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Effiziente Verfahren für die Züchtung neuer Erdbeersorten (*Fragaria ×ananassa* Duch.)

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Weiler-Simmerberg, 2016

ZUSAMMENFASSUNG

Im Rahmen dieser Arbeit wurden verschiedene Verfahren zur effizienten Züchtung neuer Erdbeersorten untersucht, die sowohl obstbauliche Merkmale wie Frühzeitigkeit und Ertrag als auch die Widerstandsfähigkeit gegenüber den für den Erdbeeranbau bedeutenden Krankheitserregern *Botrytis cinerea* und *Xanthomonas fragariae* umfassen. Hoher Ertrag und eine Ausdehnung der Reifezeit im extrem früh- bzw. spätreifenden Segment stellen in erster Linie die Grundlagen für einen wirtschaftlich erfolgreichen Anbau dar, die in Kombination mit hoher Widerstandsfähigkeit gegenüber Krankheiten auch in Zukunft zu einem konkurrenzfähigen und nachhaltigen Erdbeeranbau in Deutschland beitragen können. In einem klassischen diallelen Kreuzungsversuch mit 13 Erdbeersorten entstanden 144 F1-Nachkommenschaften, die in einem zweijährigen Feldversuch hinsichtlich ihrer obstbaulichen Merkmale evaluiert wurden, um die allgemeine und spezifische Kombinationseignung der Kreuzungseltern hinsichtlich der Merkmale Ertrag und Reifezeit zu ermitteln. Hierzu wurde im Rahmen dieser Arbeit ein statistisches Modell entwickelt, mit dem die Komponenten der genetischen Varianz erklärt werden können und der züchterische Wert der Kreuzungseltern auf Basis der Kombinationseignungen wiedergegeben wird. Hierbei zeigte sich zunächst, dass es keine Auswirkung auf die Nachkommenschaft hat, ob eine Sorte als Kreuzungsmutter oder Kreuzungsvater verwendet wird. Die genetische Varianz im Kreuzungsversuch basiert hauptsächlich auf der allgemeinen Kombinationseignung (GCA) der verwendeten Sorten (additive Effekte). Spezifische und reziproke Kombinationseignungen (nicht-additive Effekte) erwiesen sich als weniger relevant. Die Sorten ‘Clery’ bzw. ‘Yamaska’ eignen sich somit am besten, um besonders früh- bzw. spätreifende Nachkommenschaften zu erhalten. Kreuzungen mit der Sorte ‘Polka’ ergeben Nachkommenschaften mit besonders hohem marktfähigem Ertrag. Diese Ergebnisse fließen in die weitere Entwicklung des Zuchtprogramms ein, bei dem, neben den obstbaulichen Merkmalen, die gezielte Einkreuzung von Resistenzen gegenüber der Grauschimmelfäule und der eckigen Blattfleckenkrankheit im Vordergrund steht. Die Grauschimmelfäule, verursacht durch den nekrotrophen Pilz *Botrytis cinerea* Pers. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel], ist die wichtigste Pilzkrankheit im Erdbeeranbau und erfordert einen häufigen Einsatz chemischer Pflanzenschutzmittel. Die eckige Blattfleckenkrankheit, die durch den Erreger *Xanthomonas fragariae* Kennedy & King verursacht wird, ist die weltweit bedeutendste bakterielle Krankheit im Erdbeeranbau und es gibt derzeit in der EU keine geeigneten chemischen Pflanzenschutzmittel zur Bekämpfung. Der Anbau widerstandsfähiger Sorten gilt als wichtige Grundlage einer erfolgreichen Bekämpfungsstrategie und die Ergebnisse dieser Arbeit sollen zur gezielten Züchtung geeigneter Sorten beitragen. Für jede der beiden Krankheiten wurden zunächst Resistenztests etabliert, die unter kontrollierten

Bedingungen mittels künstlicher Inokulation und geeigneter Bonitурskalen reproduzierbare Ergebnisse über die Widerstandsfähigkeit verschiedener Genotypen liefern. So konnten im Laufe von drei Versuchsjahren insgesamt mehr als 100 Erdbeersorten, Wildartenakzessionen und Zuchtklone aus dem Bestand der Obstgenbank des JKI in Dresden-Pillnitz evaluiert werden. Als teilweise resistent gegenüber *B. cinerea* konnten die folgenden fünf Genotypen identifiziert werden: Die Sorten ‘Diana’, ‘Joerica’ und ‘Kimberly’ sowie die Wildartenakzessionen *F. virginiana* ‘Wildmare Creek‘ und *F. vesca* subsp. *bracteata*. Parallel dazu wurden sechs teilweise resistente Genotypen gegenüber *X. fragariae* identifiziert: Die aus den USA eingeführten Zuchtklone US4808 und US4809, sowie die Wildartenakzessionen *Fragaria vesca* f. *alba*, *Fragaria nilgerrensis* ‘Yunnan’, *F. vesca* ‘Illa Martin’ und *F. moschata* ‘Bauwens’. Vollständig resistente Genotypen wurden bislang nicht gefunden. Im Zusammenhang mit dem *Xanthomonas*-Resistenztest wurde zudem die systemische Ausbreitung des bakteriellen Erregers innerhalb der Pflanzen mit molekularbiologischen Methoden untersucht. Zum einen wurde mittels eines sensitiven nested-PCR Verfahrens das Vorhandensein des Erregers in unterschiedlichen Gewebeproben inokulierter Pflanzen anfälliger und widerstandsfähiger Sorten zu unterschiedlichen Zeitpunkten nachgewiesen. Zudem wurde ein virulenter, GFP-markierter *X. fragariae*-Stamm erzeugt, mit dem ebenfalls Inokulationsversuche an unterschiedlichen Erdbeergenotypen durchgeführt werden konnten. Die systemische Ausbreitung des Erregers wurde anschließend im Fluoreszenzmikroskop visualisiert und durch die Ergebnisse des nested-PCR Nachweises bestätigt. Bereits zum Zeitpunkt drei Tage nach Inokulation verbreitet sich *X. fragariae* in der gesamten Wirtspflanze und kann in allen Gewebeproben nachgewiesen werden. Sowohl in widerstandsfähigen als auch in anfälligen Genotypen ist der Erreger bereits 3 Tage nach Inokulation in allen Pflanzenteilen zu finden. Aus den Ergebnissen der Resistenztests können einerseits Empfehlungen zur Sortenwahl abgeleitet werden und andererseits können die identifizierten widerstandsfähigen Genotypen nun für die gezielte Züchtung neuer, widerstandsfähiger Sorten eingesetzt werden. Die Ergebnisse der vorliegenden Arbeit zeigen Wege und Strategien auf, um Zuchtprogramme effektiver zu gestalten und damit die Erfolgschancen bei der Züchtung neuer Sorten für den deutschen Erwerbsobstbau zu erhöhen, um auch in Zukunft eine wettbewerbsfähige und nachhaltige Erdbeerproduktion zu ermöglichen.

SUMMARY

In this thesis, different approaches aimed on efficient breeding of new strawberry cultivars were investigated that comprise horticultural traits such as earliness and yield potential as well as resistance traits against two of the most important pathogens in strawberry cultivation, *Botrytis cinerea* and *Xanthomonas fragariae*. High yield potential and an extended ripening period in combination with disease resistance form the basis for economic success and contribute to a competitive and sustainable strawberry cultivation in Germany. In a traditional diallel cross breeding experiment, a set of 13 strawberry cultivars were crossed in a reciprocal way. The crossings resulted in a total of 144 F1-populations which were evaluated in a field trial over two years with regards to their horticultural traits in order to investigate the general and specific combining abilities of the parental cultivars concerning the traits earliness and marketable yield. Within this thesis, a statistical model was developed to explain the components of genetic variance and the breeding value of the parental cultivars based on their calculated combining abilities. It was demonstrated that there is no reciprocal effect on the progeny and it is practically irrelevant whether a cultivar is used as mother or father in the crossing experiment. The genetic variance in the breeding experiment is mainly based on the general combining ability (GCA) of the parental cultivars (additive effects). Specific and reciprocal combining abilities (non-additive effects) appeared less relevant. Crossings with the cultivars 'Clery' and 'Yamaska' lead to very early ('Clery') and very late ('Yamaska') ripening progeny. Crossings with 'Polka' lead to progeny with higher marketable yield. These findings are taken into account for the further development of the breeding approach which has also a main focus on resistance towards the grey mold disease and the angular leaf spot disease. The grey mold disease, caused by the necrotrophic fungus *Botrytis cinerea* Pers. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel], is the most important fungal disease in strawberries and requires frequent applications of chemical plant protection products. The angular leafspot disease, caused by *Xanthomonas fragariae* Kennedy & King, is the most important bacterial disease in strawberry cultivation worldwide and there are currently no plant protection products available within the EU for an effective control of the disease. In a successful control strategy, the cultivation of resistant cultivars is of fundamental importance and the results of this thesis will contribute to the development of a targeted breeding approach. Initially, resistance tests were established for both diseases. Reproducible results concerning the resistance characteristics of different cultivars were achieved in artificial inoculation experiments and adapted evaluation scales. With this approach, more than 100 strawberry cultivars, wild-types and breeding clones from the collection of the German Fruit Gene Bank of the JKI in Dresden-Pillnitz were evaluated in three years of testing. Concerning *B. cinerea*, a total of five genotypes were identified as partially

resistant: the cultivars ‘Diana’, ‘Joerica’ and ‘Kimberly’ and the wild-types *F. virginiana* ‘Wildmare Creek’ and *F. vesca* subsp. *bracteata.* In parallel, six partially resistant genotypes were found towards *X. fragariae*: The US breeding clones US4808 and US4809 and the wild-types *Fragaria vesca* f. *alba*, *Fragaria nilgerrensis* ‘Yunnan’, *F. vesca* ‘Illa Martin’ and *F. moschata* ‘Bauwens’. No completely resistant genotyped were identified until now. Additionally to the resistance test against *X. fragariae,* the systemic dispersal of the bacteria within the plant was further investigated with molecular-biological methods. On the one hand, bacterial DNA was detected by a sensitive nested-PCR method in different plant tissues of inoculated plants from partially resistant and susceptible cultivars at different time points. Additionally, a GFP-tagged virulent *X. fragariae* strain was produced and used for inoculation experiments in different strawberry genotypes as well. The systemic dispersal of the bacteria was visualized under the fluorescent microscope and the results were confirmed by the nested-PCR detection method. Already three days after the inoculation, *X. fragariae* spreads systemically throughout the entire host plant and can be detected in all plant tissue samples. The bacteria were detected in all plant parts of partially resistant cultivars after three days post-inoculation as well. The results from the resistance tests lead to direct recommendations for the choice of cultivars on the one hand and on the other hand, the identified partially resistant genotypes can be used in further targeted breeding approaches. The results of this thesis show new ways and strategies for the improvement of strawberry breeding programs. They increase the success for targeted breeding of new cultivars for strawberry cultivation in Germany in order to maintain a competitive and sustainable strawberry production in the future.

INHALTSVERZEICHNIS

ABKÜRZUNGEN

B.c.	*Botrytis cinerea*
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
BÖLN	Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft
CFU	Koloniebildende Einheit (colony forming unit)
ddH2O	Doppelt destilliertes Wasser
DNA	Desoxyribonukleinsäure
dpi	Tage nach Inokulation (days postinoculation)
ELISA	Enzyme-linked Immunosorbent Assay
EPPO	European Plant Protection Organization
GCA	Allgemeine Kombinationseignung (general combining ability)
GFP	Grün fluoreszierendes Protein
JKI	Julius Kühn-Institut
LSD	Least significant difference
MDR	Multiple Drug Resistance
NaClO	Natriumhypochlorit
PCR	Polymerase-Kettenreaktion
PDA	Kartoffel-Dextrose-Agar (potato dextrose agar)
RGCA	Reziproke allgemeine Kombinationseignung
RSCA	Reziproke spezifische Kombinationseignung
SCA	Spezifische Kombinationseignung (specific combining ability)
SE	Standard Error
T3SS	Typ 3-Sekretionssystem
USDA	United States Department of Agriculture
X.f.	*Xanthomonas fragariae*
YDC	Yeast Extract Dextrose Calcium

1 EINLEITUNG

Die Herausforderungen im nachhaltigen und zukunftsorientierten Obstbau steigen unmittelbar mit den Erwartungen des Handels und der Verbraucher an makellose Optik und gleichbleibend hohe Qualität der Früchte bei gleichzeitig möglichst geringen Pestizidrückständen. Hinzu kommt die in den letzten Jahren steigende Nachfrage nach hochqualitativem Obst zu jeder Jahreszeit, was in Zukunft auch im deutschen Erdbeeranbau eine wichtige Rolle spielen wird. Um diesen Ansprüchen gerecht zu werden, sind unweigerlich neue Strategien zur weiteren Verbesserung der Qualität und Wirtschaftlichkeit im Obstanbau notwendig. Die vorliegende Arbeit beschäftigt sich insbesondere mit einem effizienten züchterischen Ansatz, um ertragreiche und krankheitsresistente Sorten mit erweiterter Reifezeit zu entwickeln. Der Ansatz, der in dieser kumulativen Dissertation präsentiert wird, untergliedert sich in drei Teile, die einen wesentlichen Beitrag für einen nachhaltigen und zukunftsorientierten Erdbeeranbau liefern. Zunächst wurde mittels einer Methodik der klassischen Kreuzungszüchtung in einem vollständigen Diallel die Vererbung von Ertragsleistung und Erntezeitpunkt untersucht, um geeignete Kreuzungseltern als Basis für die gezielte Sortenzüchtung zu identifizieren. Die Ergebnisse aus dem diallelen Kreuzungsversuch mit insgesamt 144 Kreuzungspopulationen wurden unter dem Titel „A diallel crossing approach aimed on selection for ripening time and yield in breeding of new strawberry (*Fragaria* ×*ananassa* Duch.) cultivars“ veröffentlicht und bilden den ersten Teil der kumulativen Arbeit (Bestfleisch et al., 2014). Die Widerstandsfähigkeit von Erdbeersorten gegenüber der mit Abstand wichtigsten Pilzkrankheit im Erdbeeranbau, der Grauschimmelfäule, die durch *Botrytis cinerea* verursacht wird, ist Bestandteil des zweiten Teils mit dem Titel „Evaluation of strawberry (*Fragaria* L.) genetic resources on resistance to *Botrytis cinerea*“ (Bestfleisch et al., 2015a). Hierbei wurde ein einfacher und reproduzierbarer Resistenztest entwickelt und mehr als 100 Erdbeergenotypen evaluiert, darunter ein Spektrum aus historischen Sorten und Wildartenakzessionen, sowie den aktuell im Anbau verbreiteten Sorten. In mehrjährigen Versuchen konnten hierbei besonders widerstandsfähige Genotypen identifiziert werden, die teilweise schon heute im Anbau eingesetzt werden können, sowie als Kreuzungseltern für die Züchtung neuer, widerstandsfähiger Sorten Verwendung finden. Parallel dazu wurde im dritten Teil der Arbeit ein weiterer Resistenztest gegenüber der an Bedeutung immer mehr zunehmenden eckigen Blattfleckenkrankheit etabliert, die durch den hochspezifischen Erreger *Xanthomonas fragariae* hervorgerufen wird. Auch hier konnten über 100 Sorten, Wildarten und Zuchtklone in Gewächshausversuchen hinsichtlich ihrer Resistenz untersucht werden. Darüber hinaus wurden Versuche zur systemischen Ausbreitung des Erregers innerhalb der Pflanzen durchgeführt und mittels eines GFP-markierten Erregerstammes visualisiert. Dadurch konnte die schnelle Ausbreitung des Erregers innerhalb der Pflanze nachgewiesen werden und die

fluoreszenzmikroskopische Auswertung erlaubt Rückschlüsse auf die Verbreitungswege. Die Ergebnisse werden in der Publikation „Resistance and systemic dispersal of *Xanthomonas fragariae* in strawberry germplasm (*Fragaria* L.)" vorgestellt (Bestfleisch et al., 2015b). Im Hinblick auf eine zukünftige Anschlussfähigkeit der Versuchsergebnisse behandelt die Diskussion die direkte Nutzbarkeit im Anbau, sowie die zukünftige züchterische Nutzbarkeit und Erfolgsaussichten zur gezielten Züchtung neuer, widerstandsfähiger Erdbeersorten.

1.1 Motivation und Zielsetzung dieser Arbeit

Langfristig dienen die durch das Vorhaben gewonnenen Ergebnisse zur gezielten Züchtung neuer resistenter Erdbeersorten, wodurch insbesondere im ökologischen Landbau stabilere Erträge und höhere Fruchtqualität unter reduziertem Einsatz von Pflanzenschutzmitteln den Anbau in Deutschland nachhaltig und langfristig sichern.

Kurzfristig werden durch die Evaluierung neue Informationen über Sorten, Wildtypen und Zuchtklone gewonnen, welche vom Anbauer und von Züchtern direkt genutzt werden können.

1.2 Bedeutung von Ertrag und Ausdehnung der Reifezeit als Zuchtziele für den Erdbeeranbau

Die aus einer eher zufälligen Hybridisierung der Wildarten *F. virginiana* und *F. chiloensis* entstandene Kulturerdbeere *Fragaria* ×*ananassa* Duch. zählt derzeit zu den wirtschaftlich bedeutendsten Beerenobstarten (Hummer und Hancock, 2009) und wird heute in über 70 Ländern weltweit angebaut. Insgesamt werden jährlich Erntemengen von schätzungsweise 4.1 Millionen Tonnen produziert (Flachowsky et al., 2011). Die große wirtschaftliche Bedeutung, der weltweit steigende Wettbewerbsdruck, die sich verändernden Umweltbedingungen und die Vermarktung der Früchte, sowohl auf dem Frischmarkt als auch in der verarbeitenden Industrie führen zum weiterhin steigenden Bedarf an neuen Erdbeersorten mit verbesserten Eigenschaften. In gleichem Maße wie die Anzahl neuer Sorten jährlich rapide zunimmt, steigen auch die Anforderungen, die an moderne Sorten gestellt werden. Derzeit liegen die wichtigsten Schwerpunkte der Erdbeerzüchtung auf komplexen Eigenschaften wie der Ertragsmenge, der Fruchtqualität in Bezug auf Geschmack, Festigkeit, Farbe, Größe, Gesamterscheinung und Transportfähigkeit. Zudem wird die Widerstandsfähigkeit gegenüber Schädlingen, Krankheiten und abiotischem Stress ein immer wichtigeres Kriterium bei der Sortenwahl. Eines der ökonomisch interessantesten Zuchtziele liegt in der Ausdehnung der Reifezeit durch ein breites Spektrum an möglichst früh, beziehungsweise

möglichst spät reifenden Sorten. In Deutschland werden im Mittel ca. 80% der Erdbeeren auf dem Frischmarkt in den Monaten Juni und Juli verkauft, was in dieser Zeit niedrige Marktpreise von im Mittel ca. 150€ pro 100kg zur Folge hat. Besonders zu Beginn der Saison in den Monaten April/ Mai werden deutlich bessere Preise erzielt, die um das 2.5-Fache höher liegen können (Behr, 2012). Aber auch zum Ende der Saison im August steigen die Marktpreise wieder, daher ist die gezielte Züchtung von extrem früh und extrem spät reifenden Sorten von besonderem ökonomischem Interesse. Um dieser Problematik gezielt zu begegnen, beschäftigt sich die vorliegende Arbeit mit einem Ansatz aus dem Bereich der klassischen Kreuzungszüchtung, der häufig als sogenannter dialleler Kreuzungsversuch bezeichnet wird. Dieser relativ aufwändige Versuchsansatz dient dazu, die Vererbung komplexer Eigenschaften der als Kreuzungseltern verwendeten Sorten an die Nachkommenschaften zu untersuchen, was eine wichtige Voraussetzung zur gezielten Sortenzüchtung darstellt und Rückschlüsse auf die züchterische Verwertbarkeit einzelner Elternsorten in Züchtungsprogrammen zulässt. Zur Untersuchung der beiden Eigenschaften Ertragsmenge und Reifezeit wurden hierfür 13 im Anbau verbreitete Erdbeersorten als Kreuzungseltern ausgewählt, die das gesamte Reifespektrum abdecken und mittlere bis gute Erträge liefern. Als früh reifende Sorten wurden ‘Antea’, ‘Clery’, ‘Daroyal’, ‘Darselect’, und ‘Madeleine’ ausgewählt, für den mittleren Reifezeitraum ‘Arosa’, ‘Elsanta’, ‘Galia’, ‘Marmolada’, und ‘Sonata’ und die spät reifenden Sorten ‘Florence’, ‘Polka’ und ‘Yamaska’. Aus diesen 13 Sorten gingen nach reziproker Kreuzung 144 F1-Populationen hervor, die unter Freilandbedingungen auf den Versuchsflächen in Dresden-Pillnitz über 2 Jahre hinweg evaluiert wurden. Der Erfolg von Züchtungsprogrammen wird maßgeblich durch die Wahl der geeignetsten Kreuzungseltern für die zuvor definierten Züchtungsziele beeinflusst und grundlegende Kenntnisse über die Eigenschaften einzelner Sorten und ihrer Vererbbarkeit werden zu einer wichtigen Voraussetzung. Hierbei können diallele Kreuzungen durchgeführt werden, um sowohl die Vererbbarkeit einzelner Eigenschaften zu untersuchen und überlegene Genotypen als Eltern für nachfolgende Kreuzungen zu identifizieren. Mit Erdbeeren wurde dieser Versuchsansatz schon mehrmals durchgeführt. Die Vererbung von quantitativen Eigenschaften wie marktfähiger Ertrag, Einzelfruchtgewicht, Anfälligkeit gegenüber Grauschimmel, Fruchtfarbe und Fruchtfestigkeit (Masny et al., 2005, 2008), die Vererbung der Reifezeit (Zurawicz et al., 2006) und Ertrag (Aalders und Craig, 1974; Zurawicz et al., 1995) wurde schon häufiger in diallelen Kreuzungsversuchen untersucht. Weitere diallele Kreuzungsversuche (Davik und Honne, 2005; Gimenez und Ballington, 2002; Melville et al., 1980) beschäftigten sich mit der Vererbung von Krankheitsresistenzen gegenüber *Colletotrichum acutatum, Sphaerotheca macularis* und *Phytophthora fragariae*. Jedoch ist der hier durchgeführte Versuch durch die große Anzahl an Kreuzungen, sowie die weiterentwickelte statistische Auswertung in einem Mixed-

Model Ansatz, der im Statistikprogramm SAS 9.3 implementiert wurde und zur zuverlässigeren Berechnung der spezifischen (SCA) und allgemeinen Kombinationseignung (GCA) führt, bislang einmalig in der Erdbeerzüchtung. Der klassische diallele Kreuzungsversuch, der in dieser Arbeit präsentiert wird, liefert statistisch gesicherte Ergebnisse, die direkt von Erdbeerzüchtern praktisch angewendet werden können. Für die Eigenschaften „marktfähiger Ertrag“ und „Reifezeit“ von Erdbeeren ist die Selektion vielversprechender Kreuzungseltern auf Basis ihrer GCA-Werte eine zuverlässige Methode.

1.3 *Botrytis cinerea* – Ausbreitung, Verlauf und mögliche Resistenzquellen gegen die Grauschimmelfäule

Die Grauschimmelfäule wird durch den nekrotrophen Pilz *Botrytis cinerea* Pers. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel] ausgelöst, der große Schäden in mehr als 200 verschiedenen Wirtspflanzenarten hervorruft (Dean et al., 2012; Williamson et al., 2007). Die Krankheit lässt sich nur schwer kontrollieren, da der Pilz mehrere unspezifische Infektionswege nutzt (Williamson et al., 2007). Die Applikation von Fungiziden ist nach wie vor die effektivste Art der Bekämpfung in den meisten wirtschaftlich bedeutenden Wirtspflanzen (Dean et al., 2012), jedoch sind Fungizidapplikationen sehr kostenintensiv und das Auftreten von Fungizidresistenzen nimmt immer mehr zu, wie schon häufig wissenschaftlich belegt wurde (Amiri et al., 2012; Leroch et al., 2011; Leroch et al., 2013; Leroux et al., 2010; Veloukas et al., 2011). Allein im Jahr 2001 wurden die Gesamtkosten für Botrytizide mit 540 Mio. € veranschlagt, was in etwa 10% des weltweiten Fungizidmarktes ausmachte (Dean et al., 2012). Für den ökologischen Anbau sind zurzeit keine wirksamen Botrytizide zugelassen. Hier wird vor allem mit Kupfer- und Schwefelspritzungen sowie mit indirekten Maßnahmen, wie einem ein- statt zweireihigen Pflanzsystem oder durch konsequentes Entfernen verdorrter Blätter im Frühjahr und fauler Früchte während der Ernte aus der Anlage versucht, den Befallsdruck zu minimieren. Ein Ausweg aus dieser Situation wird vor allem im Anbau resistenter/toleranter Sorten gesehen. Die Infektion mit Grauschimmelfäule erfolgt hauptsächlich schon im Feld (Braun und Sutton, 1987), wo abgestorbenes Laub und befallene Früchte und Blüten Quellen für das natürliche Inokulum sind. Die Sporulation des Pilzes findet ihren Ursprung in infiziertem Pflanzengewebe und wird durch kühle, feuchte Witterung im Frühjahr begünstigt (Bulger et al., 1987; Sosaalvarez et al., 1995). Die Konidien werden durch Wind und Spritzwasser verbreitet und führen zu Primärinfektionen in den Blüten, wobei die Kron- und Staubblätter vom Pilz besiedelt werden. Anschließend wächst das Pilzmycel in die sich entwickelnde Frucht, wo es quieszent bleibt bis zur Fruchtreife. Die

Sekundärinfektionen gehen von bereits infizierten Blättern oder Früchten aus, die benachbarte Pflanzenteile durch den direkten Kontakt mit dem Pilz infizieren.

Für den Verlauf der Infektion während der Reifephase der Früchte spielen die biochemischen Vorgänge innerhalb der Frucht eine entscheidende Rolle. Während der Fruchtreife steht die Zunahme von Einfachzuckern, dem Anthocyangehalt, und flüchtiger Aromastoffe der Abnahme von organischen Säuren und Phenolen gegenüber (Nunes, 2009). In der Nachernte-Periode geht die abnehmende Fruchtfestigkeit einher mit dem Abbau von Zellwandbestandteilen im Kortexgewebe (Koh und Melton, 2002). Außerdem kommt es während der Reife zu Veränderungen in der Flavonoidstruktur (Halbwirth et al., 2006). In den frühen Entwicklungsstadien der Fruchtentwicklung finden sich größere Mengen verschiedener 3', 4'-hydroxylierter Flavan 3-ole und daraus hervorgegangene Proanthocyanidine, die im Gewebe akkumuliert werden. Es wird angenommen, dass dadurch das Wachstum des Botrytis-Mycels in ein Ruhestadium versetzt wird, welches bis zur Fruchtreife anhält (Jersch et al., 1989). Bei fortschreitender Fruchtreife werden die Erdbeeren hellrot bis dunkelrot, was durch die unterschiedlichen Anthocyanidin-Derivate Pelargonidin und Cyanidin hervorgerufen wird, den wichtigsten Pigmenten bei Erdbeeren. Flavonole werden in diesen Reifestadien ebenfalls gebildet und dienen als Co-Pigmente (Henning, 1981; Hertog et al., 1992; Häkkinen und Auriola, 1998; Häkkinen und Törrönen, 2000; Olsson et al., 2004). Gleichzeitig kommt es zu einem signifikanten Abbau der 3', 4'-hydroxylierten Flavan 3-ole und der Proanthocynidine. Diese strukturellen und biochemischen Veränderungen führen zu optimalen Bedingungen für die weitere Entwicklung des Schaderregers *Botrytis cinerea* und starker Ausprägung der Grauschimmelkrankheit. Es wird angenommen, dass hierin eine mögliche Ursache für die großen Sortenunterschiede hinsichtlich der Krankheitsanfälligkeit liegt.

Auch wenn es bei den heute verbreiteten Erdbeersorten große Sortenunterschiede bei der Anfälligkeit gibt, so ist derzeit trotzdem noch keine vollständig resistente Sorte verfügbar (Droby und Lichter, 2004; Maas, 1998). Hohe Widerstandsfähigkeit mit nur 3.3 - 3.6% befallener Oberfläche pro Frucht/Blatt durch den Pilz wurden für die Erdbeerwildart *Fragaria chiloensis* beschrieben (Gonzalez et al., 2009). Jedoch gibt es noch keine beschriebene Resistenz gegenüber *B. cinerea,* die spezifisch in Form einer Gen-für-Gen Interaktion nutzbar sein könnte. Die Widerstandsfähigkeit gegenüber *B. cinerea* zeigt sich insgesamt als stark umweltabhängig und eher unspezifisch. Es wurden in der Vergangenheit zahlreiche Studien zur Charakterisierung von verschiedenen Erdbeergenotypen hinsichtlich der Resistenz gegenüber *B. cinerea* durchgeführt (Barritt, 1980; Bestfleisch et al., 2013; Bristow et al., 1986; Chandler et al., 2006; Irvine und Fulton, 1959; Jarvis, 1962; Seijo et al., 2008; Simpson, 1991). Die Studien fanden größtenteils unter Freilandbedingungen unter dem dort herrschenden natürlichen Infektionsdruck statt, wodurch die

Vergleichbarkeit der Ergebnisse schwierig ist. Es herrschen unterschiedliche Umweltbedingungen mit diversen Quellen und Konzentrationen des Inokulums vor. Aus diesem Grund hat die vorliegende Studie das Ziel, ein möglichst großes Spektrum an Erdbeergenotypen unter kontrollierten Bedingungen mittels einer standardisierten Testmethodik zu evaluieren, welche im Rahmen der Arbeit etabliert wurde. In diesen Inokulationsversuchen konnten im Laufe von 3 Jahren 82 Erdbeersorten, 11 Wildartenakzessionen und 14 Zuchtklone auf ihre Anfälligkeit gegenüber *B. cinerea* untersucht werden, was als Grundlage für Sortenempfehlungen und weitere Züchtungsexperimente dient.

1.4 *Xanthomonas fragariae* – Die zunehmende Bedeutung der eckigen Blattfleckenkrankheit im Erdbeeranbau

Die derzeit bedeutendste bakterielle Krankheit im Erdbeeranbau ist die eckige Blattfleckenkrankheit, die durch den Erreger *Xanthomonas fragariae* Kennedy & King verursacht wird und sich schnell verbreitet, sodass diese Krankheit mittlerweile ein weltweites Problem darstellt (Maas, 1998). Seit der Erstbeschreibung (Kennedy und King, 1962a) und der taxonomischen Klassifizierung (Kennedy und King, 1962b) in Nordamerika hat sich *X. fragariae* weltweit durch den Handel mit Jungpflanzen über nahezu alle Erdbeer-Anbaugebiete ausgebreitet. Hierbei stellen latente, nicht sichtbare Infektionen ein schwer zu kontrollierendes Risiko dar, daher ist die eckige Blattfleckenkrankheit mittlerweile eine große Herausforderung in der Jungpflanzenproduktion (Jamieson et al., 2013).

Trotz der Bestrebungen der Pflanzenschutzorganisationen in Europa und den USA, die weitere Ausbreitung einzudämmen, findet das erstmalig in Nordamerika beschriebene Pathogen *X. fragariae* zunehmende Verbreitung (Epstein, 1966; Howard, 1971; Kennedy und King, 1962a, b; Mahuku und Goodwin, 1997; Ritchie et al., 1993). Seit den 1970er Jahren treten Befallsherde auch in Europa auf (EPPO, 2006; Ustun et al., 2007; Zimmermann et al., 2004) und mittlerweile wurde *X. fragariae* auch in Erdbeerbeständen in Australien diagnostiziert (Gillings et al., 1998; Young et al., 2011). Vor diesem Hintergrund kommt der Entwicklung von Nachweisverfahren, insbesondere an äußerlich symptomfreien Pflanzen, eine besondere Bedeutung zu. In den vergangenen Jahren wurden die molekularbiologischen Verfahren hierzu ständig weiterentwickelt, beginnend mit spezifischen „Enzyme Linked Immunoabsorbent Assays“ (ELISA) über konventionelle Polymerase Kettenreaktionen (PCR) hin zu hochsensitiven nested-PCR Methoden, welche aktuell in standardisierten Resistenzscreenings verwendet werden (Civerolo et al., 1997; Hildebrand et al., 1990; Hsu et al., 2006; Podishetty et al., 2011; Roberts et al., 1998; Rowhani et al., 1994; Stoger und Ruppitsch, 2004; Turechek et al., 2008; Vandroemme et al., 2008; Zimmermann et al., 2004).

Der Krankheitsverlauf der eckigen Blattfleckenkrankheit beginnt mit dem Eintritt der *X. fragariae*-Bakterien in die Wirtspflanze durch natürliche Öffnungen, wie den Stomata und Hydathoden oder durch verwundetes Pflanzengewebe. Die Bakterien besiedeln den Interzellularraum, vermehren sich dort und binden, unterstützt durch die Bildung von Exopolysacchariden, an die Wirtszellen. Einer der wichtigsten Mechanismen, den die Bakterien zur Manipulation der Wirtszellen einsetzen, ist das Typ-3-Sekretionssystem (T3SS), welches die Zellwand sowie die Zellmembran durchdringt. Es dient als eine Art Tunnel, um die mehr als 25 Effektorproteine in das Cytosol einzuschleusen (Kay und Bonas, 2009). Die ersten sichtbaren Symptome sind wässrige, bakterielle Läsionen mit einem Durchmesser von 0.5 – 2 mm, die im frühen Stadium von kleinen Blattadern begrenzt werden und daher durch einen eckigen Umriss charakteristisch identifizierbar sind. Im weiteren Verlauf der Krankheit verbreiten sich diese Läsionen über das gesamte Laub und bilden größere nekrotische Stellen. Letztlich erleiden die infizierten Pflanzen einen vaskulären Kollaps (Hildebrand et al., 1967). Die Früchte sind zwar meist nicht direkt durch die Bakterien betroffen, jedoch nimmt der Anteil marktfähiger Früchte durch bräunliche Verfärbungen der Kelchblätter ab und es kommt zu Ertragsverlusten, die durch eine generelle Schwächung der Pflanzen bedingt sind. Über das Ausmaß der Ertragsverluste durch *X. fragariae* berichten Studien in Größenordnungen von 8% (Roberts et al., 1997) bis zu 80% (Epstein, 1966) in Nordamerika, jedoch gibt es derzeit keine exakten Daten, in denen die wirtschaftlichen Verluste genauer quantifiziert wurden. Die Bekämpfung des Krankheitserregers durch chemische Pflanzenschutzmittel gestaltet sich nach wie vor problematisch. Behandlungen mit antibiotischen Wirkstoffen wie Streptomycin und Oxytetracyclin sind zwar wirksam gegen das Pathogen *in vivo*, jedoch besteht unter Feldbedingungen ein hohes Risiko der Resistenzbildung und die Wirkstoffe sind im Erdbeeranbau nicht zugelassen (Roberts et al., 1997). Mischungen aus Kupferverbindungen und dem fungiziden Wirkstoff Mancozeb zeigten sich als wirksam gegen *X. fragariae,* jedoch traten bei diesen Anwendungen phytotoxische Symptome auf (Conover und Gerhold, 1981; Marco und Stall, 1983; Roberts et al., 1997). Derzeit gibt es auf dem weltweiten Markt kein Pflanzenschutzmittel, das wirksam gegen *X. fragariae* an Erdbeeren ist. Eine vielversprechende Alternative liegt im Anbau widerstandsfähiger Erdbeersorten. Leider sind derzeit alle Sorten, die auf dem europäischen Markt erhältlich sind, mehr oder weniger anfällig gegenüber der eckigen Blattfleckenkrankheit. Die Züchtung neuer, widerstandsfähiger Sorten mit gleichzeitig hoher Fruchtqualität ist daher von großer Bedeutung. Der Erfolg solcher Zuchtprogramme hängt ab von der Verfügbarkeit geeigneter, resistenter Kreuzungseltern, welche die Resistenzeigenschaften zuverlässig an eine hohe Zahl der Nachkommen weitervererben. In diesem Zusammenhang wurden bereits zahlreiche Sorten, Wildartenakzessionen und Zuchtklone hinsichtlich ihrer Widerstandsfähigkeit untersucht (Hildebrand et al., 2005; Jamieson et al., 2013;

Maas et al., 2002; Maas et al., 2000; Perez-Jimenez et al., 2012; Roberts et al., 1997; Xue et al., 2005). Dabei wurden vom USDA National Germplasm Repository zwei resistente, oktoploide Genotypen identifiziert, US4808 und US4809 (Hartung et al., 2003). Neuere Züchtungsexperimente mit diesen Genotypen haben ergeben, dass die Resistenzeigenschaft genetisch auf drei bis vier unabhängige Loci verteilt ist (Lewers et al., 2003). Vermutlich ist dies ein Grund, warum die Resistenz nur an einen kleinen Anteil an Kreuzungsnachkommen weitervererbt werden kann. Um nun die genetische Ausgangsbasis für die weitere gezielte Züchtung widerstandsfähiger Sorten mit pyramidisierten Resistenzeigenschaften zu erweitern, ist die weitere Identifikation von Resistenzgebern in oktoploidem Zuchtmaterial eine wichtige Voraussetzung. In der vorliegenden Arbeit wird eine große Anzahl an Erdbeersorten, Wildartenakzessionen und Zuchtklonen hinsichtlich ihrer Resistenz-Eigenschaften gegenüber *X. fragariae* evaluiert und zudem die systemische Ausbreitung des Krankheitserregers innerhalb der Pflanzen genauer untersucht.

2 PUBLIKATIONEN

2.1 A DIALLEL CROSSING APPROACH AIMED ON SELECTION FOR RIPENING TIME AND YIELD IN BREEDING OF NEW STRAWBERRY (*FRAGARIA* × *ANANASSA* DUCH.) CULTIVARS

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Kurzfassung:

Die Ausweitung der Reifezeit von Erdbeeren im Freilandanbau ist für die Anbauer von hoher wirtschaftlicher Bedeutung. Daher ist die gezielte Züchtung extrem früh- bzw. spätreifender Erdbeersorten mit hohem Ertragspotential von besonderem Interesse. Dreizehn Erdbeersorten wurden reziprok ohne Selbstungen miteinander gekreuzt und die daraus resultierenden 144 F1-Populationen wurden in einem Feldversuch in zwei aufeinanderfolgenden Versuchsjahren evaluiert. Die Versuchsdaten wurden mit Hilfe eines Mixed-Model Ansatzes unter SAS 9.3 statistisch ausgewertet, der hier für diallele Kreuzungsversuche angepasst wurde. Die Variabilität im Kreuzungsversuch basiert hauptsächlich auf der allgemeinen Kombinationseignung (GCA) der verwendeten Sorten (additive Effekte). Spezifische und reziproke Kombinationseignungen (nicht-additive Effekte) erwiesen sich als weniger relevant. Die höchsten GCA's für die Eigenschaft „Marktfähiger Ertrag" wurden für die Sorten 'Polka' und 'Yamaska' ermittelt. Die Eigenschaft „Frühzeitigkeit" ist bilateral mit signifikant niedrigen GCA's für die früh reifenden Sorten 'Clery' und 'Daroyal' sowie signifikant hohen GCA's für die spät reifenden Sorten 'Yamaska' und 'Florence'. Kreuzungen mit diesen Sorten ergeben mit hoher Wahrscheinlichkeit Nachkommenschaften mit hohem Ertragspotenzial und ausgedehnter Reifezeit.

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A diallel crossing approach aimed on selection for ripening time and yield in breeding of new strawberry (*Fragaria* × *ananassa* Duch.) cultivars

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With 3 figures and 4 tables

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Abstract

The extension of the ripening season in open field production is of high economic interest for strawberry growers. Therefore, targeted breeding for extreme early or late ripening cultivars with high yield potential is of particular interest. Thirteen strawberry cultivars were crossed in a reciprocal way without selfing, and the 144 resulting F_1 populations were evaluated in a field trial over a period of two consecutive years. The data were analysed using a mixed-model approach adapted for diallel crossing designs using SAS 9.3. The variability in the crossing approach is mainly based on the general combining ability (GCA) of the cultivars (additive effects). Specific and reciprocal combining abilities (non-additive effects) appear less important. The highest GCAs for the trait *Marketable Yield* were found for the cultivars 'Polka' and 'Yamaska'. The trait *Earliness* is bilateral with significantly low GCAs for early ripening in 'Clery' and 'Daroyal' and significantly high GCAs for late ripening in 'Yamaska' and 'Florence'. Crosses with these cultivars are likely to deliver populations with both high yield and an extended ripening period.

Key words: Fragaria — ripening period — yield — general combining ability — specific combining ability

The cultivated strawberry *Fragaria* × *ananassa* Duch., arisen from accidental hybridization between *F. virginiana* and *F. chiloensis* (Darrow 1966), is the economically most important berry fruit crop today (Hummer and Hancock 2009). Strawberries are produced in more than 70 countries worldwide with a yearly fruit yield of approximately 4.1 Million metric tons (Flachowsky et al. 2011). Resulting from its economic importance, the pressure of competition on the marketplace, environmental conditions and production systems used in a given area as well as the utilization of fruit for fresh market or processing always increase the demand for new strawberry cultivars with improved traits. Breeders around the globe are focused onto a huge number of traits or trait complexes such as yield capacity, fruit quality (fruit appearance, size, colour, shipping quality, shelf life and flavour), and resistance or tolerance towards important pests, pathogens and abiotic stress conditions (Flachowsky et al. 2011). One of the economically most important objectives is the extension of the ripening season. In Germany, most strawberries are sold between June and July (74% and 85% in 2009 and 2010, respectively) where only minimum prices (141 to 193 € per 100 kg) can be realized. Better prices can be realized at the beginning (April/May) of the season and in August. In April/May, prices up to 2.5-fold are possible (Behr 2012). Therefore, breeding of cultivars, which ripen very early or very late in the season, is of particular interest.

Since the early eighteenth century when strawberry breeding started on a more scientific basis, crosses and selections were made in a rather experimental way, which took an enormous amount of manpower, spatial resources and time, leaving a good lot of breeding progress by chance. The success of breeding depends strongly on the selection of the best suitable breeding parents to achieve the formerly defined goals. Fundamental knowledge about cultivars (genotypes) and their traits as well as the heritability of individual traits is therefore indispensable. Diallel crosses can be performed to determine the inheritance of specific traits and to identify superior parents among a number of genotypes. In strawberry, such diallel crosses have been conducted to study the inheritance of quantitative traits such as marketable fruit yield, fruit weight, susceptibility to grey mould, fruit colour and fruit firmness (Masny et al. 2005). Further diallel crosses were performed to study the inheritance of late ripening time (Zurawicz et al. 2006), fruit yield and related factors (Aalders and Craig 1974, Zurawicz et al. 1995), and resistance to *Colletotrichum acutatum*, powdery mildew caused by *Sphaerotheca macularis*, and *Phytophthora fragariae* (Melville et al. 1980, Gimenez and Ballington 2002, Davik and Honne 2005).

In the present study, a diallel was established to evaluate 13 commonly used strawberry cultivars for their combining abilities and reciprocal combining abilities. The study was aimed on providing knowledge for the selection of parental varieties to be incorporated into future programmes for the improvement of strawberry breeding. The diallel cross included early ripening cultivars ('Antea', 'Clery', 'Daroyal', 'Darselect' and 'Madeleine') as well as middle ripening cultivars ('Arosa', 'Elsanta', 'Galia', 'Marmolada' and 'Sonata') and late ripening cultivars ('Florence', 'Polka' and 'Yamaska'). Twelve cultivars are hermaphrodite, whereas the cultivar 'Yamaska' has female flowers. The 13 cultivars were crossed in all possible combinations to provide a total of 144 F_1 hybrids (including reciprocals). These hybrids were grown in the experimental field at the Julius Kuehn-Institute in Dresden-Pillnitz (Germany) for two consecutive years and were evaluated for a number of traits. Special attention has been paid to fruit yield and ripening time as well.

Materials and Methods

Procedure of crossing: Thirteen cultivars of the species *Fragaria* × *ananassa* Duch. were selected as parents for the diallel crossing

approach. They are commonly cultivated in an open field production in Germany, and they cover a wide range of the ripening season. The 13 cultivars were crossed in all possible combinations to provide a total of 144 F_1 populations. The diallel crosses with the cultivars 'Antea', 'Arosa', 'Clery', 'Daroyal', 'Darselect', 'Elsanta', 'Florence', 'Galia', 'Madeleine', 'Marmolada', 'Polka', 'Sonata' and 'Yamaska' were carried out in March 2009 in the greenhouse. Prior to crossing, the potted plants were raised and overwintered in an unheated sand bed compartment to provide the obligatory frost stimulus needed for flower formation. In January, the plants designated to be used as fathers were induced to flowering in a heated compartment with 20°C (day), 18°C (night) and minimal light requirements of 160 kLh/day. Pollen was collected four weeks later by picking the anthers manually with forceps, air-dried at room temperature and stored in glass vials. With two weeks retard, the mother plants were induced to flowering, too. To prevent self-fertilization in the hermaphrodite flowers, the anthers were removed at BBCH 59, when the flowers reached the balloon stage. After a targeted brush-pollination with the respective pollen, the plants were covered with fine-meshed domes in order to prevent them from any other unintended pollination. Subsequently, the seed-containing outer layers of the fully ripened fruits were peeled and dried on filter paper, so that the seeds for each cross could be collected easily. They were sown into boxes with potting substrate and stratified for two weeks at 2°C under high humidity. Hundred seeds of each combination were planted, and the number of germinated seedlings was recorded. If necessary, planting of seeds continued to generate the required number of plants. The seedlings were raised in the greenhouse, and 30 randomly selected plants per combination were transplanted in the field in August 2009. These 30 plants form one F_1 population.

Experimental set-up: The field trial area was located near Dresden, Germany at an altitude of 113–118 m above sea level, an average temperature of 9.2°C and 647 mm average annual precipitation. The soil belongs to the soil class Luvisol, and the soil type can be characterized as a sandy loam with a pH of 6.0 (2010) and an average soil value of 65. The previous crops were a grass mixture followed by *Tagetes*. The experimental plots were fertilized by 30 t/ha manure in spring 2010 and supplemented with 60 kg/ha P (TSP) and 40 kg/ha N (CAN) in spring 2011. The plant protection strategy followed the guidelines of integrated pest management. In 2011, fungicides were avoided to evaluate fungal diseases in the experimental plots.

For each cross, 30 seedlings were planted in a randomized complete block design with two replicates and 15 plants per plot. Plots were arranged in five columns with 68 rows in columns one to four and 16 rows in column five. The columns were separated into two subcolumns by a path between row 31 and 32. The parental cultivars were also planted in a close-by plot with 15 plants per cultivar, except for 'Madeleine' and 'Marmolada'. These cultivars were not included in this investigation, because of the inappropriate plant quality of the parental plant material at that time. Total yield, marketable yield and the harvest period have been measured and evaluated in 2010 and 2011. Marketable yield is a part of the total yield with fruit diameter >18 mm, regular external appearance and without pest or disease infestations. Additionally in 2011, the common leaf spot disease caused by *Mycosphaerella fragariae* and the red spot disease caused by *Diplocarpon earliana* were evaluated for each plant on a score from 1 (0-0.5% coverage) to 9 (>64% coverage).

Plots were harvested up to 15 times in the period from 11th June to 6th July 2010 and up to 13 times in the period from 31st May to 30th June in 2011.

Statistical modelling: As diallel crossing systems are a widely used approach in plant breeding (Christie and Shattuck 2010), different designs of crossing have been approved and lead to the calculation of general and specific combining abilities. The general combining ability (GCA) describes the performance of the parents used for the crosses for specific traits (Hayman 1954, Griffing 1956a,b, Vieira et al. 2009). General combining ability effects can be interpreted as additive genetic effects. We used each parent both as mother and as father. The combination between two parental cultivars occurs in two possible crosses, denoted as cross and reciprocal cross. The specific combining ability (SCA) effect is the difference of a combination from its expected theoretical value calculated from parental GCA values. It can be interpreted as the non-additive genetic effect.

In our experiment, *n* parents are being crossed using each parent both as mother and as father in a reciprocal way. The total number of crosses made is summed up by $(n^2 - n)/2$ crosses and $(n^2 - n)/2$ reciprocal crosses. Neither the parents themselves nor inbred lines of selfed parents are included, so this corresponds to Griffings Method 3 (Griffing 1956a). An anomaly occurs due to the fact that one of the parental strawberry cultivars, 'Yamaska', forms exclusively female flowers and therefore cannot be used as father. Hence, the total number is partitioned into 78 crosses (n = 13), and 66 reciprocal crosses (n = 12). For analysing the data, we used the mixed-model approach (model 2) presented by Möhring et al. (2011) for each year and across years. For the first, we treated traits of different years as different traits, because traits in the first year describe the potential in establishing plants while in the second year the traits characterize mature strawberry plants. For the latter, we assume that traits of different years are identical traits. In both cases, the mixed-model approach allows a separation of additive and non-additive effects. For the analysis, we added effects for the randomization process [replicate (R)] and physical units within the field [column (C), subcolumn (SC)]. The model for the single-year analysis in the syntax of Patterson (1997) is given by:

$$\begin{aligned} \mathrm{R} + \mathrm{R}\cdot\mathrm{C} : \mathrm{R}\cdot\mathrm{C}\cdot\mathrm{SC} + \mathrm{GCA_m} + \mathrm{GCA_f} \\ + \mathrm{SCA_{mf}} + \mathrm{RGCA_m} - \mathrm{RGCA_f} + \mathrm{RSCA_{mf}} \end{aligned} \quad (1)$$

where R, C and SC denoted factors replicate, column und subcolumn. We assumed that the effects $GCA_i \sim N(0, \sigma^2_{GCA})$, $RGCA_i \sim N(0, \sigma^2_{RGCA})$, $SCA_{mf} \sim N(0, \sigma^2_{SCA})$ and $RSCA_{mf} \sim N(0, \sigma^2_{RSCA})$. Observations were weighted by the inverse number of growing plants per plot. For the analysis across years model [1] is extended to:

$$\begin{aligned} \mathrm{R} + \mathrm{R}\cdot\mathrm{J} + \mathrm{R}\cdot\mathrm{C} + \mathrm{R}\cdot\mathrm{C}\cdot\mathrm{J} : \mathrm{R}\cdot\mathrm{C}\cdot\mathrm{SC} + \mathrm{R}\cdot\mathrm{C}\cdot\mathrm{SC}\cdot\mathrm{J} + \mathrm{GCA_m} \\ + \mathrm{GCA_m}\cdot\mathrm{J} + \mathrm{GCA_f} + \mathrm{GCA_f}\cdot\mathrm{J} + \mathrm{SCA_{mf}} + \mathrm{SCA_{mf}}\cdot\mathrm{J} \\ + \mathrm{RGCA_m} + \mathrm{RGCA_m}\cdot\mathrm{J} - \mathrm{RGCA_f} - \mathrm{RGCA_f}\cdot\mathrm{J} + \mathrm{RSCA_{mf}} \\ + \mathrm{RSCA_{mf}}\cdot\mathrm{J} \end{aligned} \quad (2)$$

where *J* is the effect of the year and all other effects are denoted as in model [1]. While we use non-transformed yield data for both years, we decided to use a square root transformation across years. In this case, given values were back-transformed for presentation only, and the delta method is used to get the standard error. Both models were implemented into SAS code (Table 1).

Germination of the seeds was analysed using [1] with the MIXED procedure in SAS 9.3 (SAS Institute Inc. 2011) on logit-transformated data. For this case, residual variance and RSCA variance were confounded, and one is dropped from the model. Presented values were

Table 1: Implementation of the single-year diallel model into SAS program code

```
proc mixed data=a1
class sca rsca wdh column subcolumn plot;
model y=wdh column*wdh/ddfm=kr;
random gca1-gca13/type=toep(1);
random rgca1-rgca13/type=toep(1);
random sca rsca;
random subcolumn;
weight w;
repeated plot/sub=subcolumn;
run;
```

back-transformed for presentation only. Variances were back-transformed using the delta method.

As dependent variables, the traits *Marketable Yield* and *Earliness* were chosen. The *Marketable Yield* is defined as the total amount of marketable fruits over all harvest dates of one year and one plot, whereas *Earliness* equates to the point of time when 50% of the marketable yield of one plot in one year is attained. The exact day is calculated by interpolating linearly between the last date with less than 50% and the first date with more than 50%.

Results

The practical outcome of the intraspecific crosses made by 13 strawberry cultivars was assessed for the fertility of the resulting seeds. Moreover, a closer look onto the yield structure and finally the comprehensive study of the general and specific combining abilities form the main objectives of the investigation.

Seed germination in intraspecific crosses

We observed different germination rates of the analysed populations. Intraspecific crossing using 13 strawberry cultivars revealed differences in the percentage of seeds that successfully emerged to seedlings. Due to the design of the investigation, an estimation of reciprocal SCA effects is impossible. Therefore, reciprocal crosses got the same estimate. The range of observations went from 3% in the cross 'Madeleine' × 'Sonata' up to 100% in the crosses 'Antea' × 'Daroyal', 'Arosa' × 'Daroyal', 'Daroyal' × 'Darselect', 'Darselect' × 'Galia', 'Darselect' × 'Florence' and 'Polka' × 'Florence'. The overall mean of germination was 77%, and 121 of 144 crosses reached germination rates ≥50%. We found difference in maternal germination rates (Fig. 1), for example, cultivars 'Madeleine' and 'Marmolada' used as mothers led to a mean germination rate of only 43.6% and 50.4% which is significantly lower at 0.05 level of probability compared to the crosses with 'Polka' as maternal parent, where 90.1% of the seedlings emerged. No significant differences were found between cultivars used as pollen donors.

Yield structure in the field experiment

In contrast to other crops, the yield structure for strawberry harvest is more complicated. During the ripening period, the plants were harvested every 1 to 3 days, that is, not only on one particular date. Naturally, the strawberry yield per harvest date increases to a maximum and declines until the end of the season. Figure 2 gives an overview of the quantity and the scatter of the yield at the particular day of harvest for the years 2010 and 2011. In comparison with the year 2010, the harvest period was 10 days earlier and 5 days longer in 2011. Additionally, we found a harvest maximum on 15th June, where the graph points out that the variance on this harvest day is remarkably wide. The graph for year 2010 was less characterized by a maximum of yield, whereas quantity and variance were more or less constant. The yield per plot ranged from 0.13 kg up to 8.86 kg and from 10 to 1106 strawberries. In 2011, the highest yields per plant were obtained by the crosses 'Yamaska' × 'Polka' (460 g/plant), 'Yamaska' × 'Daroyal' (425 g/plant) and 'Polka' × 'Daroyal' (381 g/plant).

Elements of the genetic variance

As previously described, the total genetic variance is divided into four components: GCA, RGCA, SCA and RSCA. The relative importance of these components for the traits *Marketable Yield* and *Earliness* is shown in Table 2. In the first year of cultivation, 44% of the total variance of *Marketable Yield* can be explained by GCA and 32% by SCA. Furthermore, the GCA effects explain 71% of the variance in the second year of cultivation whereas the contribution of SCA declines to only 11%. The components RGCA and RSCA appear less important of this trait. Assessing the variance components for *Earliness,* the GCA explains 63% of the variance in 2010 and 86% in 2011. The RSCA explains 31% in 2010 to 12% in 2011, whereas the impact of SCA and RGCA is down to zero.

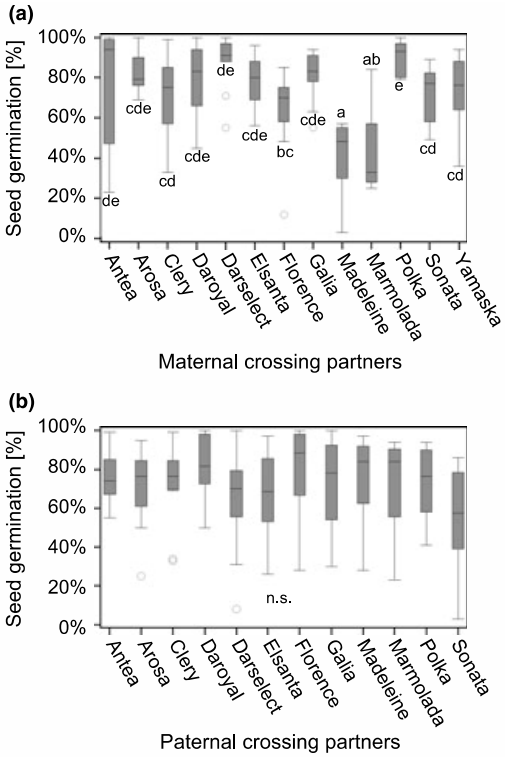

Fig. 1: Comparison of germination rates in a diallel crossing design with (a) 13 strawberry cultivars used as mothers and (b) 12 cultivars used as fathers, respectively. The respective mean values indicated with the same letters are not significantly different (F-Test, P = 0.05)

Breeding value of the parental strawberry cultivars

Because of the finding that the genetic variance is mainly explained by GCA, the breeding value of the parental strawberry cultivars can be centralized by their GCA values. In Table 3, the GCA effects for the traits *Marketable Yield* and *Earliness* are presented for the years 2010 and 2011. The GCA values for the trait *Marketable Yield* were calculated in g/plant and indicated a positive or negative effect on the yield in crosses with a particular parent. We found only a few cultivars which crosses produced significant results for both consecutive years. The combined analysis over both years resulted in non-significant GCA values. In detail, with the cultivar 'Polka' as parent, we found an average of 68.9 g higher yield per plant compared to the total mean of all crosses in 2010 and 107.4 g per plant in 2011. The highest positive effect on the yield was observed in crosses with the cultivar 'Yamaska' in the year 2011 with a benefit of 144.7 g/plant. In contrast to this, the same cross reached

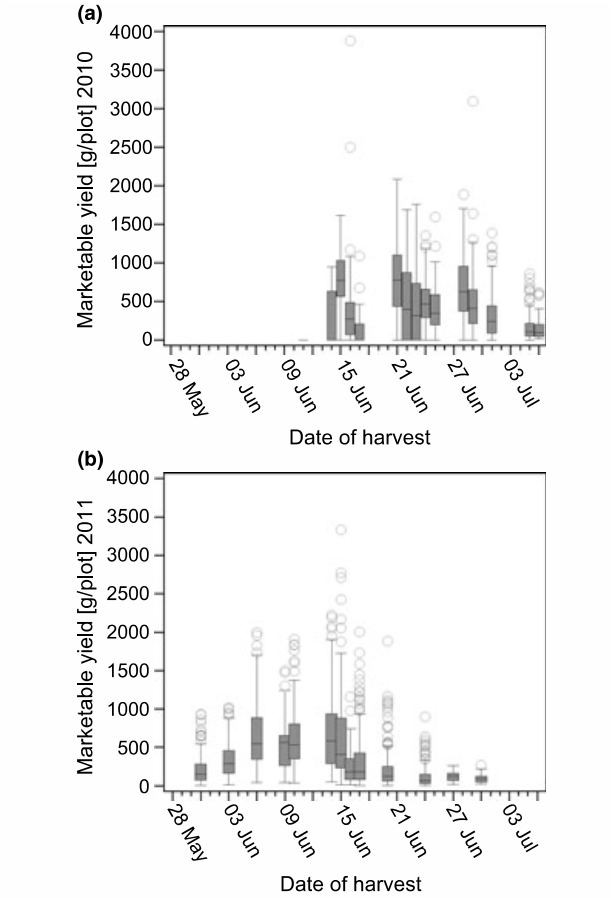

Fig. 2: Progress of harvest in field trial in the years (a) 2010 and (b) 2011. Outliers are plotted with a circle.

a not significant GCA value of only 5.6 g/plant in the year 2010. A non-significant correlation between yields of the two consecutive years is found ($r = 0.296$).

Besides the GCA effects, we also found single combinations with significant SCA values in 2011. The combinations 'Sonata' × 'Arosa' with a SCA of −46.1 g/plant and 'Sonata' × 'Darselect' with −50.0 g/plant, respectively, have significantly lower yields than estimated by the GCA values of their parents.

The trait *Earliness* was specified as the day of 50% of total yield and refers to the importance of an extended ripening period. Therefore, highly negative as well as positive GCA values can be interesting for further breeding progress. In opposition to the yield data, the GCA values *Earliness* for 2010 and 2011 correlated strongly ($r = 0.848$). For early ripening, the cultivars 'Daroyal' and 'Madeleine' seemed to be the most promising breeding parents due to their significant and very low GCA values in both years. In 2010, crosses with 'Daroyal' reached the point of 50% yield 2.4 days earlier and 'Madeleine' 1.7 days earlier than the average. Comparably, crosses with 'Florence' and 'Yamaska' resulted in a very late ripening progeny indicated by highly significant positive values in both trial years. All crosses with 'Yamaska' reached 50% of the total yield more than 4 days later than the other combinations in 2011. The earliest combination was 'Clery' × 'Daroyal' which reached 50% yield at 3rd June 2011. In contrast, the latest combination was 'Yamaska' × 'Florence', where the point of 50% yield was reached 13 days later on 16th June 2011 (Fig. 3). At the research site in Dresden-Pillnitz, the date of 50% yield of 2011 for 'Elsanta' was 6th of June, for 'Clery' 3rd of June and for 'Yamaska' and 'Florence' 9th of June. For the additionally evaluated traits fruit firmness, leaf health, position of inflorescences and fruit colour the differences between the populations were negligibly small (data not shown), as well as for the common leaf spot disease caused by *Mycosphaerella fragariae* and the red spot disease caused by *Diplocarpon earliana*. Besides, the avoidance of fungicides in 2011 did not cause visibly clear symptoms of grey mould (*Botrytis cinerea*) and mildew (*Sphaerotheca macularis*) on plants and fruits. Therefore, this study shows no relevant breeding values for those traits.

Discussion

The classical diallel crossing approach used in this study delivers good applicable results that can be directly used by strawberry breeders. For the traits *Marketable Yield* and *Earliness* in strawberries, the selection of promising breeding parents on the basis of GCA values is reasonable.

Implications about the ripening time based on GCA values

As shown in Table 3, the cultivars 'Clery' and 'Daroyal' have significantly negative GCA values of −2.5 and −2.4 for the trait *Earliness* in 2011, which means that crosses with these cultivars will result in progeny that ripen 2.5 or 2.4 days earlier in theory. As a matter of fact, the combination 'Clery' × 'Daroyal' (25) is the earliest combination in the diallel, where the point of 50% yield is 4.6 days earlier than the average. Together with the analysis of the variance components (Table 2), this leads to the assumption that the trait *Earliness* was almost exclusively inher-

Table 2: Variance components for the traits *Marketable Yield* and *Earliness* in the years 2010 and 2011 and in a combined analysis over both years

	Marketable Yield (g/plant)			*Earliness* (days to 50% harvest)		
Source	2010	2011	Combined analysis	2010	2011	Combined analysis
GCA	1015.34	4927.65	462.10	3.41	3.23	4.04
RGCA	50.27	10.56	88.21	0.00	0.00	0.00
SCA	742.35	731.75	447.75	0.24	0.00	0.00
RSCA	0.00	0.00	0.00	1.70	0.44	0.00
Subcolumn	115.64	1075.85		0.06	0.08	
Residual Error	388.86	217.93		0.04	0.01	
GCA × year			1841.71			0.01
RGCA × year			0.00			0.00
SCA × year			602.95			0.60
RSCA × year			316.61			0.83

Table 3: General combining abilities (GCAs) for the traits *Marketable Yield* and *Earliness* of 13 strawberry cultivars used as parents in a diallel cross

	GCA-effects											
	Marketable Yield (g/plant)						*Earliness* (days to 50% harvest)					
Parent	2010	SE	2011	SE	Combined analysis[1]	SE[1]	2010	SE	2011	SE	Combined analysis	SE
'Antea'	4.0	14.7	−46.5	23.0	−5.4	33.6	−1.13	0.71	−0.52	0.72	−0.85	0.65
'Arosa'	11.2	15.1	−18.2	23.2	0.3	34.3	1.59*	0.73	0.90	0.73	1.20	0.66
'Clery'	−22.4	14.4	−24.1	23.2	−5.2	33.6	−1.42	0.70	**−2.48***	0.73	**−2.08***	0.65
'Daroyal'	−8.3	14.6	64.3*	23.9	−6.2	33.5	**−1.63***	0.71	**−2.37***	0.76	**−2.06***	0.66
'Darselect'	5.2	15.0	−77.1*	23.0	9.1	35.2	−1.43	0.74	−1.01	0.73	−1.27	0.66
'Elsanta'	7.6	14.9	−81.6*	22.8	−9.6	33.0	0.01	0.71	−1.37	0.72	−0.85	0.65
'Florence'	−32.5*	14.5	16.5	23.5	−11.1	32.9	**2.89***	0.73	**3.45***	0.74	**3.32***	0.66
'Galia'	−55.5*	14.2	5.6	23.2	−2.9	33.9	1.29	0.71	**1.88***	0.73	**1.70***	0.65
'Madeleine'	−7.9	14.6	−50.2*	22.8	−9.2	33.2	**−1.79***	0.70	**−1.67***	0.72	**−1.78***	0.65
'Marmolada'	−3.8	14.6	−41.6	23.0	−9.5	33.1	−0.09	0.71	−0.30	0.72	−0.20	0.65
'Polka'	**68.9***	16.0	**107.4***	24.7	27.9	37.2	−0.73	0.76	−0.59	0.78	−0.71	0.68
'Sonata'	23.2	15.4	0.8	23.3	4.1	34.6	−0.43	0.74	−0.36	0.74	−0.42	0.66
'Yamaska'	10.2	17.5	**144.7***	27.8	20.6	36.6	**2.87***	0.90	**4.44***	0.92	**4.00***	0.77

bold values: suggested recommendation for further breeding experiments.
[1]Includes Transformation.
*P < 0.05.
SE, Standard error.

ited due to additive genetic effects which is consistent over the two consecutive years and can also be found in the combined analysis.

A comparison of the harvest progress of the earliest combination 25 with the weekly average market price for strawberries in Germany is shown in Fig. 3. The point of 50% yield is already reached in the beginning of the season when the prices are high. Assuming that this is just the basis population from where the best individuals will still be selected, we can already guess the potential of the approach. Furthermore, cultivation techniques such as the application of plastic mulch preponed the ripening season on the field for several days (Hancock 1999) and can be combined with early ripening cultivars in order to hit the most profitable period.

For late ripening time, the cultivars 'Yamaska' and 'Florence' show he highest GCA values. In fact, the combination 'Yamaska' × 'Florence' (140) is the latest ripening family in the diallel with 50% yield on 16th June which is 8 days later than the overall average. The calculated SCA for this population is very small with −0.07 and indicates that the theoretical sum of $GCA_{Florence}$ and $GCA_{Yamaska}$ with 7.89 is mainly influenced by additive genetic effects of the parents which corresponds to the analysis of variance components (Table 2) and the results for

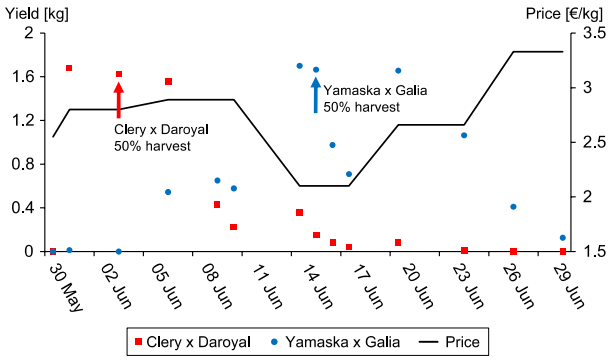

Fig. 3: Progress of yield for the earliest ('Clery' × 'Daroyal') and latest ('Yamaska' × 'Galia') combinations in 2011 in relation to the German market price for strawberries (BLE 2011)

early ripening. Eleven of the 13 parental cultivars have been tested for their 'per se performance' in plots of 15 plants each in a field trial in the year 2011. There is a strong positive correlation ($r = 0.80$; $P = 0.003$) for the trait earliness between the GCA and the per se performance. In contrast to this, there is no significant correlation for the trait marketable yield. These findings strongly meet the theory of Masny et al. (2008), who found the highest heritability for the trait *Ripening Time* among a range of traits studied in strawberry. Growers can profit by combining late ripening cultivars with cultivation techniques for a later ripening period (Rowley et al. 2011).

Implications about the yield potential based on GCA values

Furthermore, we found that crosses with the cultivars 'Yamaska' and 'Polka' lead to progeny with significantly increased yield potential in the two consecutive years but not in the combined analysis. For targeted selection of early ripening cultivars with high yield potential, a combination of the cultivars 'Daroyal' and 'Polka' might lead to the most promising success. For late ripening in combination with high yield we assume the best chances for combinations based on cultivars 'Yamaska' and 'Polka', respectively. The compatibility of these cultivars in crosses is proved by good germination rates between 77% and 90%, whereas crosses with the cultivars 'Marmolada' and 'Madeleine' as mothers show lower compatibility due to significantly lower germination rates (Fig. 1).

Consequences for further breeding approaches

The aim of an extended ripening season can be reached by selection of very early and very late ripening cultivars. Crosses with the cultivars 'Clery' and 'Daroyal' lead to early ripening progeny, and crosses with 'Yamaska' and 'Florence' deliver late ripening populations that form a basis for the site-specific selection of new strawberry cultivars. Significantly, higher yields can be obtained in crosses with 'Yamaska' and 'Polka'. 'Yamaska' was also used as parent in the diallel approach of Davik and Honne (2005) and showed good results for resistance against powdery mildew (*Sphaerotheca macularis*).

Table 4: Parentage of the selected strawberry cultivars for the diallel crossing approach

Cultivar	Parentage (Faedi et al. 2009)
'Antea'	FB6L-3 × 'Marmolada'
'Arosa'	No information available
'Clery'	'Sweet Charlie' × 'Marmolada'
'Daroyal'	'Elsanta' × 'Parker'
'Darselect'	'Elsanta' × 'Parker'
'Elsanta'	'Gorella' × 'Holiday'
'Florence'	{'Tioga' × ['Redgauntlet' × ('Wiltguard' × 'Gorella')]} × ('Providence' × self)
'Galia'	No information available
'Madeleine'	No information available
'Marmolada'	'Gorella' × Sel. Salvi n.15
'Polka'	'Induka' × 'Sivetta'
'Sonata'	'Polka' × 'Elsanta'
'Yamaska'	'Pandora' × 'Bogota'

Considering the sources of variance, there are only little reciprocal effects. The traits *Earliness* and *Marketable Yield* are mainly based upon the main genetic effects (GCA). Therefore, it is less important if the hermaphrodite crossing partners are either used as mother or father in the crossing process. As flowers of 'Yamaska' are typically female, crosses with this cultivar could only be carried out in one direction. For strawberries, it can be regarded as sufficient to design further diallel crossing approaches as a half diallel without reciprocal crosses like it has been done in previous approaches (Davik and Honne 2005, Masny et al. 2005, 2008, Shim et al. 2007). Further proof for this assumption could be given by extended field trials at different locations.

Until now, the cultivar 'Elsanta' is dominating strawberry production in Europe (Hancock et al. 2008) but it is unlikely, that crosses with this cultivar raise the breeding success due to its mostly insignificant GCA values (Table 3). A look on the parentage of the cultivars used in the diallel approach reveals that 'Daroyal' and 'Darselect' are offspring from the same crossing of 'Elsanta' × 'Parker' (Table 4), but 'Daroyal' has a higher value for further breeding progress concerning its GCA values (Table 3). The improved statistical analysis presented in this study delivered reliable results and can be easily adapted to other diallel crossing experiments as shown in Möhring et al. (2011). Despite of the high efforts concerning spatial and human resources, a diallel approach can still be seen as a targeted and effective element in temporary strawberry breeding.

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2.2 EVALUATION OF STRAWBERRY (*FRAGARIA* L.) GENETIC RESOURCES ON RESISTANCE TO *BOTRYTIS CINEREA*

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Kurzfassung:

Die Grauschimmelkrankheit, verursacht durch *Botrytis cinerea,* führt weltweit zu substanziellen wirtschaftlichen Verlusten im Erdbeeranbau. Die Bekämpfung der Krankheit erfordert einen hohen Einsatz an Fungiziden, die in wechselnden Wirkstoffkombinationen angewendet werden, da das Pathogen sehr schnell Resistenzen gegenüber einzelnen Aktivsubstanzen entwickelt. Der Anbau widerstandsfähiger Sorten scheint eine erfolgversprechende Alternative zu sein. Jedoch gibt es derzeit keine Sorten auf dem Markt, die Widerstandsfähigkeit gegenüber *B. cinerea* mit guten obstbaulichen Merkmalen verbinden. Die Züchtung neuer Sorten erfordert eine effektive Methode zur Identifikation widerstandsfähiger Erdbeergenotypen. Die vorliegende Studie hat zum Ziel, obstgenetische Ressourcen der Erdbeere unter standardisierten Bedingungen zu evaluieren. Hierzu wurde eine künstliche Inokulationsmethode entwickelt, bei der reife Früchte mit einer definierten Sporensuspension unter Laborbedingungen inokuliert werden. Die Ergebnisse zeigen, dass diese Methode schnell und einfach durchzuführen ist und zu reproduzierbaren Ergebnissen führt, die mit Feldversuchen korrelieren. Über drei Jahre hinweg wurden insgesamt 107 Erdbeergenotypen der Deutschen Genbank Obst am JKI Dresden-Pillnitz hinsichtlich ihrer Widerstandsfähigkeit evaluiert. Fünf teilresistente Genotypen konnten identifiziert werden: die Sorten ‘Diana’, ‘Joerica’ und ‘Kimberly’ sowie die Wildartenakzessionen *F. virginiana* ‘Wildmare Creek’ und *F. vesca* subsp. *bracteata*. Diese Genotypen weisen zum Zeitpunkt sechs Tage nach Inokulation einen Krankheitsbefall von < 20% auf. Die erzielten Ergebnisse werden diskutiert im Hinblick auf ihre Nutzbarkeit in zukünftigen Züchtungsversuchen.

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Evaluation of strawberry (Fragaria L.) genetic resources for resistance to Botrytis cinerea

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The grey mould disease caused by Botrytis cinerea leads to substantial economic losses in strawberry production all over the world. Control of the disease requires an extensive amount of fungicide that is applied in varying complexes because the pathogen easily develops resistance against the active compounds. Planting of resistant cultivars seems to be a promising alternative for fruit growers, but there are currently no cultivars available combining resistance to B. cinerea with attractive horticultural traits. Breeding of new cultivars requires the effective identification of resistant strawberry genotypes; therefore the current study was aimed at the evaluation of strawberry genetic resources under controlled conditions by establishing an artificial inoculation assay. The method presented in this study is an artificial inoculation of ripe fruits with a defined spore suspension under laboratory conditions. The results show that this assay is fast and simple and leads to reproducible results that correlate with field observations. Over 3 years a total of 107 strawberry genotypes of the German National Fruit Genebank at the JKI in Dresden-Pillnitz were evaluated. Five partly resistant genotypes, cultivars Diana, Joerica and Kimberly, and Fragaria virginiana 'Wildmare Creek' and F. vesca subsp. bracteata, were identified with mean disease levels of <20% at 6 days post-inoculation. The obtained results are discussed with regard to future breeding activities.

Keywords: Botrytis, grey mould, postharvest disease, resistance, strawberry

Introduction

The cultivated strawberry Fragaria 9 ananassa is one of the most important fruit crops worldwide. It ranked first within berry crops with a worldwide fruit yield of c. 4·1 million tonnes per year (Flachowsky et al., 2011). Strawberries are produced in more than 70 countries and organic fruit production is becoming increasingly important (Wilbois et al., 2012). Strawberry production is mostly hampered by plant diseases, one of the most important being grey mould disease. Grey mould is an airborne disease, caused by the necrotrophic fungus Botrytis cinerea (teleomorph Botryotinia fuckeliana), which causes severe damage in more than 200 host plant species (Williamson et al., 2007; Dean et al., 2012). The disease is difficult to control because the grey mould fungus has several modes of attack (Williamson et al., 2007). The application of fungicides is still the method of choice to control B. cinerea in most of the commercially important host species (Dean et al., 2012), but fungicide applications are expensive and fungicide resistance has been found several times (Leroux et al., 2010; Leroch et al., 2011, 2013; Veloukas et al., 2011; Amiri et al., 2012a,b). In 2001 the total costs for botryticides were calculated to be c. €540 million, representing 10% of the world fungicide market (Dean et al., 2012).

Botrytis fruit rot is one of the most important preharvest diseases, but it is also an important postharvest concern (Freeman & Pepin, 1977; Williamson et al., 2007; Lewers et al., 2012). Botrytis fruit rot is mainly initiated in the field (Braun & Sutton, 1987). Senescent foliage as well as diseased flowers and fruits are sources of inoculum. Sporulation takes place on infected plant tissue and the infection of flowers is favoured by cool wet conditions in spring (Bulger et al., 1987; Sosa-Alvarez et al., 1995). The conidia are spread by wind and water and primary infestation takes place when petals and stamens are colonized. Subsequently, the fungus grows into the developing fruit where it remains quiescent until the fruit becomes ripe. Secondary infestation results from infected fruits or leaves that infect neighbouring intact fruits by the dispersal of conidia and direct contact.

Although grey mould is one of the economically most important diseases in strawberry production and many strawberry breeding programmes are aimed at breeding cultivars with improved resistance to B. cinerea (Roudeillac, 2003), there is currently no standard test for resistance

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available, which would allow more goal-oriented breeding. Until now, genotypes were often classified by visual scoring of their susceptibility to *B. cinerea* in the field. Most of the published studies have been performed under different environmental conditions and at different field trial sites. In many cultivar descriptions, grey mould susceptibility is only mentioned as an additional observation. The main focus of cultivar testing is mostly related to traits such as yield and ripening time. Therefore, the results of most of these studies and cultivar descriptions cannot be properly compared and some are even contradictory. Most field experiments have only led to the identification of very susceptible genotypes, whereas tolerant genotypes were often overlooked due to overall low infection levels. In a field trial by Seijo *et al.* (2008), the authors observed an infestation of *Botrytis* fruits that ranged from 5 to 38% in the first year and from 0·3 to 8% in the second year. Based on their results, Seijo *et al.* (2008) concluded that the identification of resistant genotypes within the tested material was difficult. Thus, field experiments have only a limited suitability for a standardized evaluation of genetic material because of high variation in environmental conditions in the field. Research that considers spatial, temporal and personnel resources is necessary to conduct trials in which genotypes can be accurately compared.

Grey mould disease is of increasing importance particularly in organic strawberry production where pesticides are not permitted and alternative indirect plant protection measures, such as cultivation methods and the application of plant strengthening products, show only a limited efficacy (Balci & Demirsoy, 2008). Under such conditions, planting of resistant cultivars seems to be the most promising strategy. Although commercially cultivated strawberry cultivars differ in their susceptibility to this disease, no fully resistant cultivars are currently available on the market (Maas *et al.*, 2000; Droby & Lichter, 2004). Highly tolerant genotypes with 3·3–3·6% fruit/leaf surface coverage by the pathogen were recently described for *Fragaria chiloensis* (González *et al.*, 2009), but no resistance to *B. cinerea* in strawberry has been described that acts specifically in a gene-for-gene manner (Chandler *et al.*, 2006). Several reasons for this, such as the narrow genetic basis of *Fragaria* × *ananassa*, the number of factors affecting the disease progress and the unspecific way in which the pathogen reacts, have been considered.

Numerous studies have been published which focus on characterization of strawberry genetic resources and breeding material for their resistances towards *Botrytis* fruit rot (Irvine & Fulton, 1959; Jarvis & Borecka, 1968; Barritt, 1980; Bristow *et al.*, 1986; Simpson, 1991; Chandler *et al.*, 2006; Seijo *et al.*, 2008; Bestfleisch *et al.*, 2013). Most of these investigations have used natural infections in the field; meaning their comparability is very limited. For example, the studies were performed under different climatic and/or environmental conditions, with different sources and concentrations of inoculum. Therefore, the current study aimed to evaluate strawberry genetic resources for resistance to *B. cinerea* under controlled conditions. This required the establishment of an artificial fruit inoculation assay, which was efficient and reproducible, so that isolates of *B. cinerea,* which varied in their virulence and fungicide resistance, could be used to evaluate strawberry cultivars, wild species accessions and advanced breeding clones. In addition, a field trial was performed where strawberry genotypes were grown for 2 years without any fungicide treatments and evaluated for disease symptoms caused by *B. cinerea*. The obtained results are discussed in relation to the potential for increasing resistance to *B. cinerea* when breeding new strawberry cultivars.

Materials and methods

Plant material and experimental field plot design

Strawberry plants were propagated as misted tips from the collection of the German Fruit Gene Bank in Dresden Pillnitz. The misted tips were cut from runners of the mother plants in July 2010/2011/2012 and planted in Quick-Pot 35 potting plates in a Brill Type1 substrate mixed with sand in a 4:1 ratio for rooting. After 3–4 weeks in the greenhouse, the strawberry plants were transplanted into the open field near Dresden, Germany at an altitude of 113–118 m a.s.l. The soil type was characterized as a sandy loam with a pH of 6·0 (measured in 2010). The average annual precipitation is 647 mm with an average yearly temperature of 9·2°C. The previous crops were a grass mixture followed by *Tagetes*. The experimental plots were fertilized with 60 kg ha^{-1} P (triple superphosphate) and 40 kg ha^{-1} N (calcium ammonium nitrate) in spring. The plants for the artificial fruit inoculation were planted fresh each year. The plant protection strategy followed the guidelines of integrated pest management. For artificial inoculation of fruits, 15 plants per genotype were planted into the field. The characterization of fungal isolates was carried out on cultivars Clery, Elsanta, Florence, Darselect and Senga Sengana. A total of 107 strawberry genotypes from the fruit gene bank in Pillnitz were evaluated for their resistance to *B. cinerea*. The genotypes consisted of 11 strawberry wildtype accessions of the species *Fragaria moschata*, *Fragaria nilgerrensis*, *Fragaria vesca*, *Fragaria virginiana* and *Fragaria viridis* and, 96 genotypes of the species *Fragaria* × *ananassa* with 14 breeding clones from the Pillnitz strawberry breeding programme and 82 cultivars, including both modern and historical genotypes. The evaluation was carried out cumulatively over 3 years; a set of 14 genotypes was tested every year including the economically important cultivars Arosa, Clery, Darselect, Elsanta, Florence, Galia and Honeoye, the cultivars Mieze Schindler and Senga Sengana, which are known to be susceptible towards numerous fungal diseases, and the advanced breeding clones P7201, P8024, P8043, P8049 and P9042. In addition, a field trial with 60 genotypes was performed for two consecutive years in an open field production system without application of fungicides. The field trial was conducted in a randomized complete block design with two blocks, where one block represents one replication. The blocks were arranged lengthwise on a plot of 3 × 180 m with five rows at a distance of 0·5 m from each other. One block consisted of 60 units, one for each genotype, with 30 plants per genotype that were planted over five rows with a planting

distance of 0·25 m, resulting in 120 units of 3 × 1·5 m. For each harvest date, the weight and number of marketable fruits, *B. cinerea*-infected fruits and residual fruits were recorded for each unit and the proportions of fruit with typical symptoms of *B. cinerea* were subsequently calculated for each genotype.

Fungal isolates

For the artificial inoculation, a *B. cinerea* strain collection with different single spore isolates from different years and locations was established. In total, 24 isolates were collected of which five strains (Bc62083, Bc62084, Bc62085, Bc62086, Bc68731) were donated by Professor Deml (JKI, Braunschweig, Germany), four strains (Bc63444, Bc63451, Bc63453, Bc63592) were donated by Dr Stammler (BASF, Limburgerhof, Germany), two strains were field isolates from the year 2011 (Bc111 and Bc112) and 13 strains were field isolates from the year 2012 (Bc1G1, Bc2G2, Bc3G2, Bc4G2, Bc5G2, Bc6G2, Bc7G3, Bc8G3, Bc9G3, Bc10G3, Bc11G3, Bc12G4, Bc13G4). All strains originated from strawberries. The strains were cultivated in plastic Petri dishes on potato dextrose agar (PDA) and transferred onto new medium every 2 months. For sporulation, the freshly transplanted spores were allowed to colonize the plate at 18°C in darkness for 8 days followed by UV-exposure for 24 h and a sporulation period of 7 days. To produce inoculum, the spores were washed from the plates with 10 mL sterile tap water, filtered with 50 μm-mesh sieves, adjusted to a concentration of 10^7 conidia mL^{-1} with a Neubauer haemocytometer and stored for several days at 7°C until inoculations took place. Previous to use for inoculation, the spore suspensions were vortexed thoroughly to ensure the homogeneous dispersal of conidia in the solution. Additionally, the conidial concentrations were checked directly before inoculations took place and corrected if necessary. The strains Bc111, Bc62084, Bc63453 Bc68731 and Bc9G3, which cover a wide range of origins, were used to investigate cultivar × isolate effects. To evaluate strawberry genotypes for resistance to *B. cinerea*, an inoculum mixture with equal proportions of the strains Bc62084, Bc111 and Bc9G3 was used in all three years of the study and was prepared freshly each year prior to the inoculation.

Molecular characterization

For DNA extraction, 40–80 mg of mycelium was harvested from fully colonized PDA plates of each strain. The genomic DNA was extracted using the DNeasy Plant Mini kit (QIAGEN). Nucleic acid concentrations were measured with a NanoDrop 2000c UV-Vis spectrophotometer (Thermo Fisher Scientific Inc.) and adjusted to a concentration of 10 ng μL^{-1}. The molecular identification of *B. cinerea* was carried out with primer pair C729+/− (Rigotti *et al.*, 2002) with slightly modified PCR conditions. The strains were tested for benzimidazole resistance with Bcf and BctubR primers and the products were digested with *Bsa*I as described by Malandrakis *et al.* (2011). The strains were tested for multidrug resistance (MDR1) according to Leroch *et al.* (2013) with primers BcHch262 and BcHch520L and *Hha*I digestion. The MDR1 resistance group includes fenhexamid resistance. Resistance to boscalid was tested with the method described by Veloukas *et al.* (2011) using primers H272R-fw and H272-revc followed by *Hha*I digestion. All digested amplification products were separated by agarose gel electrophoresis in 2% agarose gels for 1·5 h at 125 V.

Artificial inoculation

As a standardized method for the determination of resistance of strawberries towards *B. cinerea*, the method previously described by Bestfleisch *et al.* (2013) was modified for high throughput and exact daily measurement of fruits and fungal infestation. The fruits that had fully ripened, according to Commission Regulations (EU) No. 543/2011 and (EEC) No. 1234/2007 where quality standards for trade class 'Extra' are specified, were harvested. Non-uniform, unripe and overripe strawberries were discarded. Fruits from strawberry wildtype accessions were selected for the resistance test as soon as the fruit colour indicated the ideal ripening stage had been reached. The degree of ripeness was crucial for the experiment due to inhibition of *Botrytis* fruit rot in immature fruits and an undesirable increase of other mould fungi in overripe fruits (Nunes, 2009). After harvest and acclimatization at 15°C, the sepals were removed and the fruits were surface-sterilized in 1% sodium hypochlorite (NaClO) and washed three times with fresh double distilled H_2O under sterile laboratory conditions. For inoculation, the fruits were placed into aluminium boxes on filter paper.

Inoculation was performed on 12–30 fully ripened fruits per genotype by pipetting a 5 μL droplet of inoculum on the fruit surface, without damaging the exocarp. Afterwards the boxes were covered with transparent plastic lids and the inoculated fruits were incubated at 20°C (14 h light/10 h dark) in a climate cabinet for up to 9 days. One box contained up to six inoculated strawberries and constituted one replicate. The boxes were randomized and their positions changed within the cabinet. In the first year, the degree of fruit rot by *B. cinerea* was evaluated on a scale from 0 (no symptoms), 1 (symptoms <10%), 2 (symptoms 11–25%), 3 (fruit rot 26–50%) to 4 (full fruit rot >50%). For better comparison, these scale values were recalculated into percentage of grey mould per fruit. In the following two years, the grey mould infestation was assessed by daily measurement of each lesion with a caliper. Additionally, the diameter of each fruit was recorded and the proportion of infestation (DI, disease infestation) was calculated with the following formula:

$$DI = A_{Bc}/A_S$$

where A_{Bc} is the area of the *B. cinerea* expansion zone and A_S is the strawberry surface area. The fruit surface and the expansion zones of the *B. cinerea* lesions were estimated with the presumption of a circular shape, which was the best approximation in most cases.

Statistical analysis

Statistical analyses were carried out using the software package SAS ENTERPRISE GUIDE v. 4.3 (SAS Institute). The disease infestation values from the artificial inoculation experiments were analysed with an analysis of variance using ANOVA with an overall level of probability of 0·05. Variance components were calculated for the factors 'cultivar' and 'isolate' in the virulence test with the MIXED procedure and the comparison of the mean values was carried out using Fisher's least significant difference (LSD) test with $\alpha = 0{\cdot}05$. The data from the evaluation of strawberry genotypes was analysed with the Newman–Keul's method (SNK test, $\alpha = 0{\cdot}05$) and with Duncan's test ($\alpha = 0{\cdot}05$) to identify significant differences. Correlations were calculated with the CORR procedure using Spearman's rank correlation coefficient r_S.

Results

Molecular characterization of fungal isolates

The objectives of this characterization were to demonstrate the widespread occurrence of fungicide resistance within the collection of isolates and to confirm that the isolates used for the inoculation experiments were *B. cinerea*. The results showed that 23 out of the 24 fungal strains belonged to the species *B. cinerea*, whereas strain Bc68731 was identified as *Botrytis pseudocinerea*.

All isolates were tested for resistance to different fungicides. The *B. pseudocinerea* strain Bc68731 was found to carry multidrug resistance of the MDR1 type and Bc63444 was resistant towards boscalid. Furthermore, resistance towards the benzimidazole fungicide, carbendazim, was found in Bc111, Bc112, Bc62084, Bc62086, Bc63451, Bc63453 and Bc63444.

Characterization of fungal isolates for virulence

To evaluate the virulence of different *B. cinerea* isolates and to study whether the virulence depended on a specific genotype × isolate interaction, the strains Bc111, Bc62084, Bc63453, Bc68731 and Bc9G3 were used in artificial inoculation experiments with the strawberry cultivars Darselect, Elsanta and Senga Sengana. The fruits were also inoculated with double distilled H_2O as a negative control. There were no statistically significant differences in the virulence of the different strains in general (Fig. 1). However, disease symptoms were always greater after inoculation of Darselect and Elsanta with Bc62084 and Bc68731 compared to the control, although differences were not statistically significant. This contrasts with the highly susceptible cultivar Senga Sengana, where disease symptoms after artificial inoculation were always statistically significantly different from the control. Surprisingly, individual fruits of the negative controls also showed slight disease symptoms 9 days after inoculation, which was assumed to be a result of contamination or natural infection of flowers that had occurred previously in the open field.

The analysis of variance components (Table 1) showed that the cultivar effect is 19 times higher than the isolate effect. A genotype × isolate interaction was not found. The residual error is still remarkably high for the investigation.

Determination of the best time point for measuring disease symptoms

Under laboratory conditions the method of artificial inoculation of fruits resulted in development of typical disease symptoms. With an incubation temperature of 20°C, the first visible symptoms were recorded on inoculated fruits 1–2 days post-inoculation (dpi), depending on the genotype. The growth of the lesions increased rapidly during the first week and reached a plateau of full fruit rot at 8 dpi. During this time, the lesions spread

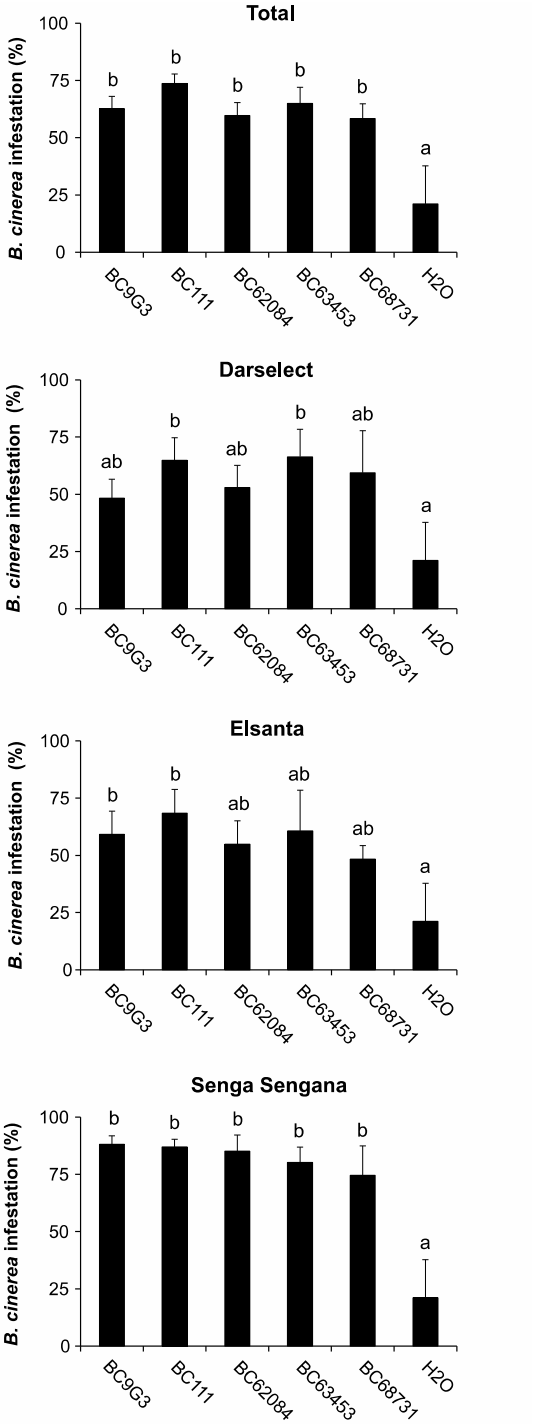

Figure 1 Virulence of the *Botrytis cinerea* strains Bc9G3, Bc111, Bc62084, Bc63453 and Bc68731 tested on three different strawberry cultivars. Infestation is shown as the percentage of grey mould-infected fruit surface at 6 days post-inoculation. Results are presented combined for all cultivars (Total) and as individual cultivars. Error bars show standard error of the mean. Different letters above bars indicate a statistically significant difference in the mean percentage of grey mould infestation at $\alpha = 0{\cdot}05$ using Fisher's least significant difference (LSD) test.

Table 1 Variance components in an artificial inoculation experiment with the strawberry cultivars Clery, Darselect, Elsanta, Florence and Senga Sengana, and *Botrytis cinerea* isolates Bc111, Bc112, Bc62084, Bc63453, Bc68731 and Bc9G3, 6 days post-inoculation

Variance component	
Parameter	Estimate
Cultivar	0·0247
Isolate	0·0013
Cultivar × Isolate	0
Residual Error	0·0603

in a circular shape, beginning from the centre of the inoculation droplet, all over the fruit of susceptible cultivars. Figure 2 shows the typical curve of the overall mean *B. cinerea* infestations in the experiment. The differences between the strawberry genotypes were greatest at 6 dpi (Fig. 2). Therefore, this time point was chosen as ideal for measuring the disease symptoms and further comparison experiments were carried out at 6 dpi.

The overall mean of *Botrytis* fruit rot was 61% of the fruit surface with a mean standard error of 0·24 and a coefficient of determination of 0·52. Comparison of four selected strawberry genotypes revealed the characteristic infection process for susceptible and tolerant genotypes (Fig. 2). For the highly susceptible cultivar Polka, *B. cinerea* infestations of >50% were found as early as 4 dpi. Full fruit rot of >80% occurred only 1 day later. In contrast, the symptoms on *F. vesca* subsp. *bracteata* remained <20% throughout the whole experiment. The more tolerant cultivar Joerica showed comparably low disease infestation until 6 dpi then increased rapidly at 7 and 8 dpi.

Evaluation of genetic resources for resistance to *B. cinerea*

Table 2 shows the results for the resistance screening of all genotypes. The highest infestation was found for the cultivars Königin Luise and Marloun as well as for the advanced breeding clones P8093, P9017 and P5072, which are not significantly different from each other and form the group of highly susceptible genotypes. The cultivars Crimson King, Mrak, Rosella, Diana, Joerica, Kimberly and the wildtype accessions *F. virginiana* 'Wildmare Creek' and *F. vesca* subsp. *bracteata* showed significantly less symptoms than the group of highly susceptible genotypes. From the 107 genotypes investigated, 47 had an infestation ≥70% and were considered to be highly susceptible, 42 were classified as susceptible (fungal infestation of ≥40%), and at least 18 had a mean disease infestation of ≤40% (less susceptible). There were no completely resistant genotypes found, but the wildtype accessions *F. virginiana* 'Wildmare Creek' and *F. vesca* subsp. *bracteata* and the cultivars Diana, Joerica and Kimberly could be regarded as partly resistant genotypes due to their mean disease infestation of ≤20%.

The number of genotypes per infestation class was neither spread equally nor followed a normal distribution (Fig. 3). There were considerably more highly susceptible cultivars than those classified as tolerant. None of the advanced breeding clones were classified as tolerant. Cultivars and wildtypes were found in nearly all infestation classes.

Fourteen lines (nine cultivars and five advanced breeding clones) were evaluated each year (Fig. 4). The overall mean at 6 dpi was 60·2% grey mould decay with a range from 30·8% for Arosa to 93·2% for Senga Sengana. Besides Arosa, the cultivars Florence (36·5%) and Darselect (38·9%) and the breeding clone P8043 were less susceptible towards *B. cinerea*. The cultivars Senga Sengana (93·2%), Mieze Schindler (91·4%), Honeoye (77%) and Elsanta (75·9%) and the breeding clone P8024 (76·7%) showed the greatest disease infestation and were therefore categorized as highly susceptible. The comparison of the mean values indicates that there is no significant difference (Duncan's test, $P < 0·05$) within the highly susceptible genotypes. This was also true for the less susceptible genotypes, whereas the two groups differed significantly in their mean disease infestation (Fig. 4). Comparative analysis over the three years showed that the results from year three were significantly correlated with year 2 ($r_S = 0·55$, $P < 0·05$) and year 1 ($r_S = 0·81$, $P < 0·05$).

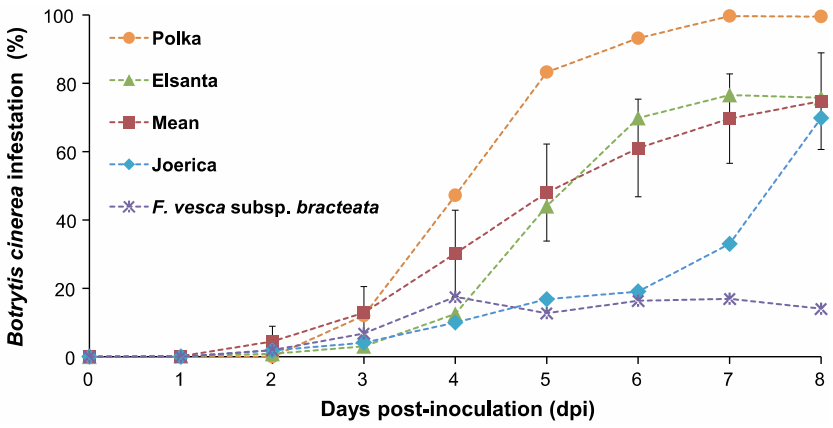

Figure 2 Development of *Botrytis cinerea* infection on strawberries after artificial inoculation of fruits under laboratory conditions. Infestation was measured daily as the percentage of grey mould-infected fruit surface. Error bars show standard error of the mean. The graph shows overall means of 107 different strawberry genotypes for each day post-inoculation and the differential infestation progress of four selected strawberry genotypes: Polka and Elsanta (highly susceptible), Joerica (tolerant) and *F. vesca* subsp. *bracteata* (partly resistant).

Table 2 Disease rating of strawberry genotypes inoculated with *Botrytis cinerea* in laboratory artificial inoculation experiments

Type	Genotype	n^a	DI mean ± SEM[b]	SNK[c]
C	Königin Luise	6	1·00 ± 0·00	a
C	Marloun	4	1·00 ± 0·00	a
P	P8093	2	1·00 ± 0·00	a
P	P9017	2	1·00 ± 0·00	a
P	P5072	2	0·98 ± 0·02	a
W	*Fragaria moschata* 'Capron'	5	0·98 ± 0·01	ab
C	Avant Tout	2	0·98 ± 0·02	ab
C	Templar	2	0·97 ± 0·03	abc
C	Späte Leopold	8	0·94 ± 0·04	abcd
C	Polka	4	0·93 ± 0·03	abcd
C	Prinz Julius Ernst	4	0·92 ± 0·04	abcd
C	Tufts	5	0·92 ± 0·04	abcd
C	Wunder von Köthen	2	0·91 ± 0·09	abcd
P	P6241	2	0·90 ± 0·10	abcd
C	Peltata	4	0·90 ± 0·06	abcde
C	Cambridge Late Pine	6	0·89 ± 0·07	abcdef
C	Sweet Eve	3	0·89 ± 0·10	abcdef
C	Hansa	3	0·88 ± 0·07	abcdefg
P	P7095	4	0·87 ± 0·03	acbdefgh
C	Senga Sengana	8	0·87 ± 0·05	acbdefgh
C	Mieze Schindler	8	0·86 ± 0·05	acbdefgh
C	Yamaska	5	0·86 ± 0·07	abcdefghi
C	Papa Lange	5	0·85 ± 0·05	abcdefghi
P	P9016	6	0·85 ± 0·09	abcdefghi
C	Panther	5	0·83 ± 0·09	abcdefghi
P	P8024	7	0·82 ± 0·08	abcdefghi
W	*F. moschata* 'Franken'	14	0·82 ± 0·07	abcdefghi
C	Solotaya	5	0·82 ± 0·08	abcdefghi
W	*F. moschata* 'Marie Charlotte'	5	0·82 ± 0·04	abcdefghi
C	Juline	6	0·81 ± 0·05	abcdefghi
C	Elsanta	14	0·81 ± 0·06	abcdefghi
C	Mara des Bois	7	0·80 ± 0·06	abcdefghi
C	Cambridge Pricewinner	4	0·79 ± 0·06	abcdefghi
C	Sturms Zuckersüße	5	0·79 ± 0·09	abcdefghi
C	Benamil	4	0·78 ± 0·08	abcdefghi
W	*F. moschata* 'Bauwens'	10	0·78 ± 0·10	abcdefghi
W	*Fragaria vesca* 'Illa Martin'	5	0·77 ± 0·09	abcdefghi
C	Aroma	5	0·75 ± 0·12	abcdefghi
C	Mainperle	5	0·75 ± 0·05	abcdefghi
C	Duretta	3	0·74 ± 0·14	abcdefghi
C	Everest	7	0·73 ± 0·07	abcdefghi
C	Red Coat	4	0·73 ± 0·00	abcdefghi
C	Vikat	5	0·72 ± 0·02	abcdefghi
C	Antea	6	0·71 ± 0·10	abcdefghi
C	Senga Dulcita	3	0·71 ± 0·05	abcdefghi
C	Honeoye	10	0·70 ± 0·07	abcdefghi
C	Frabella	3	0·70 ± 0·11	abcdefghi
C	Oberschlesien	5	0·69 ± 0·06	abcdefghi
C	Arista	5	0·69 ± 0·15	abcdefghi
C	Fructarina	4	0·68 ± 0·21	abcdefghi
C	Pyretta	2	0·68 ± 0·05	abcdefghi
C	Karla	4	0·66 ± 0·16	abcdefghi
C	Rheingold	5	0·66 ± 0·07	abcdefghi
C	Heidekind	3	0·65 ± 0·33	abcdefghi
C	Georg Soltwedel	7	0·65 ± 0·13	abcdefghi
C	Prelude	4	0·64 ± 0·10	abcdefghi
C	Symphony	3	0·64 ± 0·25	abcdefghi
P	P9036	2	0·64 ± 0·16	abcdefghi
C	Joly	3	0·60 ± 0·20	abcdefghi
P	P7077	5	0·60 ± 0·13	abcdefghi
C	Clery	18	0·56 ± 0·08	abcdefghi
P	P8049	7	0·56 ± 0·11	abcdefghi
C	Charlotte	8	0·55 ± 0·08	abcdefghi
C	Sentinel	3	0·54 ± 0·15	abcdefghi
P	P7188	11	0·53 ± 0·09	abcdefghi
C	Bella	2	0·52 ± 0·02	abcdefghi
P	P7201	12	0·52 ± 0·08	abcdefghi
C	Paula	2	0·50 ± 0·20	abcdefghi
C	Dorena	6	0·50 ± 0·08	abcdefghi
C	Alba	3	0·49 ± 0·25	abcdefghi
C	Galia	9	0·48 ± 0·10	abcdefghi
C	Eve's Delight	5	0·48 ± 0·15	abcdefghi
C	Salsa	2	0·47 ± 0·12	abcdefghi
C	Florence	16	0·47 ± 0·08	abcdefghi
W	*Fragaria nilgerrensis* 'FRA078'	5	0·46 ± 0·10	abcdefghi
C	Lambada	3	0·46 ± 0·04	abcdefghi
W	*F. nilgerrensis* 'Yunnan'	5	0·46 ± 0·07	abcdefghi
C	Asia	4	0·45 ± 0·06	abcdefghi
P	P8043	10	0·44 ± 0·09	abcdefghi
C	Albion	3	0·44 ± 0·22	abcdefghi
P	P8071	3	0·44 ± 0·20	abcdefghi
C	Sieger	3	0·44 ± 0·14	abcdefghi
C	Macherauchs Frühernte	2	0·44 ± 0·21	abcdefghi
C	Roxana	5	0·42 ± 0·12	abcdefghi
P	P9042	7	0·42 ± 0·08	abcdefghi
C	Elianny	3	0·41 ± 0·08	abcdefghi
C	Apetita	5	0·40 ± 0·11	abcdefghi
C	Fraroma	4	0·40 ± 0·08	abcdefghi
C	Malwina	6	0·40 ± 0·10	abcdefghi
W	*F. vesca* f. *alba*	13	0·39 ± 0·09	abcdefghi
C	Daroyal	4	0·39 ± 0·17	abcdefghi
C	Darselect	13	0·38 ± 0·08	abcdefghi
C	Dely	3	0·37 ± 0·09	abcdefghi
W	*F. viridis* 'Neddesitz'	5	0·37 ± 0·09	abcdefghi
C	Viktoriana	8	0·36 ± 0·05	abcdefghi
C	Reusraths Allerfrüheste	3	0·35 ± 0·12	abcdefghi
C	Arosa	12	0·35 ± 0·08	abcdefghi
C	Donna	7	0·33 ± 0·13	abcdefghi
C	Figaro	7	0·29 ± 0·09	abcdefghi
C	Crimson King	3	0·27 ± 0·07	bcdefghi
C	Mrak	4	0·26 ± 0·03	cdefghi
C	Rosella	3	0·24 ± 0·03	defghi
C	Diana	8	0·19 ± 0·07	efghi
C	Joerica	7	0·19 ± 0·04	fghi
W	*F. virginiana* 'Wildmare Creek'	5	0·17 ± 0·10	ghi
C	Kimberly	4	0·17 ± 0·07	hi
W	*F. vesca* subsp. *bracteata*	5	0·15 ± 0·08	i

C, cultivar; P, Pillnitz breeding clone; W, strawberry wildtype accession.

[a]Number of replicates, where one replicate consists of six inoculated fruits.

[b]Mean *B. cinerea* fruit rot disease infestation over all replications ± SE of the mean.

[c]Significance according to the SNK test, where mean values with the same letters are not significantly different.

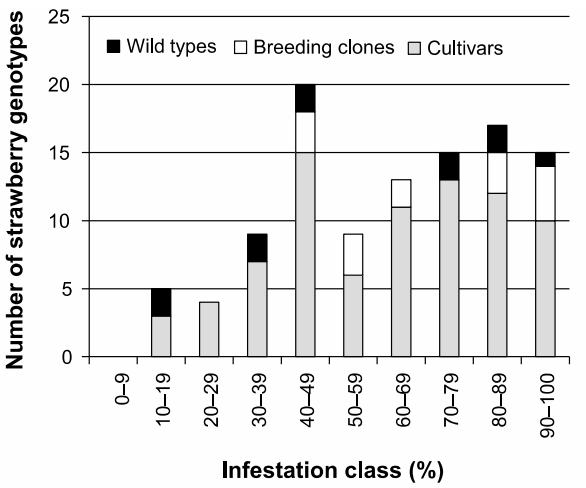

Figure 3 Distribution of strawberry genotypes after artificial inoculation with *Botrytis cinerea*, 6 days post-inoculation. Genotypes were classed according to the level of infestation observed. The number of wild type, breeding clones and cultivars in each class are shown. Total $n = 107$ genotypes.

Field experiments

The susceptibility of 60 genotypes towards *B. cinerea* was assessed in a 2-year field experiment under natural conditions. At each harvest date, the harvested strawberries of each unit were counted, weighed and grouped into the three categories: marketable, *B. cinerea*-infected, and waste. Figure 5 shows the percentage of *Botrytis*-infected fruits per genotype for the first year of the trial. The disease severity in year 1 resulted in a mean *B. cinerea* infestation of 11·5%, whereas in the second year it was only 4·9%. The correlation analysis revealed that disease infestation levels for year 2 did not correlate with the results from year 1 ($r_S = 0{\cdot}11$, $P = 0{\cdot}5$). In early summer of year 2, just before beginning of the harvest season, there was a big flood in Dresden and the field was partially affected by the increased level of ground water for *c.* 2 weeks followed by a subsequent period of drought. Due to the abnormal weather conditions and the insufficient overall disease expression in year 2, further data analyses were carried out with the results from year 1. The highest infestation was found in cultivars Vikat (26%), Georg Soltwedel (26%), Panther (32%), Mainperle (36%) and Papa Lange (44%). More than half of the 60 genotypes (33) showed disease symptoms <10%. Cultivars Segal and Hood, and breeding clones P8043 and P5518, showed only minor symptoms with <5%. Furthermore, a significant medium linear correlation ($r_S = 0{\cdot}53$, $P < 0{\cdot}001$) was found between the results obtained by artificial inoculation and those obtained from the field experiment in year 1. All genotypes from the field trial that were classified as highly susceptible also showed high infestation rates in the artificial inoculation experiment. In contrast, not all of the less susceptible genotypes of the open field experiment also had low disease infestation values in the inoculation experiment.

Discussion

A standardized test under laboratory conditions can lead to more reproducible data and can therefore be used effectively for evaluation of strawberry germplasm and cultivar descriptions. Different inoculation methods and incubation conditions tested revealed that the best reproducible results could be obtained by artificial inoculation of fruits under laboratory conditions (Bestfleisch *et al.*, 2013). The resistance to *B. cinerea* is quantitative and influenced by environmental factors as well as by general plant and fruit robustness. To compare the resistance of different genotypes, it is essential to use only fruits that are at the ideal ripening stage. During the ripening of strawberry fruits, an increase in simple sugars, anthocyanin contents, red colour pigments and aroma volatiles contrasts with a decrease in organic acids and phenolics (Nunes, 2009). In the postharvest period, the softening of strawberry fruits is accompanied by the loss of cell wall material, which seems to be more pronounced in the cortical tissue than in the pith tissue (Heng Koh & Melton, 2002). During fruit ripening there is also a two-phase and dual mode flavonoid biosynthesis (Halbwirth *et al.*, 2006) resulting in a significant shift in the amount of different flavonoid classes. During early stages of fruit development large amounts of different 3′,4′-hydroxylated flavan-3-ols and proanthocyanidin derivatives accumulate. They are thought to suppress the grey mould pathogen, which remains quiescent after infecting the flower until fruit ripening (Schlösser, 1994). At later stages of fruit development, strawberry fruits become bright to dark red, coloured by different derivatives of the anthocyanidins pelargonidin and cyanidin, which are the main pigments of strawberry fruits (Goiffon *et al.*, 1999; Nyman & Kumpulainen, 2001; Andersen *et al.*, 2004). Flavonols are also formed at these stages, which were shown to serve as co-pigments (Henning, 1981; Hertog *et al.*, 1992; Häkkinen & Auriola, 1998; Häkkinen & Törrönen, 2000; Olsson *et al.*, 2004). At the same time there is a significant decrease of 3′,4′-hydroxylated flavan-3-ols and proanthocyanidins. These biochemical and structural changes lead to optimal conditions for the development of the grey mould fungus. They are thought to be responsible for the differences in susceptibility of the strawberry genotypes.

An artificial test of strawberry genetic resources for resistance to *B. cinerea* was successfully established within the present study. This test was shown to be reproducible and gave results that were comparable to results obtained in the field. The field trial results included both primary and secondary infections with *B. cinerea*, whereas the artificial inoculations simulated secondary infections. Secondary infections are becoming more important because they can occur during transportation, storage and trade of fruits. Furthermore, the test is simple and can be used for high-throughput screening.

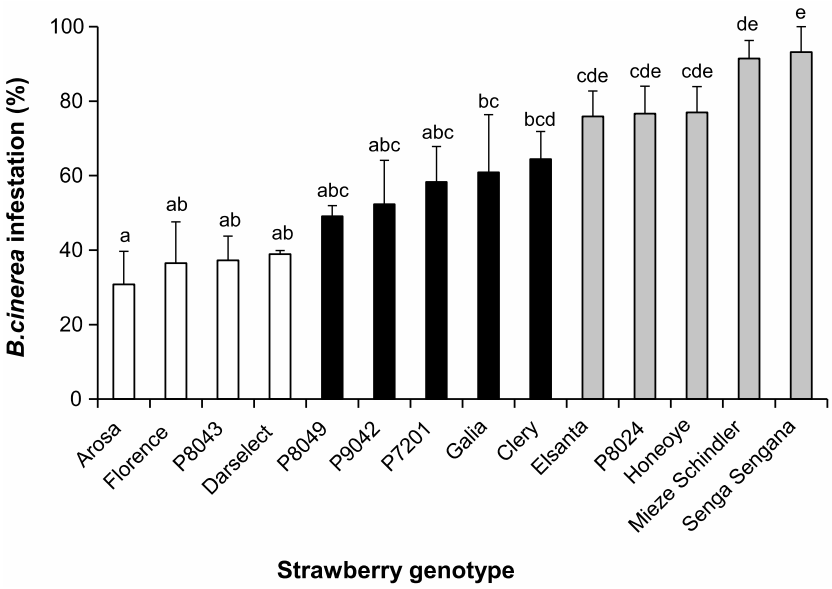

Figure 4 Comparison of percentage infestation with *Botrytis cinerea* of selected strawberry genotypes in 3-year artificial inoculation experiments. Error bars show standard error of the mean. Values indicated with the same letters are not significantly different (Duncan's test, $\alpha = 0{\cdot}05$). Grouping: less susceptible genotypes (white) and highly susceptible genotypes (grey).

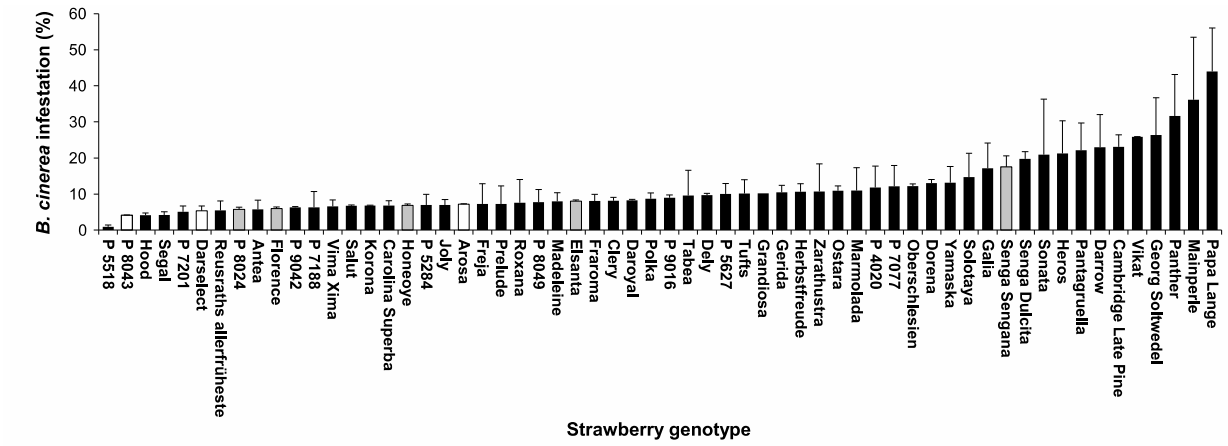

Figure 5 *Botrytis cinerea* fruit rot in open field production of strawberries in 2012 for 60 strawberry genotypes. Error bars show standard error of the mean. Shading indication according to the results from a 3-year artificial inoculation experiment: grey, highly susceptible genotypes; white, less susceptible genotypes.

The artificial test was used to evaluate 107 strawberry genotypes of the National Fruit Genebank belonging to the Julius Kühn-Institute in Dresden (Germany). Most strawberry cultivars were susceptible or highly susceptible towards grey mould, but there were less susceptible cultivars such as Joerica, Arosa, Florence and Darselect with adequate fruit size and yield that can be recommended for strawberry cultivation in Europe to reduce losses due to *B. cinerea* and which might be profitable for both organic and conventional farming. As *B. cinerea* can rapidly develop resistance against fungicides, often as a result of point mutations at the fungicide target site, the strategy of breeding resistant strawberry cultivars is of high importance. The difference in susceptibility of the genotypes in the study is extremely pronounced and there is high potential in the genetic pool of strawberry. However, there was no evidence from the present study for monogenic resistance, which presents a challenge for targeted breeding of new resistant cultivars. The partly resistant wildtype accession *F. vesca* subsp. *bracteata* might be the best donor for resistance within the material investigated but, because of its diploid nature, it could not be used directly for crossing with the octoploid *F.* × *ananassa*. Interspecific hybridization of *F. vesca* subsp. *bracteata* with *F.* × *vescana* (decaploid) could result in a number of hexaploid progeny, which could then be used for backcrossing with *F.* × *vescana* again to obtain octoploids. As the fruits of the wildtype accession *F. vesca* subsp. *bracteata* are very small (1·0–1·5 cm diameter), many backcrosses may be necessary to obtain fruits of marketable size and quality. Positive traits such as the superior taste and aroma of *F. vesca* subsp. *bracteata* fruits could possibly be lost during such a long breeding procedure. It is questionable whether such a breeding

strategy is wise and/or feasible. Therefore, future experiments will include further strawberry wildtype accessions of the octoploid species *F. virginiana* and *F. chiloensis*. However, the enhancement of the genetic pool of cultivated strawberry using *F. vesca* subsp. *bracteata* could potentially be of benefit for both resistance and the aroma of strawberry.

Further evaluation of more than 300 additional strawberry genotypes from the collection in the fruit gene bank of Dresden Pillnitz will continue. Test crossings with the partly resistant genotypes have already been conducted in order to study the inheritance of the resistance trait.

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2.3 RESISTANCE AND SYSTEMIC DISPERSAL OF *XANTHOMONAS FRAGARIAE* IN STRAWBERRY GERMPLASM (*FRAGARIA* L.)

Die Veröffentlichung von Kapitel 2.1 erfolgt mit freundlicher Genehmigung des Wiley Verlags.
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Kurzfassung:

Die eckige Blattfleckenkrankheit, verursacht durch *Xanthomonas fragariae*, ist eine wichtige Pflanzenkrankheit mit gravierenden Auswirkungen auf die Erdbeer-Jungpflanzenproduktion. Zur Zeit ist kein chemisches Pflanzenschutzmittel verfügbar, um die Krankheit effektiv zu bekämpfen. Der Anbau widerstandsfähiger Sorten scheint eine erfolgversprechende Strategie zu sein, jedoch sind alle kommerziell angebauten Sorten anfällig und es konnte noch kein potenzieller Resistenzdonor identifiziert werden. Daher wurden im Rahmen dieser Studie insgesamt 145 Genotypen aus der Sammlung der Obst-Genbank des JKI in Dresden-Pillnitz mittels künstlicher Inokulation hinsichtlich ihrer Resistenz gegenüber *X. fragariae* evaluiert. Davon wurden sechs Genotypen als teilweise resistent eingestuft, von denen zwei Zuchtklone (US4808 und US4809) oktoploid sind. *Fragaria vesca* f. *alba*, *Fragaria nilgerrensis* 'Yunnan', *F. vesca* 'Illa Martin' und *F. moschata* 'Bauwens' wurden ebenso als teilweise resistent eingestuft, können aber aufgrund ihres Ploidiegrades nur bedingt in Zuchtprogrammen verwendet werden. Völlig resistente Genotypen wurden nicht gefunden. Die systemische Ausbreitung der Bakterien in der Pflanze wurde nach einer Inokulation der Blätter mit den Stämmen XF3.9.C und dem GPF-markierten Stamm XF3.9.$C_{(pKAN)}$ untersucht. Die systemische Ausbreitung wurde an 3, 7, 14 und 28 Tagen nach Inokulation mit einem nested-PCR Nachweis und mittels Fluoreszenzmikroskopie genauer untersucht. Bereits drei Tage nach Inokulation konnte *X. fragariae* in allen getesteten Gewebeproben des inokulierten Blattes, dessen Blattstiel, dem Rhizom, der Herzknospe, bis hin

zum jüngsten vollständig entfalteten Blatt und dessen Blattstiel nachgewiesen werden. Die systemische Ausbreitung konnte auch in den teilweise resistenten Genotypen nachgewiesen werden.

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Resistance and systemic dispersal of Xanthomonas fragariae in strawberry germplasm (Fragaria L.)

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The angular leaf spot disease caused by Xanthomonas fragariae is an important plant disease with major impact for the strawberry nursery industry. Currently there is no plant protection product available for controlling the disease effectively. Planting of resistant cultivars seems to be promising, but all commercially used cultivars are susceptible and no donor with a high level of resistance has yet been found. Therefore, a total of 145 genotypes from the Fruit Genebank Dresden (Germany) were evaluated for resistance to X. fragariae by artificial inoculation. Six genotypes were classified as partly resistant, out of which only two (US4808 and US4809) are octoploid. Fragaria vesca f. alba, Fragaria nilgerrensis 'Yunnan', F. vesca 'Illa Martin' and F. moschata 'Bauwens' were also classified as partially resistant, but they are only of limited use for breeding because of their variable ploidy level. Fully resistant genotypes could not be detected. The systemic dispersal of the bacteria in strawberry plants was investigated after inoculation of leaves with X. fragariae strain XF3.9.C and the GFP-tagged strain XF3.9.C$_{(pKAN)}$. The systemic spread was evaluated after 3, 7, 14 and 28 days post-inoculation (dpi) by nested PCR and fluorescence microscopy. After 3 dpi, X. fragariae could be found in all tissues tested including the inoculated leaf, its petiole, the rhizome, the heart bud up to the youngest fully expanded leaf and its petiole. The systemic spread was also detectable in partially resistant genotypes.

Keywords: angular leaf spot, Fragaria, resistance, strawberry, Xanthomonas fragariae

Introduction

In the cultivation of strawberries (Fragaria 9 ananassa), the angular leaf spot disease caused by Xanthomonas fragariae is currently the most important and widespread bacterial disease worldwide (Maas, 1998). Since its first description (Kennedy & King, 1962a) and taxonomic classification (Kennedy & King, 1962b) in North America, X. fragariae has spread worldwide among the strawberry growing regions by trade of runner plants with latent invisible infections. Angular leaf spot disease is now of great concern to the strawberry nursery industry (Jamieson et al., 2013). Even though plant protection organizations have tried to prevent further increase of the disease, X. fragariae is still spreading, originally starting from North America (Kennedy & King, 1962a; Epstein, 1966; Howard, 1971; Ritchie et al., 1993; Mahuku & Goodwin, 1997), to all over Europe during the 1970s (Zimmermann et al., 2004; EPPO, 2006; Ustun et al., 2007), and is now present even in Australia (Gillings et al., 1998; Young et al., 2011). Detection of the pathogen in symptomless plants is therefore of high importance and has been improved in numerous molecular investigations with enzyme-linked immunosorbent assays (ELISA), conventional polymerase chain reactions (PCR) and highly sensitive nested PCR methods, which are used in standardized screening to date (Hildebrand et al., 1990; Rowhani et al., 1994; Civerolo et al., 1997; Roberts et al., 1998; Stoger & Ruppitsch, 2004; Zimmermann et al., 2004; Hsu et al., 2006; Turechek et al., 2008; Vandroemme et al., 2008; Kumar et al., 2012).

Disease progress of angular leaf spot begins with the invasion of X. fragariae through natural openings, such as stomata and hydathodes or wounds. The bacteria enter the intercellular space for multiplication and bind to host cells, supported by exopolysaccharides. One of the most important mechanisms for the manipulation of host cells is the type 3 secretion system (T3SS) which penetrates the host cell wall and cell membrane. It serves as a hollow needle-like structure to infiltrate more than 25 effector proteins into the host cytosol (Kay & Bonas, 2009). The first visible symptoms occur as water-soaked bacterial lesions of 0·5–2 mm diameter, which are restricted by small leaf veins in early stages and appear angular in shape. Later, these lesions spread over the

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foliage and form larger necrotic spots. Finally, plants suffer from vascular collapse (Hildebrand *et al.*, 1967). Fruits are not primarily affected by the bacteria, but their marketability can suffer from discolouration of the calyx and yield loss through constitutional weakness of the plants. Yield losses due to *X. fragariae* have been reported to range from 8% (Roberts *et al.*, 1997) up to 80% (Epstein, 1966) in North America, but exact data for the quantification of economic losses are not available at present. Chemical control of the disease is difficult. Treatments with streptomycin and oxytetracycline were effective against the pathogen *in vivo*, but there is a risk of formation of resistant populations under field conditions and the antibiotics are not registered for use in strawberry production (Roberts *et al.*, 1997). Mixtures of copper compounds and the fungicide mancozeb were found to be effective against *Xanthomonas* but they show/can show phytotoxicity (Conover & Gerhold, 1981; Marco & Stall, 1983; Roberts *et al.*, 1997). Currently there is no plant protection product on the market that is effective against *X. fragariae* in strawberries and therefore the planting of resistant cultivars represents an important alternative strategy. Unfortunately, all cultivars currently available on the European market are more or less susceptible to the disease. Therefore, breeding of durable resistant cultivars with excellent fruit quality is urgently needed. The success of such breeding programmes depends on the availability of resistance donors that pass on the trait effectively and to a high number of progeny.

Numerous cultivars, wild species and advanced breeding clones from different strawberry breeding programmes have been evaluated for resistance (Roberts *et al.*, 1997; Maas *et al.*, 2000, 2002; Hildebrand *et al.*, 2005; Xue *et al.*, 2005; Perez-Jimenez *et al.*, 2012; Jamieson *et al.*, 2013), but until now only two resistant octoploid genotypes, designated as US4808 and US4809, have been detected by the USDA national germplasm repository (Hartung *et al.*, 2003). Recent breeding experiments with these genotypes showed that their resistance is based on three or four unlinked loci (Lewers *et al.*, 2003). Therefore, the resistance can be inherited by only a small percentage of the progeny. To broaden the availability of durable resistant cultivars by pyramiding different resistance mechanisms, further resistance donors in octoploid breeding material are urgently required.

The main objective of this study was therefore the evaluation of strawberry genotypes for resistance to *X. fragariae* within the strawberry germplasm collection of the National Fruit Genebank at the Julius Kuehn-Institute (JKI) in Dresden-Pillnitz, Germany. In experiments with potted plants inoculated and grown under greenhouse conditions, a total of 119 cultivars, eight wildtype accessions and 18 advanced breeding clones from the Pillnitz strawberry breeding programme were evaluated over three years. In addition, the US breeding clones US4808 and US4809 were included in the resistance screening to confirm their formerly described resistance.

To provide more detailed information on pathogen development and spread within its host plant, inoculation with a fluorescent reporter strain of *X. fragariae* and detection by PCR were also used. Different plant tissues, with and without symptoms, were analysed separately and over time by fluorescence microscopy and nested PCR (Zimmermann *et al.*, 2004).

Materials and methods

Plant material

The strawberry genotypes used in this study (Table S1) were selected from the collection of the National Fruit Genebank at the Institute for Breeding Research on Fruit Crops Dresden-Pillnitz, Germany. For each genotype, 10 healthy runner-plants were propagated and transplanted to the field in August 2010/2011 as mother plants. The plants for resistance screening in the greenhouse were propagated from these mother plants grown in the experimental field, which is located near Dresden-Pillnitz. The average temperature at the location is 9·2°C with 647 mm of annual average precipitation at an altitude of 113–118 m a.s.l. and the soil is a sandy loam Luvisol with a pH of 6·0 (2010). The mother plants were treated according to the rules of integrated pest management. Before the experiments, plants were examined randomly for the latent presence of *X. fragariae* by nested PCR.

In total, 145 strawberry genotypes were evaluated, including 119 cultivars (*Fragaria* × *ananassa*). The cultivar selection included the currently commercially available cultivars as well as a large number of cultivars that were released in past centuries. Furthermore, eight wildtype accessions of *Fragaria moschata*, *Fragaria nilgerrensis*, *Fragaria vesca*, *Fragaria virginiana* and *Fragaria viridis* as well as 18 advanced breeding clones (*Fragaria* × *ananassa*) from the Dresden-Pillnitz strawberry breeding programme were tested. The cultivars Elsanta, Clery, Honeoye and Galia were tested in three subsequent years and a further 48 genotypes were tested repeatedly in 2 years.

For the investigation of the spatial and temporal distribution of *X. fragariae* within individual strawberry plants, a set of selected genotypes was used including 16 plants each of Elsanta, Clery, *F. moschata* 'Bauwens' (FRA0048) and *F. vesca* f. *alba* (FRA0185).

Cultivation of bacteria and artificial inoculation

Artificial inoculation of strawberry plants was performed with the *X. fragariae* strains Xf3.9.C, Xf3.9.F and strain 315 from the strain collection of the Julius Kühn-Institute, Institute for Resistance Research and Stress Tolerance (Quedlinburg, Germany). All strains originate from strawberry plantations in southwest Germany. The bacteria were cultivated on YDC medium containing 2% glucose, 1% yeast extract, 1·5% agar and 2% calcium carbonate diluted in sterile tap water with a pH of 7·0. Inoculum was prepared from YDC plates after 7 days' incubation by resuspending the bacteria in 5 mL sterile tap water and adjusting the cell density in a haemocytometer to a concentration of 10^9 colony-forming units (CFU) per mL.

Potted strawberry plants were inoculated 3–4 weeks after planting, when they had developed at least three fully expanded trifoliate leaves. Different inoculation techniques were compared in the first year of the investigation. All plants were inoculated both by brushing the abaxial sides of the leaves with a paint-

brush dipped in inoculum and by cutting one leaf per plant with scissors that had been dipped into inoculum before. In the second and third year of the investigation all plants were inoculated with a customary pressure sprayer (Prima 5 Type 39 TE, GLORIA®, Witten, Germany) at 200–300 kPa. The spray was held at a distance of 20–30 cm from the leaves to achieve good coverage.

For a separate experiment investigating the systemic dispersal of *X. fragariae* within individual plants, only the youngest fully expanded trifoliate leaf of each plant was inoculated on the abaxial side with a dipped paintbrush and labelled with a coated wire.

All inoculation experiments were conducted under greenhouse conditions. Directly after inoculation, the plants were covered with plastic tunnels and the humidity was increased by flooding the irrigation mats on the tables for several minutes. The relatively high daytime temperatures of 25°C were lowered to 10°C overnight to induce guttation in the plants, which opens the hydathodes, a natural entrance for the bacteria. After 3–5 days, the plastic tunnels were removed and the plants were cultivated under normal greenhouse conditions with plant protection measures following the guidelines of integrated pest management.

Symptom evaluation and statistical analysis

The plants were evaluated for symptom severity on a scale from 1 to 9. The rating was based upon the percentage of visible leaf area with symptoms from 1 (0–0·5%), 2 (0·5–1%), 3 (1–2%), 4 (2–4%), 5 (4–8%), 6 (8–16%), 7 (16–32%), 8 (32–64%) to 9 (64–100%). Plants were evaluated 21, 28, 35, 42 and 112 days post-inoculation (dpi). Each experiment was arranged in a fully randomized complete block design with three repetitions, with a single repetition consisting of 3–10 plants per genotype. Evaluation data were analysed with the statistical software package SAS ENTERPRISE GUIDE v. 4.3 (SAS Institute). Analysis of variance was also carried out with the ANOVA procedure. For the comparison of means, the LSD test with $\alpha = 0{\cdot}05$ was chosen. Correlations were computed within the CORR procedure using Spearman's rank correlation coefficient r_s.

Sample preparation and analysis with nested PCR

For investigation of systemic dispersal of *X. fragariae*, four plants of each genotype were sampled, once before inoculation and subsequently after 3, 7 and 14 days. Plants were dissected in the laboratory under sterile conditions. All instruments such as scissors, forceps, scalpels and razor blades that were used for preparation were sterilized by flaming with 99% ethanol before and after each sample was taken. From each plant, 80–90 mg of the following parts were removed, transferred into 2-mL reaction tubes and frozen in liquid nitrogen: a 1–1·5 cm circular piece from the middle of both the youngest fully expanded leaf without visible symptoms and from the inoculated leaf, a 1–2 cm piece of petiole from the same leaves, the inner part of the heart bud and the inner part of the peeled rhizome.

Genomic DNA was isolated from the samples using the DNeasy Plant Mini kit (QIAGEN) to remove PCR inhibitors, quantified with a NanoDrop 2000c UV-vis spectrophotometer (Thermo Fisher Scientific Inc.) and adjusted to a concentration of 10 ng mL^{-1} for further analysis.

Detection of *X. fragariae* DNA in the samples was carried out with nested PCR (Zimmermann *et al.*, 2004) using the primers 245A, 245B, 245.5 and 245.267 that result in the amplification of a 286 bp product. The PCR conditions were slightly modified using Phusion high-fidelity DNA polymerase (New England Biolabs) and separating the amplification products by gel electrophoresis with 1·5% agarose gels for 2 h at 120 V. The PCR analysis was repeated three times to prevent the risk of picking contaminated samples.

Analysis of *X. fragariae* spread using a GFP-tagged reporter strain

Electrocompetent cells of Xf3.9.C were produced according to the following method. Bacteria were cultivated on YDC agar and incubated at 28°C for 6 days. From these plates, the bacteria were densely restreaked on YDC medium and incubated at 28°C for 48 h to obtain a reasonable amount of fresh cells. Cells were washed from the plates with 6 mL sterile ddH_2O and harvested by centrifugation at 4°C at 18 407 *g* for 5 min. Pellets were washed twice in 3 mL ice-cold sterile ddH_2O and once in 1 mL ice-cold sterile 15% glycerol. Finally, the pellets were resuspended in 100 μL ice-cold 15% glycerol. The electrocompetent cells were stored until use at −80°C. A fluorescent reporter strain of *X. fragariae* was created by transformation with the GFP expression plasmid pKAN (Joyner & Lindow, 2000). Aliquots of 40 μL of cells were electroporated with 1 μL plasmid preparation. After electroporation with pKAN, cells were incubated at 28°C for 2 h and plated on YDC medium containing 50 μg mL^{-1} kanamycin. Transformants were selected after 5 days' incubation at 28°C for their ability to grow on kanamycin and expression of GFP. Positive colonies were verified as *X. fragariae* with nested PCR. Fifteen positive colonies were evaluated for fluorescence intensity by fluorescence microscopy and strain $Xf3.9.C_{(pKAN)}\_11$ was selected for subsequent plant experiments. The strain was cultivated further for 6 months on YDC agar and periodically examined visually under the fluorescence microscope. During this time, no obvious decline in the flurorescence intensity could be observed. It was therefore concluded that the plasmid could be considered stable. Plants were inoculated and incubated as described above. After 4, 7, 14 and 28 days, four samples of six tissues were analysed for visual fluorescence of $Xf3.9.C_{(pKAN)}\_11$ by reflected-light fluorescence microscopy.

Results

Infection progress of *X. fragariae* with different artificial inoculation techniques

The artificial inoculation of young strawberry plants under greenhouse conditions resulted in development of typical symptoms in all three years of the experiment. In year 1, different inoculation methods were compared and inoculation by brushing the abaxial side of the leaves with inoculum led to a more reliable infection of the plants. Cutting leaves with inoculum-bearing scissors caused necrosis at the wounds of the injured leaves, but successful infections were observed infrequently. Spray inoculation of the plants turned out to be the most efficient technique as it produced typical disease symptoms (Fig. 1) and was applicable in large screening experiments. The disease progress in the 40 commercially available cultivars was assessed in year 1 after 15, 21, 35 and 62 days. First symptoms appeared after 15 days and the mean disease incidence at that time point was 2·7 progressing up to 3·6 at 21 dpi, 4·9 at 35 dpi and 6·4 at

Figure 1 Typical disease symptoms of *Xanthomonas fragariae* in artificial inoculation experiments at 35 days post-inoculation, cultivar Darselect.

62 dpi tested in year 1. The time point 35 dpi was selected as the best time point for the detection of differences in the disease incidence between genotypes with a range from 2·5 to 6·2 on the evaluation scale. In years 2 and 3, the respective sets of 96 and 69 genotypes were assessed for disease progress. The first symptoms appeared after 21 dpi and the mean disease incidences at 35 dpi were lower than in year 1, with 2·8 in year 2 and 2·4 in year 3.

Screening for resistance to *X. fragariae* in strawberry germplasm

In the three years of the artificial inoculation experiments a total of 145 strawberry genotypes were evaluated for their susceptibility to *X. fragariae*. According to their mean disease incidence at 35 dpi, the genotypes were classified as partly resistant, medium resistant, susceptible and highly susceptible, as shown in Table 1. Statistical analysis revealed significant differences in the mean disease incidence between all resistance classes ($P < 0·05$). A total of six genotypes were found to be partly resistant to *X. fragariae*. In particular, these included the US breeding clones US4808 and US4809 with mean disease incidences of 1·0 and the four strawberry wildtype accessions *F. vesca* f. *alba* (FRA0185) (1·3), *F. nilgerrensis* 'Yunnan' (FRA0080) (1·5), *F. vesca* 'Illa Martin' (ERB0438) (1·5) and *F. moschata* 'Bauwens' (FRA0048) (1·5). The highest infection rates were found for the cultivars Salsa (5·6), Korona (5·6), Asia (5·7), Evi2 (5·8), Polka (6·1) and Berneck1 (6·2) with heavy symptoms of angular leaf spot disease all over the inoculated plants. The complete results for the evaluation of all 145 genotypes are given in Table S1.

Moreover, 48 genotypes were evaluated in two subsequent years. The results from both years show a medium linear correlation ($r_P = 0·48$, $P < 0·05$), which is significant. Figure 2 shows their mean disease incidence in ascending order from the least susceptible genotypes *F. vesca* f. *alba* and *F. moschata* 'Bauwens' to the highly susceptible genotypes Zarathustra and Salut. The mean disease incidence for this set of genotypes was 2·6 on the evaluation scale with a standard deviation of 0·32. Among these genotypes, the majority can be classified as susceptible.

Table 1 Number of strawberry genotypes according to their resistance to *Xanthomonas fragariae* (Xf) in artificial inoculation experiments, 35 days post-inoculation

	Strawberry genotype[b]					
Resistance class[a]	C	B	W	Total	Mean Xf	LSD[c]
R (1·0–1·5)	0	2	4	6	1·30	a
MR (1·6–2·5)	31	5	4	40	2·23	b
S (2·6–3·5)	52	7	0	59	2·89	c
HS (≥3·6)	36	4	0	40	4·75	d
Total	119	18	8	145	3·16	

[a]Classes are given according to the mean value of disease incidence evaluation from 1 = no symptoms to 9 = fully necrotic leaves. R, partly resistant; MR, medium resistant; S, susceptible; HS, highly susceptible.
[b]C, cultivar of *Fragaria* × *ananassa*; B, breeding clone; W, strawberry wildtype accession.
[c]Significant differences between the resistance classes are indicated by different letters (LSD test, $\alpha = 0·05$).

During the further course of the experiment in year 2, a set of 32 genotypes was investigated up to 112 dpi. The final evaluation showed a noticeable change in the disease progress. While the primarily inoculated leaves were withered away, not all of the young leaves showed symptoms as well. The difference in the proportion of plants with symptoms per genotype is shown in Figure 3 in combination with the mean disease of the plants with symptoms after 112 dpi. While the evaluation of the mean disease incidence of plants with typical symptoms ranges between 2 and 3 for most genotypes, the proportion of plants with symptoms per genotype ranges from 0% for *F. vesca* f. *alba* and *F. moschata* 'Bauwens' up to 100% for Arosa and Joerica. Altogether, the disease incidence decreased compared to the previous evaluation at 35 dpi. In most cases, the originally inoculated leaves were withered away at that time point and not all of the young leaves showed symptoms.

Investigations of systemic dispersal of *X. fragariae* within strawberry plants

A detailed analysis of the systemic dispersal of *X. fragariae* within the plant tissue after artificial inoculation was carried out with specific molecular detection and by visualizing the disease progress in living plant material after inoculation with the strains XF3.9.C and Xf3.9.C$_{(pKAN)}$_11.

Figure 4 shows the abaxial side of leaf samples of the highly susceptible cultivar Darselect under the reflected-light microscope and with UV light in comparison at 4 dpi with Xf3.9.C$_{(pKAN)}$_11. At that time point, the plants

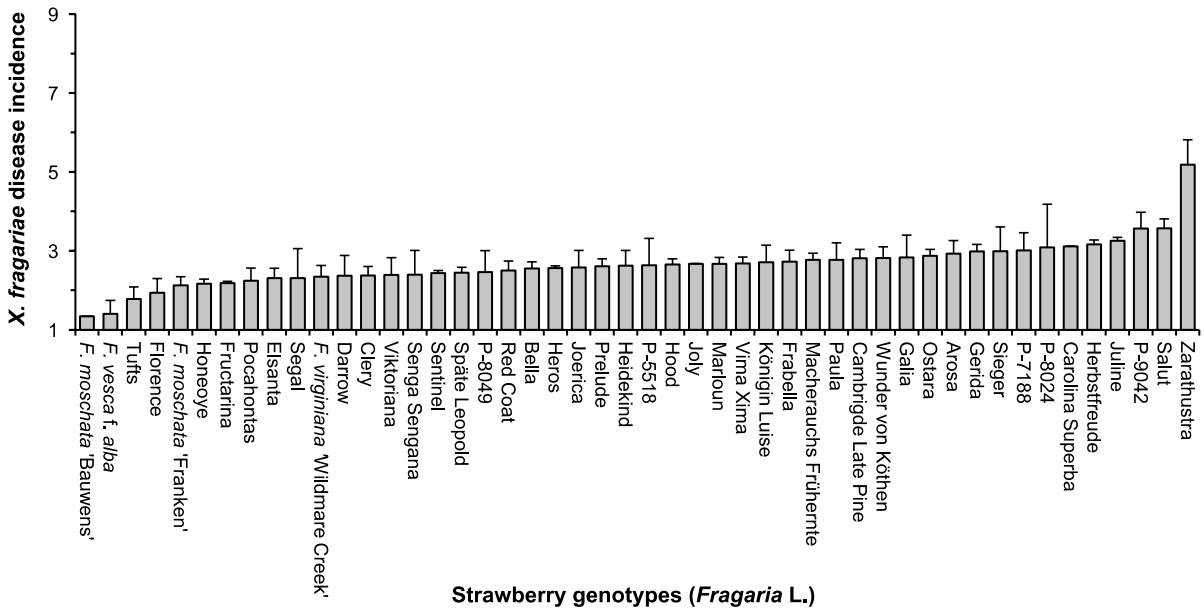

Figure 2 Disease incidence on 48 different strawberry genotypes at 35 days post-inoculation with *Xanthomonas fragariae* in artificial inoculation experiments over two consecutive years. The scale for disease incidence evaluation is from 1 (no symptoms) to 9 (fully necrotic leaves). For each genotype, 20–30 plants were evaluated. Error bars show the standard error of the mean.

had not yet developed any visible symptoms. Presence of GFP-tagged *X. fragariae* is visualized by strong fluorescence in the leaf veins in Figure 4a. In contrast, there is no fluorescence in the leaf veins of the water- inoculated control in Figure 4b. The higher magnification of the stomatal area in Figure 4c shows green fluorescence of the bacteria in the stomata, the adjacent guard cells and in the intercellular space of epidermal cells.

The results of the PCR detection of the pathogen in samples of the inoculated leaf and its petiole, the rhizome, heart bud and the youngest fully expanded uninoculated leaf and its petiole are represented in Figure 5. No *X. fragariae* infection could be detected in the samples before inoculation. At 3 dpi, *X. fragariae* DNA could be found in all tissue samples. Also at 7 dpi and 14 dpi *X. fragariae* DNA was present in all samples.

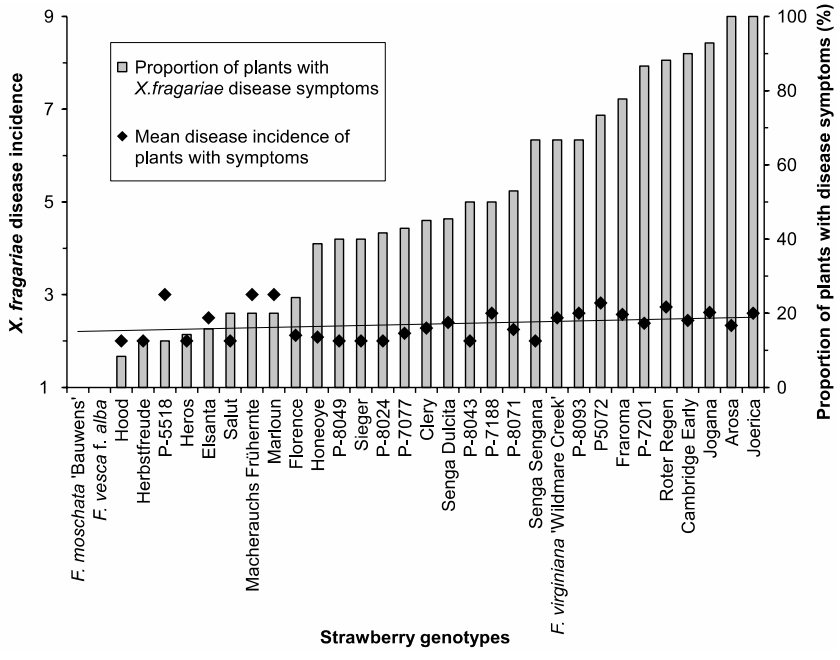

Figure 3 Proportion of plants with symptoms and disease incidence on 32 different strawberry genotypes at 112 days post-inoculation with *Xanthomonas fragariae* in artificial inoculation experiments. For each genotype, 30 plants were evaluated and the proportion of plants with symptoms is indicated by bars. The disease incidence of the plants with symptoms was evaluated on a scale from 1 (no symptoms) to 9 (fully necrotic leaves) and shown as rhombuses. Additionally, a partial regression line was determined.

The plants of Darselect developed typical symptoms of angular leaf spot disease on their leaves around 28 dpi. The GFP-tagged *X. fragariae* strain Xf3.9.$C_{(pKAN)}$_11 showed the same virulence as the *X. fragariae* wildtype strain, resulting in translucent bacterial lesions, bounded by small leaf veins, that appeared in the typical angular shape. The first lesions were visible along the main leaf vein as shown in Figure 6a. The lesions and parts of the leaf veins were filled completely with the bacteria resulting in strong fluorescence under UV light (Fig. 6b). Figure 6c shows an area of high lesion density between two main leaf veins with a high amount of fluorescence activity (Fig. 6d). A higher magnification of an emerging lesion adjacent to a leaf vein, seen under white light, is shown in Figure 6e. The same lesion under UV light (Fig. 6e) shows high densities of green fluorescing *X. fragariae* cells in the leaf vein and also in tissue adjacent to the visible lesion, where no symptoms are visible under white light. Molecular diagnostics with the nested PCR verified the presence of *X. fragariae* DNA in all samples at 28 dpi. Moreover, there is no difference in the presence of the bacteria in the plant tissues of different susceptible and partly resistant genotypes, as shown in Figure 7. The typical 286 bp fragments indicate the presence of *X. fragariae* DNA in all samples of the cultivars Clery and Elsanta and the wildtype accessions *F. vesca* f. *alba* and *F. moschata* 'Bauwens'.

Discussion

This study has investigated a total of 145 genotypes belonging to the collection of strawberry genetic resources at JKI Dresden (Germany) for resistance to *X. fragariae* in artificial inoculation experiments. Most of the genotypes that were evaluated in this study had never been investigated for resistance to angular leaf spot disease. The spray inoculation led to very strong symptom development in highly susceptible plants, apparent lesion occurrence in susceptible plants, minor disease incidence in medium resistant plants and absent or rare appearance of angular leaf spots in partly resistant genotypes. Hypersensitive reactions as a consequence of inoculation with the pathogen as described in Xue *et al.* (2005) for the *Fragaria pentaphylla* accession Pen-5 could not be observed for any of the genotypes investigated in this study. Using spray inoculation, Elsanta was classified as susceptible, which is in agreement with the findings of Kastelein *et al.* (2013). Also the two breeding clones US4808 and US4809, which were described as resistant by Maas *et al.* (2002), appeared at least partly resistant in the current experiments.

Compared to the methodology in previous inoculation experiments with *X. fragariae*, where inoculum is infiltrated by syringe (Maas *et al.*, 2000; Xue *et al.*, 2005), the approach described here is easier and applicable for large experiments with more than 2000 plants. As shown in a repetition of inoculation in two subsequent years with the same genotypes, the inoculation method leads to reproducible results.

A similar approach was chosen by Kastelein *et al.* (2013) who showed, that the pathogen invades the plant tissue rapidly through the stomata on the abaxial side of the leaves but can hardly survive on the leaf surface. The results of the present investigation show that the *X. fragariae* bacteria not only invade the leaves rapidly but also spread systemically in the whole plant within 3 dpi. Observations under the UV light stereomicroscope of a GFP-tagged *X. fragariae* strain enabled a closer examination of the bacterial invasion within the first 4 dpi. From the results (Fig. 4), it can be confirmed that

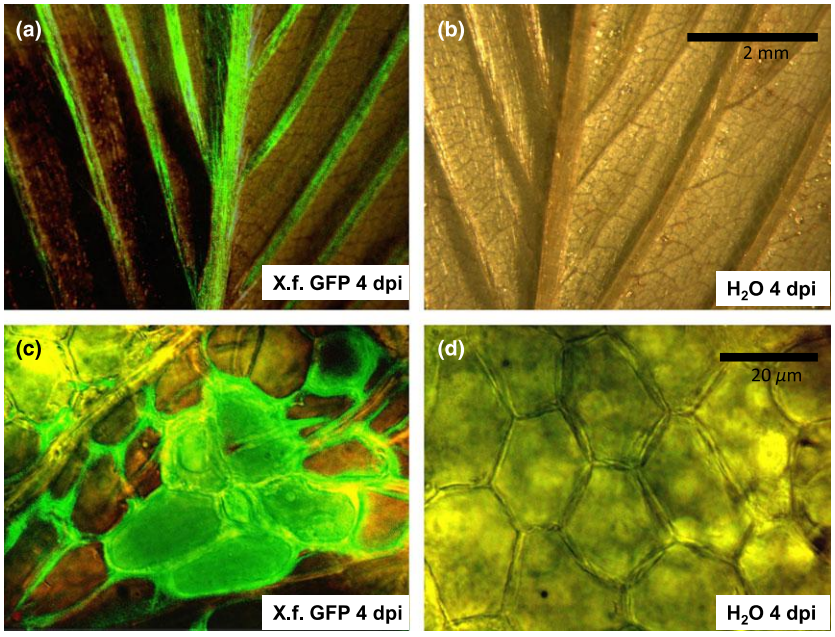

Figure 4 Histological investigations of systemic dispersal of *Xanthomonas fragariae* (X.f.) in strawberry cultivar Darselect with the GFP-tagged strain Xf3.9.$C_{(pKAN)}$_11 at 4 days post-inoculation (dpi). (b, d) the abaxial sides of different water-inoculated leaves under the reflected light microscope; (a, c) leaves inoculated with Xf3.9.$C_{(pKAN)}$_11 viewed by reflected light fluorescence microcopy. UV light activates the GFP fluorophores.

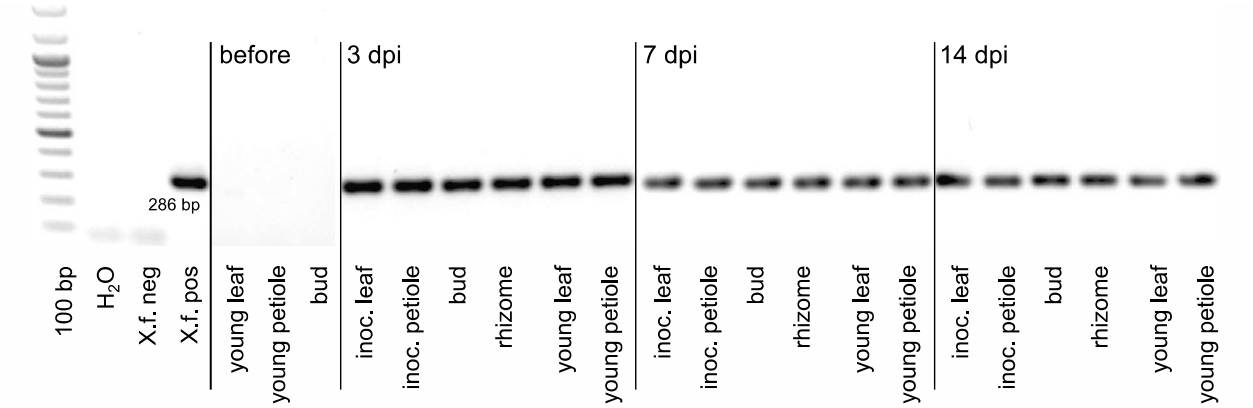

Figure 5 Molecular detection of *Xanthomonas fragariae* (X.f.) in different plant tissues of strawberry cultivar Darselect at time points before inoculation and 3, 7, 14 days post-inoculation (dpi). Molecular size standard of a 100 bp ladder and a non-template H_2O control were applied in lanes 1 and 2. Lanes 3 and 4 are samples from previously tested X.f.-positive and X.f.-negative strawberry plants. The samples in lanes 5–25 were taken from the different tissues of the inoculated plant. Amplification products were obtained in a nested PCR with primer pairs 245A, 245B and 245.5, 245.267 as described in Zimmermann *et al.* (2004) and were separated via agarose gel electrophoresis. Fragments of 286 bp indicate presence of X.f. DNA.

the pathogen invades through the stomata, colonizes the intercellular space for multiplication and spreads systemically throughout the whole plant via the leaf veins. Electroporation with plasmid DNA on other *Xanthomonas* species has been carried out in previous investigations for the purpose of specific detection of the pathogen (Atkins *et al.*, 1987; Yang *et al.*, 1991). In a previous study with GFP-tagged *X. fragariae* by Kaste-

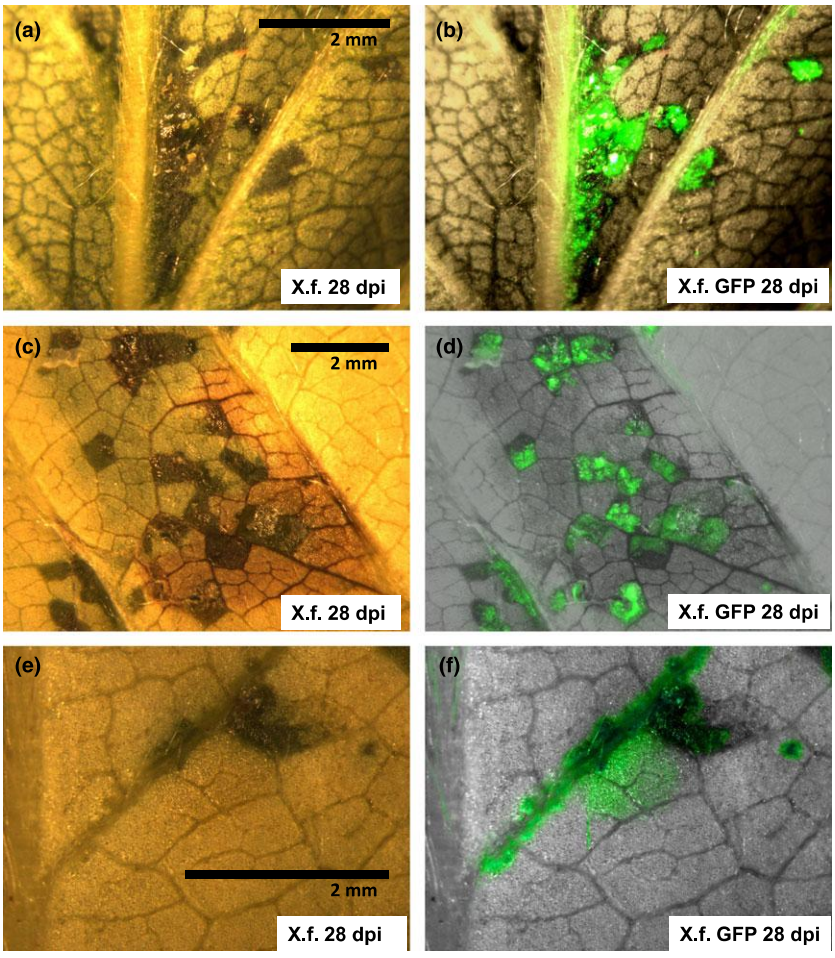

Figure 6 Histological investigations of systemic dispersal of *Xanthomonas fragariae* (X.f.) in strawberry cultivar Darselect with GFP-tagged strain Xf3.9.$C_{(pKAN)}$_11 in artificial inoculation experiments, 28 days post-inoculation (dpi). (a, c, e) the abaxial sides of different leaves with typical symptoms of X.f. under the reflected light microscope; (b, d, f) the same image sections with UV light, which activates the GFP fluorophores.

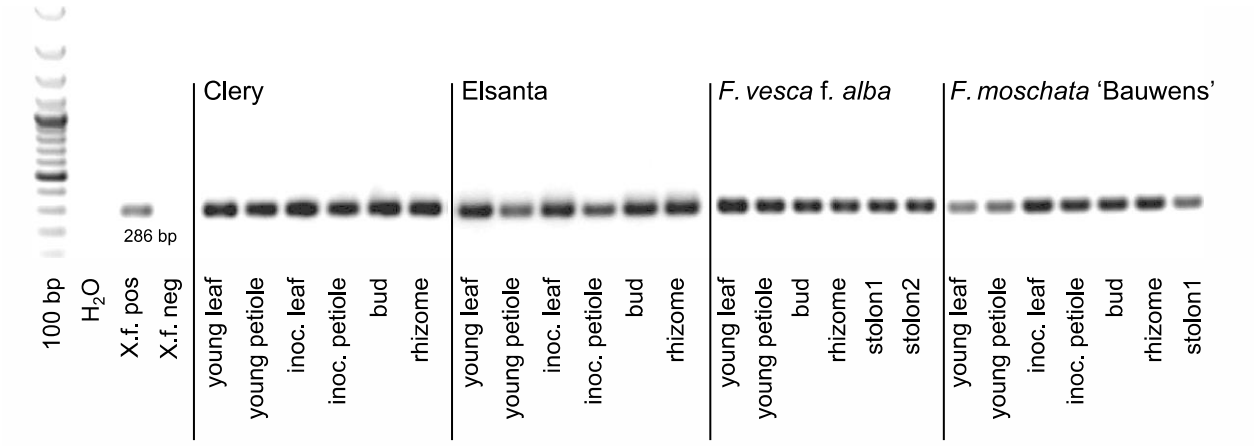

Figure 7 Molecular detection of *Xanthomonas fragariae* (X.f.) in different plant tissues of strawberry cultivars Clery, Elsanta and wildtype accessions *Fragaria vesca* f. *alba* and *Fragaria moschata* 'Bauwens' after artificial inoculation, 28 days post-inoculation (dpi). Molecular size standard of a 100 bp ladder and a non-template H_2O control were applied in lanes 1 and 2. Lanes 3 and 4 are samples from previously tested X.f.-positive and X.f.-negative strawberry plants. The samples in lanes 5–29 were taken from different tissues of inoculated plants. Additionally, 1–2 stolons from the wildtype accessions were analysed. Amplification products were obtained in a nested PCR with primer pairs 245A, 245B and 245.5, 245.267 as described in Zimmermann *et al.* (2004) and were separated via agarose gel electrophoresis. Fragments of 286 bp indicate presence of X.f. bacteria.

lein *et al.* (2013), a slightly lower virulence of the tagged strain was reported, but this was not found in the current investigation. The advantage of this approach over simply staining bacteria is that the plant material can be investigated in a non-destructive and immediate way. However, the autofluorescence of remaining chlorophyll and cell wall compounds can disturb the identification of true GFP fluorescence. The subsequent molecular detection with nested PCR in different plant tissues of inoculated plants revealed clearly the rapid systemic dispersal within plants both with and without symptoms. The experiment on the systemic dispersal was carried out with very susceptible as well as putatively resistant genotypes, but no differences in the rapidity and systemic dispersal of bacteria were found.

The results obtained at 112 dpi (Fig. 3) lead to the assumption of a putative genotype-dependent adult plant resistance. At this time point, leaves that had been inoculated had withered away and none of the wildtype accessions *F. vesca* f. *alba* and *F. moschata* 'Bauwens' developed symptoms typical of angular leaf spot disease. In contrast, all plants of the cultivars Arosa and Joerica had symptoms. Molecular detection by nested PCR at 112 dpi (data not shown) revealed that the bacteria had spread systemically in the inoculated plants of all genotypes. Ontogenic resistance has been previously described for *Xanthomonas campestris* pv. *oryzae* in different genotypes of rice (Qi & Mew, 1984). This mechanism is very common in numerous pathosystems (Develey-Riviere & Galiana, 2007) and may be important for growers because in many annual production systems strawberry plants are very susceptible to *X. fragariae* in the first year of cultivation. Further trials with inoculation of resistant and susceptible strawberry genotypes at different developmental stages might yield further useful information about this type of resistance.

The findings of this study have important implications for both direct use in strawberry cultivation and for targeted breeding of new resistant cultivars. Although there was no completely resistant cultivar among the set of genotypes in this evaluation, at least 31 cultivars were found to be of medium resistance towards *X. fragariae*. For targeted resistance breeding, the availability of octoploid plant material is important. An octoploid could be obtained by interspecific hybridization of the hexaploid *F. moschata* 'Bauwens' and the diploid *F. vesca* f. *alba*. However, such an approach would require a very large crossing population and the transfer of resistance traits through this process is of low probability. Therefore, cross breeding with the currently available octoploid resistance sources in the breeding clones US4808 and US4809, which were confirmed in this investigation, seems to be the most promising strategy in creating resistant cultivars. In the previous breeding experiments of Jamieson *et al.* (2013), crossings of US4808 × Rosie (susceptible) led to 5·5% resistant F_1 seedlings. In theory, cross breeding of the US breeding clones with medium resistant cultivars described in this study should lead to a higher proportion of genotypes with improved resistance to *X. fragariae* in addition to attractive horticultural traits. The medium resistant genotypes found in this study also cover a wide phenotypical range from early to late ripening. As the ripening time trait is inherited as a main effect directly from the breeding parents to the progeny (Bestfleisch *et al.*, 2014), it is feasible to create genotypes that are less susceptible to *X. fragariae* with an extended ripening period.

Future research activities will continue the evaluation of further strawberry genotypes in the collection of the Fruit Genebank in Dresden-Pillnitz for resistance towards angular leaf spot disease and other important diseases. Although it is a challenge to integrate the

resistance traits into the cultivated strawberry *Fragaria* × *ananassa*, it is of great interest to further breeding experiments as broadening the genetic diversity of strawberry may also lead to the improvement of fruit quality.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Susceptibility of 145 strawberry genotypes to *Xanthomonas fragariae*. The incidence of *X. fragariae* in an artificial inoculation experiment at 35 days post-inoculation is given for each genotype with the mean, minimum and maximum values and the standard deviation (STD).

3 DISKUSSION

3.1 ZÜCHTUNGSERFOLG MIT KLASSISCHEN KREUZUNGSMETHODEN FÜR HÖHEREN ERTRAG UND EINEN ERWEITERTEN ERNTEZEITRAUM IM ERDBEERANBAU

Die vorliegenden Untersuchungen im diallelen Kreuzungsexperiment haben gezeigt, dass diese Methode der klassischen Kreuzungszüchtung zuverlässige Ergebnisse im Hinblick auf die wichtigen Zuchtziele der frühzeitigen Reife und Ertragssteigerung liefert. Die Ausdehnung des Reifezeitraums kann durch die gezielte Selektion besonders früh- bzw. spätreifender Sorten als Kreuzungseltern mit großer Zuverlässigkeit erreicht werden, wie die Analyse der allgemeinen (GCA) und spezifischen (SCA) Kombinationseignung zeigt. Hierbei spielt es nach den vorliegenden Ergebnissen letztendlich keine Rolle, ob eine Sorte als „Mutter“ oder „Vater“ in der Kreuzung verwendet wurde. Kreuzungen mit den Sorten ‘Clery’ und ‘Daroyal’ führten zu besonders frühzeitig reifenden Nachkommen, wohingegen die Kreuzungen mit ‘Yamaska’ zu besonders spät reifenden Nachkommenschaften führten. Diese Kreuzungspopulationen konnten bereits als Basis zur weiteren Selektion neuer Sorten verwendet werden. Statistisch signifikant höhere Erträge konnten mit den Sorten ‘Yamaska’ und ‘Polka’ als Kreuzungseltern erreicht werden. ‘Yamaska’ wurde auch schon in früheren diallelen Kreuzungsversuchen bei Davik und Honne (2005) verwendet, wobei hier zudem gute Eigenschaften hinsichtlich der Widerstandsfähigkeit gegenüber *Sphaerotheca macularis* zu beobachten waren. Dies spricht dafür, diese Sorte in weitere Züchtungsprogramme mit einzubeziehen. Bis heute ist die Sorte ‘Elsanta’ europaweit die am häufigsten angebaute Erdbeersorte (Hancock et al., 2008), doch es ist unwahrscheinlich, dass Kreuzungen mit dieser Sorte den Züchtungserfolg steigern, wie die vorliegenden Versuche gezeigt haben, da hier keine signifikanten Werte für die allgemeine und spezifische Kombinationseignung feststellbar waren.

Die verbesserte statistische Analyse des diallelen Kreuzungsversuchs, die in dieser Arbeit vorgestellt wird, kann auch auf Kreuzungsversuche in anderen Kulturen angewendet werden (Möhring et al., 2011). Trotz des relativ großen zeitlichen, räumlichen und personellen Aufwands kann ein dialleler Kreuzungsversuch auch aus heutiger Sicht noch immer als wichtiges Element in einer zielgerichteten und effizienten Züchtungsstrategie bei Erdbeeren eingesetzt werden. Der Versuchsansatz zeigte praktisch verwertbare Ergebnisse, die direkt in der Erdbeerzüchtung verwertet werden können. Für die züchterisch wertvollen Eigenschaften Reifezeit und Ertrag von Erdbeeren ist die Selektion geeigneter Kreuzungseltern auf Basis ihrer allgemeinen Kombinationseignung (GCA) eine erfolgversprechende Herangehensweise.

3.2 Evaluierung von Genotypen zur Identifizierung von Resistenzen gegenüber *Botrytis cinerea* und *Xanthomonas Fragariae*

Neben den wichtigen Zuchtzielen Reifezeit und Ertrag wurden als phytopathologische Aspekte die Resistenzeigenschaften verschiedener Sorten und Wildarten gegenüber zwei bedeutender Krankheiten in die Arbeit mit einbezogen und hinsichtlich des züchterischen Nutzens bewertet. Nach der Entwicklung, Etablierung und Optimierung der Resistenztests gegenüber den Erregern *Xanthomonas fragariae* und *Botrytis cinerea* sollten jeweils über 100 Sorten und Wildartenakzessionen innerhalb von drei Jahren auf ihre Resistenzeigenschaften hin untersucht werden. Aus den daraus gewonnenen Erkenntnissen sollen einerseits direkt Empfehlungen zur Sortenwahl abgeleitet werden können, andererseits dient die Identifizierung resistenter Genotypen als Basis zur gezielten Züchtung neuer und widerstandsfähiger Sorten. Mit diesen Zielvorstellungen im Hintergrund wurden die Anforderungen an die Entwicklung und Optimierung der Resistenztests klar definiert. Die Schwerpunkte wurden hierbei auf hohe Replizierbarkeit sowie hohen Durchsatz an Proben bei begrenztem Personalaufwand gelegt. Im Falle des Resistenztests gegenüber *B. cinerea* musste ein solcher Test erst entwickelt werden, beginnend mit dem Aufbau einer Erregerstammsammlung. Da *B. cinerea* - Stämme sich lokal schnell verändern können und sich beispielsweise durch Punktmutationen rasch fungizidresistente Populationen durchsetzen können, ist die Auswahl geeigneter Isolate für die Durchführung des Resistenztests eine besondere Herausforderung. Im Rahmen dieser Arbeit wurden fünf Isolate unterschiedlicher geographischer Herkunft verwendet und es konnte statistisch keine Genotyp × Isolat – Interaktion festgestellt werden. Die künstliche Inokulation der Früchte erfolgte anschließend mit Inokulum aus einem Gemisch dieser Stämme. Für die weitere Durchführung des Resistenzscreenings stellt dies jedoch einen Kompromiss dar zwischen möglichst guter Replizierbarkeit, welche mit einer geringeren Anzahl verschiedener Isolate besser zu realisieren wäre, und einer möglichst hohen Diversität an Isolaten, um insgesamt die Aussagekraft des Tests zu erhöhen. Mit der gewählten Methodik konnten innerhalb der untersuchten Genotypen große Unterschiede in der Widerstandsfähigkeit ermittelt werden, wie der Vergleich zwischen der im Mittel über drei Jahre widerstandsfähigsten Sorte ‘Arosa’ mit der anfälligsten Sorte ‘Senga Sengana’ zeigt. Eine Überprüfung der Testergebnisse wurde zudem über 2 Jahre hinweg in einem Feldversuch ohne Fungizid-Applikation mit insgesamt 60 Genotypen durchgeführt, wobei auch hier große Unterschiede zwischen anfälligen und widerstandsfähigen Genotypen festgestellt wurden und die im Labor anhand der Fruchtinokulationen ermittelten Testergebnisse insgesamt bestätigt werden konnten. So zeigte der Großteil der als widerstandsfähig getesteten Sorten, wie z.B. ‘Arosa’, ‘Florence’, ‘Darselect’ sowie die Zuchtklone P8043 und P5518 auch im Feldversuch sehr geringen Befall und umgekehrt fanden

sich in der Gruppe der anfälligen Sorten des Feldversuchs auch die im Resistenztest als anfällig getesteten Sorten ‘Senga Sengana’, ‘Cambridge Late Pine’, ‘Papa Lange’ und ‘Sonata’.

Im Falle des *Xanthomonas*-Resistenztests wurden drei unterschiedliche Bakterienisolate verwendet, wobei hier von einer deutlich geringeren regionalen Diversität der Isolate ausgegangen werden kann, eine ähnlich schnelle genetische Veränderung der Populationen, wie beispielsweise bei *B. cinerea,* ist hier bisher nicht bekannt.

Die Inokulationstechnik wurde bei beiden Resistenztests an das Ziel eines möglichst hohen Pflanzendurchsatzes angepasst, um in einem Versuchsjahr möglichst viele unterschiedliche Genotypen untersuchen zu können. Für den *Botrytis*-Resistenztest zeigte sich, dass die Auswahl geeigneter Früchte im korrekt definierten Reifestadium hier eine größere Bedeutung hat als die Inokulationstechnik.

Für den *Xanthomonas*-Resistenztest wurde ebenfalls ein Verfahren gewählt, das geeignet ist für einen möglichst hohen Durchsatz an Versuchspflanzen. Eine ähnliche Methode der Sprühinokulation wurde auch von (Jamieson et al., 2013) verwendet und lieferte deutliche Krankheitssymptome in anfälligen Pflanzen. Die dadurch gewonnenen Ergebnisse weisen eine gute Reproduzierbarkeit auf, begründet durch eine statistisch signifikante mittlere lineare Korrelation von r = 0.48 über zwei Versuchsjahre. Die Sorte ‘Elsanta’ wurde als hochanfällig eingestuft, was die Ergebnisse früherer Studien (Kastelein et al., 2013) bestätigt. Ebenso wurden die zuvor als resistent beschriebenen Zuchtklone US4808 und US4809 (Maas et al., 2002) hier als teilweise resistent getestet und damit die Ergebnisse zumindest teilweise bestätigt. Die meisten Genotypen, die im Rahmen dieser Arbeit untersucht wurden, sind noch nie zuvor hinsichtlich ihrer Resistenz gegenüber *X. fragariae* getestet worden. Hierbei konnte die ganze Bandbreite von hoch anfällig mit starker Symptomausprägung bis hin zu toleranten Genotypen mit kaum oder nicht sichtbaren Symptomen beobachtet werden. Hypersensitive Reaktionen, die in früheren Studien (Xue et al., 2005) bei einzelnen Akzessionen von *Fragaria pentaphylla* Losinsk. auftraten, wurden hier nicht beobachtet. Trotzdem wird im weiteren Verlauf der zukünftigen Untersuchungen ein Hauptaugenmerk auf dieses Kriterium gelegt, um eine deutlich abgrenzbare Resistenzreaktion nachweisen zu können. Besondere Beachtung gilt zudem den Boniturergebnissen zum Zeitpunkt 112 dpi. Einerseits, da bislang noch keine Ergebnisse zu einem so späten Boniturtermin veröffentlicht wurden und andererseits, da sich eine unerwartete Befallsentwicklung abzeichnet. Der Anteil überlebender Pflanzen je Genotyp unterscheidet sich erheblich. Da die ursprünglich inokulierten Blätter zu diesem Zeitpunkt nicht mehr vorhanden sind, können die beobachteten Symptome auf eine systemische Verbreitung des Pathogens in der Pflanze zurückgeführt werden. Die genauere Untersuchung der systemischen Verbreitung wurde parallel dazu mit einem GFP-

markierten *X. fragariae* Stamm durchgeführt, aus welchem Inokulum gewonnen werden konnte, um gezielt Einzelpflanzen punktuell auf der Blattunterseite zu inokulieren. Ein ähnlicher Ansatz (Kastelein et al., 2013) zeigte bereits, dass die Bakterien schnell durch die Stomata auf der Blattunterseite ins Pflanzengewebe eindringen, während sie an der Blattoberfläche kaum überlebensfähig sind. Des Weiteren konnte im Rahmen dieser Arbeit gezeigt werden, dass dies innerhalb der ersten drei Tage nach Inokulation geschieht und sich die Bakterien zu diesem Zeitpunkt bereits systemisch in der gesamten Pflanze verbreiten konnten, was sowohl im Fluoreszenzmikroskop sichtbar gemacht, als auch mit Hilfe des spezifischen nested-PCR Nachweises bestätigt werden konnte. Um genauere Hinweise zum Ausbreitungsverhalten von *X. fragariae* zu erhalten, die zur Entwicklung von Prognose- und Bekämpfungsstrategien notwendig sind, sollten zukünftige Studien zur systemischen Ausbreitung mit anfälligen und widerstandsfähigen Sorten unter verschiedenen klimatischen Bedingungen (vor allem bzgl. Temperatur und Luftfeuchte) durchgeführt werden. Zudem ist es für den Anbauer von besonderer Bedeutung, wie sich eine Infektion zu unterschiedlichen Entwicklungsstadien auswirkt, da insbesondere das erste Jahr nach der Pflanzung in dieser Hinsicht eine kritische Phase im Anbau darstellt.

Obwohl keine vollständig resistenten Genotypen gegenüber *B. cinerea* und *X. fragariae* identifiziert werden konnten, wurde aus den Ergebnissen des Resistenzscreenings ein breites Spektrum an toleranten und widerstandsfähigen Sorten, Wildarten und Zuchtklonen ermittelt, die in weitere Zuchtprogramme mit einfließen können.

Weiterhin werden die in dieser Arbeit etablierten Resistenztests verwendet, um in den nächsten Jahren die gesamte Sammlung an Erdbeergenotypen des JKI Dresden-Pillnitz zu evaluieren, die insgesamt mehr als 300 Wildartenakzessionen, historische und aktuelle Sorten sowie verschiedene Zuchtklone umfasst. Es wird erwartet, dass bei diesem Screening noch weitere potentielle Resistenzdonoren identifiziert werden können.

3.3 Ausblick und zukünftige Nutzbarkeit der Ergebnisse

Anhand der gewonnenen Ergebnisse konnten bereits erste Rückschlüsse auf den möglichen Erfolg eines Züchtungsprogramms geschlossen werden, wofür die Identifikation potentieller Resistenz-donoren sowie die Ergebnisse zur Vererbung wichtiger züchterischer Eigenschaften wie Reifezeit und Ertrag wichtige Grundlagen darstellen. Für die weitere züchterische Arbeit muss nun erhoben werden, ob die identifizierten Genotypen für eine gezielte Züchtung geeignet sind. Dazu muss geklärt werden, wie stabil diese Eigenschaften an Nachkommen weitergegeben werden und wie hoch der Anteil der zu erwartenden widerstandsfähigen Sämlinge in der Nachkommenschaft sein wird. Dazu wurden über die in dieser Arbeit präsentierten Ergebnisse hinaus Testkreuzungen mit

den identifizierten Genotypen durchgeführt und die Nachkommen evaluiert und bewertet. Die Ergebnisse dieser Testkreuzungen sollen wiederum als Basis dienen, um ausgewählte Genotypen gezielt zu kombinieren und neue Sorten zu schaffen, die besser an die Bedingungen des ökologischen Anbaus angepasst sind. Diese Arbeiten dauern derzeit noch an und gehen über den zeitlichen Rahmen der hier präsentierten Forschungsarbeiten hinaus.

Von besonderer Bedeutung ist in diesem Kontext auch die Möglichkeit, die identifizierten widerstandsfähigen Wildarten mit niedrigerem Ploidiegrad für die gezielte Züchtung von typischerweise oktoploiden Kulursorten zu verwenden. Betrachtet man die hinsichtlich ihrer Resistenzeigenschaften gegenüber *X. fragariae* besonders interessanten Wildarten, so zeigen die Ergebnisse, dass die hexaploide *F. moschata* ‘Bauwens’ sowie die diploide *F. vesca* f. *alba* vielversprechende Resistenzdonoren sein könnten, wenn ihre Eigenschaften mittels interspezifischer Hybridisierung in oktoploides Zuchtmaterial überführt werden. Im Falle der *F. moschata* ‘Bauwens’ würde eine Kreuzung mit dem dekaploiden Genotyp *Fragaria* ×*vescana* direkt zu einigen oktoploiden Nachkommen in der F1 Nachkommenschaft führen. Im Falle der diploiden *F. vesca* f. *alba* würde eine drei-Wege Hybridkreuzung mit (*F. vesca* x *F.* ×*vescana*) x *F.* ×*vescana* zu dem gewünschten Ploidiegrad führen. Über diese Arbeit hinaus wurden Testkreuzungen durchgeführt, in denen im ersten Schritt aus Kreuzungen von (*F. vesca* x *F.* ×*vescana*) bereits ein geringer Prozentsatz (ca. 1%) fertiler hexaploider Nachkommen erzeugt werden konnten, welche für den nächsten Schritt in einer weiteren Kreuzung mit dem dekaploiden Genotyp *Fragaria* ×*vescana* verwendet werden können. Der beschriebene Ansatz würde jedoch sehr große Kreuzungspopulationen erfordern, da nur eine geringe Wahrscheinlichkeit besteht, dass die Resistenzeigenschaften durch den gesamten Kreuzungsprozess weitervererbt werden. Daher scheint die Aussicht, mit den widerstandsfähigen oktoploiden US-Zuchtklonen US4808 und US4809 direkt widerstandsfähige Nachkommen zu erhalten, deutlich vielversprechender. Zur Auswahl geeigneter weiterer Kreuzungspartner schaffen nun sowohl die Ergebnisse des diallelen Kreuzungsversuchs als auch die Ergebnisse der *Xanthomonas*-Resistenztests eine breite Basis. Aufgrund der Erkenntnis, dass es nur eine untergeordnete Rolle spielt, ob der jeweilige Genotyp als Kreuzungs-Mutter oder Kreuzungs-Vater eingesetzt wird, könnte vor allem die Eigenschaft der frühzeitigen bzw. besonders späten Reifezeit durch Kreuzungen der rein weiblichen US-Zuchtklone mit Pollen der Sorten ‘Clery’ bzw. ‘Yamaska’ gefördert werden. Zur Erweiterung des Resistenzspektrums wäre es außerdem von großem Interesse, Sorten einzukreuzen, welche eine hohe Widerstandsfähigkeit gegenüber *B. cinerea* aufweisen. Insgesamt zehn dieser Testkreuzungen konnten im Winter 2013 erfolgreich durchgeführt werden, wobei die Zuchtklone US4808 und US4809 jeweils mit Pollen der Sorten ‘Clery’, ‘Malwina’, ‘Arosa’, ‘Darselect’, und ‘Florence’

bestäubt wurden. Aus den daraus resultierenden Früchten konnten je Kreuzung bereits ca. 300 – 900 Samen gewonnen werden, von denen jeweils ca. 100 Samen ausgesät und in den Feldbestand überführt wurden. Im weiteren Verlauf werden diese Nachkommenschaften nun hinsichtlich ihrer Resistenz gegenüber *X. fragariae* und *B. cinerea* untersucht sowie hinsichtlich ihrer obstbaulichen Merkmale bewertet. Außerdem könnte es für die weitere Züchtungsarbeit von großem Interesse sein, falls sich in einer der Populationen eine Aufspaltung der Krankheitsresistenz ergeben würde. Eine spaltende Kreuzungspopulation könnte nach erfolgreicher Phänotypisierung und Genotypisierung als Grundlage zur Entwicklung genetischer Marker dienen. Mittels markergestützter Selektion können so schon zu einem sehr frühen Wachstumsstadium resistente Pflanzen aus großen Kreuzungspopulationen identifiziert werden, was wiederum zu einer höheren Erfolgswahrscheinlichkeit führt und auch schon seit einigen Jahren in anderen Obstarten wie z.B. Apfel durchgeführt wird. Die besondere Schwierigkeit beim Erdbeergenom liegt darin, dass aufgrund des komplexen Genoms und der oktoploiden Struktur nur sehr schwer geeignete Sequenzen gefunden werden können. Daher wird die Durchführung von Resistenztests auch weiterhin von großer Bedeutung zur gezielten Züchtung krankheitsresistenter Erdbeersorten sein.

Abschließend bleibt festzuhalten, dass die gezielte Züchtung neuer, krankheitsresistenter Erdbeersorten mit guten obstbaulichen Eigenschaften nach wie vor von großem Interesse für Anbauer weltweit ist. Im Falle von *X. fragariae* ist eine direkte chemische Bekämpfung in Europa nach wie vor nicht möglich, während im Falle von *B. cinerea* durch den Einsatz widerstandsfähiger Sorten eine Einsparung chemischer Pflanzenschutzmittel möglich ist sowie die Eignung für den ökologischen Landbau verbessert wird. Zudem stellt die *Botrytis*-Resistenz ein wichtiges Qualitätskriterium dar, welchem auch nach der Ernte eine übergeordnete Bedeutung bei der anschließenden Vermarktung zukommt.

Mithilfe der gewonnenen Ergebnisse wurden in dieser Arbeit neue Wege und Strategien aufgezeigt, um Zuchtprogramme effektiver zu gestalten und damit die Erfolgschancen bei der Züchtung neuer Sorten für den deutschen Erwerbsobstbau zu erhöhen, um auch in Zukunft eine wettbewerbsfähige und nachhaltige Erdbeerproduktion zu ermöglichen.

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5 ANHANG

Table S1 Susceptibility of 145 strawberry genotypes to *X. fragariae*. Types are indicated with 'C'= cultivar of *F.* ×*ananassa*, 'B'= Breeding clone and 'W'= strawberry wild type accession. Classes are given according to the resistance to *X. fragariae* with 'R'= partly resistant, 'MR'= medium resistant, 'S'= susceptible and 'HS'= highly susceptible. 'N' indicates the number of experiments with yxz inoculated plants per experiment. The incidence of *X. fragariae* in an artificial inoculation experiment at the time point 35 dpi is given for each genotype with the mean, minimum and maximum values and the standard deviation (STD)

				Incidence of X. fragariae, 35dpi			
Genotype name	***Type***	***Class***	***N***	***Mean***	***Min***	***Max***	***STD***
Adria	*C*	*S*	2	3.25	3.00	3.50	0.35
Alba	*C*	*HS*	2	5.14	4.78	5.50	0.51
Albion	*C*	*MR*	2	2.50	2.50	2.50	.
Anabelle	*C*	*HS*	2	4.60	3.75	5.44	1.20
Anita	*C*	*HS*	2	5.00	4.75	5.25	0.35
Antea	*C*	*HS*	2	4.88	4.75	5.00	0.18
Apetita	*C*	*S*	4	2.92	2.13	3.57	0.62
Arista	*C*	*S*	5	3.26	2.80	4.00	0.53
Aroma	*C*	*S*	4	3.03	2.00	3.80	0.76
Arosa	*C*	*S*	4	3.02	2.33	4.00	0.71
Asia	*C*	*HS*	2	5.68	5.25	6.11	0.61
Asiropa	*C*	*S*	1	3.33	3.33	3.33	.
Avant Tout	*C*	*HS*	2	4.60	3.86	5.33	1.04
Bella	*C*	*S*	5	2.56	2.00	3.00	0.52
Benamil	*C*	*HS*	4	3.55	3.00	4.50	0.72
Berneck 1	*C*	*HS*	2	6.17	6.00	6.33	0.24
Bäumchen	*C*	*S*	3	3.09	2.86	3.40	0.28
Cambride Pricewinner	*C*	*MR*	1	2.25	2.25	2.25	.
Cambridge Early	*C*	*S*	3	2.58	2.00	3.00	0.52
Cambrigde Late Pine	*C*	*S*	4	2.67	2.00	3.10	0.50
Carolina Superba	*C*	*S*	4	3.17	3.00	3.50	0.24
Charlotte	*C*	*HS*	2	5.43	4.71	6.14	1.01
Clery	*C*	*MR*	9	2.48	1.10	3.25	0.76
Cornelia Pötschke	*C*	*S*	3	2.59	2.50	2.71	0.11
Crimson King	*C*	*S*	4	2.70	2.40	2.89	0.21
Daroyal	*C*	*HS*	5	4.22	2.13	6.38	2.00
Darrow	*C*	*MR*	4	2.49	2.00	3.00	0.49

				Incidence of X. fragariae, 35dpi			
Genotype name	**Type**	**Class**	**N**	**Mean**	**Min**	**Max**	**STD**
Darselect	*C*	*HS*	5	4.24	3.00	6.11	1.52
Dely	*C*	*HS*	1	1.00	1.00	1.00	.
Diana	*C*	*S*	5	2.89	1.86	4.86	1.23
Donna	*C*	*S*	5	3.19	1.75	5.67	1.63
Dorena	*C*	*S*	2	2.83	2.25	3.40	0.81
Duretta	*C*	*S*	4	3.06	2.33	4.33	0.95
Elianny	*C*	*HS*	2	4.67	4.33	5.00	0.47
Elsanta	*C*	*S*	9	3.10	1.00	6.25	2.01
Elsinore	*C*	*HS*	2	4.26	3.86	4.67	0.57
Everest	*C*	*HS*	2	5.07	4.80	5.33	0.38
Eve´s Delight	*C*	*HS*	2	4.50	4.33	4.67	0.24
Evi 2	*C*	*HS*	2	5.78	5.44	6.11	0.47
F. moschata "Bauwens"	*W*	*R*	4	1.51	1.00	1.88	0.37
F. moschata "Franken"	*W*	*MR*	4	2.07	1.40	2.63	0.51
F. nilgerrensis "Yunnan"	*W*	*R*	3	1.50	1.00	2.38	0.76
F. vesca "Illa Martin"	*W*	*R*	2	1.50	1.00	2.00	0.71
F. vesca f. alba	*W*	*R*	4	1.25	1.00	1.71	0.34
F. vesca ssp. Bracteata	*W*	*MR*	3	2.07	1.30	3.00	0.86
F. virginiana "Wildmare Creek"	*W*	*MR*	3	2.52	1.83	3.00	0.61
F. viridis"Neddesitz"	*W*	*MR*	3	1.95	1.71	2.13	0.21
Figaro	*C*	*HS*	2	4.26	4.14	4.38	0.16
Florence	*C*	*S*	9	2.61	1.29	6.50	1.67
Florin	*C*	*HS*	2	4.96	3.67	6.25	1.83
Frabella	*C*	*S*	7	2.70	2.00	3.67	0.56
Fraroma	*C*	*MR*	4	2.54	2.00	3.00	0.43
Freja	*C*	*S*	1	2.63	2.63	2.63	.
Fructana	*C*	*MR*	2	1.75	1.50	2.00	0.35
Fructarina	*C*	*MR*	4	2.02	1.13	2.60	0.68
Galia	*C*	*S*	6	3.33	2.50	5.40	1.09
Georg Soltwedel	*C*	*MR*	3	1.55	1.00	2.25	0.64
Gerida	*C*	*S*	4	2.68	1.67	3.60	0.80
Grandiosa	*C*	*S*	1	3.00	3.00	3.00	.
Hansa	*C*	*S*	1	2.64	2.64	2.64	.
Heidekind	*C*	*MR*	4	2.28	1.00	3.30	0.95
Herbstfreude	*C*	*S*	4	3.13	2.25	4.00	0.72

				Incidence of X. fragariae, 35dpi			
Genotype name	**Type**	**Class**	**N**	**Mean**	**Min**	**Max**	**STD**
Heros	*C*	*MR*	4	2.35	1.00	3.40	1.08
Honeoye	*C*	*S*	9	2.83	1.40	6.22	1.59
Hood	*C*	*S*	4	2.65	2.25	3.00	0.31
Höltges Rheingauperle	*C*	*MR*	3	2.45	2.25	2.60	0.18
Joerica	*C*	*S*	7	2.60	1.88	3.00	0.41
Jogana	*C*	*S*	3	2.87	2.33	3.29	0.49
Joly	*C*	*S*	4	2.75	2.00	3.50	0.65
Juline	*C*	*S*	4	3.05	2.38	3.67	0.56
Karla	*C*	*S*	2	3.29	3.00	3.57	0.40
Kimberly	*C*	*HS*	2	5.33	4.80	5.86	0.75
Komet	*C*	*S*	3	2.69	2.40	3.00	0.30
Korona	*C*	*HS*	2	5.60	5.00	6.20	0.85
Königin Luise	*C*	*S*	4	2.56	1.63	3.00	0.63
Lambada	*C*	*HS*	2	5.00	5.00	5.00	0.00
Lina	*C*	*S*	3	3.20	2.75	3.44	0.39
Macherauchs Frühernte	*C*	*S*	4	2.56	1.80	3.20	0.65
Mainperle	*C*	*MR*	3	2.39	1.75	3.14	0.70
Malwina	*C*	*HS*	5	4.24	2.29	6.33	1.89
Mara des Bois	*C*	*HS*	2	5.36	5.22	5.50	0.20
Marloun	*C*	*S*	4	2.59	1.89	3.33	0.63
Marmion	*C*	*MR*	1	1.67	1.67	1.67	.
Mrak	*C*	*HS*	1	5.00	5.00	5.00	.
Oberschlesien	*C*	*S*	3	2.58	1.33	3.67	1.18
Orion	*C*	*MR*	2	2.50	2.00	3.00	0.71
Ostara	*C*	*S*	4	2.71	1.33	3.50	0.96
P-5072	*B*	*S*	1	2.93	2.93	2.93	.
P-5518	*B*	*S*	4	2.57	2.00	3.00	0.43
P-6241	*B*	*HS*	1	4.00	4.00	4.00	.
P-7077	*B*	*S*	1	3.14	3.14	3.14	.
P-7095	*B*	*HS*	1	4.00	4.00	4.00	.
P-7188	*B*	*MR*	4	2.46	1.14	4.10	1.39
P-7201	*B*	*S*	1	2.87	2.87	2.87	.
P-8024	*B*	*S*	4	3.10	2.70	3.60	0.37
P-8043	*B*	*MR*	1	2.33	2.33	2.33	.
P-8049	*B*	*MR*	4	2.34	1.50	3.44	0.90

				Incidence of X. fragariae, 35dpi			
Genotype name	**Type**	**Class**	**N**	**Mean**	**Min**	**Max**	**STD**
P-8071	*B*	*S*	1	3.13	3.13	3.13	.
P-8093	*B*	*S*	1	3.40	3.40	3.40	.
P-9016	*B*	*MR*	1	2.36	2.36	2.36	.
P-9017	*B*	*MR*	1	2.50	2.50	2.50	.
P-9036	*B*	*HS*	1	4.22	4.22	4.22	.
P-9042	*B*	*HS*	4	3.68	3.00	5.00	0.90
Pantagruella	*C*	*S*	1	2.60	2.60	2.60	.
Panther	*C*	*HS*	3	4.25	2.67	5.75	1.54
Papa Lange	*C*	*S*	3	3.12	2.75	3.60	0.44
Paula	*C*	*S*	4	2.67	2.00	3.33	0.61
Peltata	*C*	*S*	1	3.00	3.00	3.00	.
Pocahontas	*C*	*MR*	4	2.37	1.75	3.44	0.75
Polka	*C*	*HS*	2	6.11	6.11	6.11	0.00
Prelude	*C*	*MR*	4	2.42	1.33	3.00	0.79
Pyretta	*C*	*HS*	1	3.70	3.70	3.70	.
Red Coat	*C*	*MR*	4	2.42	1.50	3.75	1.02
Reihngold	*C*	*MR*	3	2.09	1.40	2.88	0.74
Reusraths Allerfrüheste	*C*	*HS*	1	5.00	5.00	5.00	.
Rosella	*C*	*S*	1	3.20	3.20	3.20	.
Roter Regen	*C*	*S*	3	2.89	2.67	3.00	0.19
Roxana	*C*	*MR*	3	2.18	1.00	3.20	1.11
Rumba	*C*	*HS*	2	3.67	3.22	4.11	0.63
Salinas	*C*	*MR*	1	2.00	2.00	2.00	.
Salsa	*C*	*HS*	2	5.56	4.78	6.33	1.10
Salut	*C*	*S*	4	3.26	2.13	4.20	1.00
Segal	*C*	*MR*	4	2.17	1.25	3.33	0.97
Senga Dulcita	*C*	*S*	1	2.73	2.73	2.73	.
Senga Sengana	*C*	*MR*	4	2.43	2.20	3.00	0.38
Sentinel	*C*	*MR*	6	2.43	1.50	3.50	0.76
Sieger	*C*	*S*	4	2.77	2.00	3.44	0.68
Siletz	*C*	*MR*	2	2.00	2.00	2.00	0.00
Solotaya	*C*	*S*	3	2.64	1.75	3.67	0.97
Sonata	*C*	*HS*	2	4.15	3.75	4.56	0.57
Späte Leopold	*C*	*MR*	4	2.18	1.20	3.00	0.96
Sveva	*C*	*HS*	2	5.11	5.00	5.22	0.16

				Incidence of X. fragariae, 35dpi			
Genotype name	**Type**	**Class**	**N**	**Mean**	**Min**	**Max**	**STD**
Sweet Eve	*C*	*HS*	2	4.20	2.40	6.00	2.55
Symphonie	*C*	*HS*	2	5.11	5.00	5.22	0.16
Tabea	*C*	*MR*	4	2.05	1.17	3.00	0.80
Templar	*C*	*S*	4	2.89	2.75	3.00	0.13
Tufts	*C*	*MR*	4	1.60	1.25	2.14	0.41
US 4808	*B*	*R*	3	1.04	1.00	1.13	0.07
US 4809	*B*	*R*	3	1.00	1.00	1.00	0.00
Vikat	*C*	*S*	1	3.00	3.00	3.00	.
Viktoriana	*C*	*MR*	4	2.08	1.00	3.00	0.83
Vima Xima	*C*	*S*	5	2.77	1.00	5.00	1.64
Wunder von Köthen	*C*	*MR*	4	2.53	1.63	3.38	0.80
Yamaska	*C*	*HS*	2	4.78	4.56	5.00	0.31
Zarathustra	*C*	*HS*	4	4.94	4.00	5.86	0.95

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