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Evaluation of film forming polymers to control apple scab (*Venturia inaequalis* (Cooke) G. Wint.) under laboratory and field conditions

Glynn C. Percival*, Shirley Boyle

R.A. Bartlett Tree Research Laboratory, Plant Science Laboratories, School of Biological Sciences, Whiteknights, The University of Reading, Reading RG6 6AS, UK

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ABSTRACT

A detached leaf bioassay was used to determine the influence of several film forming polymers and a conventional triazole fungicide on apple scab (*Venturia inaequalis* (Cooke) G. Wint.) development under laboratory *in vitro* conditions, supported by two field trials using established apple cv. Golden Delicious to further assess the efficacy of foliar applied film forming polymers as scab protectant compounds. All film forming polymers used in this investigation (Bond, Designer, Nu-Film P, Spray Gard, Moisturin, Companion PCT12) inhibited germination of conidia, subsequent formation of appressoria and reduced leaf scab severity using a detached leaf bioassay. Regardless of treatment, there were no obvious trends in the percentage of conidia with one to four appressoria 5 days after inoculation. The synthetic fungicide penconazole resulted in the greatest levels of germination inhibition, appressorium development and least leaf scab severity. Under field conditions, scab severity on leaves and fruit of apple cv. Golden Delicious treated with a film forming polymer (Bond, Spray Gard, Moisturin) was less than on untreated controls. However, greatest protection in both field trials was provided by the synthetic fungicide penconazole. Higher chlorophyll fluorescence Fv/Fm emissions in polymer and penconazole treated trees indicated less damage to the leaf photosynthetic system as a result of fungal invasion. In addition, higher SPAD values as measures of leaf chlorophyll content were recorded in polymer and penconazole treated trees. Application of a film forming polymer or penconazole resulted in a higher apple yield per tree at harvest in both the 2005 and 2006 field trials compared to untreated controls. Results suggest application of an appropriate film forming polymer may provide a useful addition to existing methods of apple scab management.

1. Introduction

Apple scab caused by *Venturia inaequalis* (Cooke) G. Wint. is the most economically devastating pathogen of ornamental and fruiting apples world wide (Blaedow et al., 2006). As producers, suppliers and vendors of apples generally adopt a zero tolerance policy towards scab on fruit, conventional orchard and production systems rely heavily on repeated fungicide sprays throughout the growing season (Berrie and Xu, 2003; Percival and Boyle, 2005). Public demands to reduce pesticide use, stimulated by greater awareness of environmental and health issues, as well as development of strains of *V. inaequalis* (Cooke) G. Wint. resistant to synthetic fungicides limit the effectiveness of conventional pesticide management strategies (Akbudak et al., 2006; Hagan and Akridge, 2007). Likewise increased government legislative restrictions regarding the use and application of pesticides further emphasize the need to develop alternative pathogen management systems (Marco et al., 1994; Walters, 2006).

Film forming polymers are widely used as spray adjuvants within the agricultural, forestry and horticultural industries (Backman, 1978). Their main functions are to reduce weathering and extend pesticide efficacy, act as stickers/spreaders to improve distribution and adherence of agrochemicals, and decrease water loss and wilting of young transplants (Gale and Hagan, 1996). Studies using film forming polymers as an alternative to fungicides have found several to be effective in controlling foliar pathogens of cereals, vegetables, fruit and ornamentals, including rusts (*Puccinia*, *Uromyces* spp.), grey mould (*Botrytis cinerea* Pers.), eyespot (*Septoria nodorum* Berk.) and leaf spot (*Pyrenophora* spp.) (Blaedow et al., 2006; Sutherland and Walters, 2001; Sutherland and Walters, 2002). Film forming polymers act as a physical protective barrier against invading foliar pathogens at the leaf surface and prevent adherence of spores that subsequently inhibited germ tube development (Han, 1990; Osswald et al., 1984). As film forming polymers act by physical and not chemical means, they are not subject to the stringent government legislative restrictions that relate to the use

* Corresponding author. Tel.: +44 780 891 5865.
E-mail address: gpercival@bartlettuk.com (G.C. Percival).

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and application of conventional pesticides (Anon., 2008). Current film forming polymers are also less phytotoxic than synthetic fungicides to leaf tissue, permeable to atmospheric gases and allow penetration of solar radiation so consequently have little detrimental effects on tree biology when applied at high concentrations (Fuller et al., 2003). Consequently, film forming polymers potentially offer a cheaper (polymers cost 40–80% less than conventional fungicides) and environmentally more acceptable system compared with current fungicide spray methodology for reducing scab related yield losses. The efficacy of film forming polymers against the fungal pathogen apple scab (*V. inaequalis* (Cooke) G. Wint.) has received little attention. Due to this lack of information a detached leaf bioassay was used to determine the influence of several film forming polymers on apple scab development under laboratory *in vitro* conditions, supported by two field trials using established apple cv. Golden Delicious trees to further assess the efficacy of foliar applied polymers as scab protectant compounds.

2. Materials and methods

2.1. Laboratory in vitro experiments

Sixty fully expanded leaves were excised from actively growing shoots of field grown apple cv. Golden Delicious 28 days after leaf flush (mid-May), a time when leaf material shows maximum photosynthetic performance (Kitao et al., 1998) with no visible symptoms of scab development. All leaf material was prepared within 2 h of collection. Inoculation experiments were done under sterile conditions using a laminar flow transfer hood. Leaves were surface sterilized by immersing in sodium hypochlorite for 30 s and then rinsed in sterile distilled water for 1 min prior to drying on Whatman filter paper (Muhammed et al., 1996). Immediately following drying, leaves were immersed in a 10 ml l^{-1} (1% solution) film forming polymer solution (Table 1) and allowed to dry for 1 h at ambient temperature. In addition, a comparative evaluation of the fungicide Topas (a.i. penconazole 10% emulsfiable concentrate) a protectant triazole fungicide with antisporulant activity (Syngenta Crop Protection UK Ltd, Whittlesford, Cambridge, UK) commercially used for apple scab control was conducted by immersing leaves at the manufacturers recommended rate of 1.5 ml l^{-1} of water. Twenty non-polymer leaves acted as controls. Leaves were then placed abaxial surface down in plastic Petri-dishes lined with moist (sterile distilled water) Whatman filter paper. Five plates with 12 detached leaves per plate (60 leaves per treatment) were inoculated by spraying with an axenic conidial suspension (10^6 conidia/ml) that included an mixture of races 1–5 of *V. inaequalis*. The fungus was grown in wick cultures on 4% malt extract, and spores were collected, centrifuged (2000*g*, 5 min), and re-suspended in distilled water. After inoculation, all plates were sealed with a thin polythene film (Parafilm) permeable to air but not water and incubated in a growth chamber at 19 ± 1 °C, 16 h light/8 h dark photoperiod from white fluorescent tubes at 40 µmol m^{-2} s^{-1} light intensity (Yepes and Aldwinckle, 1993a).

At day 5 post inoculation the percentage of conidia that had germinated, the percentage that had formed appressoria and the number of appressoria per conidium were determined on 100 spores from 20 leaves, 5 spores per leaf (Yepes and Aldwinckle, 1993b). Leaves were decolorized overnight by immersing in 99% cold methanol and stained with periodic acid–basic fuchsin. Whole leaves were mounted on glass slides in glycerol and examined by light microscopy. The remaining 40 leaves per treatment were assessed at day 35 after inoculation using the leaf scab severity rating for field trial evaluations. *In vitro* laboratory experiments occurred in 2005 and were repeated in 2006.

2.2. Field trials

The apple trial site consisted of a 0.75 ha block of apple cv. Golden Delicious interspersed with individual trees of Golden Crown, Red Delicious and Gala as pollinators. Golden Delicious was chosen for experimental purposes due to its sensitivity to apple scab infection. Planting distances were based on 2×2 m spacing. The trees were planted in 2003 and trained under the central-leader system to an average height of 1.5 ± 0.15 m with mean trunk diameters of 10 ± 1.2 cm at 45 cm above the soil level. The trial site was located at the University of Reading Shinfield Experimental Site, University of Reading, Berkshire (51°43′N, −1°08′W).

The soil was a sandy loam containing 4–6% organic matter, pH of 6.2, available P, K, Mg, Na and Ca were 52.0, 659.1, 175.2, 49.4 and 2188 mg l^{-1}, respectively. Weeds were controlled chemically using glyphosate (Roundup; Green-Tech, Sweethills Park, Nun Monkton, York, UK) throughout experiments. No watering or fertilization was applied during the trials. Historically the apples annually suffered heavily from apple scab infection. Consequently, prior to the trial commencing in 2005 and 2006 trees were inspected in September 2004 and 2005 and only those trees with 50–80% of leaves affected, severe foliar discolouration, and subsequent scab infection were used in the trial. The treatments (three film forming polymers, one fungicide and a water control) were applied in eight randomized complete blocks with a single tree as the experimental unit, giving a total of 40 observations per response variable. A minimal insecticide program based on the residual pyrethroid insecticide deltamethrin (Product name Bandu, Headland Agrochemicals Ltd, Saffron Walden, Essex, UK) was applied every 2 months during the growing season commencing in May (Nicholas et al., 2003), a standard practice followed at the University of Reading experimental site for insect pest control. All insecticide sprays were applied using a Tom Wanner Spray Rig sprayer at 40 ml deltamethrin 100 l^{-1} water. Trees were sprayed until runoff, generally 1.5 l per tree.

Prior to film forming polymer sprays, polyethylene screens 2 m high were erected around each tree to prevent dispersal of sprays and possible cross contact with other trees and the base of the tree

Table 1
Selected film forming polymers evaluated for the control of *Venturia inaequalis* (Cooke) G. Wint. on apple cv. Golden delicious under laboratory and field conditions.

Product	Active ingredient	Property	Supplier
Water (control)	–	–	–
Bond	Alkyl phenyl hydroxyl polyoxyethylene	Sticker/wetter	De'Songassee Swaffam Bulbeck, Cambridge CB5 0LU, UK
Designer	Styrene–butadiene copolymer + polyalkylene oxide modified heptamethyl trisiloxane	Super wetter/sticker	De'Songassee Swaffam Bulbeck, Cambridge CB5 0LU, UK
Nu-Film P	Poly-1-*p* menthene	Sticker/spreader	United Agri Products Ltd, Alconbury Weston, Huntingdon, UK
Spray Gard	Di-1-*p* menthene	Extender/sticker/wetter	United Agri Products Ltd, Alconbury Weston, Huntingdon, UK
Moisturin	Ammonium hydroxide + naphthalene + 1,2,4 trimethylbenzene	Anti-transpirant/desiccant	GSI Horticultural, Bend, Oregon, USA
Companion PCT12	25% Polyacrylamide	Spreader/sticker/wetter	Ciba Speciality Chemicals, Low Moor, Bradford, West Yorks, BD12 0JZ, UK