of degradation of mesotrione. A dry growing season reduces degradation of mesotrione, particularly if it is followed by a cool wet spring. Mesotrione has a relatively short half-life in soil, 34 to 50 d at 0 to 10 cm depth, depending on soil type (Rouchaud *et al.*, 2000). Mesotrione had sufficient residual activity to control weeds season long when applied at relatively low use rates (100 g/ha) in the field (Rouchaud *et al.*, 2000). However, residues may not be dissipated within a growing season resulting in carryover injury to following crops in the rotation (Maeghe *et al.*, 2002; Torma *et al.*, 2004).

Most mesotrione persistence studies have been conducted in Europe (Maeghe *et al.*, 2002; Maeghe *et al.*, 2004; Rapparini *et al.*, 2002; Torma *et al.*, 2004) but there is little published information on the potential for mesotrione to injure vegetable crops in the rotation, especially under a North American temperate climate. No Canadian studies have been published on the impact of mesotrione soil residues on vegetable crops in the rotation. The objective of this study was to evaluate the effects of soil residues of mesotrione on growth, visual injury and yield of several vegetable crops, grown in rotation with corn.

MATERIALS AND METHODS

Field studies were carried out at the Horticultural Research Station, Simcoe, Ontario, in 2002 and 2003. The soil in the 2002 site (Site 1) was a Walsher fine sandy loam (Brunisolic Gray Brown Luvisol) with pH 7.3 and 1.7 o.m. Soil textural fractions were 65% sand, 29% silt, and 6% clay. The 2003 site (Site 2) was a Fox fine sandy loam (Brunisolic Gray Brown Luvisol; Hapludolf Subgroup) with pH 6.3 and 1.9 % o.m. Soil textural fractions were 73% sand, 22% silt, and 5% clay. Spring cultivations consisted of two passes with a disc and a field cultivator. Plots were 5 m wide and 10 m long. The experimental design was a randomized complete block with four replications.

Glyphosate-tolerant corn was planted on May 26, 2002 and May 21, 2003. Mesotrione was applied pre-emergence to corn after planting in the spring of 2002 (Site 1) at 175 g a.i./ha and 350 g a.i./ha, and post-emergence at the 5-leaf stage of corn at 100 g a.i./ha and 200 g a.i./ha.

Mesotrione was also applied pre-emergence to corn in the spring of 2003 (Site 2) at 175 g/ha and 350 g/ha. Applications were made with a CO_2 pressurized back-pack sprayer delivering 300 litres/ha of spray solution at 200 kPa pressure, using 8002 flat-fan nozzles. In both years, glyphosate was applied once, post-emergence at the 5-leaf stage of corn, at 900 g/ha. Plots were also kept weed-free by cultivation, where required. The crop was grown to maturity and harvested in the fall each year. Prior to planting vegetable crops in the spring, the land was shallow disked followed by two passes with a field cultivator. In the spring of 2003, tomato, potato and green beans were planted into Site 1 and in the spring of 2004, peas, black beans and green beans were planted into the same site. In the spring of 2004 broccoli, onion, carrot, pepper and cucumber were planted into Site 2. At each planting date, soil samples were collected for a bioassay study (Hall *et al.*, 2000). Labeled, double, styrofoam cups (237 ml) were filled with 205 g of treated soil, collected from the field at the time of planting. Each cup received 180 g of soil, 15 seeds of sugar beet and another 25 g of soil to cover the seeds. The cups were placed in flat plastic trays to which a known amount of water was added to bring the soil to field capacity and water was added as required to keep the soil at 80% field capacity. Light intensity was at 500 $\mu mol/m^2/s$. Temperature was kept at approximately 24°C by day, 16°C by night. The photo period was 16 hours light and eight hours dark. Each cup was thinned to five sugar beet plants, about 10 to 12 d after planting. Emergence was recorded and the cups were fertilized weekly. Harvesting took place 26 d after planting. A visual rating system of the shoots was made to determine injury as compared to the controls. The plants from each cup were removed from the soil and washed to remove soil from the roots. The shoots and roots from each cup were removed, separated from each other and placed in separate envelopes. The envelopes were placed in a drying chamber 55-65C° for 48 h and dry weights recorded.

For the vegetable crops, sub-plots were used consisting of 5 m-long rows of crops spaced 1.5 m apart, planted across the main plot, perpendicular to the length of the plots. The experimental design in all experiments was a randomized complete block with a split-plot arrangement, with herbicide treatment as main plot and rotational crops as sub-plots. Plots were replicated four times for each of the application rates of mesotrione. Cultivation and hand hoeing were used to keep plots weed free. Crops were grown according to standard crop production practices recommended in Ontario. The effect of mesotrione residues on the rotation crops was monitored using injury ratings and final yield measurements. Visible crop injury ratings, using a 0 to 100% scale, where 0 = no effect and 100 = crop death, were made at 28 and 56 d after planting (DAP). Crops were harvested by hand from the middle 3 m of each crop row and total yield assessed (t/ha). Visual estimates of crop injury were subjected to an arcsin transformation ARCSIN $(X \times 10^{-2})^{1/2}$, where X is the visual injury. Data analyses were performed on transformed and non-transformed data. Transformation did not change the results so only non-transformed data are presented. The data were analyzed by ANOVA and a Fisher's Protected LSD Test at $P = 0.05$ level of probability was used to determine the effect of herbicide residue on crop injury and yield.

RESULTS AND DISCUSSION

Vegetable crops responded differently to soil residues of mesotrione. At Site 1, potatoes and tomatoes showed no significant injury in 2003 while green beans showed significant injury, especially at 350 g/ha, one year after pre-emergence mesotrione application to corn (Table 1). Injury symptoms consisted of stunting and foliar chlorosis or bleaching and crop injury generally increased as mesotrione rate increased from 175 to 350 g/ha. In 2003, only green beans showed significant yield reductions. In 2004, two years following mesotrione application to corn, none of the crops showed any significant injury or yield loss. In 2004 at Site 2, broccoli and peppers showed no significant injury while onion, carrot and cucumber showed significant injury at 350g/ha (Table 2). Only carrot showed a significant yield reduction, one year following pre-emergence mesotrione application to corn. The greenhouse bioassay also showed significant injury to sugar beet as well as reduced plant dry weight, one year following mesotrione application to corn (data not shown). Bioassays conducted on soil two years following mesotrione application to corn showed no significant injury or growth reduction to sugar beet. The approximate concentration of herbicide residues in the soil was estimated by comparing the test plants with standard dose response curves. Sugar beets were injured at mesotrione levels as low as 75 ppb of mesotrione in the soil while snap beans were affected only by residues of 100 ppb of mesotrione or higher in the soil.

Table 1. Effect of mesotrione soil residues, one and two years after application to corn on injury (%) and yield (t/ha) of potato, tomato, pea, black bean, green bean

Mesotrione (g/ha)	Timing	Potato 2003	Tomato 2003	Green Bean 2003	Pea 2004	Black Bean 2004	Green Bean 2004
				Injury (%)			
0	Pre-emergence	0 a	0 a	0 b	0 a	0 a	0 a
175	Pre-emergence	0 a	0 a	3 b	0 a	0 a	0 a
350	Pre-emergence	0 a	3 a	31 a	0 a	0 a	0 a
100	Pre-emergence	0 a	0 a	3 b	0 a	0 a	0 a
200	Pre-emergence	0 a	0 a	10 ab	0 a	0 a	0 a
LSD		NSD	NSD	27.0	NSD	NSD	NSD
				Yield (t/ha)			
0	Pre-emergence	6.3 a	85.6 a	7.3 a	2.6 a	2.0 a	9.6 a
175	Pre-emergence	7.9 a	100.1 a	7.7 a	2.6 a	2.1 a	8.7 a
350	Pre-emergence	7.0 a	91.5 a	5.1 b	2.7 a	1.8 a	6.5 a
100	Pre-emergence	8.6 a	93.9 a	7.1 a	3.5 a	1.7 a	6.8 a
200	Pre-emergence	6.7 a	101.4 a	6.3 a	2.5 a	1.9 a	8.9 a
LSD		NSD	NSD	2.9	NSD	NSD	NSD

This study shows which vegetable crops, commonly grown in rotation with corn, can be grown without injury and yield effects following mesotrione application in prior years. Results suggest that mesotrione has the potential to carryover and cause injury to sensitive crops in the rotation, one year after application: green beans, onion, carrot and cucumber showed significant injury one year after mesotrione application to corn at 350 g/ha. Green beans and carrots were more sensitive to mesotrione soil residues than the other vegetable crops evaluated and were the only crops that showed significant yield reductions. Potato, tomato, broccoli and pepper showed no significant injury or yield reduction due to mesotrione carryover. Such information could be used to change the cropping restrictions that are currently on the label for this herbicide. The potential for mesotrione to injure sensitive crops in the rotation depends, not only on the mesotrione application rate but also on soil pH, soil o.m., rainfall in the year of application and soil texture. All affect the rate of mesotrione breakdown in the year of application. While potato, tomato, broccoli and pepper were unaffected by mesotrione soil residues in this study, with low soil pH, high clay and high soil o.m. and reduced microbial activity due to temperature and moisture conditions, mesotrione may persist to injure these crops one year after mesotrione application. Degradation of mesotrione is greater in soil of high pH and low adsorption since the amount available in the soil solution for microbial breakdown is increased. Hence, mesotrione herbicide carryover is greater when soil pH is less than 6.5. Higher soil pH likely resulted in the reduced mesotrione carryover in Site 1, compared to Site 2. In conclusion, this work supports the current label guidelines for mesotrione where a 2 year interval before vegetable crops can be planted is required to protect rotational vegetable crops grown in Ontario.

Table 2. Effect of mesotrione soil residues, one year after application to corn on injury (%) and yield (t/ha) of broccoli, onion, carrot, pepper and cucumber

Mesotrione (g/ha)	Timing	Broccoli	Onion	Carrot	Pepper	Cucumber
				Injury (%)		
0	Pre-mergence	0 a	0 b	0 b	0 a	0 b
175	Pre-mergence	0 a	5 ab	0 b	0 a	5 ab
350	Pre-mergence	0 a	13 a	19 a	0 a	18 a
LSD		NSD	11.9	2.5	NSD	16.1
				Yield (t/ha)		
0	Pre-emergence	5.9 a	16.0 a	38.8 a	15.3 a	17.7 a
175	Pre-emergence	5.9 a	15.9 a	39.3 a	14.5 a	14.7 a
350	Pre-emergence	5.9 a	12.2 a	31.7 b	17.9 a	14.7 a
LSD		NSD	NSD	20.8	NSD	NSD

Finally, the sugar beet bioassay was a sensitive indicator of injury risk to field-grown vegetables from mesotrione soil residues and has the potential to aid better decisions about crop rotation. Identifying the magnitude of mesotrione residues in soil prior to planting sensitive vegetable crops in the rotation will help minimize economic losses due to mesotrione residue damage. It will also allow crops to be planted in the rotation that are not affected by the residue levels present at planting and thus allow appropriate strategies to be taken to avoid any residue problems with these crops.

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