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Genetic parameters of husk spot resistance in macadamia breeding families

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Abstract Husk spot caused by the *Pseudocercospora macadamiae* fungus induces premature abscission of fruit in many industry standard macadamia cultivars. Fungicides and other management strategies add to farm costs, thus breeding for varietal resistance is important. Genetic parameters of husk spot symptom expression had not previously been estimated. To guide selection methods for field resistance, over 300 open-pollinated seedlings of 32 families and 24 parent genotypes were inoculated, and seven symptom expression traits were evaluated. Narrow-sense and broad-sense heritabilities were estimated, breeding values were predicted, and correlations between breeding values of trait pairs were tested for significance. The traits with the highest heritabilities were necrotic lesion number per fruit ($H^2 = 0.41–0.59$; $h^2 = 0.21–0.30$) and necrotic incidence ($H^2 = 0.19–0.27$; $h^2 = 0.17–0.24$). Breeding values of the two traits were highly correlated ($r = 0.98$; $p < 0.001$), suggesting that either trait could be used to indirectly select for the other. All genotypes expressed symptoms to some degree, however, breeding values for necrotic traits and symptom-induced premature abscission were low for clones and progeny of cultivar 'HAES791'. Necrotic trait breeding values were also promising for progeny of cultivar 'HAES246' and clones of Australian Macadamia Breeding Program elite selection, 'BAM263'. Having been identified as potentially partially resistant, these selections can now be further evaluated and used as parents of new progeny populations.

Keywords Macadamia · Husk spot · *Pseudocercospora macadamiae* · Fruit tree breeding · Disease resistance

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Introduction

Husk spot is one of the four most limiting diseases to production in Australian macadamia farms (Department of Agriculture and Fisheries 2019). Symptoms induced by the causal fungus, *Pseudocercospora macadamiae* occur only on macadamia pericarps (husks) (Beilharz et al. 2003). In

many industry-standard cultivars, symptomatic fruits abscise earlier than asymptomatic fruits, which can be problematic for farmers (Akinsanmi and Drenth 2012; Akinsanmi et al. 2007; Akinsanmi et al. 2012; Akinsanmi et al. 2016; Mayers 1997; Mayers et al. 1999; Miles et al. 2010b).

Most macadamia trees flower heavily, though fruit-set is typically below 10% of initial flower numbers (O'Connor 2019). While macadamia is predominantly outcrossing, several cultivars are partially self-compatible (Howell et al. 2016; Langdon et al. 2019; Pisanu et al. 2009). Historically, heterozygosity and allelic richness in *M. integrifolia*, *M. tetraphylla* and cultivars was reported as being high relative to other fruit crops (Nock et al. 2008, 2015; O'Connor et al. 2015; Peace 2005). More recently, genetic diversity of 32 progeny families of crosses between 29 cultivars based on heterozygosity was found to be generally lower than, or comparable to other fruit crops (O'Connor et al. 2019). Chloroplast genomic variation identified in *M. integrifolia* indicates further potential for genetic improvement (Nock et al. 2019). To produce new genetic combinations, seedling families are typically produced via open-, or controlled pollination among accessions with high breeding values for favoured selection traits (Topp et al. 2016, 2012, 2020). Clonal propagation of cultivars is predominantly conducted via grafting or rooted cuttings (Topp et al. 2020).

Commercially valuable traits of macadamia nuts such as size and kernel oil content develop over time while fruit are attached in the tree (McConchie et al. 1996). In Australia, most flower anthesis and fruit-set occurs during August–September (Boyton and Hardner 2002). Nut kernels typically attain commercial levels of size and oil content by March (McConchie et al. 1996; Trueman et al. 2000). Cultivars vary in their nut-drop pattern (the quantity of nuts that abscise per month over the season) (Topp et al. 2020), but nuts that have abscised prior to development of commercial traits may be classed as 'immature' and rejected by commercial processors (Australian Macadamia Society 2016). Accelerated abscission caused by husk spot can result in significant increases in immature rejects and, consequently, significant reductions in saleable yield (Akinsanmi and Drenth 2012; Akinsanmi et al. 2007).

The major source for reinfection from season to season is from husks infected with *P. macadamiae* that have failed to abscise (Miles et al. 2010b). Macadamia husks that have senesced without abscising, and thus remain in the tree canopy, are known as stick-tights (Hardner et al. 2009). Rain-splash can disperse conidia from diseased stick-tights to nearby fruit, where germinating conidia can penetrate husk stomata (Miles et al. 2009). Most infection occurs in fruit of approximately 2 mm and larger (Miles et al. 2010a). Symptoms become visible 5–8 weeks post-pathogen penetration (Wright 1993), commencing as chlorotic flecks that become sclerified, and progress to tan, brown or black necrotic lesions (Miles et al. 2009). The concentration of *P. macadamiae* DNA in infected tissue increases during early stages of symptom development (Ong et al. 2017). Sporulation can occur on chlorotic tissue (Wright 1993), though the density of mycelia and conidial production appear to increase as lesions progress (Miles et al. 2009). Early work identified that the disease is predominantly asexual (Akinsanmi and Drenth 2010), with the most recent genetic study finding P. macadamiae isolates to be clonal (Ong et al. 2017).

Husk spot is predominantly managed with prophylactic fungicide applications (Akinsanmi and Drenth 2012; Akinsanmi et al. 2007). Synthetic abscission-promoting hormones (e.g. ethephon), and/or tree-shaking are also used to increase fruit abscission (Salter et al. 2005; Trueman 2003; Trueman et al. 2002) and, consequently, reduce stick-tight prevalence (Akinsanmi and Drenth 2016; Drenth and Akinsanmi 2012). However, fungicides add to farm costs, and efficacy varies with cultivar and environmental conditions (Akinsanmi and Drenth 2012), as do tree-shaking and hormonal applications (Salter et al. 2005). Resistance or tolerance to husk spot is recognised by industry as a valuable selection trait when breeding for new varieties (O'Hare and Topp 2010). Three mechanisms of plant defense to, or avoidance of husk spot have been suggested, including: (1) resistance to infection by *P. macadamiae*, (2) resistance to accelerated abscission of infected fruit, and (3) non-retention of stick-tights (Miles et al. 2010b). While stick-tight prevalence is currently selected against in breeding programs due to its provision of husk spot inoculum, the trait appears to be partially influenced by environment (Hardner et al. 2009; Miles et al. 2010b; Nunn et al. 2022b). Thus, additional forms of resistance could strengthen protection against husk spot.

Phenotypic variation has been observed in husk spot symptom expression among cultivars (Akinsanmi et al. 2012; Wright 1993), but genetic parameters of husk spot resistance traits have not previously been estimated. The degree of genetic variance in a trait, relative to its phenotypic variance indicates the potential for improved performance in future generations through selection (Falconer 1960). Broad-sense heritability (H^2), the proportion of phenotypic variance due to both additive and non-additive genetic effects (Falconer 1960), can be exploited through clonal propagation of macadamia genotypes (Hardner et al. 2001). The proportion of phenotypic variance due to additive genetic effects (narrow-sense heritability; h^2) is indicative of how well progeny phenotypes can be predicted from parent phenotypes (Falconer 1960). Thus, the breeding values (additive genetic effects) of parents, being the average effects of their genes that are transmitted to their progeny, can influence the genetic gain achieved over generations (Falconer 1960). To optimise genetic gain and the efficacy of breeding efforts, the choice of selection traits and breeding strategies should be based on the genetic parameters of selection traits (Acquaah 2012; Hansche 1983). Estimation of H^2 and h^2 and prediction of breeding values for husk spot resistance traits within current breeding germplasm will help to guide selection. Effective selection is particularly important, given that husk spot inoculations are laborious and time consuming (Nunn et al. 2022a) due to the large size of macadamia trees (Miles et al. 2010b), and that the long juvenility periods of macadamia further impede breeding efficiency (Topp et al. 2020).

Inoculated trees that express low husk spot incidence (proportion of fruit with symptoms) and low disease severity (quantity and/or intensity of symptoms per fruit) indicate disease resistance. In previous studies, genotypes with high disease severity (numbers of lesions) have been described as tolerant to disease-induced abscission, based on their ability to retain fruit with high levels of disease before abscission (Akinsanmi et al 2012; Wright 1993). It is unknown whether genotypes with low disease severity in abscised fruit indicates high sensitivity to disease-induced abscission, or low susceptibility to infection. Thus, in this study, we define genotypes with low disease incidence and severity and low premature abscission as resistant to husk spot, primarily due to the genotype's ability to limit pathogen multiplication, even after infection.

To identify genotypes that are resistant to husk spot under high inoculum pressure, a diverse range of genotypes were inoculated and assessed in this study. Genetic parameters of several husk spot symptom traits were estimated to inform which offered the most potential for genetic gain. Specifically, to guide selection of field resistance to husk spot, this study aimed to: (1) inoculate and evaluate husk spot resistance in a genetically diverse macadamia breeding population; (2) estimate the heritability of husk spot symptom traits used for evaluating host resistance; (3) examine the relationships among these traits; and (4) identify the most resistant parents and progeny families within the population according to their breeding values.

Methods

Macadamia genotypes and trial site

This study was conducted on a subset of trees within an Australian macadamia breeding program population, located at Maroochy Research Station, Nambour, Queensland (Alam et al. 2018c; Topp et al. 2016). The population was planted in-field in 2011 at high density (1 m × 4 m spacing) and was drip-irrigated. Trees utilised in this study consisted of 316 open-pollinated seedling progeny made up of 32 half-sib families (each family consisted of open-pollinated seedlings from the same maternal parent) and replicated clonal cuttings of 24 of the maternal parent genotypes (Table 1). The range and mean number of seedlings per family was 2–14 and 9, respectively, and the range and mean number of clonal cuttings per parents was 0–7 and 3, respectively (Table 1). Maternal parents of all seedling progeny were known (Table 1). Parents consisted of a diverse range of cultivars and elite selections from Australian and USA breeding programs, as well as from the non-commercial species *M. jansenii* (Table 1). All trees were free of husk spot until the trial commenced and were treated with a standard insecticide schedule but no fungicides. To account for heterogeneity in slope and soil-type within the site, trees included in the study were divided into 25 incomplete blocks to be included in analyses.

Table 1 Numbers of clonal macadamia cuttings per parent genotype and open-pollinated seedlings per family inoculated with *Pseudocercospora macadamiae* and evaluated for husk spot symptoms

Origin of parent	Parent	Parentage of parent	No. of clones per parent	No. of seedlings per family
Australian heritage cultivar	D4	–	4	13
	Daddow	–	3	12
Australian Macadamia Breeding Program	BAM263	NG8 × HAES762	5	12
	C	HAES814 × A16	7	2
	E	HAES246 × A16	2	9
	F	HAES816 × A4	5	4
	G	Daddow × HAES246	6	6
	H	Daddow × A16	0	14
	I	A16 × HAES814	1	5
	J	A16 × HAES781	2	5
	K	HAES842 × Daddow	2	6
	L	HAES842 × Daddow	4	10
	M	Daddow × A16	2	8
	N	HAES842 × A16	0	11
	O	Daddow × A4	0	5
	P	A16 × HAES814	6	6
	Q	HAES246 × A16	4	7
	R	HAES842 × Daddow	5	6
	S	A16 × HAES814	0	9
	T	HAES849 × Daddow	5	10
Hawaiian Agricultural Experiment Station	HAES246	*M. integrifolia*	2	9
	HAES344	*M. integrifolia*	0	8
	HAES788	*M. integrifolia*	0	14
	HAES791	Tri-species hybrid	3	12
	HAES814	*M. integrifolia*	1	12
Hidden Valley Plantations	A268	HAES344 OP	0	11
	A376	–	2	10
	A38	Own choice OP	6	8
	A4	Renown OP x Own choice	4	11
	A538	–	4	11
Macadamia Conservation Trust	M141	–	1	6
Wild *Macadamia jansenii* germplasm	*M. jansenii*	*M. jansenii*	0	3

OP, Open-pollinated

Inoculation

Inoculations and phenotyping were carried out over the three fruiting seasons (October to April) of 2017–18, 2018–19 and 2020–21. Due to variation in flowering and fruit set among the breeding population, the number of trees inoculated varied among seasons, as did the number of fruit per tree. Inoculation methods were as described by Nunn et al. (2022a), which was based on the process described by Miles et al. (2010b), with some modification. Briefly, macadamia husks with visible husk spot lesions were collected from several cultivars including A16, HAES344 and HAES741 during July–August each year from orchards in Bundaberg and Gympie, Queensland. The fresh diseased husks were air dried and then stored in an airtight tub at 9.5 °C until use in October each

year. Approximately 75 g of husks were placed in each individual netted bag. To induce sporulation in lesions prior to use, the husk bags were dipped in water for 10 s, then placed in an enclosed container to maintain high humidity and kept at room temperature (25 °C ± 2 °C) for 24 h.

The husk bags were attached to branches above clusters of developing fruit of match-head—to pea-size stage in each tree canopy in October of each fruiting season. Positions were chosen to capture the maximum number of fruit underneath each husk bag within an approximately 50 cm radius to enable dispersal of conidia via water-splash (Fitt et al. 1989). At least three husk bags were inserted per tree each year, based on the number of clusters of fruit available to be inoculated and assessed. Old husk bags from previous years remaining on the trees were not removed, to provide potential additional inoculum. Trees that received husk bags for more than one season were noted for inclusion in statistical analyses.

To capture abscised fruit for assessment, inoculated fruit clusters were enclosed in net bags in mid-December. The net bag enclosure date was chosen to be post the typical abscission of many small nuts that occurs independent of husk spot (Trueman and Turnbull 1994), but prior to appearance of husk spot symptoms (Miles et al. 2009,2010a) to lessen the effect of husk spot-independent abscission on assessment results. The field trials were maintained under natural environmental conditions, but to support conidial dispersal and germination in the absence of rainfall, trees were supplemented with overhead sprinkler irrigation for 30 min at 18:00, three times per week for four weeks in November each season.

Phenotyping

Each season, visual symptoms of inoculated fruit were evaluated twice at 4–6 week intervals starting at the full fruit-size stage during late-January. At both evaluation dates, abscised fruit within the net bags were collected and assessed, as were any fruit that were still attached at the final evaluation date. The mean number of inoculated fruit evaluated per tree per season was 17, with a range of 1–74. Symptoms were confirmed to be caused by *P. macadamiae* as per visual characteristics and tissue hardness (Beilharz et al. 2003; Drenth et al. 2009; Miles et al. 2009; Ong et al. 2017). To enable rapid phenotyping of hundreds of fruit every season, symptoms were evaluated using simple and efficient methods. Descriptions of husk spot symptom expression variables and the numbers of genotypes and trees per genotypes that each variable was assessed in are described in Table 2.

To account for variation in the timing of fruit development and nut-drop, and to avoid confounding such variation with other forms of disease resistance, the mode (most common) fruit size class at inoculation and the nut-drop pattern of asymptomatic fruit were noted for inclusion as fixed effects in analyses. Mode fruit size (FruitSize) was recorded as per stages modified from Miles et al. (2010a): 1 (match-head-size to pea-size); 2 (match-head-size to 50% expanded); and 3 (50% expanded to full size). For nut-drop pattern (DropPattern), the month when 50% of asymptomatic fruit had abscised was recorded and scored as 1 (February); 2 (March); 3 (April); or 4 (post-April).

Statistical analysis

Linear mixed models were fitted for each symptom expression variable using the asreml function in the ASReml-R package v4 (VSN International Ltd) (Butler et al. 2017) in R v4.1.0 (R Core Team 2021). To determine whether previous inoculations influenced current observations, the effect of the tree having been previously inoculated in an earlier season (PrevInoc) was fitted as a fixed effect within preliminary models for each tree in each season. FruitSize and DropPattern were also fitted as fixed effects in preliminary models for reasons previously explained. Block within season was fitted as a random term in all models to account for trial design. A relationship matrix was calculated from the available pedigree information, and its inverse computed in R package ASReml-R using the algorithm of Meuwissen and Luo (1992). Using this information on the relatedness of genotypes, together with the clonal replication of parental lines enabled estimation of total genetic effects and their partitioning into additive and non-additive components (Oakey et al. 2006). Interaction terms additive × season and non-additive × season were also fitted as random effects, reflecting additive and non-additive genetic variance across the seasons respectively, in all preliminary models.

Table 2 Husk spot symptom expression variables assessed in macadamia parents and progeny

Symptom variable	Assessment method	Season	No. of genotypes assessed (no. of clones per genotype)
Incidence (Inc)	Total proportion of inoculated fruit with any visible husk spot symptoms (ranging from chlorosis to necrosis) by the final evaluation date per tree	2017–18 2018–19 2020–21	88 (1–2) 333 (1–7) 144 (1–6)
Premature abscission of diseased fruit (PremAbs)	Proportion of fruit that had abscised with husk spot symptoms by late-January/early-February per tree		
Area affected (AreaAff)	Percentage of surface area of husk with symptoms per fruit. Each fruit was classified as 0, 1–30%, 31–60%, and 61–90% of surface area with symptoms and converted to midpoint proportions of each class (0, 0.15, 0.45 and 0.75) per fruit for analysis	2018–19 2020–21	333 (1–7) 144 (1–6)
Worst lesion intensity score (Intensity)	Most progressed stage of lesion development observed per tree as per classes modified from Miles et al. (2009) and Ong et al. (2017): 0 (asymptomatic (not shown)); 1 (chlorotic flecking (Fig. 1A)); 2 (chlorosis with tan-brown center (Fig. 1B)); 3 (necrotic dark tan spot (Fig. 1C)); 4 (necrotic tan/brown/black spot (Fig. 1D))	2018–19 2020–21	9 (3) 144 (1–6)
Necrotic incidence (NecInc)	Total proportion of inoculated fruit with tan, brown, or black lesions (Fig. 1C and D) by the final evaluation date per tree		
Lesion number score (LesNo)	As per classes modified from Akinsanmi et al. (2012), lesions per fruit were classified as: asymptomatic (0 lesions), low (1–4 lesions), medium (5–10 lesions) and high (> 10 lesions), and these scores were converted to 0, 1, 2, and 3 per fruit for analysis		
Necrotic lesion number score (NecLesNo)	As per LesNo, except only for tan, brown, or black lesions		

To confirm model assumptions of homogeneity of variance and normality of residuals, distributions of residuals for each symptom expression variable were visually checked, and transformations of observations were implemented where required. Inc, NecInc and PremAbs proportions were transformed with the empirical logit transformation (elogit), and as the number of fruit available for phenotyping differed among trees, inverse variances were included as weights in the models as per Cox and Snell (1989). Square-root-transformed (sqrt) LesNo and NecLesNo were used when fitting the final models. AreaAff and Intensity were not transformed as the distributions of the residuals were relatively normal. As Intensity data consisted of one score per tree, the observations were weighted as per the total number of fruit evaluated per tree to give more weight to observations based on higher numbers of fruit. This was unnecessary for LesNo, NecLesNo and AreaAff, as the data consisted of individual fruit observations.

For PremAbs, the data consisted of the proportions of symptomatic and asymptomatic fruit that had abscised by the first evaluation date, per tree for each fruiting season. An effect of disease status (DisStatus) was added to the PremAbs model to distinguish symptomatic from asymptomatic fruit proportions and to enable comparisons between the two effect levels. DropPattern was omitted from the PremAbs model, as the model only considered early-season abscission and the proportion of asymptomatic fruit

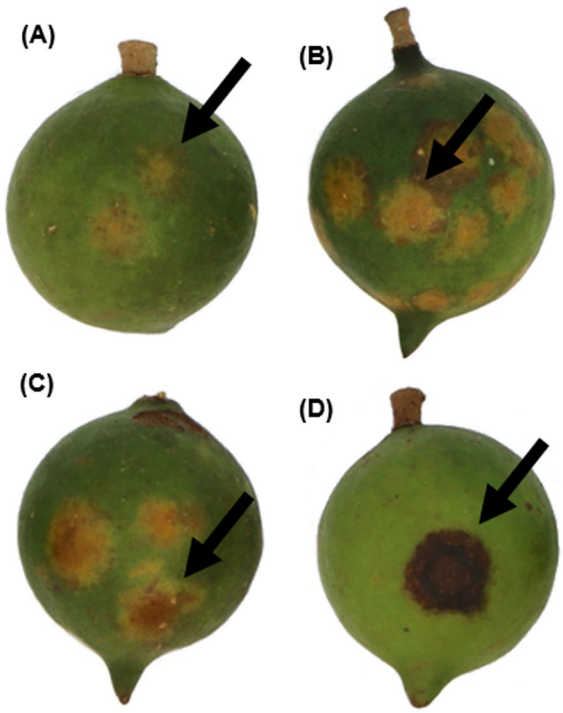

Fig. 1 Stages of husk spot lesion intensity progression indicated by black arrows: **A** chlorotic flecking; **2** chlorosis with tan-brown center; **C** necrotic dark tan spot; **D** necrotic tan/brown/black spot

was instead included to account for early-season drop pattern that was independent of husk spot.

The significance of fixed effects was tested for each model using Wald tests. The significance of additive × season and non-additive × season interaction effects was tested by generating full and reduced models for each term sequentially and performing Residual Maximum Likelihood Ratio Tests (adj-REMLRT) (Stram and Lee 1994) between each pair of full and reduced models. For variables where REMLRTs were not significant, the reduced models were interpreted as being as effective as the full model in modeling the response variable. To estimate variance components for each variable, the most parsimonious models were chosen, being:

Inc and NecInc

For proportion traits Inc and NecInc, the experimental units were defined by two factors, namely the individual tree (i) and the season (k). In addition, the trees were grouped into blocks (b) with each tree assigned a genotype (j) with a DropPattern (p). Hence the data can be represented by $y_{ik(bjp)}$, denoting the observation for the ith tree in the kth season, with associated block (b), genotype (j) and DropPattern (p), and the model for $y_{ik(bjp)}$ is given by:

$$y_{ik(bjp)} = s_k + d_p + b_{kb} + a_j + g_j + t_i + ts_{ik} + e_{ik}$$

where s_k is the fixed effect of the kth season, d_p is the fixed effect for the pth DropPattern, b_{kb} represents the random effect of the bth block in the kth season, with the vector of block effects, $\boldsymbol{b}$ having distribution, $\boldsymbol{b} \sim N(0, \sigma_b^2 \boldsymbol{I})$, a_j is the random additive genetic effect of the jth genotype across seasons (with distribution $\boldsymbol{a} \sim N(0, \sigma_a^2 \boldsymbol{A})$ where $\mathbf{A}$ is the known additive relationship matrix based on pedigree and σ_a^2 is the additive genetic variance), g_j is the random non-additive genetic effect (dominance and epistatic) for the jth genotype across seasons (with $\boldsymbol{g} \sim N\left(0, \sigma_{na}^2 \boldsymbol{I}\right)$, where $\boldsymbol{I}$ is the identity matrix and σ_{na}^2 is the non-additive genetic variance), t_i and ts_{ik} are random tree effects (with $\boldsymbol{t} \sim N\left(0, \sigma_t^2 \boldsymbol{I}\right)$ and $\boldsymbol{ts} \sim N\left(0, \sigma_{ts}^2 \boldsymbol{I}\right)$), an overall effect and an effect specific to seasons, reflecting the fact that the trees are repeatedly measured over time, and $e_{(ik)}$ are the residual effects, assumed to be normally distributed with mean zero. Here, the vector $\boldsymbol{e}$ of residual effects is the known error that reflects the variance of the empirical logit (Cox and Snell 1989). This term is $\boldsymbol{e} \sim N(0, \boldsymbol{V})$ where $\boldsymbol{V}$ is a diagonal variance matrix with elements $v_{ik} = \frac{(M_{ik}+1)(M_{ik}+2)}{M_{ik}(X_{ik}+1)(M_{ik}-X_{ik}+1)}$ where M_{ik} is the total number of fruit inoculated and X_{ik} is the number of symptomatic fruit for the ith tree in the kth season. This is fitted in ASReml by specifying weights which are the inverses of v_{ik}.

Intensity

For Intensity, the experimental units and all effects were as per Inc and NecInc, except for the vector $\boldsymbol{e}$, which was a vector of residuals based on weights which were the total counts of fruit assessed per tree.

AreaAff and LesNo

For AreaAff and LesNo, the experimental units were as per Inc and NecInc, and the model for $y_{ik(bjp)}$ is given by:

$$y_{ik(bjp)} = s_k + d_p + b_{kb} + a_j + as_{jk} + g_j + t_i + ts_{ik} + e_{ik}$$

where as_{jk} is the random additive genetic effect of the *jth* genotype in the *k*th season not explained by the additive main effects (with $\boldsymbol{as} \sim N\left(0, \sigma^2_{as}\boldsymbol{A}\right)$, where σ^2_{as} is the variance of additive genetic-by-season effects), and all other effects were as per Inc and NecInc, except for the vector $\boldsymbol{e}$, which was a vector of residuals with $\boldsymbol{e} \sim N\left(0, \sigma^2_e\boldsymbol{I}\right)$, and no weights were included.

NecLesNo

For NecLesNo, the experimental units and all effects were as per Inc and NecInc, except for the vector $\boldsymbol{e}$, which was a vector of residuals with $\boldsymbol{e} \sim N\left(0, \sigma^2_e\boldsymbol{I}\right)$, and no weights were included.

PremAbs

For proportion trait PremAbs, the experimental units were defined, and trees were grouped into blocks and assigned genotypes as per Inc and NecInc., with the additional factors FruitSize (*f*) and DisStatus (*d*). Hence the data can be represented by $y_{ik(bjfd)}$, denoting the observation for the *i*th tree in the *k*th season, with associated block (*b*), genotype (*j*), FruitSize (*f*) and DisStatus (*d*), and the model is given by:

$$y_{ik(bjfd)} = dfs_{dfk} + b_{kb} + a_{jk} + ad_{jkd} + g_j + gd_{jd} + gs_{jk} + gds_{jdk} + t_i + ts_{ik} + e_{ik}$$

where dfs_{dfk} is the fixed effect of the DisStatus × FruitSize × season interaction, b_{kb},a_{jk}, g_j are block, additive and non-additive genetic effects, defined previously, ad_{jkd} is the random additive genetic effect × DisStatus interaction, gd_{jd} is the random non-additive genetic effect x DisStatus interaction, g s_{jk} is the random non-additive genetic effect of the *jth* genotype in the *k*th season, not explained by the non-additive main effects and gds_{jdk} is the random non-additive genetic effect × DisStatus × season interaction, t_i and ts_{ik} are random tree effects and $e_{(ik)}$ are the residual effects, assumed to be normally distributed with mean zero that reflect the variance of the empirical logit, as per Inc and NecInc.

To identify preferred strategies for selection for future trials, individual narrow-sense (h^2) and broad-sense (H^2) heritabilities for all incidence and severity variables (Inc, NecInc, Intensity, AreaAff, LesNo, NecLesNo) were estimated for different theoretical sampling strategies ('selection units') using variance components obtained from the final models. As macadamia progeny trials typically consist of un-replicated seedlings, all heritabilities were estimated for the individual tree level. For symptom expression variables recorded as one value per tree (Inc, NecInc, and Intensity), heritabilities were estimated for observations recorded for one and two seasons. For symptom expression variables recorded as one value per fruit (AreaAff, LesNo and NecLesNo), heritabilities were estimated for observations recorded for one, and two seasons based on 10 and 20 fruit per observation.

Heritabilities and their standard errors were estimated using the ASReml-R vpredict function for Intensity, AreaAff, LesNo and NecLesNo. The same function was used to estimate Inc and NecInc heritabilities, but standard errors were not obtained for such, due to the inclusion of weights in the models. Equations used to estimate heritabilities were as follows:

Inc, NecInc and Intensity

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{na} + \sigma^2_t + \sigma^2_{ts} + \sigma^2_e} \quad (1)$$

$$H^2 = \frac{\sigma^2_a + \sigma^2_{na}}{\sigma^2_a + \sigma^2_{na} + \sigma^2_t + \sigma^2_{ts} + \sigma^2_e} \quad (2)$$

where σ^2_a was the additive genetic variance, σ^2_{na} was the non-additive genetic variance, σ^2_t was the individual tree variance, σ^2_{ts} was the variance of the tree × season interaction, and σ^2_e was the residual error variance (where $\sigma^2_e = \overline{v}$, where $\overline{v}$ is the mean of v_i). To estimate heritabilities based on one season of observations for Inc and NecInc, Eqs. 1 and 2 were used as presented, and for two seasons of observations, σ^2_{ts} and σ^2_e were each divided by two.

AreaAff and LesNo.

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{na} + \sigma^2_{as} + \sigma^2_t + \sigma^2_{ts} + \sigma^2_e} \quad (3)$$

$$H^2 = \frac{\sigma^2_a + \sigma^2_{na}}{\sigma^2_a + \sigma^2_{na} + \sigma^2_{as} + \sigma^2_t + \sigma^2_{ts} + \sigma^2_e} \quad (4)$$

where *n* was the number of fruit per sample and all other terms were as described for Eqs. 1 and 2. To estimate heritabilities based on one season of

observations for Area and LesNo, Eqs. 3 and 4 were used as presented, and for two seasons of observations, σ^2_{as}, σ^2_{ts} and σ^2_e/n were each divided by two.

NecLesNo

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{na} + \sigma^2_t + \sigma^2_{ts} + \sigma^2_e/n} \quad (5)$$

$$H^2 = \frac{\sigma^2_a + \sigma^2_{na}}{\sigma^2_a + \sigma^2_{na} + \sigma^2_t + \sigma^2_{ts} + \sigma^2_e/n} \quad (6)$$

where n was the number of fruit per sample and all other terms were as described for Eqs. 1 and 2. To estimate heritabilities based on one season of observations for NecLesNo, Eqs. 5 and 6 were used as presented, and for two seasons of observations, σ^2_{ts} and σ^2_e/n were each divided by two.

To enable selection of parents for use in creating future progeny populations, breeding values, being additive genetic effects, were estimated for all genotypes using best linear unbiased predictions (BLUPs). For variables where the additive genetic × season interaction term was significant, breeding values were predicted for individual years from the interaction term. For variables where the additive genetic × season interaction term was not significant, and thus, was omitted from final models, breeding values were predicted from the additive genetic term. To obtain predicted values on the scale of assessment, additive genetic predictions that included overall means and that were adjusted for fixed effects were made for each genotype using the predict function in ASRreml and any transformed values were then back-transformed for interpretability, as per Kostick et al. (2021).

To explore relationships among incidence and severity variables, Pearson's correlation coefficients and their corresponding p-values based on their t-scores were calculated for breeding values of each pair of traits using the cor.test function in base R. For PremAbs, breeding values were predicted for both levels of DisStatus for each season to enable comparisons between symptomatic and asymptomatic fruit predictions within genotypes. Correlations were therefore not estimated for PremAbs breeding values, as to interpret PremAbs breeding values, it is necessary to consider both levels of predictions. For example, a genotype with a high PremAbs breeding value for symptomatic fruit, and a low PremAbs breeding value for asymptomatic fruit indicates a propensity toward premature abscission induced by husk spot symptoms (based on additive genetic effects). Whereas, a genotype with high PremAbs breeding values for both symptomatic and asymptomatic fruit, may indicate a propensity toward early fruit drop, independent of husk spot.

Results

The effects included in final models varied between measured variables. No significant effect from PrevInoc or FruitSize was detected ($p > 0.05$) for any incidence or severity variables (Inc, NecInc, AreaAff, LesNo, NecLesNo, and Intensity), thus, both effects were omitted from the six final models. DropPattern was only significant for Inc, AreaAff and Intensity ($p < 0.05$; Table 3), but was retained in all incidence and severity models, as it reduced the residual error terms. Non-additive genetic × season interaction effects were not significant for any incidence or severity variable, so were omitted from all final models. As additive genetic × season interaction effects were significant for AreaAff ($p = 0.003$; Table 3) and LesNo ($p = 0.038$; Table 3), the term was retained and used to predict breeding values for both variables (Tables 4 and 5). No significant additive genetic × season interaction effect was detected for Inc, NecInc, Intensity and NecLesNo and as such, the interaction term was omitted from those four final models and breeding values were predicted from the additive genetic term (Tables 4 and 5).

Non-additive genetic variance was low relative to additive genetic variance for most incidence and severity variables, with Inc being the only variable where that of non-additive was greater than additive (where additive includes additive genetic + additive genetic × season variance) (Table 3). Estimates of H^2 and h^2 were highest for NecLesNo based on a selection unit of two seasons of evaluations with 20 fruit evaluated per tree ($H^2 = 0.59$, $h^2 = 0.30$; Table 3). Aside from NecLesNo, the only other variables with heritability estimates of 0.20 and higher, were Inc ($H^2 = 0.20$ for a selection unit of two seasons of evaluations), and NecInc ($H^2 = 0.27$, $h^2 = 0.24$ for a selection unit of two seasons of evaluations) (Table 3).

Table 3 Wald chi-squared statistics for fixed effects (*$p<0.05$; **$p<0.01$; ***$p<0.001$), and variance components and standard errors for random effects estimated for husk spot symptom expression variables from clonally replicated macadamia parent genotypes and open-pollinated half-sib seedling progeny. Individual broad-sense (H^2) and narrow-sense (h^2) heritabilities for alternative selection units (all based on selection units being one tree, but evaluated over one or two seasons, and for AreaAff, LesNo and NecLesNo, 10 or 20 nuts per tree within seasons) are also presented

Effect		Inc		NecInc	Intensity		AreaAff	LesNo	NecLesNo
Fixed	*df*	*Wald* χ^2	*df*	*Wald* χ^2					
Season	2	85.54***	1	4.67*	0.31		1.73	3.20	9.31**
DropPattern	4	6.27***	4	0.91	2.94*		4.26**	0.60	1.02
Random		σ^2							
Block 2018[a]		$1.01\times10^{-7}\pm0.00$		NA	NA		NA	NA	NA
Block 2019		$1.32\times10^{-1}\pm0.08$		$1.40\times10^{-7}\pm0.00$	$1.99\times10^{-7}\pm0.00$		$2.84\times10^{-3}\pm0.00$	$2.74\times10^{-2}\pm0.02$	$3.15\times10^{-8}\pm0.00$
Block 2021		$4.13\times10^{-7}\pm0.00$		$9.76\times10^{-2}\pm0.09$	$7.36\times10^{-4}\pm0.01$		$5.40\times10^{-9}\pm0.00$	$4.54\times10^{-4}\pm0.00$	$1.36\times10^{-2}\pm0.01$
Additive genetic		$7.05\times10^{-3}\pm0.08$		$3.28\times10^{-1}\pm0.26$	$5.87\times10^{-2}\pm0.04$		$1.18\times10^{-3}\pm0.00$	$9.13\times10^{-9}\pm0.00$	$3.67\times10^{-2}\pm0.03$
Non-additive genetic		$2.04\times10^{-1}\pm0.11$		$3.97\times10^{-2}\pm0.24$	$1.99\times10^{-7}\pm0.00$		$1.42\times10^{-8}\pm0.00$	$2.55\times10^{-3}\pm0.00$	$1.19\times10^{-2}\pm0.03$
Tree		$7.73\times10^{-7}\pm0.00$		$4.27\times10^{-1}\pm0.28$	$1.64\times10^{-7}\pm0.00$		$7.52\times10^{-3}\pm0.00$	$7.71\times10^{-4}\pm0.01$	$3.59\times10^{-2}\pm0.03$
Tree:Season		$6.67\times10^{-1}\pm0.10$		$4.57\times10^{-1}\pm0.27$	$1.12\times10^{-1}\pm0.06$		$9.71\times10^{-3}\pm0.00$	$2.29\times10^{-2}\pm0.01$	$6.15\times10^{-2}\pm0.02$
Additive genetic:Season[b]		NA		NA	NA		$3.66\times10^{-3}\pm0.00$	$5.97\times10^{-3}\pm0.00$	NA
Residual[c]		NA		NA	NA		$4.64\times10^{-2}\pm0.00$	$9.02\times10^{-2}\pm0.00$	$3.12\times10^{-1}\pm0.01$
Heritability	*# seasons*					*# fruit*			
H^2	*1*	0.11 ± 0.05		0.19 ± 0.11	0.03 ± 0.03	*10*	0.04 ± 0.08	0.06 ± 0.10	0.41 ± 0.19
						20	0.07 ± 0.12	0.11 ± 0.17	0.56 ± 0.23
	2	0.20 ± 0.07		0.27 ± 0.15	0.05 ± 0.06	*10*	0.05 ± 0.09	0.07 ± 0.11	0.45 ± 0.21
						20	0.07 ± 0.13	0.13 ± 0.20	0.59 ± 0.24
h^2	*1*	0.00 ± 0.04		0.17 ± 0.13	0.03 ± 0.03	*10*	0.04 ± 0.08	0.00 ± 0.00	0.21 ± 0.15
						20	0.07 ± 0.12	0.00 ± 0.00	0.23 ± 0.16
	2	0.01 ± 0.07		0.24 ± 0.18	0.05 ± 0.06	*10*	0.05 ± 0.09	0.00 ± 0.00	0.28 ± 0.20
						20	0.07 ± 0.13	0.00 ± 0.00	0.30 ± 0.21

Inc, proportion of inoculated fruit with symptoms; NecInc, proportion of inoculated fruit with necrosis; Intensity, worst stage of lesion progression per tree; AreaAff, percentage of fruit surface area discoloured by husk spot; LesNo, score for number of lesions per fruit; NecLesNo, score for number of necrotic lesions per fruit; DropPattern, fruit drop pattern independent of husk spot; NA, not applicable

[a]variance components labelled as NA were not obtained as data was not collected in 2018, thus the Block 2018 effect was not included in models

[b]variance components labelled as NA were not obtained as the Additive genetic:Season was not significant ($p>0.05$) and thus, was not included in final models

[c]residual variance components labelled as NA were not obtained as models were weighted

Table 4 Predicted values (breeding value plus overall mean) for husk spot symptom expression variables for clonally replicated macadamia parents. The overall mean of all parents is provided in bold for each variable

Parent	NecInc (proportion)	Intensity (score 0–4)	NecLesNo (score 0–3)	Year	AreaAff (%)	LesNo (score 0–3)	PremAbs: Symptomatic (proportion)	PremAbs: Asymptomatic (proportion)
A376	0.58	3.3	0.9	2019	52	–	0.69	0.60
				2021	48	2.7	0.46	0.52
A38	0.52	3.5	0.7	2019	49	2.3	0.35	0.35
				2021	48	2.6	0.53	0.42
A4	0.62	3.6	0.9	2019	37	1.9	0.34	0.36
				2021	53	2.7	0.53	0.46
A538	0.57	3.4	0.8	2019	40	2.1	0.41	0.36
				2021	45	2.5	0.48	0.47
D4	0.78	3.7	1.4	2019	40	–	0.44	0.41
				2021	58	2.9	0.41	0.52
Daddow	0.59	3.3	0.9	2019	48	–	0.67	0.53
				2021	48	2.6	0.56	0.54
M141	–	–	–	2019	51	–	0.28	0.28
				2021	–	–	–	–
HAES246	–	–	–	2019	50	–	0.55	0.40
				2021	–	–	–	–
HAES791	0.40	3.2	0.4	2019	44	–	0.41	0.64
				2021	44	2.7	0.47	0.45
HAES814	–	–	–	2019	53	–	0.57	–
				2021	–	–	–	–
BAM263	0.45	3.0	0.5	2019	44	–	0.36	0.38
				2021	40	2.6	0.53	0.43
C	0.68	3.4	1.1	2019	47	2.1	0.52	0.50
				2021	53	2.9	0.48	0.51
E	0.65	3.5	1.0	2019	48	–	0.51	–
				2021	51	2.8	0.50	0.46
F	0.62	3.5	1.0	2019	46	2.2	0.29	0.34
				2021	49	2.7	0.51	0.50
G	0.56	3.4	0.8	2019	51	2.3	0.56	0.42
				2021	49	2.7	0.49	0.47
I	0.70	3.6	1.2	2019	48	–	0.5	–
				2021	52	2.8	0.49	0.50
J	–	–	–	2019	42	–	0.52	0.40
				2021	–	–	–	–
K	0.60	3.1	0.9	2019	44	–	0.67	0.48
				2021	45	2.5	0.48	0.56
L	0.54	3.2	0.7	2019	44	2.3	0.62	0.43
				2021	40	2.3	0.56	0.49
M	–	–	–	2019	50	–	0.45	0.35
				2021	–	–	–	–
P	0.71	3.6	1.2	2019	55	2.2	0.50	0.43
				2021	58	2.9	0.49	0.50

Table 4 (continued)

Parent	NecInc (proportion)	Intensity (score 0–4)	NecLesNo (score 0–3)	Year	AreaAff (%)	LesNo (score 0–3)	PremAbs: Symptomatic (proportion)	PremAbs: Asymptomatic (proportion)
Q	0.67	3.4	1.0	2019	47	–	0.55	0.30
				2021	49	2.7	0.41	0.54
R	0.55	3.3	0.7	2019	43	–	0.57	0.46
				2021	46	2.5	0.57	0.59
T	0.56	3.3	0.8	2019	52	2.4	0.48	0.37
				2021	48	2.5	0.50	0.49
All	0.60	3.4	0.9	2019	47	2.2	0.49	0.42
				2021	49	2.7	0.50	0.50

NecInc, proportion of inoculated fruit with necrosis; Intensity, worst stage of lesion progression per tree; AreaAff, percentage of fruit surface area discoloured by husk spot; LesNo, score for number of lesions per fruit; NecLesNo, score for number of necrotic lesions per fruit

Endash (–) indicate that no clones of those parents were phenotyped for that variable

While some non-additive genetic variance was detected for Inc, due to minimal additive variance, predicted parent and progeny values did not vary. All parent and family mean predicted Inc values were 0.87. For all other variables, predicted values varied among progeny within families, as did mean predicted values among families and among parents (Tables 4 and 5). For all variables, overall means of predicted values were similar between parents and their progeny (Tables 4 and 5). All Intensity (most progressed stage of lesion development observed per tree) predicted values were three (necrotic dark tan spot (Fig. 1D)) or greater (Tables 4 and 5). Whereas NecInc (proportion of inoculated fruit with necrotic symptoms) predicted values ranged from 0.39 to 0.78 and NecLesNo (mean score for number of necrotic lesions per fruit) ranged from 0.4 to 1.4 (representing low mean score ranges of 0–4 necrotic lesions per fruit) (Tables 4 and 5). Overall, LesNo (mean score for number of lesions per fruit) predicted values ranged from 1.9 to 2.9 (representing medium scores of 5 or more lesions per fruit to high scores of 10 or more lesions per fruit) (Tables 4 and 5), though less variation was observed within the 2021 season (1.9–2.9 in 2019 and 2.3–2.8 in 2021). Similarly, when considering AreaAff (mean fruit surface area with symptoms) across both seasons, predicted values ranged from 37 to 58% (Tables 4 and 5), but variation among predictions within years was slightly less (37–55% in 2019 and 40–58% in 2021).

For PremAbs, the fixed DisStatus × season and Fruitsize × season interaction effects were significant ($p < 0.05$; Table 6). The highest variance for the additive genetic × DisStatus effect was detected in 2019, followed by 2021 (Table 6). There was no overall trend in the difference between predicted PremAbs values for symptomatic and non-symptomatic fruit among families or parents; for some families and parents in some years, the mean predicted PremAbs value for symptomatic fruit was higher than for non-symptomatic fruit, and vice versa.

As almost no additive genetic variation was detected for Incidence (Table 3), predicted NecInc and severity variable (NecLesNo, Intensity, LesNo, AreaAff) values were interpreted in conjunction with PremAbs to identify potentially resistant parents and progeny families. Of the parents evaluated, 'HAES791' and 'BAM263' had the lowest predicted NecInc and NecLesNo values, their Intensity and AreaAff predicted values were below the overall means, and their 2021 LesNo predicted values were equal to, or slightly lower than the overall mean (Table 4). For 'HAES791', predicted PremAbs values for symptomatic fruit were lower than, or almost equal to those of asymptomatic fruit across the two seasons (Table 4), indicating a lack of husk spot-induced premature abscission. For 'BAM263', the predicted 2019 PremAbs value for symptomatic fruit was almost equal to that of asymptomatic fruit, however, the 2021 value for symptomatic fruit was higher

Table 5 Means and ranges of predicted values (breeding value plus overall mean) for husk spot symptom expression variables for families of open-pollinated macadamia progeny (same maternal parent). The overall range and mean of all progeny of all families is provided in bold for each variable

Family	NecInc (proportion)	Intensity (score 0–4)	NecLesNo (score 0–3)	Year	AreaAff (%)	LesNo (score 0–3)	PremAbs (proportion)	
							Symptomatic	Asymptomatic
	Mean (range)	Mean (range)	Mean (range)		Mean (range)	Mean (range)	Mean (range)	Mean (range)
A268	–	–	–	2019	47 (44–51)	–	0.56 (0.42–0.68)	0.51 (0.50–0.51)
				2021	–	–	–	–
A376	–	–	–	2019	51 (46–54)	–	0.65 (0.56–0.79)	0.60 (0.56–0.64)
				2021	–	–	–	–
A38	0.57 (0.49–0.61)	3.3 (3.3–3.5)	0.8 (0.6–1.0)	2019	50 (47–52)	–	0.41 (0.32–0.50)	0.46 (0.40–0.52)
				2021	47 (43–50)	2.6 (2.4–2.7)	0.51 (0.47–0.60)	0.46 (0.41–0.49)
A4	0.59 (0.51–0.67)	3.4 (3.3–3.5)	0.8 (0.6–1.0)	2019	43 (40–46)	–	0.40 (0.31–0.61)	0.42 (0.36–0.58)
				2021	49 (46–52)	2.7 (2.7–2.7)	0.52 (0.47–0.58)	0.48 (0.45–0.52)
A538	0.57 (0.52–0.64)	3.3 (3.2–3.4)	0.8 (0.7–1.0)	2019	45 (42–48)	–	0.45 (0.35–0.65)	0.42 (0.33–0.57)
				2021	46 (42–49)	2.6 (2.4–2.6)	0.49 (0.46–0.54)	0.48 (0.45–0.51)
D4	0.68 (0.65–0.73)	3.5 (3.4–3.6)	1.1 (1.0–1.2)	2019	45 (41–48)	–	0.46 (0.36–0.63)	0.44 (0.37–0.50)
				2021	52 (49–55)	2.8 (2.7–2.8)	0.45 (0.39–0.52)	0.51 (0.48–0.55)
Daddow	0.56 (0.49–0.61)	3.2 (3.1–3.3)	0.8 (0.6–0.9)	2019	49 (45–52)	–	0.66 (0.49–0.80)	0.56 (0.41–0.67)
				2021	47 (43–51)	2.6 (2.5–2.7)	0.53 (0.49–0.55)	0.53 (0.48–0.57)
M141	0.64 (0.62–0.66)	3.3 (3.3–3.3)	1.1 (1.1–1.1)	2019	50 (47–53)	–	0.35 (0.27–0.44)	0.34 (0.32–0.36)
				2021	44 (43–45)	2.7 (2.7–2.7)	0.46 (0.43–0.48)	0.51 (0.48–0.54)
HAES246	0.46 (0.46–0.46)	3.1 (3.1–3.1)	0.5 (0.5–0.5)	2019	50 (46–52)	–	0.56 (0.42–0.68)	0.46 (0.40–0.59)
				2021	41 (41–41)	2.5 (2.5–2.5)	0.45 (0.45–0.45)	0.50 (0.50–0.50)
HAES344	–	–	–	2019	45 (41–49)	–	0.62 (0.56–0.71)	0.59 (0.46–0.74)
				2021	–	–	–	–
HAES788	–	–	–	2019	49 (45–52)	–	0.49 (0.39–0.68)	0.51 (0.42–0.57)
				2021	–	–	–	–
HAES791	0.45 (0.39–0.54)	3.2 (3.1–3.3)	0.5 (0.4–0.7)	2019	46 (42–50)	–	0.44 (0.31–0.56)	0.58 (0.48–0.66)
				2021	45 (41–50)	2.7 (2.6–2.7)	0.49 (0.45–0.56)	0.48 (0.43–0.52)

Table 5 (continued)

Family	NecInc (proportion)	Intensity (score 0–4)	NecLesNo (score 0–3)	Year	AreaAff (%)	LesNo (score 0–3)	PremAbs (proportion)	
							Symptomatic	Asymptomatic
	Mean (range)	Mean (range)	Mean (range)		Mean (range)	Mean (range)	Mean (range)	Mean (range)
HAES814	–	–	–	2019	52 (50–53)	–	0.55 (0.44–0.65)	0.61 (0.54–0.71)
				2021	–	–	–	–
BAM263	0.49 (0.40–0.58)	3.1 (3.0–3.2)	0.6 (0.5–0.8)	2019	47 (45–50)	–	0.40 (0.33–0.53)	0.43 (0.34–0.52)
				2021	43 (40–47)	2.6 (2.5–2.7)	0.52 (0.48–0.59)	0.46 (0.42–0.49)
C	0.59 (0.54–0.63)	3.3 (3.2–3.3)	0.8 (0.7–1.0)	2019	48 (47–50)	–	0.61 (0.61–0.61)	0.59 (0.59–0.59)
				2021	48 (46–50)	2.8 (2.8–2.8)	0.50 (0.47–0.53)	0.50 (0.49–0.51)
E	0.61 (0.56–0.66)	3.4 (3.3–3.5)	0.9 (0.8–1.1)	2019	49 (45–51)	–	0.50 (0.47–0.57)	0.45 (0.40–0.49)
				2021	50 (47–52)	2.7 (2.7–2.7)	0.50 (0.47–0.54)	0.49 (0.44–0.53)
F	0.58 (0.51–0.63)	3.3 (3.2–3.3)	0.9 (0.7–1.1)	2019	48 (46–49)	–	0.39 (0.32–0.44)	0.44 (0.44–0.44)
				2021	48 (45–51)	2.7 (2.7–2.7)	0.51 (0.49–0.52)	0.50 (0.50–0.50)
G	0.58 (0.48–0.65)	3.3 (3.2–3.4)	0.8 (0.6–0.9)	2019	52 (48–54)	–	0.48 (0.37–0.56)	0.40 (0.37–0.44)
				2021	50 (46–52)	2.7 (2.6–2.7)	0.51 (0.47–0.56)	0.48 (0.47–0.49)
H	–	–	–	2019	49 (45–52)	–	0.57 (0.42–0.74)	0.48 (0.35–0.62)
				2021	–	–	–	–
I	0.65 (0.63–0.68)	3.5 (3.4–3.5)	1.0 (1.0–1.1)	2019	49 (47–52)	–	0.46 (0.42–0.55)	0.49 (0.45–0.52)
				2021	50 (49–52)	2.7 (2.6–2.8)	0.50 (0.47–0.54)	0.50 (0.47–0.53)
J	–	–	–	2019	45 (42–48)	–	0.46 (0.43–0.51)	0.40 (0.39–0.40)
				2021	–	–	–	–
K	0.57 (0.50–0.61)	3.1 (3.1–3.2)	0.8 (0.6–0.9)	2019	47 (46–50)	–	0.63 (0.47–0.79)	0.46 (0.43–0.48)
				2021	45 (41–48)	2.5 (2.3–2.6)	0.49 (0.48–0.51)	0.52 (0.50–0.55)
L	0.54 (0.46–0.60)	3.2 (3.1–3.3)	0.7 (0.5–0.9)	2019	46 (43–48)	–	0.61 (0.48–0.70)	0.52 (0.41–0.63)
				2021	42 (40–45)	2.4 (2.3–2.5)	0.53 (0.50–0.58)	0.49 (0.47–0.51)
M	0.65 (0.63–0.66)	3.4 (3.3–3.4)	1.0 (1.0–1.1)	2019	51 (48–53)	–	0.44 (0.34–0.52)	0.45 (0.41–0.49)
				2021	53 (52–53)	2.7 (2.7–2.8)	0.56 (0.54–0.59)	0.50 (0.50–0.50)

Table 5 (continued)

Family	NecInc (proportion)	Intensity (score 0–4)	NecLesNo (score 0–3)	Year	AreaAff (%)	LesNo (score 0–3)	PremAbs (proportion)	
							Symptomatic	Asymptomatic
	Mean (range)	Mean (range)	Mean (range)		Mean (range)	Mean (range)	Mean (range)	Mean (range)
N	–	–	–	2019	48 (44–50)	–	0.48 (0.35–0.66)	0.39 (0.37–0.41)
				2021	–	–	–	–
O	–	–	–	2019	45 (43–48)	–	0.50 (0.45–0.56)	–
				2021	–	–	–	–
P	0.62 (0.60–0.66)	3.3 (3.3–3.4)	0.9 (0.8–1.1)	2019	54 (52–55)	–	0.54 (0.42–0.73)	0.43 (0.43–0.43)
				2021	52 (49–55)	2.8 (2.8–2.8)	0.50 (0.48–0.54)	0.50 (0.47–0.51)
Q	0.60 (0.53–0.66)	3.3 (3.2–3.4)	0.8 (0.7–1.0)	2019	49 (45–52)	–	0.53 (0.43–0.65)	0.38 (0.33–0.44)
				2021	48 (45–50)	2.7 (2.6–2.7)	0.45 (0.41–0.49)	0.52 (0.48–0.55)
R	0.53 (0.47–0.59)	3.2 (3.1–3.3)	0.6 (0.5–0.8)	2019	46 (44–48)	–	0.52 (0.46–0.62)	0.47 (0.42–0.52)
				2021	48 (46–50)	2.6 (2.4–2.6)	0.55 (0.51–0.58)	0.57 (0.54–0.62)
S	–	–	–	2019	51 (48–53)	–	0.41 (0.33–0.49)	0.41 (0.34–0.50)
				2021	–	–	–	–
T	0.54 (0.54–0.54)	3.3 (3.3–3.3)	0.7 (0.7–0.7)	2019	52 (48–54)	–	0.45 (0.37–0.66)	0.42 (0.38–0.48)
				2021	49 (49–49)	2.6 (2.6–2.6)	0.52 (0.52–0.52)	0.49 (0.49–0.49)
M. jansenii	0.54 (0.53–0.55)	3.3 (3.2–3.3)	0.7 (0.7–0.7)	2019	48 (46–49)	–	0.49 (0.44–0.59)	0.57 (0.48–0.67)
				2021	52 (52–52)	2.7 (2.7–2.7)	0.46 (0.44–0.48)	0.52 (0.51–0.53)
All	0.57 (0.39–0.73)	3.3 (3.0–3.6)	0.8 (0.4–1.2)	2019	47 (40–55)	–	0.50 (0.27–0.80)	0.47 (0.32–0.74)
				2021	48 (40–55)	2.7 (2.6–2.8)	0.50 (0.40–0.60)	0.50 (0.41–0.62)

NecInc, proportion of inoculated fruit with necrosis; Intensity, worst stage of lesion progression per tree; AreaAff, percentage of fruit surface area discoloured by husk spot; LesNo, score for number of lesions per fruit; NecLesNo, score for number of necrotic lesions per fruit

Endash (–) indicate that no progeny from those families were phenotyped for that variable

than the asymptomatic value (Table 4), indicating premature abscission in husk spot-affected fruit.

Of the progeny families, mean predicted NecInc and NecLesNo values were lowest for 'HAES246' and 'HAES791' (Table 5). Mean predicted Intensity values for both families were below the overall mean and their mean predicted LesNo numbers were equal to or less than the overall mean (Table 5). The mean predicted AreaAff values for 'HAES791' were below the overall means for both seasons, but higher in 2019 and lower in 2021 for 'HAES246' (Table 5). For progeny of 'HAES246', the mean predicted PremAbs value for symptomatic fruit was higher than that of asymptomatic fruit in 2019, but lower in 2021 (Table 5). For 'HAES791' progeny, the mean predicted PremAbs value for symptomatic fruit was

Table 6 Wald chi-squared statistics for fixed effects (*$p<0.05$; **$p<0.01$; ***$p<0.001$), and variance components and standard errors for random effects estimated for husk spot-induced premature abscission (PremAbs) from clonally replicated parents and open-pollinated seedling progeny

Effect	Statistical parameter	
Fixed	*df*	*Wald* χ^2
DisStatus	1	44.27***
Season	2	7.94***
FruitSize	2	0.96
DisStatus:Season	2	27.83***
DisStatus:FruitSize	2	2.32
FruitSize:Season	1	4.71*
DisStatus:FruitSize:Season	1	1.59
Random	σ^2	
Additive genetic 2018	$5.66\times10^{-7}\pm0.00$	
Additive genetic 2019	$3.94\times10^{-1}\pm0.21$	
Additive genetic 2021	$7.90\times10^{-7}\pm0.00$	
Additive genetic:DisStatus 2018	$1.43\times10^{-7}\pm0.00$	
Additive genetic:DisStatus 2019	$2.18\times10^{-1}\pm0.15$	
Additive genetic:DisStatus 2021	$1.77\times10^{-1}\pm0.15$	
Non-additive genetic	$2.34\times10^{-7}\pm0.00$	
Non-additive genetic:DisStatus	$1.51\times10^{-1}\pm0.09$	
Non-additive genetic:Season	$7.66\times10^{-8}\pm0.00$	
Non-additive genetic:DisStatus:Season	$2.00\times10^{-1}\pm0.12$	
Tree	$2.08\times10^{-1}\pm0.09$	
Tree:Season	$2.89\times10^{-1}\pm0.10$	
Block 2018	$1.43\times10^{-7}\pm0.00$	
Block 2019	$2.85\times10^{-2}\pm0.04$	
Block 2021	$2.80\times10^{-1}\pm0.13$	

DisStatus, symptomatic or asymptomatic; FruitSize, mode size of fruit when inoculated

lower than that of asymptomatic fruit in 2019 and almost equal in 2021 (Table 5).

Correlations between 2019 LesNo breeding values and those of other variables were not tested, as only nine LesNo breeding values were obtained for 2019. Correlations between Inc and 2019 AreaAff breeding values were based on 333 genotypes and all other variable pairs were based on 144 genotypes. All relationships, other than that between 2019 AreaAff and Intensity were positive (Table 7). Correlations were significant at $p<0.05$ for all variable pairs, except for those between 2019 AreaAff and NecInc, Intensity and NecLesNo ($p>0.05$) (Table 7). Of the significant correlations, Pearson's correlation coefficients (r) were equal to or greater than 0.75 for NecLesNo and NecInc, Inc and 2021 LesNo, 2021 AreaAff and 2021 LesNo, Intensity and NecInc, and Intensity and NecLesNo (Table 7).

Discussion

As described by Falconer (1960), genetic gain is a function of genetic control. Thus, to guide parental selection for husk spot resistance, genetic parameters of seven husk spot symptom expression variables that could be used to infer husk spot susceptibility were explored in this study. In macadamia, variability in tree architecture (Toft et al. 2018), flowering (Alam et al. 2018a; O'Connor 2019), precocity (Alam et al. 2018c), and fruit-set and abscission (Boyton and Hardner 2002; O'Connor 2019) among seedlings can complicate screening trials. To capture and account for such variation, the current study was conducted

Table 7 Pearson's correlation coefficients among husk spot incidence and severity variables based on breeding values estimated for macadamia parents and open-pollinated progeny

	Inc	NecInc	Intensity	AreaAff 2019	AreaAff 2021	LesNo 2021
NecInc	0.51***					
Intensity	0.54***	0.77***				
AreaAff 2019	0.40***	0.05	−0.07			
AreaAff 2021	0.71***	0.71***	0.73***	0.18*		
LesNo 2021	0.80***	0.48***	0.53***	0.18*	0.78***	
NecLesNo	0.51***	0.98***	0.75***	0.10	0.70***	0.50***

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Inc, proportion of inoculated fruit with symptoms; NecInc, proportion of inoculated fruit with necrosis; Intensity, worst stage of lesion progression per tree; AreaAff, percentage of fruit surface area discoloured by husk spot; LesNo, score for number of lesions per fruit; NecLesNo, score for number of necrotic lesions per fruit

over three fruiting seasons and included a diverse range of parents and progeny. Pedigree-based mixed models were used to account for unbalanced components of the trial design and enable exploitation of all available data.

In previous studies, husk spot-induced abscission was typically measured by comparing kernel maturity of abscised nuts in symptomatic and asymptomatic trees (Akinsanmi and Drenth 2012; Akinsanmi et al. 2007, 2008; Miles et al. 2010a, 2010b). Kernel maturity assessments have been favoured because of their direct relevance to on-farm profits (Akinsanmi and Drenth 2012; Akinsanmi et al. 2007). However, the timing of kernel development varies among genotypes (Trueman et al. 2000), and it is unknown whether traits such as husk spot-accelerated abscission and the timing of kernel development are genetically linked and consequently, likely to be inherited together. Therefore, in this trial, to enable the selection of parents for future populations, breeding values were predicted for the effect of symptoms on early-season abscission, instead of for the effect on kernel maturity of abscised fruit. The effect of nut drop pattern was also retained in all incidence and severity models to assist in separating potential causes of resistance from inherent abscission patterns.

Heritabilities estimated from the variance components suggest that genetic gain could be best achieved by selecting accessions with low NecLesNo and NecInc to be used as parents for future generations. The narrow-sense heritability (h^2) of 0.30 for NecLesNo based on a selection unit of two seasons of evaluations of 20 fruit/tree indicates the highest potential for improvement via phenotypic selection. Given the strong correlation between NecLesNo and NecInc, selection for low NecInc could be used to indirectly select for NecLesNo, as NecInc is a simpler trait to phenotype. Increases observed in NecLesNo and NecInc heritability estimates between one to two-season evaluations highlights the importance of assessing husk spot susceptibility for more than one season. In all traits where residuals were estimable, residuals components were larger than all other variance component estimates, followed by the tree × season interaction. Heritabilities estimated for selection units based on two-season evaluations allowed the residuals and tree × season interaction variance component to be reduced, thus increasing the ratio of genetic control.

The NecLesNo broad-sense heritability estimates (H^2) of approximately 0.6 for 20 fruit assessments indicates that individuals (e.g. unreplicated seedlings) with low NecLesNo could be expected to perform moderately similarly if clonally propagated. The H^2 estimate of 0.45 for 10 fruit/tree for two seasons may also indicate similar potential. This is a significant finding, as the availability of fruit for evaluations in young progeny can be limited by variable fruit-set (Alam et al. 2018c). However, due to the high levels of uncertainty associated with all heritability estimates indicated by their large standard errors, such inferences are cautionary. Additionally, the trial was carried out over several seasons, but did not include multi-locations. Thus, care should be taken extending interpretations outside of the location and/or population in which they were estimated (Falconer 1960). Nevertheless, these results provide a baseline of information to support future selection strategies. Such information is important as husk spot data is often difficult to obtain due to issues such as variable nut-set (Miles et al. 2010b) and/or insufficient disease pressure (Akinsanmi et al. 2007, 2008).

Low heritabilities for fruit tree disease responses, like those observed for traits other than NecLesNo and NecInc, are not uncommon when estimated for an individual tree basis. For example, Jeff-Ego et al. (2021) reported h^2 of 0.10–0.23 for *Phytophthora* spp. stem wound severities in macadamia cultivar progeny. In a pecan study, H^2 for fruit and leaf scab ratings ranged between 0.05 and 0.16 (Bock et al. 2020). In the present study, the large residual variance for all traits relative to the genetic variances implies high variation in husk spot symptom expression among fruit within trees. As the inoculation method relied on water-splash spore dispersal from diseased stick-tights, within tree variation may have been inflated if some fruit were splashed with more, or fewer spores than others. However, the diseased stick-tight bag method mimics naturally occurring husk spot dispersal (Akinsanmi and Drenth 2010; Miles et al. 2010b). Although it is not guaranteed that all fruit will receive the same quantity of inoculum when using the stick-tight bag method, such variation is likely representative of on-farm conditions. As discussed by Miles et al. (2010b), if inoculation methods that deviate from naturally occurring processes are used for susceptibility screening, disparities between trial outcomes and on-farm observations are likely.

As such, the high within-tree variation observed in the current trial is likely representative of on-farm conditions and should be considered within resistance breeding strategies.

Under the high inoculum pressure applied in this trial, all genotypes displayed symptoms. Given the lack of additive genetic variation detected for Inc, the trait would not be useful for selecting parents to produce future generations based on phenotypes within the current population. The high breeding values for Inc across all genotypes, but varied breeding values for NecInc indicates that most inoculated fruit expressed at least chlorosis, but the proportion of fruit that expressed necrosis was more varied. Predicted NecInc and NecLesNo values were lowest for 'HAES791' and 'BAM263' clones and progeny families of 'HAES246' and 'HAES791'. In A16, sporulation has been reported to increase with lesion development (Miles et al. 2009). If sporulation is also low in early stages of lesion development in other genotypes, inoculum production may be impeded in genotypes with less necrosis. Such a trait may be of particular interest in genotypes like 'HAES791', where husk spot symptoms do not appear to increase early-season abscission. The concurrently low predictions for NecInc, NecLesNo and PremAbs for 'HAES791' clones and progeny may indicate that although symptomatic fruit are retained in the canopy, lesion development is still slow relative to other genotypes. Interestingly, genetic marker studies have identified that 'HAES791' is at least a tri-species cultivar, with *M. integrifolia*, *M. tetraphylla* and *M. ternifolia* ancestry (Alam et al. 2018b; Peace 2005). This is uncommon, as the genetic constitution of most industry standard cultivars consists predominantly of *M. integrifolia*, *M. tetraphylla*, or a mixture of both species (Hardner et al. 2009; Peace 2005). For example, 'HAES246' is described as pure *M. integrifolia* and the parents of 'BAM263', 'NG8' and 'HAES762' are *M. integrifolia* and *M. tetraphylla* hybrid and pure *M. integrifolia*, respectively (Peace 2005). As such, these results indicate that there may be value in screening *M. ternifolia,* or other wild germplasm accessions for resistance.

While the low predicted NecLesNo and NecInc values of 'BAM263' clones and the 'HAES246' progeny family may indicate some resistance, their predicted PremAbs values for symptomatic fruit were higher than for asymptomatic fruit in some seasons, implying accelerated abscission caused by husk spot infection. Thus, their low severity values may have resulted from abscission of infected fruit occurring before symptoms could progress to necrotic stages, rather than slow lesion development. Accelerated abscission of symptomatic fruit may reduce inoculum load in the canopy, but it can also result in increased proportions of commercially immature kernels and, consequently, loss of saleable yield (Akinsanmi and Drenth 2012; Akinsanmi et al. 2007). Additionally, it is unknown whether symptom-accelerated abscission is genetically linked with other forms of disease resistance. If a genotype with promising (low) breeding values for incidence and severity traits, but not for premature abscission (higher early abscission in symptomatic than asymptomatic fruit) is used as a parent, it's progeny may be likely to express higher incidence and severity if less prone to premature abscission. Further testing should be conducted to determine whether the low severity predictions for 'HAES246' and 'BAM263' progeny were due to premature abscission of infected fruit. To enable their use as parents, individual progeny of 'HAES791', 'HAES246' and 'BAM263' with low NecLesNo and NecInc predicted values (for example, those with NecLesNo predictions of 0.4 and NecInc of 0.39) should be evaluated in replicated trials to confirm their putative resistance.

To conclude, genetic parameters estimated in this study indicate that genetic gain in husk spot resistance may be achieved by selecting parents based on necrotic lesions per fruit (NecLesNo) and/or proportions of fruit with necrotic lesions (NecInc). The higher heritabilities of selection units based on two seasons demonstrated that genotypes should be evaluated for multiple seasons. Given the strong correlation between NecLesNo and NecInc breeding values, NecInc could be used to indirectly select for NecLesNo. Based on limited necrosis, varieties 'HAES791', 'HAES246' and 'BAM263' appear partially resistant, thus, should be further tested in replicated trials, with the potential for use as parents for the development of future progeny populations. Finally, the unique tri-species ancestry of the most resistant genotype, 'HAES791' suggests that investigations involving *M. ternifolia,* or other wild germplasm accessions should be undertaken.

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Data availability The datasets generated analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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