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Crop damage caused by powdery mildew on hop and its relationship to late season management

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Powdery mildew of hop (*Podosphaera macularis*) may cause economic loss due to reductions in cone yield and quality. Quantitative estimates of crop damage from powdery mildew remain poorly characterized, especially the effect of late season disease management on crop yield and quality. Field studies in Washington State evaluated cone yield, bittering acid content and quality factors when fungicide applications were ceased at different stages of cone development. The incidence of cones with powdery mildew was linearly correlated with yield of cones, bittering acids and accelerated cone maturation. In cultivar Galena, the cumulative effect of every 1% increase in cones powdery mildew incidence was to reduce alpha-acid yield by 0·33%, which was due to direct effects on cone yield but also indirect effects mediated by dry matter. In the more susceptible cultivar Zeus, alpha-acid yield was increased 20% by controlling powdery mildew through the transition of bloom to early cone development compared to ceasing fungicide applications at bloom: additional applications provided only modest improvements in alpha-acid yield. In both cultivars, the impact of powdery mildew on aroma characteristics and bittering acid content were less substantial than cone yield. The damage caused by powdery mildew to cone colour and alpha-acid yield, as well as the effectiveness of fungicide applications made to manage the disease, appears inseparably linked to dry matter content of cones at harvest. Realising achievable yield potential in these cultivars requires control of the disease through early stages of cone development and harvest before maturity exceeds *c.* 25% dry matter.

Keywords: crop loss, *Humulus lupulus*, quantitative epidemiology

Introduction

Plant diseases are managed because of their potential damage to crop yield and quality, which can lead to economic loss. Knowledge of crop damage caused by a disease is important for making appropriate strategic and tactical management decisions (Savary *et al.*, 2006; Esker *et al.*, 2012). Without this information management efforts may be insufficiently or unnecessarily applied if the costs of control measures are misaligned with the value of attainable yield lost to a disease.

Crop damage may lead to losses from reductions in attainable yield or quality of the harvested product. For diseases that primarily reduce yield, crop damage results from the cumulative effect of a disease on normal host physiological functions (Gaunt, 1995). In field crops such as cereals, powdery mildews may affect yield potential through diminished photosynthetic capacity or efficiency (Gaunt, 1995). Measurements of disease or healthy leaf area duration can be used to estimate crop damage, and empirical damage functions in several crops often indicate a negative linear relationship between powdery mildew intensity and yield (e.g. Daamen, 1988; Lipps & Madden, 1989). Crop damage is more difficult to predict for commodities used directly by buyers and evaluated by cosmetic appearance such as fruits and fresh market vegetables (Jarvis *et al.*, 2002). In these situations, assumptions about acceptable crop quality can be made to allow calculation of the economic value of powdery mildew management (Yoder, 2000) or sensory evaluations can be conducted to determine consumer preference (Calonnec *et al.*, 2004). However, quality assessments are often subjective and may be confounded by penalties for crop quality being dependent on market conditions and demand (Zadoks, 1985; Esker *et al.*, 2012), resulting in ambiguous and complicated damage thresholds.

The latter situation is characteristic of powdery mildew on hop (caused by *Podosphaera macularis*). Hops are produced primarily for brewing, either directly or as bittering acids removed by a supercritical carbon dioxide extraction process. By far the most important bittering acids are the alpha-acids (Hysert, 2009). For cultivars intended for extraction, yield of alpha-acids is generally the most important factor in gross revenue per hectare. Other cultivars may be used directly in brewing and therefore both direct yield loss and quality impacts are important because the crop worth is determined in

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part by cone appearance, organoleptic factors and their bittering acid content (Royle, 1978; Neve, 1991).

Cone appearance, specifically colour, is related in part to maturation. Maturation of hop cones is commonly assessed by measurement of dry matter content. The optimal dry matter for maximizing yield of alpha-acids and quality factors is cultivar dependent, but typically in the range of 22–24% or greater (Rybáček, 1991; Murphey & Probasco, 1996). Cone aroma and colour are often assessed as indicators of brewing quality and storage stability. These assessments are visual and subjective, and often use an ordinal scale (e.g. 1–10). For hop cultivars grown for production of alpha-acids, aroma and appearance of cones is typically of less concern than yield of alpha-acids because the latter is the final product sold to brewers. The situation for cultivars sold, evaluated and used as whole cones is more complicated. The lack of any perceptible colour or aroma defect is optimal, but defining lower thresholds of acceptability is problematic because the ratings are subjective and may depend on market conditions and the end use of the hop.

Although *P. macularis* may infect most aerial plant tissues, crop loss is thought to be associated primarily with occurrence of the disease on cones (Royle, 1978; Gent *et al.*, 2008). Indirect reductions of cone yield due to occurrence of powdery mildew on leaves, resulting in reduced solar radiation interception or radiation use efficiency, have not been estimated. However, the relationship between biomass production and healthy area absorption of radiation is asymptotic and generally affected less in plants with high green leaf areas than plants with low green leaf area (Johnson, 1987; Gaunt, 1995). In commercial hop production, powdery mildew is assumed to have negligible effects on radiation interception because the plant produces superfluous foliage and disease management practices typically result in a low incidence of leaves being affected by the disease (Neve, 1991; Gent *et al.*, 2008). In contrast, occurrence of powdery mildew on cones may lead to their abortion, malformation, and discolouring. The potential effects of powdery mildew on both yield quantity and quality remain poorly defined (Royle & Griffin, 1973; Royle, 1978). The few estimates of crop damage available suggest the potential for complete loss in cone yield and marketable yield with severe occurrence of powdery mildew on cones (Neve, 1991; Mahaffee *et al.*, 2009), and some regional epidemics of the disease illustrate this damage potential (e.g. Mahaffee *et al.*, 2003).

It is generally assumed that the most severe and damaging powdery mildew outbreaks are associated with occurrence of the disease during bloom and on young developing cones (Royle, 1978). The effect of powdery mildew on development of alpha-acids is largely unknown but believed to be most substantial when the disease occurs during the early stages of cone development because lupulin gland (the location of biosynthesis of bittering acids) development is arrested. Limited experimental data exists to support this assumption and decreases in yield and bittering acids are not always incurred when the disease attacks. Royle & Griffin (1973) did not detect differences in cone yield or alpha-acid content of cones when the incidence of cones with powdery mildew varied from 2·3 to 17%. In Washington State, powdery mildew may cause substantial crop damage if not controlled adequately, with some estimates of 50–80% or more loss of attainable yield in highly susceptible cultivars such Galena and Zeus if no control measures are applied (G. Probasco, John I. Haas Inc., Yakima, Washington, USA, personal communication). However, this situation rarely occurs in practice and provides little guidance on the appropriate intensity with which the disease should be managed for optimal economic returns in susceptible cultivars.

The occurrence of powdery mildew on cones has also been associated with premature ripening (Hammond, 1900; Coley-Smith, 1964; Mahaffee *et al.*, 2009). Premature ripening and the concomitant deterioration of cone colour are apparently related to powdery mildew development on the internal surfaces of bracts and bracteoles. This cryptic phase of the disease may not result in malformation of the cone and can be difficult to diagnose with casual observations. The degree of cone discoloration appears related to the severity of powdery mildew on the cone (Coley-Smith, 1964). How such infections impact yield, quality and brewing characteristics has not been fully characterized.

Hysert *et al.* (1998) reported that the effect of powdery mildew on harvested hop cones was similar to the effect of early maturation and prolonged storage, namely reduced alpha-acid and essential oil content, and increased levels of hard resins. They reported 'off-aroma' characteristics in hop cones when 80% of the cones had powdery mildew, but these impacts were not detected when 50% or less were affected. Brewing trials and sensory evaluations did not indicate any major flavour defects or preference for beers made with cones with slight versus severe levels of powdery mildew. However, conflicting information exists on the impacts of powdery mildew on brewing quality (Rybáček, 1991).

In the absence of rigorous experimental data on crop damage from powdery mildew, growers often assume the potential for severe crop damage and loss from the disease. Cultural and chemical control measures are applied prophylactically beginning early in the season with the goal of reducing inoculum levels on leaves to minimize the incidence of cones with powdery mildew (Turechek *et al.*, 2001; Mahaffee *et al.*, 2009; Gent *et al.*, 2012). Crop damage then becomes a mixture of both yield and quality losses depending on the severity of the disease outbreak and efficacy of management efforts. Disease management strategies and deployment could be refined with better knowledge of the yield and quality damage caused by powdery mildew. In this research, quantification of the association between the incidence of powdery mildew on cones, crop yield and measures of cone quality was sought.

Materials and methods

The general approach taken was to create varying levels of powdery mildew intensity on leaves and cones by applying a range of fungicide treatments and staggering the date of the last application. The resultant impact of powdery mildew on cone yield, levels of bittering acids and cone quality factors was quantified. This was carried out in experimental plots planted with cultivar Galena and in a commercial hop yard planted with cultivar Zeus. Galena matures during early September in Washington State and Zeus matures in approximately mid-September (Kostelecky, 2009). Details of the experimental design, disease assessments and yield and quality measurements are given separately for each experiment.

Experimental plots

Experiments were conducted during 2009–2011 in a research hop yard located at the Washington State University Irrigated Agriculture Research and Extension Center near Prosser, Washington. During each year, a set of fungicide treatments were designed consisting of trifloxystrobin (Flint 50W, Bayer CropScience) and quinoxyfen (Quintec, Dow AgroSciences) applied in a rotational or a blocking programme (i.e. two sequential applications of each product) beginning in mid-May and continued through bloom stage in mid-July. After mid-July, subsequent applications were ceased at 2-week intervals up to 10–17 days before harvest (detailed in Table 1). A non-treated control was included in each experiment.

Each plot consisted of nine plants in a single row arranged in 1 × 3 m spacing under a 3 m-tall trellis with one string per plant. During all years, treatments were arranged in a randomized complete block design with four replications. Fungicides were applied with a backpack mist blower (Stihl, model no. SR420) in an application volume ranging from 327 to 1300 L ha^{-1} water, depending on plant size. To minimize the effects of potential drift of fungicides or inoculum onto adjacent plots, only the middle seven plants in each plot were evaluated for disease. The incidence of leaves with powdery mildew was assessed by arbitrarily selecting four leaves from both the east and west side of each plant and examining each leaf for signs of powdery mildew. In 2009 foliar disease evaluations began on 8 July and continued at approximately biweekly intervals until the end of August. In 2010 and 2011 initial foliar evaluations were 8 and 15 June, respectively, followed by evaluations approximately 1 month later then continuing at approximately biweekly intervals until late August. There was a minimum of five evaluations each year. Individual disease ratings were used to calculate standardized area under the disease progress curve (AUDPCS), which is the area under the disease progress curve standardized by duration of time over which disease assessments were conducted (Madden *et al.*, 2007).

The incidence of cones with powdery mildew was assessed at harvest by arbitrarily selecting one lateral branch from the top 2–3 m of each of 7 plants per plot. The cones were counted and visually inspected for signs of powdery mildew to determine disease incidence. An average of 163, 168 and 143 cones were assessed per plot in 2009, 2010 and 2011, respectively.

Plots were harvested in early September using a hop-picking machine to estimate cone yield on a per plant basis. The fresh weight of the cones for each plot was recorded, and a subsample of cones (*c.* 70 g) was collected and dried for 48–72 h at 60°C in order to determine percentage dry matter. This value was then used to standardize yields to dry weight per plant.

A second subset of cones collected was also dried overnight from *c.* 67–75% moisture content (treatment dependent) to 8–10% moisture. From these cones, bittering acid content was determined by standard spectrophotometric methods (ASBC, 2009). A subsample of the dried cones were provided to a hop brokerage company and rated using their standard rating scale of 1–10 for cone colour and aroma. In the colour scale, a rating of 10 indicates the best possible colour and smaller values indicate increasing degrees of brown discoloration, with 1 being the worst or lowest possible score. An aroma rating of 10 indicates an aroma typical of a given cultivar, and perception of uncharacteristic aromas leads to progressively lower ratings. The ratings were conducted subjectively, although this is a standard process for hop quality evaluations. In all years, evaluations were conducted in a blind manner where the cone samples were coded so that the rater was unaware of the treatment each sample of cones received. The same person performed the ratings in all years.

Commercial hop yard plots

During each year, subsets of fungicide treatments were also applied to a commercial hop (cultivar Zeus) near Toppenish, Washington. The crop was produced using standard production practices, including weekly to biweekly applications of sulphur fungicides applied to the entire hop yard up to the first week of July. Plots were then established in the yard and arranged as a randomized complete block design with five replications, with each replicate plot consisting of three rows each containing 13 plants. Plots were separated by three rows that were left non-treated after 15 July, which was approximately the beginning of bloom stage (Kavalier *et al.*, 2011). On 15 July of each year, the entire field received an application of quinoxyfen at the rate of 146 g ha^{-1}. The subsequent date of the last fungicide application was staggered at 2-week intervals in each treatment so that the last fungicide application dates were 15 July (no additional applications after bloom), 29 July (one additional application), 12 August (two additional applications) and 27 August (three additional applications) during 2009. During 2010 and 2011, a fifth treatment was added so that the dates of the last fungicide applications were 29 July, 12 August, 24 August and 8 September for both years. Thus, the last fungicide application corresponded approximately to cone developmental stages I, II, III, IV and V, respectively, as described by Kavalier *et al.* (2011). Detailed description of the developmental stages can be found at http://pubs.acs.org/doi/suppl/10.1021/jf1049084/suppl_file/jf1049084_si_001.pdf. In brief, stage I corresponds to the late stages of bloom; stage II is a transition stage from bloom to cone development wherein stigmas begin to abscise and bract and bracteole development become conspicuous; in stage III, cone volume and mass begins to increase linearly, stigmas are fully senesced and abscised, and bracteoles are approximately half the length of the bract; in stage IV, development of bracteoles proceeds such that bracteoles are slightly open and no longer closely enclose the ovary; stage V is full maturity, wherein cone mass is maximal and bracteoles have fully elongated to approximately the same length as the bracts.

In all years, the application made in stage II of cone development (late July) was quinoxyfen applied at the rate of 146 g ha^{-1}. The application made in stages III and IV of cone development (mid- and late August) consisted of a mixture of pyraclostrobin and boscalid (Pristine, BASF) applied at the rate of 251 and 496 g ha^{-1}, respectively. In 2010 and 2011, the fungicide application made at stage V (early September) was

Table 1 Yield, bittering acid content and incidence of hop cones with powdery mildew in relation to fungicide programme and date of the last application in cultivar Galena, Prosser, WA, USA 2009–2011[a]

					Yield (kg/string)[d]			Bittering acids (%)[e]		
Year	Fungicide treatment[b]	Last appl.	AUDPCS[c]	Diseased cones (%)	Cone weight	Alpha-acid	Dry matter (%)	Alpha	Beta	HSI
2009	Non-treated	–	0·49a	96·5a	0·37	0·037a	27·2	10·05	6·25	0·25
	TQTQTQTQ	24-Aug	0·20cb	36·7de	0·44	0·046ab	24·7	10·39	6·20	0·24
	QTQTQTQT	24-Aug	0·14c	38·0cde	0·43	0·048bc	25·1	11·29	6·79	0·24
	QQTTQQTT	24-Aug	0·24bc	40·0cde	0·51	0·057c	25·6	11·22	6·57	0·25
	TTQQTTQQ	24-Aug	0·24bc	42·3cde	0·47	0·049bc	25·4	10·48	6·38	0·23
	TTQQTTQ	10-Aug	0·22bc	41·9cde	0·43	0·050bc	25·4	11·56	6·85	0·24
	QQTTQQT	10-Aug	0·24b	43·8de	0·42	0·047bc	25·2	11·13	6·57	0·25
	TTQQTT	27-July	0·17bc	48·8bcd	0·38	0·042ab	24·7	11·10	6·78	0·24
	QQTTQQ	27-July	0·22bc	32·5e	0·39	0·043ab	25·9	11·00	6·54	0·24
	TTQQT	13-July	0·19cb	62·5b	0·44	0·047bc	26·0	10·67	6·29	0·24
	QQTTQ	13-July	0·26b	53·3bc	0·43	0·046b	25·9	10·70	6·28	0·24
	P-value		<0·001	<0·001	0·126	0·006	0·078	0·200	0·383	0·494
2010	Non-treated	–	0·19a	42·8a	0·21	0·024	27·3	11·26	6·33	0·20
	TQTQTQTQ	24-Aug	0·16ab	27·1ab	0·28	0·032	24·5	11·84	6·58	0·19
	QTQTQTQT	24-Aug	0·18a	26·5ab	0·24	0·027	26·4	11·11	6·18	0·21
	QQTTQQTT	24-Aug	0·11bc	20·0b	0·31	0·035	26·6	11·37	6·40	0·21
	TTQQTTQQ	24-Aug	0·11bc	16·2b	0·26	0·031	26·5	11·58	6·32	0·19
	QTQTQTQT	17-Aug	0·14abc	27·2ab	0·21	0·026	26·1	12·24	6·69	0·20
	TQTQTQTQ	17-Aug	0·15abc	24·3ab	0·23	0·025	26·8	10·92	5·95	0·17
	TTQQTTQ	10-Aug	0·11bc	16·1b	0·25	0·028	26·6	11·34	6·36	0·20
	QQTTQQT	10-Aug	0·10c	14·4b	0·30	0·034	26·0	11·17	6·34	0·21
	TTQQTT	27-Jul	0·12bc	23ab	0·25	0·027	26·9	10·96	6·20	0·21
	QQTTQQ	27-Jul	0·11bc	15·3b	0·22	0·026	24·3	11·71	6·51	0·21
	TTQQT	13-Jul	0·12bc	40·7a	0·26	0·031	27·2	11·73	6·61	0·20
	QQTTQ	13-Jul	0·12bc	39·9a	0·28	0·034	26·3	12·19	6·73	0·20
	P-value		0·036	0·021	0·752	0·680	0·344	0·769	0·772	0·293
2011	Non-treated	–	0·08ab	96·7a	0·03	0·0036	29·5	12·05	7·41	0·19
	TQTQTQTQ	29-Aug	0·04cd	75·3abc	0·03	0·0035	28·3	11·94	7·18	0·20
	QTQTQTQT	29-Aug	0·04cd	59·9c	0·04	0·0047	28·5	11·86	7·18	0·19
	QQTTQQTT	29-Aug	0·08abcd	81·3abc	0·03	0·0039	29·3	12·26	7·38	0·19
	TTQQTTQQ	29-Aug	0·06abcd	86·3a	0·03	0·0036	28·8	12·34	7·31	0·20
	QQTTQQTT	24-Aug	0·04bcd	69·4abc	0·03	0·0041	28·0	12·08	7·38	0·21
	TTQQTTQQ	24-Aug	0·03d	86·2a	0·03	0·0036	28·5	12·30	7·65	0·22
	TTQQTTQ	17-Aug	0·06abcd	83·3ab	0·03	0·0040	29·3	11·84	7·54	0·21
	QQTTQQT	17-Aug	0·10a	81·4abc	0·03	0·0039	28·8	12·28	7·49	0·18
	TTQQTT	4-Aug	0·07abcd	87·5a	0·03	0·0032	28·8	12·84	7·99	0·20
	QQTTQQ	4-Aug	0·07abcd	75·5abc	0·03	0·0038	28·3	12·06	7·63	0·22
	TTQQT	20-Jul	0·10a	88·9a	0·03	0·0031	28·0	12·35	7·82	0·21
	QQTTQ	20-Jul	0·08abc	79·9abc	0·03	0·0039	28·5	11·51	7·23	0·20
	P-value		0·049	<0·001	0·211	0·396	0·767	0·540	0·149	0·870

[a]Treatments within a column and the same year followed by different letters are significantly different based upon an *F*-protected pairwise comparison of least-square means ($\alpha = 0{\cdot}05$).

[b]Sequence of applications. Q: quinoxyfen; T: trifloxystrobin.

[c]Standardized area under the disease progress curve for the incidence of leaves with powdery mildew based on assessments during June–August. AUDPCS is area under the disease progress curve standardized by duration of time over which disease assessments were conducted (page 108 in Madden *et al.*, 2007). In 2011, treatments with AUDPCS = 0·04 have identical numerical values due to rounding at the third decimal place; however, significant mean differences denoted by letters are correct.

[d]Yield was estimated by harvesting seven plants per plot, with each plant having one string.

[e]Determined by American Society of Brewing Chemists spectrophotometric method (ASBC, 2009). HSI: hop storage index, a measure of loss of bittering acids.

tebuconazole (Monsoon, Loveland Products, Inc.) applied at the rate of 252 g ha^{-1}. Fungicides were applied at the highest rate allowed by the manufacturers' label with an air blast orchard sprayer (LectroBlast, Progressive Ag., Inc) in an application volume of 375 L ha^{-1}, which was the standard practice of the cooperating grower. Disease assessments and yield measurements were conducted as described above, except that 10 leaves were assessed per plant during disease assessments and measurements were always taken on 10 plants in the middle of the centre row of each plot. Harvest dates were 14 or 15 September in all years. Chemical analysis and cone evaluations were conducted similarly to the experimental plots.

Simulated late harvest

During 2010 and 2011, the impact of simulated late harvests on cone quality factors was also assessed. Lateral branches bearing cones that remained after the harvest on 14 September were collected from heights of *c.* 3, 4 and 5 m from each of 10 plants per plot. These later harvests occurred on 21 September and 28 September in both 2010 and 2011. The cones were collected from the branches using a hop-picking machine to simulate mechanical harvest, and cone samples were collected for determination of dry matter, bittering acids and quality evaluations as described previously.

Statistical analysis

For both the experimental and commercial hop yard plots, differences among means of the response variables for each treatment were analysed in a mixed model using the MIXED or GLIMMIX procedures in SAS v. 9.3 (SAS Institute; Littell *et al.*, 2006). Repeated measures analyses were applied for the

Table 2 Effect of fungicide treatments and last application date on hop cone colour and aroma of cv. Galena in experimental plots at Prosser, WA, USA 2009–2011

Year	Fungicide treatment[a]	Last application	Colour[bc]			Aroma[bc]		
			Median	Mean rank	Relative marginal effect	Median	Mean rank	Relative marginal effect
2009	Non-treated	–	4·5	3·3	0·07 (0·05–0·17)	9	8·0	0·18 (0·07–0·56)
	TQTQTQTQ	24-Aug	9	35·8	0·84 (0·67–0·91)*	10	24·0	0·56 (0·52–0·60)
	QTQTQTQT	24-Aug	8	27·5	0·64 (0·56–0·72)*	10	24·0	0·56 (0·52–0·60)
	QQTTQQTT	24-Aug	8	23·5	0·55 (0·37–0·71)*	10	24·0	0·56 (0·52–0·60)
	TTQQTTQQ	24-Aug	9	35·8	0·84 (0·67–0·91)*	10	24·0	0·56 (0·52–0·60)
	TTQQTTQ	10-Aug	8	27·3	0·64 (0·42–0·80)*	10	24·0	0·56 (0·52–0·60)
	QQTTQQT	10-Aug	7·5	21·5	0·50 (0·36–0·64)*	10	24·0	0·56 (0·52–0·60)
	TTQQTT	27-July	7·5	24·3	0·57 (0·33–0·77)*	10	18·9	0·44 (0·25–0·65)
	QQTTQQ	27-July	7	16·4	0·38 (0·23–0·56)*	10	18·9	0·44 (0·25–0·65)
	TTQQT	13-July	6	9·8	0·22 (0·13–0·37)	10	24·0	0·56 (0·52–0·60)
	QQTTQ	13-July	6	9·1	0·21 (0·14–0·31)	10	24·0	0·56 (0·52–0·60)
		P-value			<0·0001			0·0711
2010	Non-treated	–	4·5	9·8	0·18 (0·10–0·35)	10	22·8	0·44 (0·24–0·66)
	TQTQTQTQ	24-Aug	5·5	27·8	0·53 (0·28–0·77)	10	29·0	0·56 (0·52–0·60)
	QTQTQTQT	24-Aug	5·5	27·8	0·53 (0·28–0·77)	10	29·0	0·56 (0·52–0·60)
	QQTTQQTT	24-Aug	5	19·9	0·38 (0·23–0·56)	10	29·0	0·56 (0·52–0·60)
	TTQQTTQQ	24-Aug	6	32·1	0·62 (0·37–0·81)	10	29·0	0·56 (0·52–0·60)
	QTQTQTQT	17-Aug	6	36·5	0·71 (0·55–0·82)	10	29·0	0·56 (0·52–0·60)
	TQTQTQTQ	17-Aug	6·5	40·0	0·77 (0·58–0·88)	10	29·0	0·56 (0·52–0·60)
	TTQQTTQ	10-Aug	6	32·1	0·62 (0·37–0·81)	10	29·0	0·56 (0·52–0·60)
	QQTTQQT	10-Aug	6	27·6	0·53 (0·18–0·85)	9·5	15·8	0·30 (0·12–0·62)
	TTQQTT	27-July	6	28·6	0·55 (0·38–0·71)	10	29·0	0·56 (0·52–0·60)
	QQTTQQ	27-July	6	22·8	0·44 (0·15–0·80)	9·0	12·3	0·23 (0·07–0·64)
	TTQQT	13-July	5·5	21·7	0·42 (0·21–0·67)	10	29·0	0·56 (0·52–0·60)
	QQTTQ	13-July	4·5	10·5	0·20 (0·11–0·34)	10	22·8	0·44 (0·24–0·66)
		P-value			0·132			0·168
2011	Non-treated	–	4	18·38	0·34 (0·15–0·63)	10	27·5	0·52 (0·49–0·55)
	TQTQTQTQ	29-Aug	4	24·88	0·47 (0·21–0·75)	10	27·5	0·52 (0·49–0·55)
	QTQTQTQT	29-Aug	5	35·63	0·68 (0·39–0·86)	10	27·5	0·52 (0·49–0·55)
	QQTTQQTT	29-Aug	4	21·88	0·41 (0·24–0·62)	10	27·5	0·52 (0·49–0·55)
	TTQQTTQQ	29-Aug	4·5	30·25	0·57 (0·29–0·81)	10	27·5	0·52 (0·49–0·55)
	QQTTQQTT	24-Aug	4	21·88	0·41 (0·24–0·62)	10	27·5	0·52 (0·49–0·55)
	TTQQTTQQ	24-Aug	5	35·63	0·68 (0·39–0·86)	10	21·0	0·39 (0·21–0·63)
	TTQQTTQ	17-Aug	4	27·25	0·51 (0·30–0·72)	10	27·5	0·52 (0·49–0·55)
	QQTTQQT	17-Aug	5	32·63	0·62 (0·40–0·79)	10	27·5	0·52 (0·49–0·55)
	TTQQTT	4-Aug	4	18·38	0·34 (0·15–0·63)	10	27·5	0·52 (0·49–0·55)
	QQTTQQ	4-Aug	5	35·63	0·68 (0·39–0·86)	10	27·5	0·52 (0·49–0·55)
	TTQQT	20-July	4·5	27·25	0·51 (0·30–0·72)	10	21	0·39 (0·21–0·63)
	QQTTQ	20-July	3·5	14·88	0·28 (0·09–0·64)	10	27·5	0·52 (0·49–0·55)
		P-value			0·387	–	–	0·399

[a]Sequence of applications. Q: quinoxyfen; T: trifloxystrobin.

[b]Colour and aroma ratings were conducted by a commercial hop merchant. Values are a 1–10 scale, where 10 is the highest quality.

[c]Ordinal data was analysed using a nonparametric ANOVA-type statistic. Mean rank is the mean mid-rank of the ordinal value for each experimental unit. Relative marginal effect ranges from 0 to 1 and provides a probability measure that one random variable is larger than another random variable (Brunner *et al.*, 2002). The 95% confidence intervals for a given experiment are provided parenthetically. Asterisks indicate significant differences compared to non-treated plots.

bittering acid, dry matter and hop storage index values for the commercial hop yard data collected over time during 2010 and 2011. Each experiment was analysed separately and block was considered a random factor. Ordinal ratings for cone colour and aroma were analysed using a nonparametric ANOVA-type statistic appropriate for designed experiments and repeated measurements (Shah & Madden, 2004). In this analysis, a relative marginal effect is a statistic that ranges from 0 to 1 and is calculated for each treatment, based on an empirical distribution function of ranks of the medians (explained in Shah & Madden, 2004). Relative marginal effects represent probabilities that one random variable is larger than another. (Although the statistical terminology refers to this statistic as a 'relative' marginal effect, relative in this sense should not be confused with the calculation of relative yield to standardize data collected in separate experiments.) The data were ranked and a mean rank was calculated to obtain a single value for each experimental unit. Differences between treatments were considered statistically significant when 95% confidence intervals for the relative treatment effects did not overlap. Analyses were conducted in PROC MIXED in SAS using macros developed by Brunner *et al.* (2002) and available on the website of L. Madden, The Ohio State University.

For the data from experimental plots, the fungicide treatments resulted in a range of cones with powdery mildew within and between years. To standardize the effect of disease control among years, the percentage difference in alpha-acid content, cone yield and bittering acid yield (the product of cone yield and bittering acid content) were expressed relative to that of the non-treated plot (within a replication) for a given experiment. In the commercial hop yard plots, measurements were standardized to that of the plots where fungicide applications ceased on 15 July. The general equation was percentage relative to the non-treated = 100 × [(value of treated/value of non-treated) − 1]. The standardized alpha-acid and yield variables were related to the incidence of cones with powdery mildew, percentage dry matter of cones at harvest, and cone colour through scatterplots and regression analyses. These exploratory analyses indicated that numerous variables were interrelated with possible direct

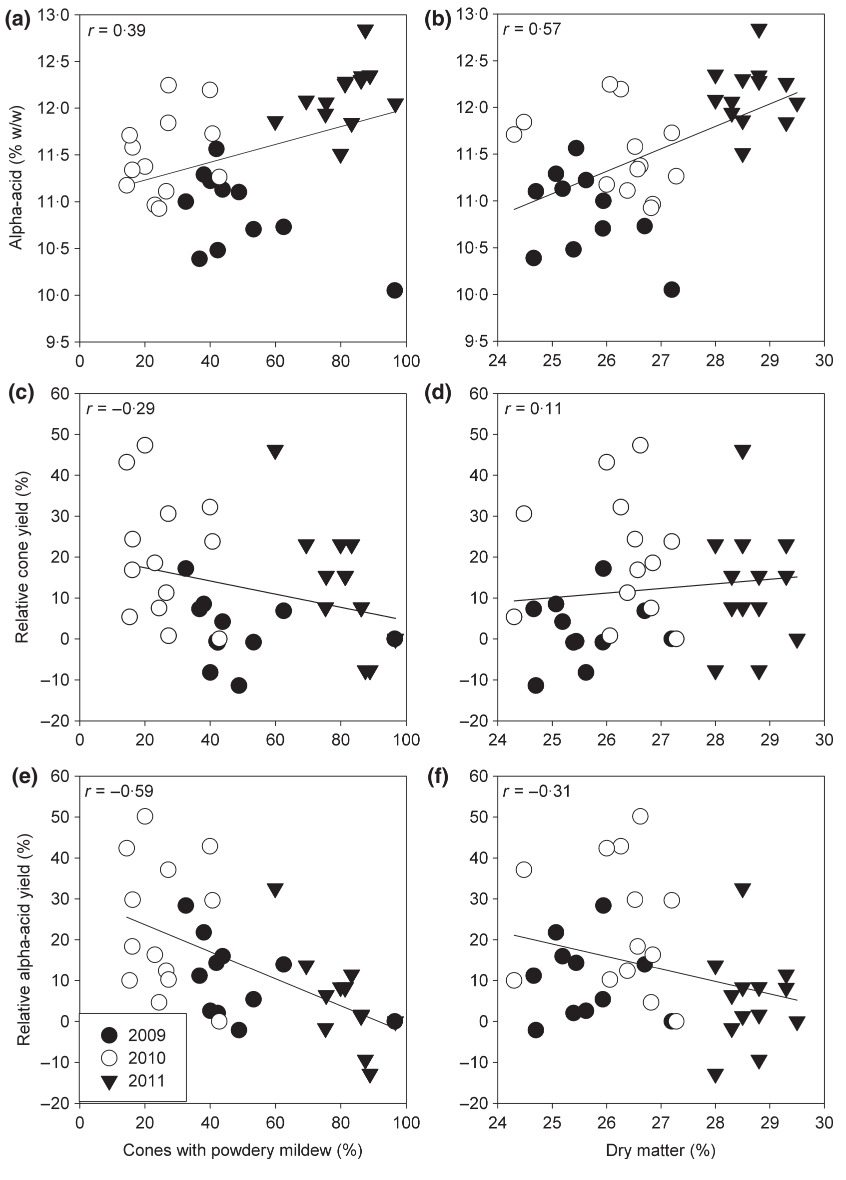

Figure 1 Association of percentage cones with powdery mildew (a, c, e) and dry matter (b, d, f) to alpha-acid content (a, b), and percentage gain/reduction in cone yield (c, d) and alpha-acid yield (e, f) relative to the non-treated control within a given experiment. The value of Pearson's correlation coefficient is shown numerically in each figure. Data is for cv. Galena in experimental plots from 2009 to 2011.

and indirect effects on components of cone yield and quality. Path analysis was conducted to quantify possible interactions among the variables and develop models of how powdery mildew occurrence on cones is associated with alpha-acid yield. Competing models were fitted, and the simplest, best fitting model was selected based on Akaike's information criterion (AIC) (Loehlin, 1987).

Path coefficients are standardized partial regression coefficients (β weights) that indicate the magnitude and sign (positive or negative) of direct effects of variables when other variables are held constant. Path coefficients can be decomposed to infer direct and indirect causal effects of an exogenous variable on an endogenous variable. Direct effects are the standardized path coefficients of a path denoted by an arrow connecting two variables. Indirect effects are the association of one variable with another mediated through one or more other variables. Indirect effects are calculated as the sum of the product of the path coefficients linking two variables. The total effect is the sum of direct and indirect effects (Loehlin, 1987).

Several plausible hypotheses for how powdery mildew affects alpha-acid yield were constructed as path diagrams (Loehlin, 1987). In all models, the effect of powdery mildew was modelled to directly affect dry matter, which in turn affected alpha-acid content of cones and the final alpha-acid yield. In the simplest model, the powdery mildew occurrence on cones was modelled to have only indirect effects on alpha-acid yield through its effect on dry matter. Other path diagrams modelled powdery mildew to have both indirect and direct effects on cone yield and/or alpha-acid content of cones.

To conduct the analysis, a correlation matrix of the variables used in the models was constructed using the CORR procedure in SAS, which was then subsequently analysed using the CALIS procedure in SAS. Goodness-of-fit of the models was assessed with a chi-square test and by inspection of residual diagrams. Each model was fitted separately and the model that minimized AIC was considered the superior, or best fitting, model. The significance of each path was assessed by *t*-tests; values >|2| indicated significant paths ($\alpha = 0{\cdot}05$).

In the commercial hop yard plots, the incidence of cones with powdery mildew was nearly 100% in most experiments and varied little among treatments (described below). Therefore, correlations between disease incidence and other variables were not considered for these data. To quantify the effect of late season fungicide applications on yield and bittering acids, the yield values were standardized among years as percentage gain or reduction relative to fungicide applications that ceased at bloom. This was done for each treatment–year combination and then each year was considered an experimental unit (replication) and individual plots subsamples within years. Treatment means were then analysed in a mixed model in SAS, with year a random effect in the analysis.

The association between relative alpha-acid increase with post-bloom fungicide applications, dry matter and cone colour were expressed by linear or non-linear regression in SIGMAPLOT 11 (Systat Software, Inc.). The best-fitting models that provided a reasonable description of the data (e.g. appropriate to the variables and biological relationships) were selected based on the pseudo-R^2, standard error of parameter estimates and inspection of residual plots.

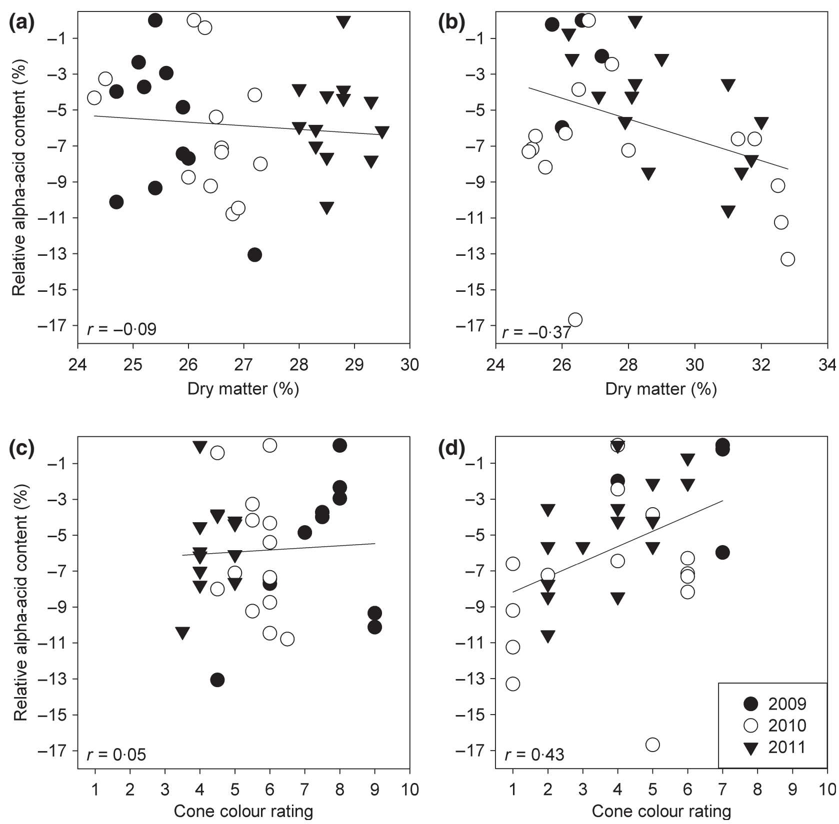

Figure 2 Relative alpha-acid content of cones in relation to percentage dry matter and cone colour. Relative alpha-acid content was calculated as percentage gain/reduction compared to a non-treated control within an experiment for cv. Galena (a, c) or relative to plots where fungicide applications ceased at bloom in cv. Zeus (b, d). The value of Pearson's correlation coefficient is shown numerically in each figure. Data is from 2009 to 2011.

Results

Experimental plots

In cultivar Galena, fungicide treatments significantly reduced powdery mildew on both leaves (summarized as AUDPCS) and cones during every year of the study (Table 1). There were no systematic increases in foliar disease incidence when six fungicide applications were made (and applications ceased during 27 July to 4 August) compared to a full season regimen of eight applications. Fungicide applications made until 27 July to 4 August (stage II of cone development) resulted in similar incidence of cones with powdery mildew compared to treatments that continued to be applied after this time, with one exception (treatments 28 in 2011, which received six fungicide applications). Alpha-acid yield was significantly affected by fungicide treatment only in 2009; however, treatment differences were not consistently related to the temporal duration of fungicide applications. In all studies, dry matter, levels of bittering acids and hop storage index were not significantly affected by fungicide treatment.

Cone colour was significantly affected by fungicide treatment during 2009 (Table 2). Median cone colour was improved by at least 2·5 colour categories when fungicides were applied through to at least 27 July (stage II) compared to ceasing applications prior to this date. In 2010 and 2011, there was a tendency for cone colour and aroma to be worse in non-treated plots compared to plots that received some level of fungicides (Table 2), although colour and aroma were numerically and statistically similar among treatments in these years.

When pooled data from 2009 to 2011 was analysed, there were significant correlations between the incidence of cones with powdery mildew and alpha-acid content ($r = 0{\cdot}39$; $P = 0{\cdot}016$), relative cone yield ($r = -0{\cdot}29$; $P = 0{\cdot}084$) and relative alpha-acid yield ($r = -0{\cdot}59$; $P < 0{\cdot}0001$; Fig. 1). There were also significant correlations among alpha-acid content of cones ($r = 0{\cdot}57$; $P = 0{\cdot}0003$) and relative alpha-acid yield ($r = -0{\cdot}31$; $P = 0{\cdot}059$), but not relative cone yield ($r = 0{\cdot}11$; $P = 0{\cdot}499$). Alpha-acid content expressed relative to the non-treated control was independent of dry matter ($r = -0{\cdot}09$; $P < 0{\cdot}0001$) and colour ($r = 0{\cdot}05$; $P < 0{\cdot}0001$; Fig. 2a,c). The incidence of cones with powdery mildew, dry matter and cone colour were interrelated (Fig. 3). The incidence of cones with powdery mildew was negatively associated with cone colour rating ($r = -0{\cdot}56$; $P < 0{\cdot}0003$) and positively associated with dry matter ($r = 0{\cdot}76$; $P < 0{\cdot}0001$). In turn, cone colour was also negatively associated with dry matter ($r = -0{\cdot}79$; $P < 0{\cdot}0001$).

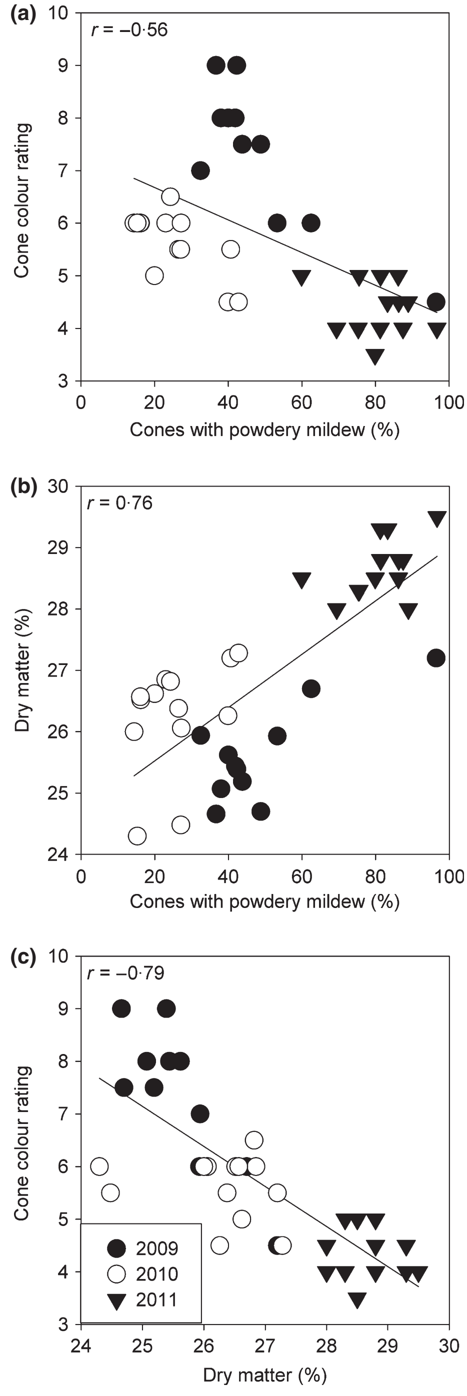

Figure 3 Associations between the incidence of cones with powdery mildew, cone colour and dry matter in cv. Galena. The value of Pearson's correlation coefficient is shown numerically in each figure. Data is from 2009 to 2011.

Path analysis

Structural equations that modelled a direct effect of powdery mildew on cone yield, as expressed in Fig. 4a,b, adequately fit the data (chi square goodness of fit $P = 0{\cdot}913$ and $0{\cdot}836$, respectively). The model that assumed powdery mildew affected alpha-acid yield only via indirect effects did not provide an adequate fit ($P = 0{\cdot}004$). The relationship between incidence of cones with powdery mildew and potential determinates of

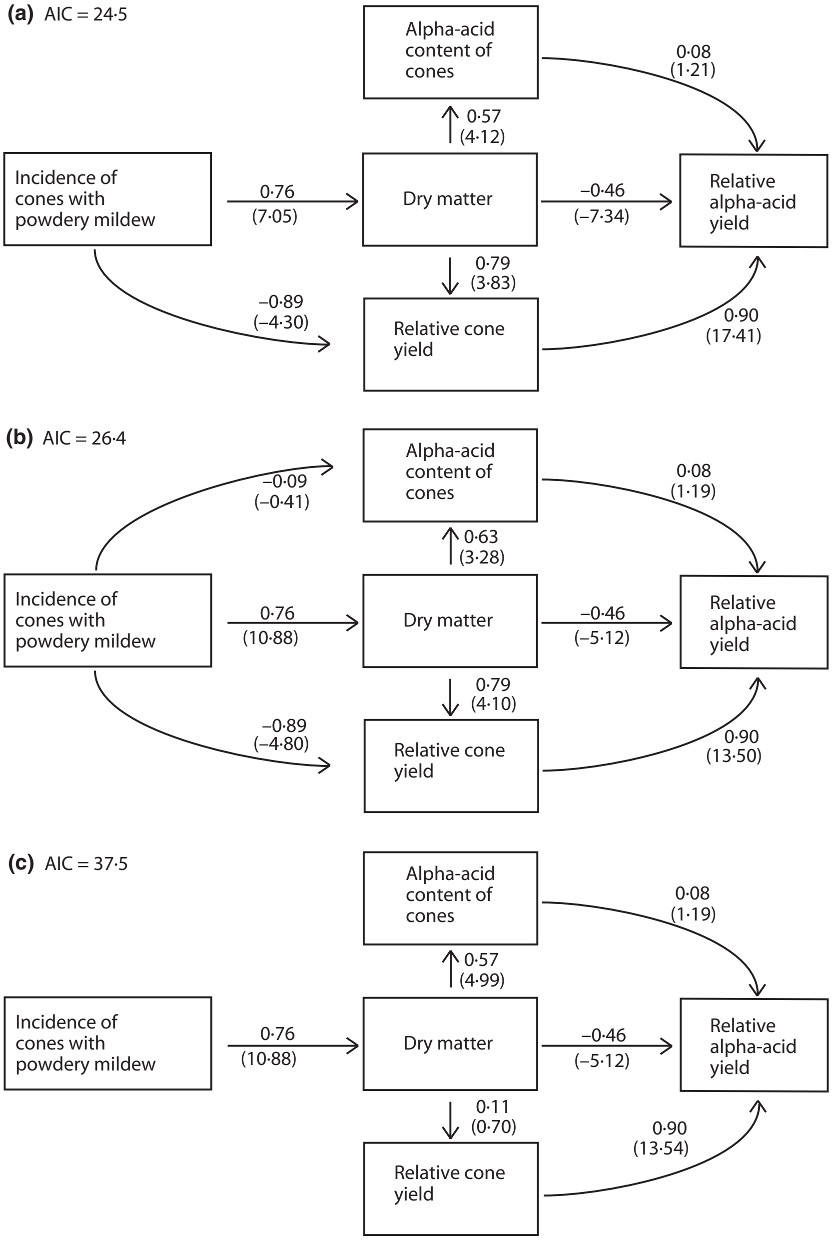

Figure 4 Path diagrams depicting three models for crop damage caused by powdery mildew on hop cones. In (a), powdery mildew is modelled to have direct effects on alpha-acid yield through decreasing cone yield and indirect effects through impacts on dry matter of cones. In (b), both direct and indirect effects on cone yield and alpha-acid content of cones are modelled. In (c), only indirect effects are modelled via impacts on dry matter. Path coefficients are shown numerically on the figures and associated *t*-statistics are presented parenthetically. Values of $t > |2|$ indicate significant ($\alpha = 0.05$) paths. The value of Akaike's information criterion (AIC) is shown numerically; the smallest value of AIC in (a) indicates this is the best-fitting model.

alpha-acid yield (expressed relative to non-treated plots) provided evidence for a direct effect of powdery mildew on cone yield, but not alpha-acid content (Fig. 4a). The AIC was lowest for this model (AIC = 24·5). Further evidence for a minimal impact of powdery mildew on alpha-acid content in cv. Galena was the non-significant path coefficient (−0·09; $t = -0.41$) for the relationship between the incidence of cones with powdery mildew and alpha-acid content in the model presented in Fig. 4b.

In the best-fitting model, powdery mildew affected alpha-acid yield through its direct effects on cone yield (path coefficient −0·89) and dry matter (path coefficient 0·76). The total effect of powdery mildew on alpha-acid yield was −0·58 ($P < 0.0001$) and entirely indirect through these variables. Cone yield, as expected, had a positive and direct effect on alpha-acid yield (total effect 0·90; $P < 0.0001$). Dry matter had a direct effect on alpha-acid yield (−0·46; $P < 0.0001$), but also large indirect effects (0·76; $P < 0.0001$) on alpha-acid yield mediated through its effect on yield and alpha-acid content of cones. In this study, the variability in alpha-acid content of cones among treatments had only a small direct effect on total alpha-acid yield (0·08; $P = 0.235$).

Commercial hop yard

In cultivar Zeus, levels of powdery mildew on leaves were similar among the treatments evaluated in all years.

In 2009, the incidence of cones with powdery mildew was significantly reduced by fungicide applications made through to at least 29 July (stage II). Disease incidence was 98% or greater during 2010 and 2011, independent of intensity of fungicide programme (Table 3).

Cone yield and alpha-acid yield were similar among treatments during 2009 (Table 3). Under greater disease pressure in 2010, there was a tendency ($P = 0{\cdot}055$) for the greatest alpha-acid yield to occur when fungicide applications were made through to at least 12 August (stage III). This was associated with a propensity for greater yield ($P = 0{\cdot}088$) and alpha-acid content of cones ($P = 0{\cdot}036$) when fungicides were applied after 15 July (Table 4). Similarly in 2011, there was a tendency ($P = 0{\cdot}085$) for greater cone yield with fungicide applications made through to at least 28 July (stage II). Beta-acid content of cones was also affected by the fungicide treatments in 2010, with an increase from 3·6 to 4·1% when fungicide applications ceased on 15 July as compared to 12 August ($P = 0{\cdot}012$; Table 4).

Table 3 Powdery mildew incidence, yield and alpha-acid content of hop cones from commercial hop yard plots in relation to the date of the last fungicide application in cultivar Zeus, Toppenish, WA, USA 2009–2011

				Yield (kg/string)[c]	
Year	Last application[a]	AUDPCS[b]	Diseased cones (%)	Cone weight	Alpha-acid
2009	27-Aug	0·002	62·9b	1·08	0·146
	12-Aug	0·001	58·8b	1·06	0·143
	29-July	0·001	62·9b	1·18	0·151
	15-July	0·002	80·0a	1·10	0·145
	P-value	0·198	0·022	0·525	0·953
2010	8-Sept	0·074	100	0·85ab	0·100bc
	24-Aug	0·063	100	0·90b	0·105c
	12-Aug	0·074	99·8	0·83ab	0·098bc
	28-July	0·073	100	0·79a	0·093ab
	15-July	0·061	100	0·72a	0·074a
	P-value	0·879	0·459	0·088	0·055
2011	8-Sept	0·006	98	0·99a	0·143
	24-Aug	0·005	99	0·85ab	0·130
	12-Aug	0·007	98	0·95ab	0·145
	28-July	0·007	98	0·99a	0·149
	15-July	0·006	98	0·82b	0·119
	P-value	0·839	0·799	0·085	0·141

[a]In all years, plots receiving applications on a given date were treated with quinoxyfen in July, pyraclostrobin + boscalid in August, and tebuconazole in September.

[b]Standardized area under the disease progress curve for the incidence of leaves with powdery mildew based on biweekly disease assessments during June to August. AUDPCS is area under the disease progress curve standardized by duration of time over which disease assessments were conducted (page 108 in Madden *et al.*, 2007).

[c]Treatments within a column and year followed by different letters are significantly different based upon an *F*-protected pairwise comparison of least-square means ($\alpha = 0{\cdot}05$). Yield was estimated by harvesting up to 10 plants per plot, with each plant having two strings. Data are means of five replications.

Although differences in yield and bittering acid content were not consistently different in individual experiments, analysis of data over all 3 years did reveal significant differences in yield and bittering acid yield with late season applications relative to plots where fungicide applications ceased on 15 July. Relative bittering acid contents were similar among fungicide treatments for alpha-acid ($P = 0{\cdot}280$) and beta-acid ($P = 0{\cdot}092$; Fig. 5a). Cone yield was improved by 11·3% when fungicide applications were made through to late July ($P = 0{\cdot}045$) compared to ceasing fungicide applications at 15 July; subsequent fungicide applications did not significantly increase cone yield (Fig. 5b). Similarly, alpha-acid yield was increased 20% ($P \leq 0{\cdot}016$) and beta-acid yield was increased 22% ($P \leq 0{\cdot}027$) with fungicide applications through to at least late July. Fungicide applications made after late July had little effect on yield as yield gains were not statistically different from those achieved by controlling powdery mildew through to late July.

Simulated late harvest

In simulated late harvest, factors that influenced alpha-acid and beta-acid content of cones varied during 2010 and 2011. In 2010, alpha-acid content of cones depended on both the date of the last fungicide application and harvest date, but these factors were not significant during 2011 (Table 4).

There was not a systematic relationship between fungicide treatment and loss of bittering acids as measured by the hop storage index. In 2010, the hop storage index increased with harvest date ($P < 0{\cdot}0001$) although this effect was not observed in 2011 ($P \geq 0{\cdot}102$).

Dry matter percentage of cones increased with maturity during 2010 and 2011, increasing 6·6 and 4·3% between 14 September and 28 September, respectively ($P < 0{\cdot}0001$; Table 5). The intensity of late season fungicide applications had no discernible effect on dry matter or maturation rate in these experiments ($P \geq 0{\cdot}193$).

Cone colour was improved in 2009 and 2010 by certain fungicide treatments made after 15 July ($P = 0{\cdot}032$; Table 6). In 2009, fungicide applications made at least through to 29 July (stage II) improved cone colour compared to ceasing fungicides at 15 July. In 2010, differences in cone colour depended both on number of fungicide applications and harvest date (fungicide treatment × harvest data interactions $P = 0{\cdot}039$). Cone colour deteriorated in all treatments with time ($P < 0{\cdot}0001$), but the rapidity of colour loss was moderated by increasing number of fungicide applications (Table 6). In 2011, cone colour was relatively poor on all harvest dates and unaffected significantly by fungicide application intensity ($P = 0{\cdot}700$). Colour was strongly affected by harvest date ($P < 0{\cdot}0001$), deteriorating with later harvests, and this deterioration of colour was not influenced by the fungicide treatments (fungicide treatment × harvest data interactions $P = 0{\cdot}240$). Aroma evaluations were not significantly associated with fungicide treatment in any year ($P \geq 0{\cdot}102$).

Table 4 Bittering acid content and hop storage index (HSI) of cones from commercial hop yard plots in relation to the date of the last fungicide application and harvest in cultivar Zeus, Toppenish, WA, USA 2009–2011

		Bittering acids (%w/w) and storage index on three harvest dates[a]								
		14-Sept			21-Sept			28-Sept		
Year	Last application[b]	Alpha	Beta	HSI	Alpha	Beta	HSI	Alpha	Beta	HSI
2009	15-July	13·3	4·8	0·27	–	–	–	–	–	–
	29-July	12·8	4·7	0·27	–	–	–	–	–	–
	12-Aug	13·6	5·0	0·27	–	–	–	–	–	–
	27-Aug	13·5	5·0	0·27	–	–	–	–	–	–
2010	15-July	10·6a	3·6a	0·22bc	11·8	3·9	0·25	11·0a	3·7	0·24
	28-July	11·9b	3·9ab	0·19a	12·4	4·2	0·24	11·5ab	3·9	0·25
	12-Aug	11·8ab	4·1b	0·23bc	12·7	4·1	0·23	11·9b	4·0	0·24
	24-Aug	11·7ab	3·9ab	0·23c	11·9	4·1	0·23	11·9b	3·9	0·24
	8-Sept	11·8ab	3·8ab	0·21ab	12·2	4·0	0·25	11·3ab	3·8	0·24
	Mean	11·6A	3·9A	0·22A	12·2B	4·1B	0·24B	11·5A	3·9A	0·24B
2011	15-July	13·6	4·5	0·24	13·6	4·8	0·24	13·0	4·2	0·23
	28-July	13·9	4·9	0·24	13·7	4·7	0·24	13·7	4·5	0·25
	12-Aug	14·1	4·9	0·25	13·4	4·6	0·24	12·7	4·1	0·24
	24-Aug	14·2	4·8	0·24	13·9	5·0	0·25	13·4	4·8	0·24
	8-Sept	13·4	4·8	0·25	13·0	4·8	0·25	13·1	4·3	0·24
	Mean	13·8A	4·8A	0·25A	13·6A	4·8A	0·24AB	13·2A	4·4B	0·24B
Type 3 tests of fixed effects P-values by year										
		2009			2010			2011		
Last application date		0·690	0·704	0·982	0·036	0·012	0·137	0·602	0·124	0·457
Harvest date					0·009	0·015	<0·001	0·618	0·002	0·102
Last application × harvest date					0·779	0·985	0·016	0·722	0·137	0·164

[a]Fungicide treatments within a column followed by different lower case letter are significantly different based upon an *F*-protected pairwise comparison of least-square means (α = 0·05). Statistical differences in the variables between sampling dates are noted by upper case letters in the row with average values. Bittering acids and HSI were measured using American Society of Brewing Chemist standard methods (ASBC, 2009).

[b]In all years, plots receiving applications on a given date were treated with quinoxyfen in July, pyraclostrobin + boscalid in August, and tebuconazole in September.

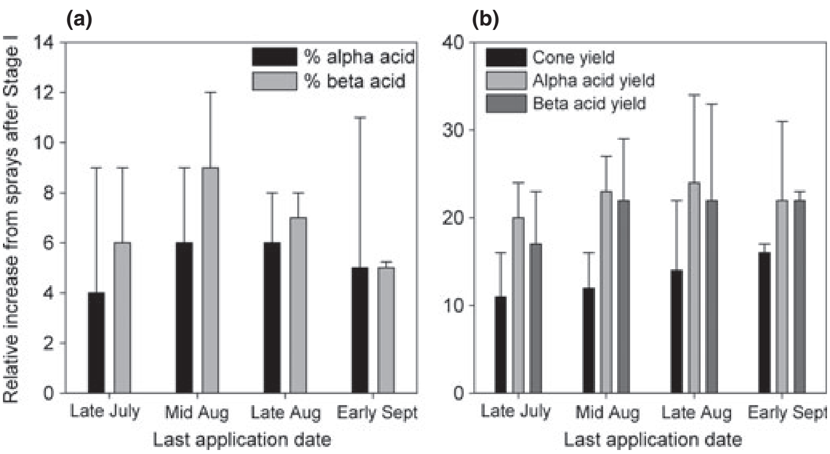

Figure 5 Mean increase in bittering acid content (a) and yield (b) resulting from late season fungicide applications (relative to ceasing fungicide applications at bloom) in cv. Zeus during 2009 to 2011. Mean increases are significantly >0 for cone yield (*P* = 0·045), alpha-acid yield (*P* = 0·016), and beta-acid yield (*P* = 0·027), but are not different among the last application dates for a given variable.

Relationships between dry matter, colour and relative alpha-acid yield

In cultivar Zeus, cone colour and dry matter were correlated with relative alpha-acid content of cones (Fig. 2b,d). Relative acid content of cones was negatively correlated with dry matter ($r = -0{\cdot}37$; $P = 0{\cdot}030$), although only relatively extreme levels of dry matter (i.e. >29%) were associated with reductions in alpha-acid. In contrast, relative alpha-acid content of cones was positively correlated with cone colour ($r = 0{\cdot}43$; $P = 0{\cdot}010$).

As with cultivar Galena, there was a clear relationship between cone colour and dry matter (Fig. 6a). Cone colour deteriorated rapidly with increasing dry matter above *c.* 25%, independent of fungicide treatment, and was

Table 5 Dry matter of hop cones from commercial hop yard plots in relation to the date of the last fungicide application and harvest date in cultivar Zeus, Toppenish, WA, USA 2011

	Dry matter (%) on three harvest dates[a]						
	2009	2010			2011		
Last application	14-Sept	14-Sept	21-Sept	29-Sept	14-Sept	21-Sept	29-Sept
15-July	27·2	26·4	28·0	32·8	27·1	28·1	31·4
28-July	26·0	26·1	27·5	32·5	26·3	28·2	31·0
12-Aug	26·6	25·1	26·8	31·8	26·2	27·9	31·0
24-Aug	25·7	25·5	25·2	31·3	28·2	29·0	32·0
8-Sept	–	25·0	26·5	32·6	27·9	28·6	31·7
	–	25·6A	26·8B	32·2C	27·1A	28·4B	31·4C
Type 3 tests of fixed effects P-values							
Last application date	0·493	0·193			0·674		
Harvest date		<0·0001			<0·0001		
Last application × harvest date		0·570			0·711		

[a]Fungicide treatments within a column followed by different lower case letter are significantly different based upon an *F*-protected pairwise comparison of least-square means ($\alpha = 0{\cdot}05$). Statistical differences in the variables between sampling dates are noted by upper case letters in the row with average values.

well-described ($R^2 = 0{\cdot}86$) as an exponential decay given by the following equation:

$$\text{Colour rating} = 33.48e^{0.042(\text{dry matter})}$$

There was a relationship between relative alpha-acid yield increase associated with fungicide treatments and dry matter content of the cones when they were harvested (Fig. 6b). The yield benefit of fungicide applications made after 15 July was dependent on the dry matter of the cones at harvest. The greatest yield benefit from fungicide treatments was realized when harvest occurred at a dry matter content of *c.* 25%, with the yield benefit of the fungicides diminishing as dry matter increased. The relationship was adequately described by a quadratic regression equation ($R^2 = 0{\cdot}62$): relative alpha-acid yield increase = 1278·3 – 87·33(dry matter) + 1·502(dry matter)^2. There was a similar decline in relative yield increase and cone colour, described by the equation: relative alpha-acid yield increase = $1{\cdot}896e^{0{\cdot}387(\text{cone colour})}$ ($R^2 = 0{\cdot}27$; Fig. 6c). The greatest yield benefit from fungicide applications occurred when cone colour was greatest at harvest, which was a value of 7 or greater on the merchant rating scale in this study.

Discussion

In this research relationships have been established between the incidence of powdery mildew on cones and resulting yield and quality factors. In a given experiment, the effect of fungicide treatments and disease on yield and quality was not always clear or statistically significant. With multiple years of observations, however, it is apparent that the incidence of cones with powdery mildew is linearly correlated with cone yield, bittering acid yield and accelerated maturation of cones as measured by dry matter content. In cultivar Galena, the cumulative effect of every 1% increase in cones with powdery mildew was to reduce alpha-acid yield by 0·33% (Fig. 1e). A similar relationship could not be derived for cultivar Zeus because the incidence of cones with powdery mildew was nearly 100% in the experiments in 2010 and 2011 despite the regular application of fungicides. Nonetheless, alpha-acid yield was increased 20% by controlling powdery mildew through stage II of cone development as compared to ceasing fungicide applications at bloom. Subsequent improvements in alpha-acid yield tended to be more modest with additional applications after stage II, which were 4·1% or less in these studies and statistically insignificant.

Impacts of the powdery mildew on perceptible aroma characteristics and bittering acid content of cones appears to be far less significant than alpha-acid yield. In cultivar Galena, powdery mildew had little effect on development of bittering acids or their stability (as measured by the hop storage index) when the disease was controlled through to bloom (or later). In the more susceptible cultivar Zeus, there was a weak trend for a greater bittering acid content of cones with control of powdery mildew through at least stage II of cone development (Fig. 5a). In this cultivar, though, alpha-acid content appears to be more strongly influenced by the maturity of cones at harvest than the number of late season fungicide applications (Fig. 2b). Alpha-acid content was negatively correlated with dry matter and positively correlated with colour. Relatively extreme over-maturity (dry matter >29%) and exceptionally poor colour were associated with 9% reductions in alpha-acid content of cones when harvested in late September as compared to harvest in mid-September. When harvested at dry matters of <29% there were no significant increases in alpha-acid content from fungicide applications made after stage II of cone development. Therefore, fungicide applications made through the transition stage of bloom to cone development appear adequate to maintain the alpha-acid content of cones, provided that cones are harvested before extreme over-maturity (i.e. dry matter >29%).

Table 6 Effect of last application date and harvest timing on hop cone colour and aroma in commercial hop yard plots at Toppenish, WA, USA 2009–2011

		Colour[bcd]			Aroma[bcd]		
Harvest date	Last application[a]	Median	Mean rank	Relative marginal effect	Median	Mean rank	Relative marginal effect
2009							
15-Sept	15-July	4	4·1	0·18 (0·14–0·35)	10	10	0·48 (0·29–0·67)
	29-July	7	11·8	0·50 (0·38–0·72)*	10	9·4	0·45 (0·26–0·67)
	12-Aug	7	12·8	0·62 (0·38–0·78)*	10	10·6	0·51 (0·32–0·68)
	27-Aug	7	13·3	0·64 (0·44–0·77)*	10	12	0·58 (0·39–0·73)
2010							
14-Sept	15-July	5	55·1	0·73 (0·63–0·81)	10	49	0·65 (0·63–0·67)
	28-July	6	62·7	0·83 (0·71–0·90)	10	49	0·65 (0·63–0·67)
	12-Aug	6	65·5	0·87 (0·79–0·91)	10	49	0·65 (0·63–0·67)
	24-Aug	6	59·9	0·79 (0·67–0·87)	10	49	0·65 (0·63–0·67)
	8-Sept	6	62·7	0·83 (0·72–0·90)	10	49	0·65 (0·63–0·67)
21-Sept	15-July	2	26·0	0·34 (0·24–0·46)	10	49	0·65 (0·63–0·67)
	28-July	4	33·1	0·43 (0·32–0·55)	10	49	0·65 (0·63–0·67)
	12-Aug	4	43·5	0·57 (0·51–0·64)*	10	49	0·65 (0·63–0·67)
	24-Aug	4	43·5	0·57 (0·44–0·70)	10	49	0·65 (0·63–0·67)
	8-Sept	5	45·5	0·60 (0·45–0·73)	10	49	0·65 (0·63–0·67)
28-Sept	15-July	1	14·5	0·19 (0·17–0·21)	5	15·6	0·20 (0·07–0·52)
	28-July	1	14·5	0·19 (0·17–0·21)	8	25·2	0·33 (0·15–0·60)
	12-Aug	1	14·5	0·19 (0·17–0·21)	9	17·0	0·22 (0·17–0·28)
	24-Aug	1	14·5	0·19 (0·17–0·21)	7	10·9	0·14 (0·09–0·23)
	8-Sept	1	14·5	0·19 (0·17–0·21)	7	11·3	0·14 (0·10–0·21)
2011							
14-Sept	15-July	5	55·6	0·73 (0·57–0·85)	10	25·5	0·33 (0·16–0·59)
	28-July	6	57·4	0·76 (0·60–0·86)	10	40·5	0·53 (0·51–0·56)
	12-Aug	6	60·5	0·80 (0·64–0·89)	10	40·5	0·53 (0·51–0·56)
	24-Aug	4	45·9	0·61 (0·38–0·79)	10	40·5	0·53 (0·51–0·56)
	8-Sept	3	39·7	0·52 (0·33–0·71)	10	33	0·43 (0·27–0·62)
21-Sept	15-July	4	40·8	0·54 (0·32–0·74)	10	40·5	0·53 (0·51–0·56)
	28-July	4	50·1	0·66 (0·46–0·81)	10	40·5	0·53 (0·51–0·56)
	12-Aug	5	47·6	0·63 (0·41–0·80)	10	40·5	0·53 (0·51–0·56)
	24-Aug	5	50	0·66 (0·49–0·79)	10	40·5	0·53 (0·51–0·56)
	8-Sept	4	44·6	0·59 (0·45–0·71)	10	40·5	0·53 (0·51–0·56)
28-Sept	15-July	2	11·8	0·15 (0·08–0·29)	10	33	0·43 (0·26–0·62)
	28-July	2	18·7	0·24 (0·13–0·43)	10	40·5	0·53 (0·51–0·56)
	12-Aug	2	18·7	0·24 (0·16–0·35)	10	33	0·43 (0·27–0·62)
	24-Aug	2	11·8	0·15 (0·08–0·31)	10	40·5	0·53 (0·51–0·56)
	8-Sept	2	16·8	0·22 (0·13–0·36)	10	40·5	0·53 (0·51–0·56)

[a]See text for detailed treatment information.

[b]Colour and aroma ratings were conducted by a commercial hop merchant. Values are a 1–10 scale, where 10 is the highest quality.

[c]Ordinal data was analysed using a nonparametric ANOVA-type statistic. Mean rank is the mean mid-rank of the ordinal value for each experimental unit. Relative marginal effect ranges from 0 to 1 and provides a probability measure that one random variable is larger than another random variable (Brunner *et al.*, 2002). The 95% confidence intervals for a given experiment are provided parenthetically.

[d]Asterisks indicate significant differences compared to applications terminated at 15 July for a given year and harvest date.

The damage caused by powdery mildew, as well as the effectiveness of fungicide applications made to manage the disease, appear closely linked to the dry matter content of cones at harvest. The individual correlation and path analyses clearly indicate that powdery mildew reduces alpha-acid yield primarily through its direct effect on cone yield but also indirectly through effects mediated by cone dry matter. Dry matter is a surrogate variable for cone maturity, and in this sense, the damage from powdery mildew on cones could be characterized as both an 'assimilate sapper' and 'senescence accelerator' (Boote *et al.*, 1983). The dry matter of cones is also related to their propensity to shatter during mechanized harvest, and the overall association between dry matter and alpha-acid yield probably reflects both accelerated senescence and cone damage during harvest activities.

Maturation of hop cones, as measured by dry matter content, is directly proportional to the incidence of powdery mildew, which in turn is inversely proportional to cone colour. As described previously, acceptable cone aroma and colour standards are subjective and vary depending on buyer standards and market conditions. This ambiguity renders definition of an economic damage

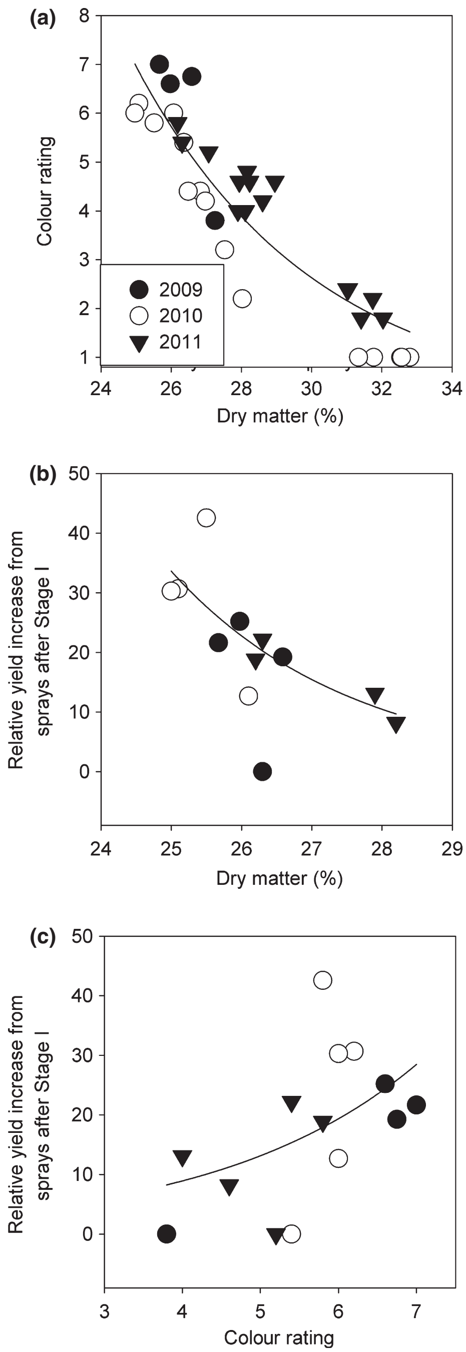

Figure 6 Association between cone colour, dry matter and relative yield increase from fungicide applications made after Stage I of cone development (bloom) in cv. Zeus from commercial hop yard plots. The lines are regression fits for the models (a) colour rating = $33{\cdot}48e^{0{\cdot}042(\text{dry matter})}$ with $R^2 = 0{\cdot}86$; (b) relative alpha-acid yield increase = $1278{\cdot}3 - 87{\cdot}33(\text{dry matter}) + 1{\cdot}502(\text{dry matter})^2$ with $R^2 = 0{\cdot}62$; and (c) relative alpha-acid yield increase = $1{\cdot}896e^{0{\cdot}387(\text{cone colour})}$ with $R^2 = 0{\cdot}27$.

threshold for quality factors exceedingly difficult. It is likely, though, that the relationships between powdery mildew, dry matter and colour exist for most cultivars. It seems reasonable that strategies that minimize powdery mildew, especially during the early stages of cone development, and lead to timely harvest could largely mitigate cosmetic defects caused by the disease.

Collectively, this research points to the critical importance of controlling powdery mildew during the early stages of cone development, specifically stages I and II, in order to minimize its impact. Improvements in disease control and yield with fungicide applications made after stage II of cone development were observed only with one treatment in the experiment with cultivar Zeus in 2010. This raises questions about the susceptibility of hop cones to powdery mildew in varying developmental stages. In many pathosystems, enhanced susceptibility of juvenile plants or tissues to powdery mildews is common (e.g. Turechek *et al*., 2001; Gadoury *et al*., 2003), a phenomenum termed ontogenic or age-related resistance. Preliminary studies indicate that juvenile hop cones are most susceptible to powdery mildew but do develop some level of ontogenic resistance (Seigner *et al*., 2003; Wolfenbarger *et al*., 2012). Studies are underway to more fully characterize ontogenic resistance in hop cones and its implications for disease management. If a period of juvenile susceptibility exists, there may be possibilities for modelling crop damage as a function of time of infection. Time of infection models have been recommended for systemic diseases such as certain viruses or soilborne pathogens that affect entire plants (Madden *et al*., 2007). An analogous situation can be considered for harvested products (hop cones) where the damage from powdery mildew is most detrimental in juvenile stages but damage is progressively reduced with later infection due to expression of ontogenic resistance.

The current study points to several strategies to mitigate crop damage caused by powdery mildew. Even under relatively low disease pressure on leaves, control of powdery mildew through the early stages of cone development appears warranted in eastern Washington State to maximize yield potential and cone colour. Additional fungicide applications may not be warranted in cultivars destined for alpha-acid extraction because alpha-acid yield was similar with fungicide applications ceasing at stages II to III of cone development versus fungicide programmes that continued until just before harvest. Importantly though, realizing achievable alpha-acid yield potential in these cultivars requires harvest before cone maturity exceeds *c*. 25% dry matter. The yield benefits of fungicide applications diminished as cones matured beyond this point. Targeted fungicide application and timely harvest are predicted to not only maximize yield, but also maximize cone colour. Such a strategy would also eliminate one or more applications of synthetic fungicides, which could have implications for fungicide resistance management. However, the optimal duration of fungicide applications may need to consider the potential for inoculum carryover into the following season. Studies are underway to clarify how late season disease management practices influence outbreaks of powdery mildew in the ensuing season.

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