SURVIVAL OF *MONILINIA FRUCTICOLA* RESISTANT TO MBC AND DICARBOXIMIDE FUNGICIDES ON TWIG CANKERS AND MUMMIFIED FRUITS

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SUMMARY

The survival of 10 isolates of *Monilinia fructicola* (Wint) Honey sensitive or resistance to MBC and dicarboximide fungicides on twig cankers and mummified fruits was compared. The ability to produce conidia on twig cankers infected in late spring 1989 was maintained for at least 1 year. The maximum potential for conidial production coincided with fruit ripening. The production of conidia on mummified fruits decreased after 2-3 months in the field. Dicarboximide resistant strains produced less inoculum than the MBC resistant and sensitive strains. The pathogenicity and fitness of all isolates was similar to the original values after 1 year.

Keywords: *Monilinia fructicola*, stone fruit, fungicide resistance, methyl benzimidazole carbamate, dicarboximide

INTRODUCTION

Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is an important disease of stone fruit in New Zealand and world wide. In 1979, strains resistant to methyl benzimidazole carbamate (MBC) fungicides were detected in the North Island (Anon. 1980), and dicarboximide fungicides replaced the MBC fungicides in many New Zealand spray programmes. Elmer and Gaunt (1986) reported that 19% of 1,292 isolates were resistant to dicarboximide fungicides, and that some showed no growth reduction on agar and sporulated profusely on host tissues. Strains resistant to MBC fungicides were present in isolates sampled from the North Island in 1988 (Sanoamuang unpublished). Penrose (1990) reported that the resistant populations of *M. fructicola* had persisted for at least 12 years since withdrawal of the MBC fungicides. Unlike dicarboximide resistant strains (Elmer and Gaunt 1990), the MBC resistant strains appear to persist in the pathogen population without selection pressure, suggesting that the strains are as fit and pathogenic as the sensitive strains.

Conidia, and possibly ascospores, are the principal initial inoculum sources in brown rot epidemics. Conidia may be produced whenever diseased tissues on twigs, branches, and mummified fruit become moist. Secondary inoculum is in the form of conidia on these diseased tissues and on freshly infected blossoms, peduncles or fruit. Little is known of the number of conidia produced from these different sources of inoculum. In this paper, we describe the potential for inoculum production on twig cankers and mummified fruit and the ability of resistant and sensitive strains to survive adverse conditions during winter and summer and to produce further inoculum.

MATERIALS AND METHODS

Two sensitive isolates, four high-resistant MBC isolates, two low-resistant MBC isolates, one dicarboximide resistant isolate and one dual dicarboximide/high-resistant MBC isolate were selected for study. These were obtained from earlier studies (Elmer and Gaunt 1990), from the PDDCC (DSIR) culture collection or from new collections from the North Island and Californian imported nectarines. Inoculum was prepared by transferring pieces of mycelium on to V-8 juice agar or potato dextrose agar on petri plates, and incubating at 25 °C for 6-8 days. A conidial suspension from these plates was shaken mechanically, filtered through lens paper to remove unbroken conidial chains and mycelium, and adjusted to 1 x 10^6 conidia/ml.

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Four year old nectarine trees (*cv* Fantasia) were potted in spring 1989 and all flowering buds were removed. The trees were inoculated in late spring 1989 by placing a mycelial plug, 4 mm diameter, on to wounded 1 year old twigs (Ogawa and English 1960). Twenty twigs in each tree were inoculated and wrapped with transparent sticky tape for 7-10 days to prevent desiccation. The inoculated trees were placed outdoors with standard row spacing and watered twice a week. The trees were arranged in a randomized complete block design with three replications and ten cankers per replicate.

Pathogenicity was assessed by measuring the length of the cankers 7 and 12 days after inoculation. Sporulation was induced (Corbin and Cruickshank 1962) on twigs with cankers sampled on four occasions after inoculation. The cankers were measured, the twigs washed under running tap water to remove old conidia, and surface sterilized with 5% (v/v) sodium hypochlorite plus 0.05% (v/v) Tween 20 for 3 min. They were then rinsed, soaked with tap water for 6 h, and placed upright individually on a polystyrene support in semi-transparent plastic pottles containing 20 ml water. The twigs were incubated at 25 °C for 48 h with a 12 h photoperiod of fluorescent and near ultra-violet light. Conidia were removed by shaking the twigs in 50% ethanol for 1 min and counted. The number of conidia produced per unit area of twig canker was calculated.

Nectarine fruit (*cv* Fantasia) of uniform maturity and size were washed thoroughly to remove fungicide residues and surface sterilized in 0.5% (v/v) sodium hypochlorite plus 0.05% (v/v) Tween 20 for 2.5 min. They were pre-incubated at room temperature for 2 days to reveal and eliminate fruit with latent infections. Twenty-five fruit were placed in 100 sterile plastic 'Plix' trays in cardboard boxes and sprayed with a conidial suspension (1 x 10^6 conidia/ml) until run off. These were incubated in a moist plastic bag at room temperature for 5-7 days or until the fruit were fully colonized. The diseased fruit were hung in open net bags (five fruit/bag) in trees to mummify naturally. Six weeks later, when the fruit were fully mummified, the bags were transferred to trees in a peach block isolated from commercial orchards. Two bags (ten fruit) per isolate were collected from May to November from each of the three replicates and sporulation was induced as described for the twig cankers.

Fitness components (Elmer and Gaunt 1990) of all isolates were tested, on fruit and blossom, at the beginning and end of the twig canker experiment. Fruit were wounded with a 4 mm diameter cork borer to a depth of 2 mm. Ten μl of a conidial suspension of the fungus (1 x 10^6 conidia/ml) was applied to the wound. Inoculated fruit were placed in plastic 'Plix' cups in moist plastic containers (2 litres capacity). They were incubated at 25 °C with a 12 h photoperiod of fluorescent and near ultra-violet light. The incubation period (IP), latent period (LP), lesion diameter and spore production were assessed. Lesion expansion rates and sporulation rates were derived from linear regressions over the linear period.

Peach (*cv* Redhaven) shoots harvested at the late calyx green to pink tip stage were induced to flower as described by Elmer (1990). Conidial suspensions (1 x 10^5 conidia/ml) were applied to run-off with a handheld airbrush at 172 kPa. The fitness components were investigated as on fruit.

RESULTS

At 7 and 12 days after inoculation, there were differences ($P<0.05$) in the length of cankers between the 10 isolates tested. One sensitive and a high resistant MBC isolate produced smaller cankers than all other isolates. One low-resistant MBC isolate produced larger ($P<0.05$) cankers than the remainder. All cankers expanded up to at least 40 mm long within 2 to 3 weeks. There was a heavy exudation of gum from injured tissues.

There were no differences ($P>0.05$) in conidial production within sensitive, high-resistant and low-resistant isolate types. The data are summarized as mean values for each isolate type in Table 1. On twig cankers the fungus produced fewer conidia per unit area in November 1989 than 2-3 months later, coinciding with fruit ripening. All fungal isolates survived in the cankers over winter and produced conidia in the following spring. Conidial production was similar in August and November 1990 to the number

produced in the previous early summer. There was no evidence for reduced conidial production in MBC resistant isolates compared to the sensitive isolates. In August and November 1990 conidial production was greater in the resistant isolates. In contrast, the dicarboximide and dual resistant isolates produced fewer conidia than the sensitive isolates.

TABLE 1: Production of conidia by *M. fructicola* isolates assessed on different dates in twig cankers and mummified fruits after exposure to field conditions.

Isolates[a]	Twig cankers (x 10^3/mm^2)					Mummified fruit (x 10^6/fruit)				
	20 Nov	10 Feb	28 Aug	24 Nov	LSD	10 May	28 Jun	20 Aug	6 Nov	LSD
S	18	42	12	6	19.8	73	64	15	4	29.9
HR	17	25	19	20	8.4	75	58	43	14	25.2
LR	39	54	26	26	14.5	103	99	71	50	33.5
D	9	12	12	2	9.9	70	49	18	5	70.0*
DMR	5	12	7	3	4.6	52	44	21	8	45.0*
LSD	16.5	28.3	9.2	6.8		52.5	42.8	24.6	5.2	

a S = sensitive, HR = high-resistant MBC, LR = low-resistant MBC, D = dual dicarboximide/high-resistant MBC and DMR = dicarboximide resistant

* significant difference at $P<0.05$, all other values are $P<0.001$

The number of conidia produced per mummified fruit by all isolates was less ($P<0.05$) in August and November than the initial number. From May to July the MBC resistant isolates produced at least as many conidia in mummified fruit as the sensitive isolates. The MBC resistant isolates survived the winter and produced more conidia in August and November than the sensitive and dicarboximide resistant isolates.

DISCUSSION

All isolates of *M. fructicola*, resistant or sensitive to MBC and dicarboximide fungicides, survived adverse conditions effectively as mycelium in both infected twig cankers and mummified fruits for at least one season. Since *M. fructicola* overwintered effectively in both twig cankers and mummified fruits in trees, these are likely to be important sources of primary inoculum in the spring., The fitness and pathogenicity on blossoms of MBC-resistant isolates re-isolated from twig cankers after 1 year were as great as the original isolates (spore production was 20.5 x 10^3 conidia/hour and 18.2 x 10^3 conidia/hour respectively). Similarly, resistant isolates re-isolated from mummified fruits (0.9 x 10^6 and 0.8 x 10^6 conidia/fruit) showed no reduction in their pathogenicity or fitness. Conidia produced from twig cankers and mummified fruits during late spring and summer will infect immature fruit.

The reduced sporulation of *M. fructicola* on mummified fruits and twig cankers after winter was possibly related to water availability. Very wet weather during winter causes leaching of nutrients from the surface layers of infected tissues (Corbin and Cruickshank 1962), especially carbohydrate reserves. The lack of carbohydrate reserves could inhibit conidial production (Budge and Whipps 1991). Willetts and Harada (1984) suggested that under moist conditions, mummified fruits and twig cankers were slowly degraded, thus reducing the potential for conidial production. We observed that with prolonged exposure the mummified fruits and twig cankers were contaminated with other organisms. *Fusarium, Cladosporium, Penicillium* and *Bacillus* species were found increasingly on the infected tissues.

Isolates resistant to dicarboximide fungicide survived and sporulated poorly compared with the sensitive isolates. This may be a further reason why such strains do not persist well in the pathogen population unless dicarboximide fungicides are used frequently (Elmer and Gaunt 1990). Caution is still required because of the potential for

recombination of dicarboximide resistance with enhanced survival and other aspects of fitness. In contrast, MBC resistant strains remain in the population. In this paper we have shown that they can survive and sporulate on survival sites at least as well as the sensitive strains.

REFERENCES

Anon., 1980. Stonefruit — Disease, pest and product summary. New Zealand Fruit Grower' Federation Technical Guide, 6.

Budge, S.P. and Whipps, J.M., 1991. Effect of sucrose concentration on sclerotia production and subsequent apothecial formation by *Sclerotinia sclerotia*. *Mycol. Res. 95(2):* 195-198.

Corbin, J.B. and Cruickshank, A.M., 1962. Environment and sporulation in phytopathogenic fungi *V. Monilinia fructicola* (Wint.) Honey: effect of water relations on regeneration of conidia in vivo. *Aust. J. Biol. Sci 16:* 99-110.

Elmer, P.A.G., 1990. The epidemiology of dicarboximide resistant strains of *Monilinia fructicola* (Wint.) Honey in stone fruit orchards. Ph.D. Thesis. Lincoln University Canterbury. 133 pp.

Elmer, P.A.G. and Gaunt, R.E., 1986. A survey of fungicide insensitivity in *Monilinia fructicola*. *Proc. 39th N.Z. Weed and Pest Control Conf.:* 166-169.

Elmer, P.A.G. and Gaunt, R.E., 1990. Population dynamics of dicarboximide resistant strains of *Monilinia fructicola* and conservation of the dicarboximides for brown rot control. *Proc. 43rd N.Z. Weed and Pest Control Conf.:* 109-113.

Ogawa, J.M. and English, H., 1960. Relative pathogenicity of two brown rot fungi, *Sclerotinia laxa and Sclerotinia fructicola*, on twigs and blossoms. *Phytopathology 50:* 550-558.

Penrose, L.T., 1990. Prolonged field persistence of resistance to benomyl in *Monilinia fructicola*. *Crop Prot. 9:* 190-192.

Willetts, H.J. and Harada, Y., 1984. A review of apothecial production by *Monilinia* fungi in Japan. *Mycologia 76:* 314-325.