EVALUATION OF ACIBENZOLAR-S-METHYL FOR INDUCTION OF RESISTANCE IN CAMELLIA FLOWERS TO *CIBORINIA CAMELLIAE* INFECTION

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ABSTRACT

The synthetic plant defence elicitor acibenzolar-S-methyl (CGA245704 50WG, Syngenta Crop Protection) was applied to *Camellia japonica* bushes to induce systemic acquired resistance for protection of flowers against ascospore infection by *Ciborinia camelliae*. Bushes were sprayed weekly at 500 mg/litre, either before flowering, during flowering, or both before and during flowering. All untreated and treated flowers became infected with camellia blight to the same degree, and produced similar numbers of sclerotia, averaging 3.4 sclerotia/flower. Thus, acibenzolar-S-methyl was not effective in reducing the incidence of camellia flower blight.

Keywords: acibenzolar-S-methyl, systemic acquired resistance, *Camellia japonica*, camellia flower blight, *Ciborinia camelliae*.

INTRODUCTION

Camellia flower blight is caused by the fungus *Ciborinia camelliae* (Kohn), and is present throughout New Zealand, in regions north of Christchurch, except north of Auckland, Bay of Plenty and the Waikato (Taylor et al. 1999). *Ciborinia camelliae* causes premature browning of flowers, without any apparent detrimental effects on the camellia plants (Baxter 1996). Repeated applications of fungicides during flowering may protect flowers against ascospore infection, but control is unreliable. Weekly applications of triadimefon (Bayleton® 5 DF) reduced blighted flowers by 31-77% over 6 years in the United States (Holcomb 1990), although four fortnightly applications with either cyproconazole (Alto® 100SL) or fluazinam (Shirlan® 500SC) gave no control of flower blight in New Zealand (Fullerton et al. 1999).

An alternative to the use of fungicides for control of fungal pathogens on flowers is chemical induction of systemic acquired resistance using salicylic acid. Although it has no effect on the pathogen itself, salicylic acid stimulates the production of glucanase and chitinase enzymes which are translocated to distal plant parts where they may degrade fungal or bacterial cell walls (Kessmann et al. 1996). Eight weekly pre-harvest foliar sprays of salicylic acid applied at 2 mg/litre to Geraldton waxflower (*Chamelaucium uncinatum*) plants reduced colonisation by *Alternaria* sp. and *Epicoccum* sp. on flower tissue, but led to an associated increase in infection by *Botrytis cinerea* and *Cladosporium* sp. (Beasley et al. 1999). An analogue of salicylic acid, the synthetic benzothiadiazole plant elicitor, acibenzolar-S-methyl, (CGA245704 500 g ai/litre WG, Syngenta Crop Protection) (CGA), sprayed at 300 mg/litre onto apple seedlings 7 d before inoculation, effectively controlled *Erwinia amylovora* (fire blight) in pipfruit (Gouk & Boyd 1999). Based on these results, the distributor recommended CGA be applied weekly to camellias at a rate of 500 mg/litre during flowering (G. Follas, pers. comm.).

This study evaluated acibenzolar-S-methyl for protection of camellia flowers from *C. camellia* ascospore infection.

New Zealand Plant Protection 54:209-212 (2001)

METHODS

Forty, 1-1.5 m tall, potted plants of *Camellia japonica*, were used in the trial. The 4 year-old plants were re-potted 2 months before treatment application in 10 litre bags with a general potting mix. The mix comprised 500 g of 12-14 month release Osmocote Plus (15% N, 3.5% P, 9.1% K, 1.2% Mg, plus traces of B, Cu, Fe, Mn, Mo and Zn) and 250 g dolomite lime in 200 litres of bark and 50 litres of sand. The four treatments were (1) an untreated control, (2) three applications of CGA sprayed at 500 mg/litre until run-off, before bud burst on 4, 18 and 25 July 2000, (3) 12 applications every 7 d during flowering commencing 1 August 2000, and (4) all 15 applications before and during flowering. The treatments were replicated 10 times in a randomised block design. Each of the 10 blocks contained four bushes of one of the eight camellia cultivars (see names below), with two blocks containing a cultivar in common with two other blocks.

The bushes were kept in a shade-house, but those intended for spraying were moved outside to eliminate spray drift to unsprayed bushes. CGA was applied using a knapsack sprayer containing a hollow cone 26 nozzle with a size D2 aperture. Once the leaves had dried, the bushes were returned to their original positions in the shade-house. At the times of spraying, in zero to light winds, temperature ranged from 7-24°C and relative humidity from 24-70%.

During winter 2000, 1512 sclerotia were buried 10 mm deep in general camellia potting mix within 56 trays (150 x 450 mm). The trays were distributed on the shade-house floor among the camellia bushes, so that the potential inoculum sources were no more than 2 m from any bush. Production of apothecia was monitored, and from 1 July 2000, 4 d before flowering, to 14 November 2000 at completion of flowering; 247 of the sclerotia produced a total of 384 apothecia that released enough ascospores to provide primary inoculum for infection of the flowers during the full flowering period.

The camellia flowers were inspected once a week for disease. Each newly emerged flower was identified with a numbered 20 mm diameter label paper-clipped to an adjacent leaf. For each flower, the number of infected petals, the proportion infected, and the number of weeks it remained on the bush, were recorded. The proportion of petals infected was calculated by dividing the number of infected petals per flower by the total number of petals (including stamenoids) per flower, for each cultivar. For 10 flowers per cultivar, Gwenneth Morey averaged 87 petals, Wilamina 64, Demi-Tasse 38, Dona Herzilia de Frietas Magalhaes 167, Little Michael 22, Nuccio's Pearl 96, Sir Victor Davis 68 and Wedding Cake 86.

The dropped flowers were placed on the soil of each pot containing the bush from which they originated, and allowed to decay. On 6 December 2000, 7 weeks after the last flowering, the rotting flowers were placed into individual plastic bags, and stored in a dark room at 15-18°C for 12 weeks to allow for development of sclerotia. Sclerotia were recovered from the rotted petals on 28 February 2001 by washing the petal matter over a 4 mm sieve. The sclerotia were air-dried on tissue until their surfaces appeared dry, then weighed, sorted into weight classes of 1-50, 51-250, 251-700 and >701 mg, representative of small to large sclerotia, and counted.

During early flowering, the scale insect *Pulvinaria floccifera* (Westwood) was observed on the underside of most leaves, averaging 11.6 (SEM = 1.79) scale per five apical leaves/bush. To prevent excessive damage by scale, all bushes were sprayed on 2 August with Attack® (25 g/litre permethrin, 475 g/l primiphos-methyl and 375.4 g/l hydrocarbon liquid) at 1 ml/litre water, until run-off.

Statistical analysis

The incidence of camellia blight and proportion of sclerotia within arbitrary weight categories were analysed by generalised linear model with a binomial logit link. The number of flowers per bush, duration of flowering, and the number and weight of sclerotia per plant were analysed by analysis of variance. Treatment means are compared at $P<0.05$, with 95% limits in brackets.

RESULTS AND DISCUSSION

CGA showed no phytotoxic effects on camellia leaves and flowers during the trial. There was no significant effect of CGA on the duration of flowering which averaged 9 weeks (SEM = 0.4), or on the number of flowers produced which averaged 19 per bush (SEM = 1.4).

Treatment with CGA had no significant effect on the incidence of camellia blight at any assessment date (Table 1). The proportion of flowers with more than 20% of the flower showing *C. camelliae*-induced necrosis during early flowering (5th assessment) averaged 65% (39-84), with no significant difference between treatments, indicating that CGA did not delay the onset of the disease.

TABLE 1: Accumulated number and proportion of flowers infected with *C. camelliae* at the 5th, 9th and 16th assessments.

Applications of CGA245704 to flowering	Early flowering 29 August Flowers /bush	% Infected /bush	Mid flowering 26 September Flowers /bush	% Infected /bush	Late flowering 14 November Flowers	% Infected
Untreated control	4.3	77(49-92)[1]	15.2	79(58-91)	19.0	100(-)
3 before	4.1	85(56-96)	14.8	78(57-90)	18.6	98(95-99)
12 during	5.4	78(53-92)	18.4	81(63-92)	22.2	100(-)
15 before & during	5.0	78(52-92)	15.2	87(67-96)	17.5	99(96-100)
LSD (P=0.05)	1.6		3.2		3.5	

[1]95% confidence limits in brackets. (-) Confidence limits cannot be calculated.

After three months incubation, 91.3% (SEM = 1.39) of flowers contained sclerotia, with no significant differences between treatments. These flowers contained sclerotia weighing on average 393 mg (SEM = 36), with no significant differences between treatments (Table 2). The number of sclerotia per flower differed ($P<0.05$) slightly between treatments, but the differences were not large enough to be important. Because the numbers of sclerotia were influenced greatly by the shape of the flowers from each cultivar, the differences in number of sclerotia per flower were probably an artefact of the blocking structure. The number of sclerotia averaged 3.4/flower (SEM = 0.60), with the proportion of sclerotia within each size category averaging 63% (SEM = 0.60), 25%

TABLE 2: Number and proportion of sclerotia per flower, and percentage of sclerotia within each weight range.

Applications of CGA245704 to flowering	Sclerotia/flower (mg)	(No.)	% of sclerotia within each weight (mg) range 0-50	51-250	251-700	> 701
Untreated control	371	3.6	69(43-87)[1]	21(9-44)	105-20)	2(1-7)
3 before	381	4.0	66(42-84)	26(12-47)	8(4-17)	2(1-6)
12 during	448	3.2	57(32-79)	28(13-52)	14(7-25)	4(1-9)
15 before & during	373	3.0	61(34-83)	26(11-51)	10(5-22)	4(2-10)
LSD (P=0.05)	93	0.6				

[1]Percentage of flowers infected with 95% confidence limits in brackets.

(0.06), 10% (0.03), 3% (0.02) for the very small, small, medium and large sclerotia, respectively.

Acibenzolar-S-methyl, in the formulation tested, was not effective in reducing the incidence of camellia flower blight, nor was it able to influence the formation of sclerotia within each flower. This is in contrast to a report of its ability to limit the growth of *Sclerotinia sclerotiorum*, a pathogen closely related to *C. camelliae*, and reduce white mould symptoms on soya bean leaves by 60% after four applications (Dann et al. 1998). Possible reasons for its lack of efficacy on camellia blight include inadequate induction of systemic resistance, or inadequate translocation of plant defence compounds or signal molecules from leaves to flower tissue. Alternatively, some induction of resistance may have occurred but it was not sufficient to overcome the high disease pressure that occurred in the trial. Although the density of apothecia present in the trial was similar to that observed in camellia gardens in the North Island, conditions in the shade-house may have allowed for a greater expression of the disease. However, based on these results, the use of acibenzolar-S-methyl for control of camellia blight does not appear promising.

ACKNOWLEDGEMENTS

Syngenta Crop Protection for supply of CGA245704 50WG; Jordan's Nursery, Ashburton for supply of the camellia bushes and Mervyn Spurway for their maintenance; Ruth Butler for statistical advice; Rosa Henderson for identification of the scale insect; Brian Mason Scientific and Technical Trust and New Zealand Camellia Society for financial assistance.

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