Diversification de la résistance quantitative à la rouille brune du blé, à partir de la caractérisation des composantes de la résistance

Gustavo Azzimonti

ParisTech
INSTITUT DES SCIENCES ET TECHNOLOGIES
PARIS INSTITUTE OF TECHNOLOGY

AgroParisTech
INSTITUT DES SCIENCES ET INDUSTRIES DU VIVANT ET DE L'ENVIRONNEMENT
PARIS INSTITUTE OF TECHNOLOGY FOR LIFE, FOOD AND ENVIRONMENTAL SCIENCES

Ecole Doctorale ABIES
Agriculture Agriculture Alimentation Food Biologie Biology Environnement Environment Santé Health

Doctorat ParisTech

T H È S E

pour obtenir le grade de docteur délivré par

L'Institut des Sciences et Industries du Vivant et de l'Environnement

(AgroParisTech)

Spécialité : Biologie des populations et écologie

présentée et soutenue publiquement par

Gustavo AZZIMONTI

21 Septembre 2012

Diversification de la résistance quantitative à la rouille brune du blé, à partir de la caractérisation des composantes de la résistance

Directeur de thèse : **Ivan SACHE**
Encadrement de la thèse : **Henriette GOYEAU, Christian LANNOU**

Jury

Mme. Claire NEEMA, PR, Unité de recherche, Ecole	Président du jury
M. Charles- Éric DUREL, DR, UMR1345 IRHS	Rapporteur
M. Jean-Benoit MOREL, DR, UMR0385 BGPI	Rapporteur
M. Olivier ROBERT, S.A.S. Florimond Desprez	Examinateur
M. Fabien HALKETT, CR, UMR 1136 IAM	Examinateur
M. Ivan Sache, CR, UMR 1290 BIOGER-CPP	Directeur de thèse
Mme. Henriette GOYEAU, IR1, UMR 1290 BIOGER-CPP	Encadrante

Thèse effectuée au sein de l'unité mixte INRA-AgroParisTECH BIOlogie et GEstion du Risque en agriculture (UMR 1290 BIOGER)
F-78850 Thiverval Grignon

SOMMAIRE

Remerciements

Cette thèse a été financée via une bourse CIFRE par l'entreprise Florimond Desprez et l'Agence nationale de la recherche. Les travaux de recherche ont été financés par le GNIS (Groupement National Interprofessionnel des Semences), projets FSOV 2005-2007 et FSOV 2008-2011.

Pendant mon doctorat, j'ai travaillé directement avec plus de 40 personnes. La thèse que vous allez lire est le fruit de leur travail. Je suis très reconnaissant envers mes encadrants, Henriette et Christian, qui ont su me guider fermement quand j'en avais besoin, mais qui m'ont laissé le temps de découvrir et d'apprendre par moi-même le métier de chercheur. Ils n'ont pas seulement encadré mon travail, ils m'ont appris une pratique de la recherche honnête, cordiale et stimulante. Je suis aussi très reconnaissant envers mon directeur, Ivan, qui a apporté ses connaissances et sa perspicacité au travail de rédaction. La collaboration étroite avec Thierry Marcel, Olivier Robert, Julien Papaix, Sophie Paillard est partie intégrante de cette thèse. Leur bonne disposition à partager leurs connaissances a permis un travail fluide et dynamique. Les membres du comité de thèse (C. Neema, S. Paillard, D. Tharreau, C. E. Durel, O. Robert, T. Marcel, et P. Lonnet), n'ont pas seulement validé le bon déroulement de la thèse, ils ont participé activement à la conception des expériences du troisième chapitre. Nicolas Lécutier et Lucette Duveau ne m'ont pas seulement aidé pendant les expériences en serre, ils ont mené avec moi ces expériences, apportant leurs connaissances, leur savoir faire, et leur cordialité. Ils ont su supporter mes exigences en termes de qualité d'expériences, parfois trop élevées, je le sais… Je suis aussi très reconnaissant envers le reste de l'équipe technique qui m'a appris le travail en serre et dont la cordialité a souvent adouci les longues journées passées à compter spores et pustules. La mise en place des expériences au champ a été assurée avec beaucoup de soin par les personnels d'Arvalis de la station Boigneville, de Florimond Desprez à Cappelle en Pévèle, et de SERASEM à Maisse. Je suis aussi reconnaissant envers toute l'équipe d'Epidémiologie, ainsi que le personnel administratif, technique et l'ensemble des membres des équipes de recherche de l'UMR BIOGER.

A Adrienne

Résumé

L'enjeu de la thèse est de proposer une stratégie de gestion durable de la résistance génétique à la rouille brune du blé, basée sur des sources de résistance quantitative. Nous proposons d'identifier des résistances se traduisant par une diminution des performances du pathogène sur les différentes phases du cycle infectieux. Ainsi, l'exercice de contraintes diversifiées sur le pathogène devraient ralentir son adaptation et augmenter la durabilité de la résistance.

La confrontation d'un ensemble de génotypes de blé à trois isolats de rouille brune a permis de mesurer le niveau de résistance pour cinq composantes en serre (efficacité d'infection, période de latence, taille de lésion, sporulation par lésion, sporulation par unité de surface sporulante), et à différentes étapes de l'épidémie au champ. Nous avons mis en évidence une grande diversité des composantes affectées, et une variabilité importante pour toutes les composantes. Le développement d'un modèle statistique a permis d'établir que l'ensemble des composantes intervient dans la détermination du niveau de résistance à l'échelle épidémique, mais l'efficacité d'infection et la latence sont les composantes qui jouent le rôle le plus important pour déterminer le niveau de résistance au champ. L'impact d'une composante sur le niveau global de résistance change selon les étapes de l'épidémie. Les trois isolats utilisés ont exprimé un profil d'agressivité contrasté vis-à-vis des différentes composantes. La cartographie des QTLs associés aux différentes composantes de résistance a permis d'établir que la diversité phénotypique observée est liée à une diversité génotypique.

Summary

The issue of this thesis is to propose a durable management of genetic resistance to wheat leaf rust, based on quantitative resistance. We propose to identify resistance factors reducing pathogen development across the different stages of the infectious cycle. Diversifying constraints exerted by host resistance on the pathogen development should slow down the pathogen adaptation, and increase resistance durability.

A set of wheat genotypes was confronted to three leaf rust isolates, and resistance level was measured for five components in the greenhouse (infection efficiency, latent period, lesion size, spore production per lesion, spore production per unit of sporulating tissue), as well as at different stages of field epidemics. Across the germplasm investigated, the resistance components involved were diversified, and their resistance level varied. Developing a statistical model, we established that all the components are involved in the resistance level observed in field epidemics, the most important components being infection efficiency and latent period. The incidence of a component on the field resistance level varied across epidemic stages. The three pathogen isolates used displayed contrasted aggressiveness profiles, according to the different resistance components. QTL mapping of resistance associated to the different components showed that phenotypic diversity corresponded to genotypic diversity.

Présentation générale

Plan et objectifs de la thèse

Introduction Générale

> Un objet que je considère en lui-même, dans sa globalité, ne peut être classé ; il est face à moi, irréductible, non soumis à mes catégories ; plus soi-même que lui-même, il m'échappe.
>
> Jean-Pierre Luminet, « L'invention du big-bang »

Présentation générale

Les programmes de recherche de l'unité BIOGER sont dédiés aux maladies fongiques des plantes, à des échelles allant du gène au paysage. L'enjeu est de concevoir et de gérer des méthodes de lutte prenant en compte aussi bien les mécanismes des interactions plante-pathogène, que leur évolution dans différents systèmes de culture. Au sein de cette unité, l'équipe d'Epidémiologie, dans laquelle s'est déroulée ma thèse, consacre une part importante de ses programmes à la résistance génétique des variétés de blé aux agents pathogènes fongiques. L'enjeu de ces programmes est de proposer des méthodes de gestion des résistances aux maladies : à partir des connaissances sur la biologie des parasites, il s'agit d'imaginer, par des approches expérimentales et théoriques, comment organiser le système cultivé pour limiter l'apparition et le développement des épidémies, et pour ralentir l'adaptation des agents pathogènes aux nouvelles variétés résistantes.

Sur le modèle de la rouille brune du blé, maladie provoquée par *Puccinia triticina*, l'obtention de variétés à résistance durable est un enjeu de poids, qui a suscité l'intérêt de nombreux partenaires sélectionneurs de la filière blé. C'est avec leur collaboration qu'un premier programme, centré sur la résistance quantitative à la rouille brune, à été développé dans l'équipe à partir de 2005. Des variétés et lignées présentant divers niveaux de résistance quantitative à la rouille brune ont ainsi été caractérisées au champ. Dans le cadre de ce même programme, la thèse de B. Pariaud (soutenue en 2008) sur l'agressivité de *P. triticina* a montré l'existence d'une adaptation du pathogène à son hôte pour des traits quantitatifs, et a permis de mettre au point un ensemble de méthodes pour mesurer ce type de traits.

En 2008, dans le prolongement de ces travaux, un second programme, avec les mêmes partenaires, a été dédié à la caractérisation de sources diversifiées de résistance quantitative et de leurs composantes, ainsi qu'à la préparation de l'analyse du support génétique, en appui à la création variétale. Mon mémoire de Master, puis ma thèse, se sont inscrits dans le cadre de ce programme, auquel était associé une bourse CIFRE dont j'ai bénéficié.

Nous disposions ainsi, au démarrage de nos travaux, de l'évaluation de la résistance quantitative au champ d'un ensemble de lignées. Par ailleurs, nous avions également une bonne connaissance des virulences des populations françaises de *P. triticina*, et des facteurs de résistance spécifiques (gènes majeurs) présents dans les variétés et le matériel génétique français.

Plan et objectifs de la thèse

Cette thèse est focalisée sur l'étude des composantes de la résistance quantitative, appliquée au pathosystème blé-rouille brune. L'enjeu est de proposer des sources pour le développement de résistance quantitative à la rouille brune du blé, efficaces et potentiellement durables. La mesure des traits de l'interaction hôte-pathogène, traduisant les mécanismes physiologiques sous-jacents, va permettre de déterminer le degré de diversification du déterminisme d'une source de résistance quantitative, et donc d'inférer sa durabilité. Nous nous sommes attachés dans ce travail à mesurer des variables qui permettent d'appréhender l'ensemble du cycle infectieux. Toutefois ces mesures, effectuées de manière précise en conditions contrôlées, ne rendent compte de l'incidence de la résistance quantitative qu'à l'échelle d'un seul cycle. L'évaluation a été complétée par des mesures au champ à l'échelle de l'épidémie. Le déterminisme génétique associé chez l'hôte a également été établi. Une attention particulière a été prêtée à la mise en évidence d'interactions spécifiques entre génotypes hôtes porteurs de résistance quantitative et isolats du pathogène, susceptibles de conduire à une érosion de la résistance quantitative.

Les objectifs du **premier chapitre** étaient 1) de déterminer la variation du niveau de résistance quantitative au champ pour un ensemble de génotypes; 2) de déterminer la diversité des composantes affectées et leurs variations quantitatives; 3) d'évaluer la spécificité de la résistance pour les différentes composantes.

Un ensemble de 86 variétés et lignées, préalablement évaluées au champ pour leur résistance quantitative, nous a permis de sélectionner un groupe de génotypes représentant une gamme de résistance quantitative. Des plantes adultes ont été inoculées en conditions contrôlées avec trois isolats, représentant trois pathotypes différents. L'efficacité d'infection, la période de latence, la taille de lésion, la sporulation par lésion, et la sporulation par unité de surface sporulante ont été mesurées.

Dans le **deuxième chapitre**, nous avons déterminé les relations entre ces composantes, ainsi que la relation entre le niveau de résistance pour ces composantes, et le niveau de résistance quantitative au champ, à différentes étapes de l'épidémie. Les objectifs étaient 1) d'analyser la relation entre les différentes composantes de la résistance mesurées à l'échelle de la plante et le niveau de résistance observé au cours d'une épidémie au champ; 2) de déterminer laquelle des deux variables, pourcentage de surface malade ou pourcentage de surface

sporulante, était la mieux corrélée aux composantes de résistance; 3) d'analyser les corrélations entre composantes de la résistance.

Le matériel végétal hôte et pathogène était le même que celui étudié dans le premier chapitre. La sévérité de la maladie au champ a été notée à trois dates successives au cours de l'épidémie. Un modèle statistique a été construit, afin d'estimer chaque composante mesurée en serre et la sévérité de la maladie au champ, en séparant les effets expérimentaux des effets de l'interaction génotype hôte-isolat, pour analyser les corrélations entre ces derniers.

Dans le **troisième chapitre**, nous avons identifié le déterminisme génétique de ces composantes. Les objectifs étaient 1) d'identifier les QTLs associés aux composantes de la résistance quantitative mesurées en conditions contrôlées; 2) de déterminer quels sont les QTLs qui ont un impact sur l'épidémie au champ; et 3) de déterminer le niveau de spécificité de ces QTLs vis-à-vis de deux isolats, en conditions d'épidémie au champ.

Une population d'haploïdes doublés, issue du croisement de deux variétés du groupe de génotypes précédemment étudié, a été évaluée pour sa résistance à la rouille brune au stade adulte, en serre et au champ. En serre, les cinq composantes de résistance quantitative ont été mesurées. Au champ, la sévérité de la maladie a été notée au cours d'épidémies initiées séparément à partir de deux isolats, appartenant à deux pathotypes différents.

Réduire l'incidence des épidémies en utilisant les résistances génétiques

Les schémas de production agricoles développés au cours du XX^{e} siècle, que ce soit en extensif ou en intensif, ont permis d'atteindre des niveaux de rendement et de productivité très élevés, mais ils commencent à montrer leurs limites (Foley *et al*., 2005). Les paysages agricoles ont évolué vers une augmentation de la taille des parcelles occupées par des plantes hôtes génétiquement homogènes, et vers une réduction de la diversité en espèces cultivées (Robinson & Sutherland, 2002). Ce contexte de forte homogénéité est en particulier très propice au développement des épidémies (Stukenbrock & Mcdonald, 2008 ; Stuthman *et al*., 2007). Une adaptation des itinéraires techniques peut permettre de limiter les pertes dues aux maladies. En particulier l'utilisation de pesticides est très efficace. Toutefois leur utilisation intensive n'est pas durable dans le long terme. L'utilisation systématique des pesticides a généré des phénomènes de résistance chez certains agents pathogènes, ainsi que des problèmes de pollution importants (Robinson & Sutherland, 2002 ; Geiger *et al*., 2009 ; Isenring, 2010), qui orientent actuellement vers des politiques publiques de limitation de leur utilisation (« Grenelle de l'Environnement » 2008). L'utilisation de variétés portant des résistances de type qualitatif est un autre élément de l'intinéraire technique qui permet de contrôler efficacement les maladies. La résistance qualitative se caractérise par un arrêt, ou un dérèglement majeur, du cycle infectieux du pathogène, qui ne produit alors plus, ou très peu, de descendants. Le phénotypage de la résistance qualitative est facile, puisque qu'elle se traduit par une absence quasi-totale de symptômes. Cette résistance est basée sur des gènes majeurs, hérités selon un déterminisme mendélien simple. Très efficace et facile à sélectionner, la résistance qualitative a été, et est encore, largement utilisée. Toutefois des cas de contournement sont apparus dès le début de son utilisation à grande échelle, avec la perte d'efficacité concomitante (Samborski, 1985 ; Parlevliet, 2002). Ces contournements résultent d'une adaptation du pathogène, facilitée par l'homogénéité du paysage variétal. La gestion de ce type de résistance peut être décrite comme une « course aux armements », entre d'une part les sélectionneurs qui introduisent de nouveaux gènes majeurs de résistance dans les variétés, et d'autre part les populations pathogènes, dont les isolats virulents sont fortement sélectionnés, générant des cycles de « boom-and-bust » (Wolfe, 1973 ; Brown & Tellier,

2011). Une gestion plus durable des résistances est envisageable, et reste un des enjeux majeurs pour l'agriculture du XXI^{e} siècle (Mundt *et al*., 2002 ; Cheatham *et al*., 2009).

La résistance quantitative est-elle durable ?

La résistance quantitative se traduit par un ralentissement de la progression et/ou une diminution de la sévérité des épidémies (Shaner & Hess, 1978 ; Shaner *et al*., 1978). Contrairement à la résistance qualitative, caractérisée par une réponse en "tout ou rien", la résistance quantitative présente un *continuum* de réponse qui va, selon le génotype hôte, d'un haut niveau de résistance à la sensibilité complète.

L'identification d'interactions différentielles entre génotypes de la plante et isolats du pathogène ont conduit à la proposition d'un modèle gène mineur-pour-gène mineur, comme base génétique de la résistance quantitative (Parlevliet & Zadoks, 1977 ; Niks & Marcel, 2009). Les gènes de résistance de la plante interagiraient avec les gènes de pathogénicité du pathogène d'une façon spécifique, qui se traduirait au niveau moléculaire par l'interaction entre les effecteurs du pathogène et les gènes ou les facteurs moléculaires de la plante. Brièvement, le niveau de résistance dépendrait, d'une part, de la capacité de la plante à reconnaître les effecteurs du pathogène et à mettre en place correctement, et en temps, des mécanismes de défense ; d'autre part, de la capacité du pathogène à émettre des effecteurs capables d'altérer les mécanismes de défense de la plante (Niks & Marcel, 2009). Notre travail ne comportant pas d'étude de l'interaction hôte-pathogène à l'échelle moléculaire, nous ne détaillerons pas d'avantage les travaux qui s'y rapportent. L'hypothèse d'une interaction gène mineur-pour-gène mineur permet de relier la diversité des gènes et mécanismes impliqués, à la durabilité de la résistance. Une grande diversité des gènes de résistance quantitative et des molécules associées commence à être mise en évidence dans plusieurs pathosystèmes (Niks & Marcel, 2009). La variété des mécanismes moléculaires impliqués peut être interprétée comme une diversité des mécanismes physiologiques, et donc des processus associés (infection, croissance du pathogène dans les tissus de l'hôte, production de spores). Ainsi, face à une diversité de contraintes physiologiques imposées simultanément par la résistance quantitative de la plante, l'adaptation du pathogène serait ralentie (Stuthman *et al*., 2007).

La résistance quantitative est, en pratique, plus durable que la résistance qualitative complète (Stuthman *et al*., 2007 ; Parlevliet, 2002). Toutefois des cas d'adaptation du pathogène à la résistance quantitative de l'hôte ont été obtenus expérimentalement, ou bien

observés au champ (Mundt *et al*., 2002). Une augmentation de l'efficacité d'infection a été mesurée dans une population de *P. graminis* f. sp. *avenae* sur deux génotypes de l'hôte, dans une expérience de sélection en conditions contrôlées, en seulement sept générations (Leonard, 1969). Au champ, Chin & Wolfe (1984) et Villaréal & Lannou (2000) ont mesuré une adaptation quantitative de la population pathogène de *Blumeria graminis* f. sp. *tritici* dans une population hôte homogène. De même, la dominance au champ d'un pathotype de *P. triticina* sur la variété Soissons a été expliquée par une aggressivité élevée pour l'efficacité d'infection, la latence, la production de spores, et la période infectieuse (Pariaud *et al*., 2009b). Ces études montrent qu'une sélection sur des traits quantitatifs peut s'opérer assez rapidement au sein des populations pathogènes, conduisant à une adaptation à l'hôte et parfois à une érosion de la résistance quantitative (Pariaud *et al*., 2009a). Une diversité des modes d'action en jeu, et des gènes qui y sont associés, pourrait conditionner la durabilité de la résistance quantitative.

Traits de l'interaction hôte-pathogène impliqués dans la résistance quantitative: les composantes de la résistance et leur mesure en conditions contrôlées

La résistance quantitative se caractérise par une diminution des performances du pathogène sur les différentes phases du cycle infectieux (Parlevliet, 1979 ; Pariaud *et al*., 2009ab). Pour la plupart des pathogènes, le cycle infectieux comprend l'infection, la colonisation des tissus de la plante, et la reproduction via la production de spores.

L'efficacité d'infection est définie comme la proportion de spores déposées sur les feuilles donnant des lésions sporulantes. Cette composante mesure l'effet de la résistance sur plusieurs processus qui vont de la germination des spores, la pénétration et la colonisation des tissus de la plante, jusqu'à la formation de lésions (Parlevliet, 1979). L'arrêt du développement du pathogène au cours de l'un de ces processus va se traduire par une diminution de la quantité de lésions. **La période de latence**, mesurée en jours ou degré-jours, est définie comme l'intervalle de temps entre le dépôt d'une spore sur une feuille, et le début de la sporulation de la lésion qui en résulte (Shaner, 1980). La latence mesure l'effet de la résistance sur les mêmes processus que l'efficacité d'infection, processus qui ne sont pas bloqués, mais dont la vitesse est réduite. **La sporulation par lésion**, mesurée en mg de spores par lésion, est définie comme le taux de production de spores par lésion dans un temps donné. Cette composante depend de deux caracteristiques des lésions : leur taille, et la quantitié de spores produites par unité de surface du tissu sporulante (Sache & de Vallavieille-Pope,

1993). **La taille des lésions**, mesurée en mm², est définie comme la surface des lésions produisant des spores. Cette composante mesure l'effet de la résistance sur la capacité de colonisation du mycélium du pathogène dans les tissus de la plante. **La sporulation par unité de surface sporulante**, mesurée en mg de spores par mm² de tissu sporulant, est définie comme le taux de production de spores du tissu sporulant dans un temps donné. Cette composante mesure l'effet de la résistance sur la capacité du pathogène à extraire et transformer les nutriments de la plante en tissus reproductifs et en spores.

L'expression des composantes de la résistance quantitative dépend des conditions climatiques, de l'état physiologique et du stade de développement de l'hôte et du pathogène, ainsi que de la densité de lésions (Parlevliet, 1979 ; Imhoff *et al*., 1982 ; Baart *et al*., 1991 ; Sache, 1997 ; Pariaud *et al*., 2009a). La mesure des composantes doit donc être effectuée dans les conditions expérimentales les plus homogènes possibles.

La quantification de toutes les composantes déterminant la résistance quantitative durant l'ensemble du cycle infectieux n'a été menée à bien que rarement. L'ensemble des composantes a été mesuré pour la rouille brune du blé, mais avec un niveau de précision variable selon les différentes composantes (Milus & Line, 1980 ; Knott & Mundt, 1991 ; Singh *et al*., 1991 ; Denissen, 1993 ; Herrera-Foessel *et al*., 2007). La période de latence et la taille des lésions ont été mesurées avec un bon niveau de précision (Broers, 1989ab ; Drijepondt & Pretorius, 1989 ; Das *et al*., 1993 ; Singh & Huerta-Espino, 2003 ; Lehman *et al*., 2005 ; Herrera-Foessel *et al*., 2007). Par contre l'efficacité d'infection n'a pas été mesurée mais seulement estimée, souvent par la densité de lésions, en supposant un dépôt de spores homogène (Knott & Mundt, 1991 ; Pariaud *et al*., 2009b), ce qui est rarement vérifié (Pariaud *et al*., 2009a). À de rares exceptions près (Johnson & Taylor, 1976 ; Milus & Line, 1980 ; Pariaud *et al*., 2009b), la seule variable mesurée pour caractériser la sporulation est la taille des lésions. Une variabilité du niveau de résistance exprimé sur les différentes composantes a ainsi été mise en évidence pour divers pathosystèmes (Singh *et al*., 1991 ; Carlisle *et al*., 2002 ; Negussie *et al*., 2005 ; Herrera-Foessel *et al*., 2007 ; Pariaud *et al*., 2009b).

Corrélations entre composantes et sévérité de l'épidémie au champ

La mesure des composantes, réalisée à l'échelle d'un seul cycle et en conditions contrôlées, ne permet pas d'évaluer directement leur effet sur le niveau de résistance en conditions épidémiques. Le niveau de corrélation, entre d'une part les composantes de la résistance mesurées en conditions contrôlées, et d'autre part la sévérité de l'épidémie au

champ, est très variable selon les études (Johnson & Taylor, 1976 ; Baart *et al*., 1991 ; Denissen, 1993 ; Carliste *et al*., 2002 ; Negussie *et al*., 2005 ; Herrera-Foessel *et al*., 2007). Une très forte corrélation de chaque composante avec le niveau de résistance au champ a été observée pour les rouilles du blé (Broers, 1989ab ; Singh *et al*., 1991), ainsi que pour le mildiou de la pomme de terre (Carlisle *et al*., 2002) ; les composantes étaient dans ces cas également très fortement corrélées entre elles. Mais dans la plupart des études, les corrélations ne sont pas si catégoriques. Une forte corrélation entre l'efficacité d'infection, la latence, et la taille de lésions, mais une faible corrélation entre chacune de ces composantes et l'AUDPC ou la sévérité finale au champ, a été mise en évidence pour un ensemble de 15 cultivars du blé confronté à deux isolats de rouille brune (Denissen, 1993). Des corrélations entre la latence et la taille de lésions, et entre ces composantes et l'AUDPC ou la sévérité finale au champ, ont été trouvées pour un ensemble de neuf cultivars de blé dur confrontés à un isolat de rouille brune (Herrera-Foessel *et al*., 2007) ; par contre, dans cette même étude, l'efficacité d'infection n'était pas corrélee avec les autres composantes, ni avec l'AUDPC ou la sévérité finale au champ.

Le support génétique des composantes de la résistance quantitative

Très peu d'études ont été faites pour déterminer les facteurs génétiques agissant sur les différentes composantes de la résistance quantitative (Tableau 1). Ceci est dû principalement à la difficulté d'obtenir des mesures précises avec peu de répétitions. En effet, dans la plupart des expériences en conditions contrôlées, le nombre de répétitions est souvent de dix au maximum, du fait des limitations d'espace et de temps, ce qui entraîne un faible précision dans les mesures, avec une répercussion dans la précision des analyses QTL. Pour augmenter le nombre de répétitions ou réduire la durée des expériences, les mesures peuvent être réalisées sur jeunes plantes (Qi *et al*., 1998 ; Richardson *et al*., 2006 ; Marcel *et al*., 2008). Toutefois une validation de l'expression au stade adulte des QTLs ainsi identifiés est nécessaire du fait de l'influence du stade de développement sur la mise en place de la résistance quantitative. Par exemple, Wang *et al*. (2010) ont déterminé, dans le cas du pathosystème orge-rouille brune, trois QTLs agissant sur la latence, l'un uniquement au stade plantule, un autre uniquement à partir du stade tallage, et le troisième à tous les stades de développement de la plante. L'expression des QTLs dépend également fortement des conditions d'environnement (Young, 1996 ; Doerge, 2002), donc des conditions expérimentales. Les composantes les plus étudiées sur des plantes adultes

sont la période de latence, la taille des lésions et la densité de lésions. À notre connaissance, il n'y a aucune étude de cartographie QTL pour la sporulation par lésion.

De même très peu d'études ont été consacrées à la cartographie QTL de traits physiologiques de l'interaction hôte-pathogène, tels que l'accumulation de callose dans les parois cellulaires (Tableau 1). Élucider le déterminisme génétique des différents processus de l'infection à l'échelle microscopique, en lien avec les variables mesurées à l'échelle macroscopique, permet une compréhension très fine de l'interaction. Toutefois cette approche implique un coût expérimental élevé, et nécessite une bonne connaissance préalable du matériel d'étude. Pour la phase de sporulation, où les connaissances des mécanismes physiologiques sont très limitées, ce type d'approche n'est pas encore envisageable.

Une validation au champ s'avère nécessaire pour préciser l'efficacité de QTLs de composantes de la résistance quantitative identifiés en conditions contrôlées : en effet l'expression des QTLs dépend de l'environnement et du stade de développement de l'hôte. De plus l'effet mis en évidence à l'échelle d'un seul cycle infectieux en conditions contrôlées peut être amplifié, ou au contraire atténué, à l'échelle de l'épidémie polycyclique. La majorité des QTLs trouvés pour des composantes en conditions contrôlées sont retrouvés à l'échelle de l'épidémie au champ (Qi *et a*l.,1998 ; Jorge *et al*., 2005 ; Marone *et al*., 2009 ; Talukder *et al*., 2004 ; Wang *et al*., 1994 ; Chung *et al*., 2010). Par contre, la part de la variance phénotypique expliquée par un QTL (estimée par le R^2) peut être très différente entre un QTL identifié sur une composante en conditions contrôlées, et un QTL identifié à partir de la sévérité de la maladie au champ (Jorge *et al*., 2005 ; Talukder *et al*., 2004).

Par ailleurs, les travaux d'identification de QTLs au champ sont basés exclusivement sur la sévérité globale de l'épidémie (mesurée par l'aire sous la courbe de maladie ou AUDPC), à deux exceptions près où les analyses QTL ont été conduites séparément pour différentes dates de notation, pour la rouille jaune du blé : le R^2 des QTLs identifiés par Ramburan *et al*. (2004), augmentait au cours de l'épidémie, tandis que Dedryver *et al*. (2009) ont trouvé des QTL spécifiques des dates de notation, dont certains non identifiés dans l'analyse QTL conduite sur l'AUDPC.

La spécificité de la résistance quantitative et de ses composantes

L'existence d'interactions spécifiques entre génotypes hôtes et isolats du pathogène pour la résistance quantitative est avérée pour plusieurs pathosystèmes, mais elle n'est pas la règle,

Tableau 1. QTLs associés aux composantes et/ou aux mécanismes physiologiques de la résistance quantitative, à différents stades de développement de la plante, pour différents pathosystèmes.

Composante de la résistance	**Stade de développement**	**Isolat - Spécificité**	**Pathosystème**	**Référence**
Efficacité de l'infection				
	Plante adulte	Oui	Rouille du peuplier	Jorge *et al*., 2005; Dowkiw & Bastien, 2007
	Plante adulte	Oui	Pyriculariose du riz	Talukder *et al*., 2004
	Plante adulte	Non	Pyriculariose du riz	Wang *et al*., 1994
	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al*., 2010
	Plantule	Non	Rouille jaune de l'orge	Richardson *et al*., 2006
Période de latence				
	Plantule et plante adulte	Non	Rouille naine de l'orge	Qi *et al*., 1998; Wang *et al*., 2010
	Plante adulte	Non	Rouille brune du blé	Xu *et al.,* 2005
	Plante adulte	Oui	Rouille du peuplier	Jorge *et al*., 2005; Dowkiw & Bastien, 2007
	Plantule et plante adulte	Non	Rouille brune du blé dur	Marone *et al.,* 2009
	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al.,* 2010
	Plantule	Non	Rouille jaune de l'orge	Richardson *et al.,* 2006
	De plantule à plante adulte	Oui	Rouille naine de l'orge	Marcel *et al.,* 2008
Taille de lésion				
	Plante adulte	Oui	Rouille du peuplier	Jorge *et al*., 2005; Dowkiw & Bastien, 2007
	Plante adulte	Oui	Pyriculariose du riz	Talukder *et al.,* 2004
	Plante adulte	Non	Pyriculariose du riz	Wang *et al.,* 1994
	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al.,* 2010
	Plantule	Non	Rouille jaune de l'orge	Richardson *et al.,* 2006
Production de spores par unité de tissu sporulant	Plante adulte	Oui	Tavelure du pommier, septoriose du blé	Calenge *et al*., 2004; Kelm *et al*., 2011
Dynamique de nécrose	Plante adulte	Non	Mildiou du piment	Thabuis *et al*., 2004
Surface foliaire nécrotique	Plante adulte	Oui	Septoriose du blé	Kelm *et al*., 2011
Mécanisme physiologique				
Avortement précoce des colonies (avec ou sans nécrose cellulaire)	De plantule à plante adulte	Non	Rouille brune du blé dur	Marone *et al*., 2009
Etablissement des colonies (avec ou sans nécrose cellulaire)	De plantule à plante adulte	Non	Rouille brune du blé dur	Marone *et al*., 2009
Formation d'appressoria multiples	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al*., 2010
Accumulation de callose et de composés phénoliques	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al*., 2010
Invasion vasculaire	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al*., 2010
Rapport de biomasse fongique	De plantule à plante adulte	Non	*Northern leaf blight* du maïs	Chung *et al*., 2010
Phénologie de l'infection et de la colonisation	De plantule à plante adulte	Non	Rouille jaune du blé	Jagger *et al*., 2011

et la spécificité de la résistance quantitative demeure donc en débat. D'après la revue bibliographique de Ballini *et al.* (2008), tous les QTLs qui ont été identifiés pour la pyriculariose du riz sont spécifiques. Dans le cas du pathosystème pommier-*Venturia inaequalis*, Calenge *et al.* (2004) ont identifé sept QTLs, tous spécifiques. La spécificité peut être délicate à mettre en évidence pour des QTLs à effet faible (Marcel *et al*., 2008). Les QTLs à effet fort sont le plus souvent non spécifiques, mais certains QTLs spécifiques à effet fort ont été identifiés (Calenge *et al*., 2004 ; Marcel *et al*., 2008).

Des interactions différentielles hôte-pathogène pour la résistance quantitative ont également pu être mises en évidence à partir de la mesure des composantes (Parlevliet, 1979). Dans le cas de la rouille brune du blé, des interactions différentielles variété-isolat ont été trouvées pour la période de latence (Broers, 1989b ; Lehman & Shaner, 1996) et pour la sporulation par lésion (Milus & Line, 1980) ; toutefois Denissen (1991) n'a mis en évidence aucune spécificité dans le niveau de résistance quantitative à la rouille brune pour ces mêmes composantes.

La spécificité des QTLs agissant sur les composantes de la résistance quantitative a été très peu étudiée (Tableau 1). Des QTLs spécifiques ont été mis en évidence pour la sporulation par unité de surface sporulante (Calenge *et al*., 2004) et pour la période de latence (Marcel *et al*., 2008). Parmi les QTLs trouvés par Jorge *et al*. (2005), agissant sur la latence, la densité et la taille de lésion, quatre étaient efficaces contre un ou deux isolats seulement, donc fortement spécifiques, et un était efficace contre quatre des sept isolats étudiés, donc moyennement spécifique. Une spécificité des QTLs peut également être révélée au niveau quantitatif, dans le cas où la magnitude de l'effet des QTLs est spécifique de l'isolat. Marcel *et al.* (2008) ont trouvé chez l'orge un QTL dont l'effet variait quantitativement selon l'isolat de rouille naine utilisé. Jorge *et al.* (2005) ont mis en évidence chez le peuplier deux QTLs spécifiques, dont les effets quantitatifs vis-à-vis de deux isolats de rouille variaient en sens opposé.

La rouille brune du blé

La rouille brune du blé est le produit de l'interaction entre le champignon basidiomycète *Pucinia triticina* et le blé tendre (*Triticum aestivum* L. subsp. *aestivum*).

Le cycle asexué comprend trois étapes : infection de la feuille, croissance du pathogène dans les tissus foliaires, et production de spores. Les processus physiologiques et moléculaires de l'infection sont bien connus (Bolton *et al*., 2008). Lorsque la feuille est couverte par une fine pellicule de gouttelettes d'eau, le tube germinatif émis par la spore s'allonge jusqu'à ce

qu'il rencontre un stomate. Sur le stomate se forme un appressorium, qui produit une cheville de pénétration dans l'espace intercellulaire du mésophylle. Les hyphes se différencient en cellules mères de l'haustorium, qui vont pénétrer dans les cellules du mesophylle et former des haustoria, structures chargées de l'assimilation des nutriments de la plante. Sept à dix jours après l'inoculation, le mycélium produit des urédies, qui libèrent des urédospores après rupture de l'épiderme foliaire. Les lésions ont une croissance finie, mais de petites lésions secondaires peuvent apparaître autour d'une lésion primaire. Autour de la partie sporulante de la lésion, les tissus envahis par le mycélium forment un halo chlorotique, qui deviendra progressivement nécrotique vers la fin du cycle.

La dispersion des spores s'effectue majoritairement par le vent. Les épidémies résultent de la succession de quatre à cinq cycles de reproduction asexuée au cours de la saison, lorsque les conditions environnementales sont favorables (Zadoks & Bouwman, 1985). Le facteur critique est une période de rosée dans le couvert végétal suffisamment longue pour permettre la germination des spores.

Champignon biotrophe strict, *P. triticina* affecte la plante en exportant des assimilats pour produire des tissus fongiques et des spores (Robert *et al*., 2002, 2004), en réduisant la surface photosynthétique, et en accélérant la sénescence foliaire. (Robert *et al*., 2005).

P. triticina possède des caractéristiques biologiques communes aux agents des rouilles des céréales, qui favorisent un développement épidémique redoutable : sporulation abondante, dissémination efficace par le vent, important potentiel de variation pour les virulences. De plus la culture de son hôte est très répandue dans le monde, sur de grandes surfaces, souvent dans des conditions environnementales propices pour le champignon (Dean *et al*., 2012). Les pertes de rendement associées aux épidémies sévères de rouille brune sont élevées, mais elles restent inférieures à celles provoquées par la rouille noire du blé (IFPRI Discussion Paper 00910). Cependant, l'aire de répartition géographique de la rouille brune est plus vaste que celle des rouilles jaune et noire du blé (Huerta-Espino *et al*., 2011). En France, de fortes épidémies se produisent régulièrement dans les régions Ouest et Sud-Ouest. La perte de rendement des parcelles non traitées avec des fongicides peut atteindre 40% par rapport aux parcelles traitées (suivi SPV Phytoma 1988-2003).

La population de *P. triticina* en France est très diversifiée en pathotypes (Goyeau *et al*., 2006), avec 30 à 40 pathotypes différents identifiés en moyenne chaque année. L'hôte alternatif, *Thalictrum speciosissimum*, qui héberge le cycle sexuel, n'est pas présent en France, et très rare en Europe. Au niveau international, pour la majorité des zones de production du blé,

l'absence d'hôte alternatif, ainsi que les données de génotypage, suggèrent que la phase sexuée du cycle n'intervient pas dans l'épidémiologie de cette maladie, et qu'elle est une source négligeable de variation génétique chez le champignon (Bolton *et al*., 2008). En France, la reproduction de *P. triticina* est strictement asexuée, et les isolats appartenant à un même pathotype ont le même génotype SSR, à l'exception de rares mutants (Goyeau *et al*., 2007). La structure de la population en pathotypes dépend fortement des variétés cultivées et des gènes majeurs qu'elles contiennent (Goyeau *et al*., 2006). Aucune structuration géographique n'a été mise en évidence à l'échelle de la France, ce qui peut être imputé à une dispersion importante qui homogénéise les populations, et à l'absence d'adaptation locale aux conditions climatiques.

Au début des années 1980, la plupart des variétés cultivées en France ne possédait pas de gènes de résistance qualitative. En réaction à de sévères épidémies, les sélectionneurs ont progressivement incorporé des combinaisons de gènes de résistance spécifique *Lr*. Les contournements successifs de ces gènes ont conduit à utiliser des combinaisons de gènes *Lr* de complexité croissante (Goyeau & Lannou 2011). Aucune sélection dirigée pour la résistance quantitative n'a pu être conduite en raison de l'absence des connaissances nécessaires correspondantes sur l'agressivité des isolats pathogènes et sur le déterminisme génétique de la résistance quantitative à la rouille brune. Toutefois les sélectionneurs ont veillé à éviter les génotypes très sensibles, et la plupart des variétés actuellement inscrites ont un niveau correct de résistance quantitative.

Chapitre 1

“Components of quantitative resistance to leaf rust in wheat cultivars: diversity, variability and specificity”

Components of quantitative resistance to leaf rust in wheat cultivars: diversity, variability and specificity

Azzimonti G*, Lannou C, Sache I, Goyeau H

INRA, UMR1290 BIOGER-CPP F-78850, Thiverval-Grignon, France

gazzimonti@versailles.inra.fr

Accepted in Plant Pathology, 13-Jul-2012

Keywords: durability of resistance, *Puccinia triticina.*

Abstract

Based on assumptions of non-specific host-pathogen interactions, a complex genetic basis and diversified underlying resistance mechanisms, quantitative plant resistance is generally expected to be more durable. This study aimed to investigate the potential diversity and pathogen-specificity of sources of quantitative resistance to leaf rust in French wheat germplasm. From a set of 86 genotypes displaying a range of quantitative resistance levels during field epidemics, eight wheat genotypes were selected and confronted in a greenhouse to three isolates, belonging to different pathotypes. Five components of resistance were assessed: infection efficiency, for which an original methodology was developed, latent period, lesion size, spore production per lesion, and spore production per unit of sporulating tissue. A high diversity and variability for all these components was expressed in the host x pathotype combinations investigated; pathotype specificity was found for all the components.

The host genotypes displayed various resistance profiles, based on both the components affected and the pathotype-specificity of the interaction. Their usefulness as sources of quantitative resistance was assessed: line LD7 likely combines diversified mechanisms of resistance, being highly resistant for all the components, but displaying isolate specificity for all the components; cultivar Apache did not show isolate specificity for any of the components, which could be related to the durability of its quantitative resistance in the field over more than 11 years.

INTRODUCTION

Durability of genetic resistance to plant pathogens is a central issue in crop breeding (Stuthman *et al*., 2007). Resistance to plant pathogens displays a continous range, from complete resistance to complete susceptibility. Complete resistance is based on the action of major resistance genes that determine an incompatible reaction between the plant and the pathogen. This type of resistance, although very effective, is often poorly durable (Parlevliet, 2002). Quantitative resistance leads to a reduction in disease, rather than the absence of disease. This resistant phenotype can be based on a few to several genes with partial effects associated to quantitative traits loci (Ballini *et al*., 2008; Marcel *et al*., 2008; Palloix *et al*., 2009; St. Clair, 2010). Empirical results suggest that quantitative resistance can be more durable than complete resistance (Mundt *et al*., 2002; Parlevliet, 2002). Highly durable quantitative resistance was observed in pathosystems where complete resistance was overcame (Stuthman *et al*., 2007). For instance, the French cultivar Apache has remained quantitatively resistant to leaf rust over a long period of time (11 years) and at a large

geographic scale (Papaïx *et al*., 2011), even though qualitative resistance is easily and repeatedly overcome in this pathosystem (Goyeau *et al*., 2006, 2011).

Although quantitative resistance is expected to be durable, adaptation of the pathogen, leading to erosion of resistance, is still possible and has been observed in an agricultural context (Mundt *et al*., 2002) or obtained experimentally (Lehman & Shaner, 1997). Many studies show that selection for quantitative traits of the host-pathogen interaction influences pathogen evolution in agricultural systems (Pariaud *et al*., 2009a). Leonard (1969) observed the selection for higher infection efficiency in a *P. graminis* f. sp. *avenae* population on two different host genotypes after seven asexual generations in controlled conditions. Chin & Wolfe (1984) and Villaréal & Lannou (2000) observed quantitative adaptation to different cultivars by comparing pathogen evolution in host mixtures vs. pure stands in field epidemics. This shows that a diversity for quantitative traits exists in pathogen populations, that selection for quantitative traits can occur within a short number of generations, and that the host genotype influences the direction of this selection. More generally, selection for quantitative traits can lead to increased pathogenicity on a host variety (Ahmed *et al*., 1996) and to the erosion of a host quantitative resistance, as suggested by Mundt *et al.* (2002). Understanding how pathogens adapt to quantitative resistance will require to go beyond these general observations and to precisely identify the traits of the host-pathogen interaction that are involved, as well as the specific or general nature of the quantitative resistance. Our study will contribute to this objective by identifying resistance components in wheat and their specificity to *Puccinia triticina* isolates.

Quantitative resistance can alter the expression of different traits of the host–pathogen interaction. For many pathogens, including *P. triticina*, these traits are infection efficiency, latent period, lesion size, and sporulation rate (Parlevliet, 1979; Pariaud *et a*l., 2009a). For the sake of simplicity, the term "resistance component" will refer to the expression of the host

resistance with regard to a specific trait of the host–pathogen interaction (e.g. the latent period). Variability in the level of resistance for each of these components has been detected for different host cultivars, and with regard to different pathogens (Singh *et al*., 1991; Carlisle *et al*., 2002; Negussie *et al*., 2005; Herrera-Foessel *et al*., 2007; Pariaud *et al*., 2009b). Breeding for quantitative resistance can make use of this variability by combining resistance genetic factors that affect different resistance components. This could render the resistance more difficult to overcome by the pathogen, which would have to adapt to different constraints at the same time.

Leaf rust, caused by *P. triticina*, is the most common and widely distributed of the three wheat rusts (Huerta-Espino *et al*., 2011). However, even for this well-known pathosystem, identification of the components of quantitative resistance and characterization of the variability in the level of resistance for each of these components remain scarce (Milus & Line, 1980; Knott & Mundt, 1991; Singh *et al*., 1991; Denissen, 1993; Herrera-Foessel *et al*., 2007). Latent period and lesion size have been most often measured (Broers, 1989ab; Drijepondt & Pretorius, 1989; Das *et al*., 1993; Singh & Huerta-Espino, 2003; Lehman *et al*., 2005; Herrera-Foessel *et al*., 2007). Infection efficiency is usually estimated by lesion density (Knott & Mundt, 1991, Pariaud *et al*., 2009b) but this estimation remains fairly rough (Pariaud *et al*., 2009a) because lesion density strongly depends on the number of spores deposited on the inoculated tissue, which is usually not well controlled. An improved procedure has been used in this study to measure infection efficiency with a better precision. Specificity of quantitative resistance with regard to the pathogen isolates remains a matter of debate. Cultivar-by-isolate differential interactions have been found for different pathosystems (Parlevliet, 1979; Carlisle *et al*., 2002, Talukder *et al*., 2004). In the case of wheat leaf rust, interactions between host genotype and pathogen genotype were found for latent period (Broers, 1989b; Lehman & Shaner, 1996) and for sporulation rate per lesion

(Milus & Line, 1980), but Denissen (1991) did not find any specificity for these components, and. Singh *et al*. (2011) stated that there was no isolate specificity for quantitative resistance in a large collection of CIMMYT breeding material for the three wheat rust diseases. Evidence provided both by QTL analyses and functional analyses suggest that at least part of the known resistance genetic factors present a specific spectrum (Ballini *et al*., 2008). Most of these studies are based on an analysis of disease severity, thus confounding the effect of the resistance on all resistance components. Some studies, however, identified the pathogenicity-related trait that is targeted by the QTL. In such cases, both large-spectrum QTLs and specific QTLs were detected (Calenge *et al*., 2004; Marcel *et al*., 2008). Whether cultivar-by-isolate differential interaction is a general feature of quantitative resistance is then still an unresolved question which justifies more efforts for identifying the traits of the host-pathogen interaction that are affected by quantitative resistance and the specificity of the resistance factors associated.

The objectives of this study were: i) to screen cultivars and lines for quantitative resistance to leaf rust, ii) to assess the resistance components,, and iii) to evaluate, based on a small set of isolates, whether the resistance components present some specificity with regard to pathogen isolates. For this, eight host genotypes displaying a range of quantitative resistance were selected out of a set of 86 cultivars and lines, and adult plants of these genotypes were confronted to three isolates belonging to different leaf rust pathotypes under controlled conditions. Infection efficiency, latent period, lesion size, sporulation rate per lesion, and sporulation rate per unit of sporulating tissue were measured.

MATERIALS AND METHODS

Field experiments: A preliminary field assessment of the level of quantitative resistance was conducted, for a set of host lines and cultivars: a series of 86 lines and cultivars was planted in 7, 9 and 4 locations in France, in 2005, 2006 and 2007 respectively, in a randomized block design. Depending on the location, the design comprised two or three blocks, and each cultivar was planted on two or three rows 1.5m-long. Wheat leaf rust epidemic was initiated by spraying spreader rows of the cultivar Buster, just before heading, with a spore suspension of isolate P3 (Table 1), in Soltrol® oil (Phillips Petroleum). Two or three diseases assessments were performed on flag leaves, using the modified Cobb scale (Peterson *et al.* 1948). The Relative Area Under the Disease Progress Curve (RAUDPC) was calculated for each line or cultivar, relatively to the most susceptible cultivar. A mean RAUDPC was calculated across years, locations and replicates. Six cultivars (Andalou, Apache, Balance, Écrin, Soissons and Trémie), and two lines (LD 00170-3, hereafter LD7 and PBI-04-006, hereafter PBI) were selected to represent the range of quantitative resistance observed in the field (Fig. 1). Morocco, considered at first as a susceptible check for greenhouse experiments, was also included.

Overview of greenhouse experiments: Components of quantitative resistance were measured on adult plants in greenhouse conditions for different host x isolate combinations. The experiments were based on a set of cultivars and three isolates, labelled P3, P4 and P5. Three greenhouse experiments were performed. For technical limitations, some of the interactions could not be tested three times. In experiment 1, conducted in 2007, five cultivars (Andalou, Apache, Écrin, Soissons and Trémie), and Morocco, were confronted to isolate P3. In experiment 2, conducted in 2008, lines LD7 and PBI, and a second isolate P4, were added to those tested in experiment 1. In experiment 3, conducted in 2009, another cultivar, Balance, and a third isolate, P5, were added to those tested in experiment 2.

Plant material: All measurements were performed on adult plants with, as much as possible, homogeneous growth stages and physiological states. Seeds were sown in Jiffy pots with two seeds per pot. Seedlings at the two-leaf stage were vernalized at 8°C in a growth chamber. To synchronize the development of different cultivars, sowing times were staggered taking into account earliness in heading time, and duration of vernalization.

After vernalization, plants were transferred to a greenhouse and left for 7 to 10 days to acclimatize. The most vigorous seedlings were then individually transplanted into pots filled with 0.7 L of commercial compost (Klasmann® Substrat 4, Klasmann France SARL) to which 6.7 g of slow release fertilizer (Osmocote® 10-11-18 N-P-K, The Scotts company LLC) were added. Moreover, once a week, all plants were watered with nutritive solution (Hydrokani C2®, Hydro Agri Spécialités) at a 1:1,000 dilution rate, starting whenever needed, which occurred one, three and four weeks before inoculation for experiments 1, 2 and 3 respectively. During plant growth, natural light was supplemented as needed with 400-W sodium vapor lamps between 6:00 a.m. and 9:00 p.m. Greenhouse temperature was maintained between 15 and 20 °C.

The plant material was standardized as much as possible, selecting homogeneous individuals for inoculation. Plants with necrotic symptoms at the stem basis (caused by *Fusarium* spp.), with apparent physiological disorders, or with extreme heights or growth stages were discarded.

Fungal material: three isolates, labelled P3, P4 and P5, with different virulence combinations (Table 1), i.e. representing three different pathotypes, were selected; given their frequency in the natural population over the period 2000-2008, respectively low, high and intermediate (Table 1), they were postulated to represent different aggressiveness levels. They had compatible interactions with all cultivars and lines used, except for isolate P4, which was

Table 1. Virulence combinations and frequency over time in France of *Puccinia triticina* isolates used in experiments 1, 2 and 3.

Isolates			Frequency per year[Z]									
Code (isolate)[W]	Virulent on genes[X]	Exp[Y]	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
166336 (P3)	*Lr1, Lr3, Lr3bg, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr27+Lr31, Lr37*	1, 2, 3	0	5.0	7.7	0.9	0	0	0	0	0.4	1.3
106314 (P4)	*Lr1, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr37*	2, 3	0	0	0	0.9	7.3	12.5	28.7	26.3	44.8	14.5
126-136 (P5)	*Lr1, Lr2c, Lr3, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr23, Lr26, Lr27+Lr31, Lr37*	3	0	0	0	0.4	2.1	8.2	8.3	13.0	3.4	4.8

[W] : Six-digit code of pathotypes based on an 18-*Lr* gene differential set (Goyeau *et al*., 2006) and name of isolates used.
[X] : Wheat leaf rust resistance genes for which the isolates are virulent at the seedling stage (Goyeau *et al*., 2006).
[Y] : Experiments where isolate was used (experiments conducted in 2007, 2008 and 2009 were named experiment 1, 2 and 3, respectively).
[Z]: Frequency of isolates, as a percentage of the total number of isolates analysed over the period 2000-2008, sampled over 50 locations in France.

avirulent on cultivar Balance. The isolates were derived from the collection of single-lesion spores kept at –80°C. Adult plants were inoculated with freshly produced spores. Spores of each isolate were increased for a single multiplication cycle on three pots of seedlings of the susceptible wheat cultivar Michigan Amber. Maleic hydrazide solution (0.25 g/L) was added into pots to prevent the emergence of secondary leaves and to increase spore production. Seven day-old seedlings were inoculated by spraying a suspension of spores in Soltrol® oil (Phillips Petroleum). Before the onset of sporulation, the pots were wrapped with cellophane bags to prevent cross-contamination between different isolates. Spores were collected 11 to 13 days after inoculation, stored for 1 to 5 days in a cabinet (9°C, 35% relative humidity), and eventually used to inoculate adult plants.

Inoculation of adult plants: Plants were cleared the day before inoculation, to leave only the main stem in experiment 1, three to four stems in experiment 2, and four stems in experiment 3. Growth stages ranged between heading and flowering. At inoculation, plants of different growth stages were evenly distributed among isolates. In experiment 1, all plants were inoculated 124 days after sowing. In experiment 2, plants were arranged in three sets, inoculated at a 7-day interval: Andalou and Écrin inoculated 121 days after sowing; Apache, Morocco, Soissons and Trémie, inoculated 121 days after sowing; and LD7 and PBI, inoculated 119 days after sowing. In experiment 3, plants were again arranged in three sets: Écrin, LD7, Morocco and Trémie, inoculated 128 days after sowing; Andalou, Apache, PBI, and Soissons, inoculated 127 days after sowing; and Balance, inoculated 146 days after sowing. In experiment 1, only the flag leaf of the main stem was inoculated. In experiment 2, two flag leaves per plant were inoculated, that of the main stem and that of the most developed secondary stem. In experiment 3, three flag leaves per plant were inoculated, that of the main stem and those of the two most developed secondary stems. Inoculation was

Table 2. Level of field quantitative resistance, estimated as RAUDPC, specific leaf rust resistance genes, and year of registration of winter wheat cultivars and lines used in experiments 1, 2 and 3.

Cultivar	RAUDPC [W]	R-genes [X]	Registration date	Experiment [Y]
Morocco	100	-	-	1, 2, 3
Ecrin	96	*Lr13, (Lr14a)*	1985	1, 2, 3
Soisson	61	*Lr14a*	1988	1, 2, 3
Trémie	53	*Lr10, Lr13*	1992	1, 2, 3
Apache	51	*Lr13, Lr37*	1998	1, 2, 3
Andalou	45	*Lr13*	2002	1, 2, 3
PBI-04-006 (PBI)	36	*Lr13, Lr14a*	Not registered	2, 3
Balance	25	*Lr10, Lr13, Lr20, Lr37*	2001	3
LD 00170-3 (LD7)	17	*Lr13, Lr37*	Not registered	2, 3

[W] : RAUDPC = Area under disease progress curve, in percentage of the AUDPC of a susceptible check measured in field experiments inoculated artificially with isolate P3.
[X] : Postulated seedling leaf rust resistance genes (Goyeau *et al*., 2011). Genes indicated in parentheses are likely to be present, but not confirmed. -: No specific resistance gene.
[Y] : Experiments in which the cultivar was used. Experiments conducted in 2007, 2008, and 2009 were named experiment 1, 2, and 3, respectively).

performed by applying a mixture of rust spores and *Lycopodium* spores on the leaf surface with a soft brush. The proportion [rust spores : *Lycopodium* spores] of the mixture was 1:80 for the flag leaves of the main stem, and 1:160 for the flag leaves of secondary stems in experiments 2 and 3. The leaf surface was inoculated along 10 cm, starting at 3 to 5 cm from the stem. The non-inoculated leaf surface was protected with a stencil. Immediately after inoculation, plants were placed in a dew chamber (15°C) for 24 h and then returned to the greenhouse until the end of the experiment, with temperature set beetwen 12°C and 18°C. All plants were placed in the same greenhouse compartment which was used for the three experiments.

Measurement of resistance components: Infection efficiency (IE) was defined as the ratio of the number of sporulating lesions to the number of deposited spores. IE was measured in experiments 2 and 3 only, using the inoculated flag leaf of secondary stems. Immediately after inoculation, the half distal portion of the inoculated zone was cut off, saved apart at –20°C, and used later to count the number of deposited spores. Spores were counted in a randomly delimitated area of 0.7 cm^2 in the inoculated zone, with a stereo binocular magnifying glass (40X). The sporulating lesions were counted on the proximal half portion of the inoculated flag leaves that stayed attached to the plants. Lesions were counted in a randomly delimited area of 1 cm^2, at the end of the latent period, *i. e.*, when the number of lesions counted each day was stabilized. For each cultivar x isolate interaction, the infection efficiency was estimated by the mean value of 15 replicates in experiment 2, and 30 replicates in experiment 3.

The latent period (LP) was measured on the flag leaf of the main stem. Sporulating lesions were counted daily, until their number stabilized, on a randomly delimited area of 1 cm^2. Latent period was determined as the time when half of the maximum number of

sporulating lesions had appeared. This time was estimated by linear interpolation around the 50% count (Knott & Mundt, 1991). Since latent period is highly dependent on temperature, LP was expressed in degree-days. For each cultivar x isolate interaction, the latent period was estimated by the mean value of 15 replicates. The last count of sporulating lesions performed for latent period was used to estimate lesion density.

Once the number of lesions had stabilized, each leaf was placed into a cellophane bag and maintained horizontally with a plastic frame. After five days of spore production, the leaf was gently brushed so that the spores fell into the cellophane bags. Spores were transferred into aluminium paper containers, desiccated for 7 to 15 days in a cabinet (9°C, 35% relative humidity), and then weighed. Digital pictures of the leaves were taken with a scanner (400 ppi). The area of the inoculated surface and the sporulating surface were calculated by image analysis (Optimas 5, Media Cybernetics).

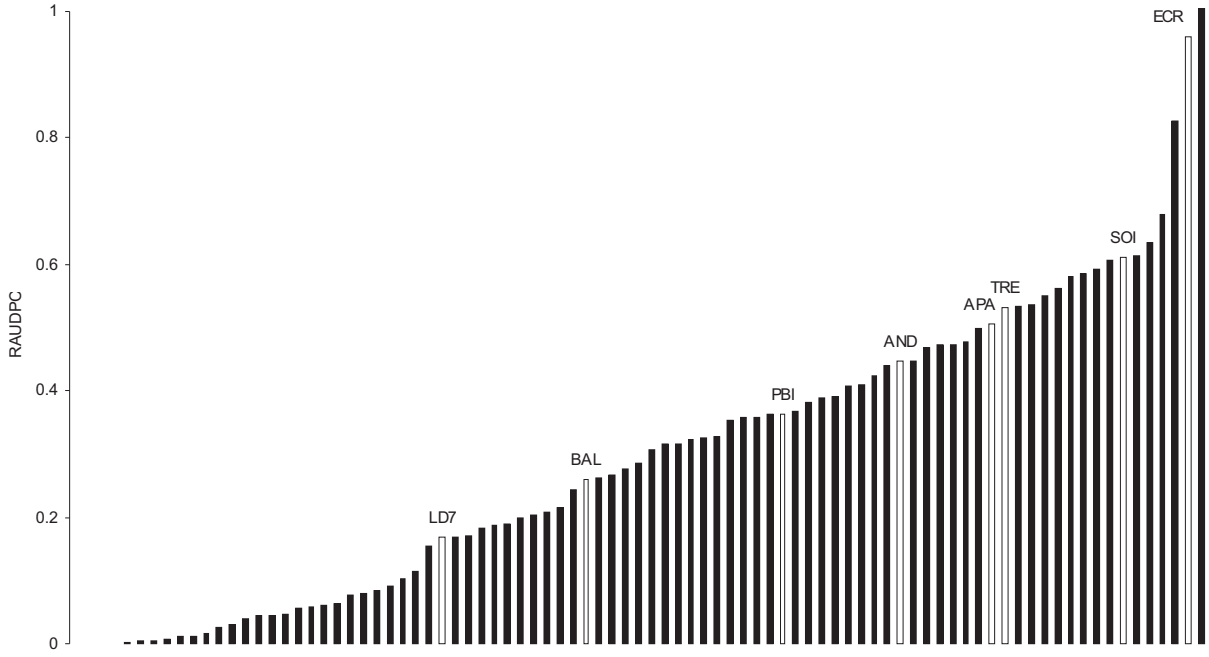
The total number of lesions in the inoculated zone was estimated by the lesion density, determined in a leaf portion of 2 cm^2, multiplied by the area of the inoculated surface. Lesion size (LS) was calculated as the sporulating surface divided by the total number of lesions. Spore production per lesion (SPL) was calculated as the amount of spores produced in five days divided by the total number of lesions. Spore production per unit of sporulating tissue (SPS) was calculated as the amount of spores produced in five days divided by the sporulating surface. LS, SPL and SPS were estimated, for each cultivar x isolate interaction, by the mean value of 15 replicates.

Statistical analyses: All statistical analyses, performed with Splus software (Lucent Technologies, Inc.), were based on linear models. The following factors, named hereafter experimental factors, were defined to take into account the variability of host and environment. Plant growth stage at inoculation was recorded according to three categories

("early heading", "late heading/early flowering", and "late flowering"). As an indication of plant nitrogen content, flag leaf color was visually recorded as "normal", "light green" or "light-green striped". The occurrence of *Fusarium spp.* at the stem basis, and of *Blumeria graminis* on leaves was also recorded.

Each variable (IE, LP, LS, SPL, SPS) was analysed as a function of the cultivar, isolate and experimental factors. For IE in experiment 3, where two secondary stems were used, a stem factor was included in the analysis. Interaction beetwen these factors was tested whenever possible. The experimental factors (plant growth stage, plant nitrogen content, occurrence of *Fusarium* spp. or *B. graminis*) and the stem factor in the analysis of IE, were never found to be significant ($P>0.01$), and were removed from the models. Lesion density was used as an additional co-variable in the ANOVA models for the analyses of LP, LS, SPL, and SPS, because lesion density can affect these components (Robert *et al*., 2004). Lesion density was found to have a significant effect on all the components ($P<0.01$). To take into account the effect of lesion density in the analyses, each component was thus estimated at a constant lesion density of 30 lesions per cm^2 of leaf, based on the ANOVA model (see Lannou & Soubeyrand, 2012). The resistance components estimated at a constant lesion density were then analysed as a function of the cultivar and isolate factors, and their interaction. Analyses were performed separately for each experiment. The differences between experiments were tested separately for isolates P3 and P4, because the overall experimental design was not balanced, due to the inclusion of new isolates and cultivars in experiments 2 and 3. The resistance components estimated at a constant lesion density were analysed as a function of the experiment, the cultivar and their interaction. In all the ANOVAs, cultivar, isolate and experimental factors were considered as fixed factors. Effects were evaluated with type III sum of squares and a significance level set at $P=0.05$. Multiple comparisons of means were performed with Tukey-Kramer range test with significance level set at $P=0.05$.

Figure 1. Disease severity in field epidemics for French wheat cultivars and lines confronted to leaf rust isolate P3. Mean values of Relative Area Under the Disease Progress Curve (RAUDPC, relative to the susceptible check) for 86 wheat genotypes. Abbreviated names of the cultivars selected for greenhouse experiments AND = Andalou, APA = Apache, BAL = Balance, ECR = Ecrin, LD7 = LD 00170-3, PBI = PBI-04-006, SOI = Soissons, TRE = Tremie).



RESULTS

From the 86 host genotypes evaluated under field epidemic with isolate P3, and displaying a continuous range of quantitative resistance level (Fig. 1), a set of six cultivars and two lines was selected, with RAUDPC values going from 0.17 to 0.96 (Fig. 1, Table 2).

Experiment 3, which included the complete set of six cultivars plus Morocco, two lines and three isolates, was the most informative experiment of the three greenhouse experiments performed; thus we present the results of this experiment first, followed by a comparison with experiments 1 and 2. Finally, the resistance profiles of the cultivars and lines were compared, taking into account the three experiments.

Resistance components estimated at a constant lesion density were analyzed as function of the main factors cultivar and isolate, and their interaction. This interaction being always significant ($P<0.0001$), the means were compared for each cultivar-by-isolate combination. For each component, i) the cultivars were ranked for each isolate, to determine if the range of cultivars studied was diversified for resistance components (Table 3), and ii) the isolates were ranked for each cultivar, to analyze the specificity of the resistance components with regard to the isolates (Fig. 2).

Resistance components

The cultivars were ranked for each of the 15 components x isolates combinations (Table 3). For each combination, two sub-groups of cultivars displaying significantly different values ($P<0.05$) for a given component were distinguished. The group of cultivars with the

Table 3. Ranking of cultivars for each component of resistance, with each isolate. Results of Tukey-Kramer tests for experiment 3.

	Isolate P3							Isolate P4							Isolate P5						
Component[W]	cv[X]	mean[Y]	Tukey[Z]					cv	mean	Tukey[Z]					cv	mean	Tukey[Z]				
IE	ld7	0.143	A					ld7	0.101	A					pbi	0.281	A				
	tre	0.230	A	B				tre	0.172	A	B				tre	0.362	A	B			
	pbi	0.446		B	C			ecr	0.179	A	B				ld7	0.438	A	B			
	ecr	0.456		B	C			pbi	0.299	A	B	C			ecr	0.498	A	B	C		
	and	0.597			C			mor	0.397		B	C			mor	0.543		B	C		
	bal	0.604			C			and	0.472			C	D		and	0.593		B	C	D	
	mor	0.614			C	D		soi	0.685				D		apa	0.680			C	D	E
	apa	0.616			C	D		apa	0.696				D		soi	0.779				D	E
	soi	0.849				D		bal	-						bal	0.830					E
LP	mor	156.4	A					soi	153.9	A					mor	157.0	A				
	soi	157.6	A	B				mor	155.7	A					bal	167.7	A	B			
	and	166.0	A	B	C			and	164.7	A	B				soi	170.1	A	B	C		
	pbi	167.9	A	B	C	D		tre	170.7	A	B				ecr	170.7	A	B	C		
	ecr	176.2		B	C	D	E	pbi	175.6		B				tre	179.7		B	C	D	
	tre	181.8			C	D	E	apa	177.3		B				and	182.1		B	C	D	
	bal	184.0				D	E	ecr	177.9		B				pbi	187.1			C	D	
	apa	189.7					E	ld7	210.9			C			ld7	193.1				D	
	ld7	214.0					F	bal	-						apa	193.2				D	
SPL	ecr	5.12	A					ld7	5.13	A					ecr	7.72	A				
	ld7	5.60	A					apa	6.18	A	B				apa	8.25	A				
	apa	6.59	A					and	6.92	A	B				soi	8.96	A				
	and	7.88	A	B				ecr	8.83	A	B	C			and	9.06	A				
	tre	8.31	A	B				soi	9.15	A	B	C			tre	9.80	A				
	soi	8.94	A	B				mor	9.46		B	C			ld7	10.54	A	B			
	bal	10.49		B				tre	11.10			C			mor	14.13		B	C		
	mor	10.99		B				pbi	17.73				D		pbi	14.81			C		
	pbi	15.83			C			bal	-						bal	20.85				D	
LS	bal	0.039	A					ecr	0.050	A					ecr	0.052	A				
	ecr	0.065	A					apa	0.122		B				bal	0.075	A				
	apa	0.118		B				ld7	0.138		B	C			apa	0.129		B			
	ld7	0.138		B	C			tre	0.152		B	C			pbi	0.163		B	C		
	pbi	0.140		B	C			and	0.161		B	C			tre	0.173			C	D	
	tre	0.166			C	D		mor	0.168			C			and	0.181			C	D	
	mor	0.180				D		pbi	0.171			C			soi	0.183			C	D	
	and	0.182				D		soi	0.211				D		mor	0.206				D	
	soi	0.200				D		bal	-						ld7	0.210				D	
SPS	tre	78.49	A					ld7	89.02	A					tre	90.73	A				
	ld7	79.53	A					and	103.99	A					and	95.15	A	B			
	and	88.65	A	B				soi	107.30	A					soi	99.57	A	B			
	soi	98.21	A	B				pbi	119.09	A					ld7	107.26	A	B	C		
	apa	114.47	A	B	C			apa	119.60	A					pbi	130.09	A	B	C	D	
	mor	118.04	A	B	C			tre	121.51	A					apa	138.24		B	C	D	
	pbi	128.99		B	C			mor	131.03	A					mor	142.88			C	D	
	ecr	152.77			C	D		ecr	292.26		B				bal	168.19				D	
	bal	186.23				D		bal	-						ecr	294.17					E

W : Component of quantitative resistance : IE = infection efficiency (number of lesions divided by number of spores deposited on leaf); LP = latent period (degree-days); SPL = spore production per lesion (mass of spores produced per lesion, in µg); LS = lesion size (square millimeters); SPS = spore production per unit of sporulating tissue (mass of spores per square millimeter of sporulating tissue, in µg/mm).

X : Cultivar names abbreviated (and = Andalou, apa = Apache, bal = Balance, ecr = Ecrin, ld7 = LD 00170-3, mor = Morocco, pbi = PBI-04-006, soi = Soissons, tre = Tremie).

Y : Mean values estimated at constant lesion density for each component of quantitative resistance, for every cultivar-by-isolate combination. Isolate P4 was avirulent to cultivar Balance, therefore this combination was not tested.

Z : Comparisons of means between cultivars within each isolate, for each component. Different letters indicate that cultivars are significantly different ($P < 0.05$, Tukey-Kramer tests).

highest values for IE, LS, SPL and SPS (lowest for LP) was labelled as susceptible, as compared to the group of cultivars with the lowest values (highest for LP), which was labelled as resistant.

For IE, Apache and Soissons were susceptible to the three isolates, while Morocco, Andalou and Balance were susceptible only to P3, P4 and P5, respectively. LD7 and Trémie were resistant to the three isolates, while Écrin and PBI were resistant to P4 and P5, respectively. For LP, Morocco and Soissons were susceptible to the three isolates, Andalou was susceptible to P3 and P4, and Balance was susceptible to P5. PBI, Trémie and Écrin were susceptible to P3, P4, and P5 respectively. LD7 was resistant to the three isolates, displaying a higher level of resistance to P3 and P4 than all others cultivars. Apache was resistant to P3 and P5. For SPL, PBI and Morocco were susceptible to the three isolates and Balance was susceptible to P3 and P5. Trémie was susceptible to P4. No cultivar was resistant to the three isolates, but LD7 was resistant to P3 and P4, and Apache and Écrin were resistant to P3 and P5. Additionally, Soissons, Andalou and Trémie were resistant to P5. For LS, Soissons and Morocco were susceptible to the three isolates, while Andalou and Trémie were susceptible to P3 and P5. PBI and LD7 were susceptible to P4 and P5, respectively. Balance, Écrin and Apache were resistant to the three isolates. Balance and Écrin displayed a particularly high level of resistance, being significantly different from all others cultivars. For SPS, Écrin was susceptible to the three isolates, while Balance was susceptible to P3 and P5. PBI and Morocco were susceptible to P3 and P5 respectively. No cultivar was found resistant to P4. Trémie was resistant to P3 and P5, LD7 was resistant to P3, and Andalou and Soissons were resistant to P5.

Several authors used lesion density as an estimation of IE (Knott & Mundt, 1991, Pariaud *et al*., 2009b). In order to compare the results obtained using lesion density, to those obtained using precise measures of IE carried out in this study, a statistical analysis of lesion

Table 4. Ranking of cultivars for lesion density, with each isolate, in experiment 3.

Isolate P3			Isolate P4			Isolate P5		
X cv	Y mean	Z Tukey	X cv	Y mean	Z Tukey	X cv	Y mean	Z Tukey
ld7	11.98	A	ld7	12.61	A	ld7	22.09	A
mor	28.07	A B	ecr	14.30	A	mor	27.91	A B
pbi	29.18	A B	pbi	18.68	A	bal	28.12	A B
bal	33.19	B	apa	19.13	A	pbi	37.07	A B C
and	34.03	B	mor	19.58	A	tre	39.74	B C
tre	36.61	B	soi	21.74	A	ecr	45.06	B C D
ecr	37.47	B	and	24.29	A	apa	49.61	C D E
soi	40.67	B	tre	24.76	A	soi	60.53	D E
apa	43.72	B	bal	-		and	63.15	E

[X] : Cultivar names abbreviated (and = Andalou, apa = Apache, bal = Balance, ecr = Ecrin, ld7 = LD 00170-3, mor = Morocco, pbi = PBI-04-006, soi = Soissons, tre = Tremie).
[Y] : Mean values for every cultivar-by-isolate combination. Isolate P4 was avirulent to cultivar Balance, therefore this combination was not tested.
[Z] : Comparisons of means between cultivars within each isolate. Different letters indicate that cultivars were significantly different ($P < 0.05$, Tukey-Kramer tests).

density was performed: lesion density was analysed as a variable, function of the cultivar and isolate, and their interaction. The cultivar-by-isolate interaction was significant ($P<0.01$). Comparisons of means yielded two and five overlapping groups of equal significance level with isolates P3 and P5, respectively (Table 4). There were no differences in lesion density between cultivars with isolate P4. The ranking of cultivars, within isolate, was different when using lesion density or IE (Tables 3 and 4). This is particularly clear for cultivars Morocco and Trémie with isolate P3, Apache and Trémie with isolate P4, and Balance and PBI with isolate P5: for all these combinations, no significant differences between cultivars were found for lesion density, whereas significant differences were found for IE.

Cultivar-by-isolate interactions for each resistance component

For each cultivar, significant differences ($P<0.05$) among isolates were found, involving all resistance components. A total of 26 significant differences were found among isolates ($P<0.05$) : six for IE (Fig. 2a), seven for LP (Fig. 2b), five for SPL (Fig. 2c), four for LS (Fig. 2d), and four for SPS (Fig. 2e).

Differences between isolates were found for all cultivars, except Apache, for the following components: IE for Balance, Écrin, LD7 and Morocco; LP for Andalou, Balance, LD7, PBI and Soissons; SPL for Balance, LD7 and Morocco; LS for Balance, LD7 and Morocco; SPS for Écrin, Morocco and Trémie.

Comparison of experiments

The components of quantitative resistance were analysed as a function of the experiment, the cultivar and their interaction. Analyses were performed separately for isolates P3 and P4.

Figure 2. Cultivar-by-isolate differential interactions for the quantitative component of resistance: infection efficiency (Fig. 2A), latent period (Fig. 2B), spore production per lesion (Fig. 2C), lesion size (Fig. 2D), and spore production per unit of sporulating tissue (Fig. 2E). For each cultivar, the mean ± confidence interval (P = 0.05) is figured in yellow, red and green for isolates P3, P4 and P5, respectively, and the letters indicate the significant differences between isolates (P < 0.05, Tukey-Kramer tests).
Values are expressed as autoscaled mean values, estimated at constant lesion density for each cultivar-by-isolate combination. Autoscaled mean was calculated by subtracting the overall mean (within each component, for each isolate) from each data entity, and dividing with the overall standard deviation. Autoscaling procedure results in a zero overall component mean and an unit of standard deviation, allowing comparison of the magnitude of variation between differents components.
The cultivar names are abbreviated as AND = Andalou, APA = Apache, BAL = Balance, ECR = Ecrin, LD7 = LD 00170-3, MOR = Morocco, PBI = PBI-04-006, SOI = Soissons, and TRE = Tremie.

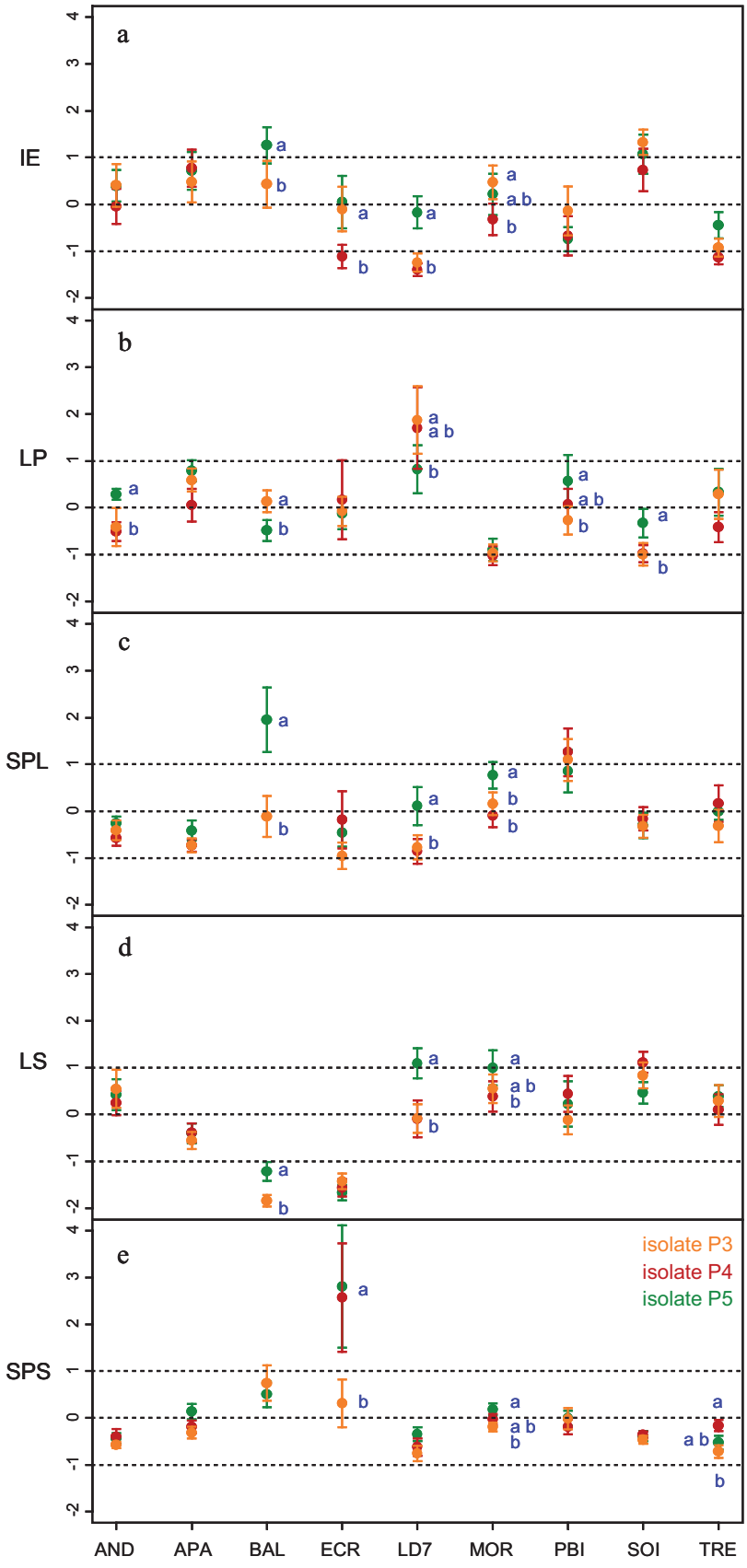

a
b
c
d
e
IE
LP
SPL
LS
SPS
isolate P3
isolate P4
isolate P5
AND
APA
BAL
ECR
LD7
MOR
PBI
SOI
TRE

Table 5. Comparison of experiments 1, 2, and 3, for isolates P3 and P4: mean values for each component of resistance and for each cultivar.

		Isolate P3			Isolate P4	
Component [W]	Cv [X]	exp 1 [Y]	exp 2	exp 3	exp 2	exp 3
IE	and	-	-0.156	0.250	0.055	-0.081
	apa	-	-0.245	0.386	-0.659	0.670
	ecr	-	1.092	-0.044	0.969 a	-1.023 b
	ld7	-	-0.246 a	-1.087 b	-0.368 a	-1.212 b
	mor	-	-0.684	0.564	-0.393	-0.411
	pbi	-	-0.027	-0.185	0.772	-0.692
	soi	-	0.742	1.156	-0.358	0.686
	tre	-	-0.946	-0.826	0.798 a	-1.000 b
LP	and	0.001	0.252	-0.245	-0.057	-0.202
	apa	1.290	0.334	0.890	0.519	0.322
	ecr	-1.152 a	-0.241 ab	0.303 b	-0.421	0.359
	ld7	-	0.812	1.910	1.086	1.775
	mor	-0.656	-0.806	-0.555	-0.180	-0.592
	pbi	-	0.441	-0.058	1.159	0.258
	soi	-0.717	-0.603	-0.552	-0.407	-0.668
	tre	0.731	-0.198	0.544	0.762	0.058
SPL	and	0.134	0.440	-0.257	0.216	-0.416
	apa	-1.119	-0.220	-0.493	-0.074	-0.579
	ecr	-0.072	-	-0.782	-	-0.027
	ld7	-	-0.672	-0.673	-0.754	-0.768
	mor	0.508	0.420	0.396	1.130	0.096
	pbi	-	-0.097 a	1.365 b	-0.036 a	1.466 b
	soi	-	0.381	-0.050	1.521	0.032
	tre	0.405	-0.366	-0.143	-0.006	0.422
LS	and	-0.027	1.427	0.721	0.611	0.290
	apa	-0.673	-0.021	-0.459	-0.099	-0.368
	ecr	-0.398	-	-1.383	-	-1.618
	ld7	-	-0.430	-0.100	-0.997 a	-0.103 b
	mor	0.295	0.033	0.618	0.914	0.417
	pbi	-	0.213	-0.066	-0.073	0.468
	soi	-	-0.111	1.004	1.055	1.174
	tre	-0.198	-0.599	0.386	-0.194	0.142
SPS	and	0.001	-1.466	-0.634	-0.464	-0.366
	apa	-0.366	-0.583	-0.192	0.073	-0.094
	ecr	-0.130	-	0.467	-	2.867
	ld7	-	-0.700	-0.782	0.439	-0.619
	mor	-0.023	0.171	-0.126	0.081	0.100
	pbi	-	-0.665	0.062	0.239	-0.133
	soi	-	1.140 a	-0.461 b	0.835 a	-0.308 b
	tre	0.850	0.126	-0.807	0.556	-0.065

[W] : Component of quantitative resistance : IE = infection efficiency (number of lesions divided by number of spores deposited on leaf); LP = latent period (degree-days); SPL = spore production per lesion (mass of spores produced per lesion, in µg); LS = lesion size (square millimeters); SPS = spore production per unit of sporulating tissue (mass of spores per square millimeter of sporulating tissue, in µg/mm).

[X] : Cultivar names abbreviated (and = Andalou, apa = Apache, bal = Balance, ecr = Ecrin, ld7 = LD 00170-3, mor = Morocco, pbi = PBI-04-006, soi = Soissons, tre = Tremie).

[Y] : Autoscaled mean values estimated at constant lesion density for each cultivar-by-isolate combination in each experiment (exp 1, 2 and 3) for isolates P3 and P4. Isolate P4 was not used in experiment 1. Autoscaled mean was calculated by subtracting the overall mean (within each component, for each isolate) from each data entity, and dividing with the overall standard deviation. Different letters indicate that cultivars, within each isolate, were significantly different ($P < 0.05$, Tukey-Kramer tests). -: cultivar-by-isolate combination not available.

Isolate P5 and cultivar Balance (used only in experiment 3) were not included in this analysis. The experiment-by-cultivar interaction was significant for all components, with both isolates ($P \leq 0.0001$, except for LS and SPS with P4 where $P < 0.01$), indicating that there were significant differences between experiments for some cultivars (Table 5). Significant differences occurred for only 10 out of the 87 comparisons performed (5 components x 9 cultivars x 2 isolates, with 3 comparisons not available). Four of these ten significant differences occurred for IE between experiments 2 and 3, involving LD7 with both isolates, and Écrin and Trémie with isolate P4 only. The remaining six significant differences involved Écrin with P3 for LP; PBI with P3 and P4 for SPL; LD7 with P4 for LS; Soissons with P3 and P4 for SPS.

Resistance profiles of the cultivars

Different resistance profiles were defined for the cultivars (Table 6), based on their ranking for each component (Table 3), and on the differential interactions between cultivars and isolates (Fig. 2). Cultivar Andalou was moderately to highly resistant for SPL and SPS, and showed isolate specificity only for LP. Cultivar Apache was highly resistant for LP, SPL and LS, but was susceptible for IE. The high level of resistance for SPL was explained by a low LS. Apache did not show isolate specificity for any of the components. Cultivar Balance was highly resistant for LS only. Balance showed isolate specificity for all the resistance components, except for SPS, being more susceptible to isolate P5 than to P3. Cultivar Écrin was moderately to highly resistant for IE, SPL, and LS, but highly susceptible for SPS. The high level of resistance for SPL was probably due to a low LS. Écrin showed isolate specificity for IE and SPS. Cultivar LD7 was highly resistant for all the components, except for SPL, LS, and SPS when tested with isolate P5. LD7 showed isolate specificity for all the

Table 6. Resistance profiles for cultivars based on the ranking for each component of quantitative resistance (Table3), and differential interactions between cultivars and isolates (Fig. 2). The level of resistance is categorized as follow: +++ for resistant combinations, + for susceptible combinations, and ++ for combinations displaying an intermediate level of resistance according to the statistical analysis presented in Table 3. For each cultivar by component combination, different letters indicate that isolates were significantly different ($P < 0.05$, Tukey-Kramer tests).

Cv [X]	Isolate	Component [W]				
		IE	LP	SPL	LS	SPS
and	P3	++	+ a	++	+	++
	P4	+	+ a	++	++	++
	P5	++	++ b	+++	+	+++
apa	P3	+	+++	+++	+++	++
	P4	+	++	++	+++	++
	P5	+	+++	+++	+++	++
bal	P3	++ a	++ a	+ a	+++ a	+
	P5	+ b	+ b	+ b	+++ b	+
ecr	P3	++ a	++	+++	+++	+ a
	P4	+++ b	++	++	+++	+ b
	P5	++ a	+	+++	+++	+ b
ld7	P3	+++ a	+++ a	+++ a	++ a	+++
	P4	+++ a	+++ ab	+++ a	++ a	++
	P5	+++ b	+++ b	++ b	+ b	++
mor	P3	+ a	+	+ a	+ ab	++ a
	P4	++ b	+	+ a	+ a	++ ab
	P5	++ ab	+	+ b	+ b	+ b
pbi	P3	++	+ a	+	++	+
	P4	++	++ ab	+	+	++
	P5	+++	++ b	+	++	++
soi	P3	+	+ a	++	+	++
	P4	+	+ a	++	+	++
	P5	+	+ b	+++	+	+++
tre	P3	+++	++	++	+	+++ a
	P4	+++	+	+	++	++ b
	P5	+++	++	+++	+	+++ ab

[W] : Component of quantitative resistance : IE = infection efficiency (number of lesions divided by number of spores deposited on leaf); LP = latent period (degree-days); SPL = spore production per lesion (mass of spores produced per lesion, in µg); LS = lesion size (square millimeters); SPS = spore production per unit of sporulating tissue (mass of spores per square millimeter of sporulating tissue, in µg/mm).

[X] : Cultivar names abbreviated (and = Andalou, apa = Apache, bal = Balance, ecr = Ecrin, ld7 = LD 00170-3, mor = Morocco, pbi = PBI-04-006, soi = Soissons, tre = Tremie).

components, except for SPS, being more susceptible to isolate P5. Morocco was always among the most susceptible cultivars, except for IE with isolates P4 and P5, and for SPS with isolate P3 and P4. Morocco showed isolate specificity for all the components except LP. It was more susceptible to isolate P4 for IE, and more susceptible to isolate P5 for SPL, LS and SPS. Cultivar PBI was moderately to highly resistant for IE, but highly susceptible for SPL. PBI showed isolate specificity for LP only. Cultivar Soissons was moderately to highly resistant for SPL and SPS, but susceptible for IE, LP and LS. Soissons showed isolate specificity for LP only. Cultivar Trémie was highly resistant for IE with all isolates, and for SPS with isolates P3 and P5. Trémie showed isolate specificity for SPS only.

These profiles, defined after the analysis of experiment 3, changed only marginally when compared to the profiles obtained from the other experiments (data not shown): with isolate P4, Écrin and Trémie were susceptible for IE, and PBI was moderatly resistant for SPL.

DISCUSSION

High variability of resistance to wheat leaf rust was found for all the resistance components measured on the set of cultivars and lines studied. Differential cultivar-by-isolate interactions were detected for all components of quantitative resistance, and all cultivars and lines tested, except Apache. Therefore, both the components of resistance involved, and their isolate specificity, have to be taken together into account, when looking for sources of quantitative resistance.

High variability in the level of quantitative resistance with isolate P3 was found in field conditions for the collection of cultivars and lines tested (Fig.1). This suggests the presence of

a potentially large diversity of resistance factors in these host genotypes. Cultivar Soissons appears here as moderately resistant, with a RAUDPC value of 0.61, whereas this cultivar is known to have been very susceptible when grown at high frequency in the field (Papaïx *et al*., 2011). On the opposite, cultivar Apache, displaying a slightly higher level of resistance than Soissons to isolate P3 (RAUDPC of 0.51), kept a good level of quantitative resistance in the field since its release in 2001. The resistance mechanisms present in Soissons and Apache are thus likely to be different and to oppose different constraints to the adaptation of the pathogen population. These observations largely justify the need for a closer examination of the quantitative resistance present in those cultivars, the resistance components considered, and the specificity of these components with regard to pathogen isolates.

All the components measured in controlled conditions were affected by the quantitative resistance in the set of cultivars and lines tested (Table 6). Infection efficiency (IE) was highly affected in Écrin, LD7, PBI, and Trémie; Latent period (LP) in Apache and LD7; spore production per lesion (SPL) in Andalou, Apache, Écrin, LD7 and Soissons; lesion size (LS) in Apache, Balance and Écrin; spore production per unit of sporulating tissue (SPS) in Andalou, LD7, Soissons and Trémie. With the exception of Morocco and Balance with P5, more than one component was affected by the resistance in all host genotypes. This suggests a diversity of underlying mechanisms of resistance in these hosts.

The originality of the present study is to perform an assessment of components of resistance all together, over all pathogen life cycle, and with a good precision. Indeed, when measurements of latent period, lesion size and lesion density are commonly found in the literature (Singh & Huerta-Espino, 2003, Jorge *et al*., 2005, Richardson *et al*., 2006), IE is discarded, arguing a lack of precision (Broers, 1989a, Pariaud *et al*.; 2009b). IE was an important component of resistance in four of the cultivars tested here (LD7, Trémie, PBI, and Écrin). Precise measurements of IE remain scarce (Denissen, 1993; Lehman & Shaner, 1997).

IE can be indirectly estimated by lesion density, assuming that spore deposition is homogeneous among leaves, an assumption that is usually not checked. Preliminary tests established that the inoculation method used in this study did not allow a homogeneous deposition among leaves. Thus the estimation of IE using lesion density was likely to be incorrect, which is consistent with the fact that the ranking of cultivars was different when using lesion density as compared to IE (Tables 3 and 4). This was especially clear for cultivars Morocco and Trémie with isolate P3, Apache and Trémie with isolate P4, and Balance and PBI with isolate P5.

LS is often the only component measured to determine the level of resistance in the sporulation process (Broers, 1989b; Singh *et al*., 1991; Denissen, 1993). Only a few studies considered all together the three components, LS, SPL and SPS, involved in the sporulation process in leaf rust (Lehman & Shaner, 1997; Pariaud *et al.*, 2009b). In the present study, the components LS, SPL, and SPS were precisely measured, and resistance differentially affected all of them. For example, cultivar Balance displaying low LS could be considered as highly resistant for sporulation when considering only this component. Nevertheless, its SPL and SPS were very high. Thus, the evaluation of the level of resistance for sporulation can be inaccurate if only LS is taken into account.

Correlations between components were calculated, for each combination cultivar/line x isolate, in order to investigate whether positive correlations could reveal some redundancy between the components measured. Correlations never exceeded 0.45 (data not shown). Therefore, the level of resistance for the different components varied, to a great extent, independently. Surprisingly, this occurred even for the three components involved in sporulation. The amount of spores produced by a single lesion (SPL) is determined by the size of the lesion (LS) and the amount of spores produced by unit of sporulating tissue (SPS). Thus, it could be expected these components to be correlated. However, it was not the case

here. High levels of resistance in SPL resulted from different combinations of LS and SPS values. The extreme cases were Écrin, which was resistant for SPL because of a small LS even if SPS was high; and Soissons, which was resistant for SPL because of a low SPS even if LS was high. Phenotypic and genetic correlations between components have been found, particularly between shortened latent period and increased lesion size (Parlevliet, 1986; Das *et al*., 1993; Herrera-Foessel *et al*., 2007; Lehman & Shaner, 2007) and pleiotropy has been argued. However, correlations rarely exceeded values of 0.70. For breeding purposes, the evaluation of the overall level of quantitative resistance can be misleading if only one, or a few, components are taken into account when correlation between components is not high.

In the present study, differential cultivar-by-isolate interactions were detected for all components of quantitative resistance, and for all the cultivars tested, except for Apache. In contrast, Broers (1989b) and Lehman & Shaner (1996) found differential interactions for latent period only, when studying respectively 11 x 5, and 5 x 7, cultivar x isolate combinations. Screening a large collection of CIMMYT germplasm with a good level of quantitative resistance to leaf, stripe and stem rusts, Singh *et al.* (2011) did not even find any race-specificity. This divergence in the occurrence of specific interactions for the same pathosystem is probably due to the genetic diversity of both, resistance in breeding sources, and aggressiveness in the pathogen populations. The present study emphasizes the need to check for specificity of quantitative resistance.

The repeatability of the three experiments was high. Most of the differences between experiments were found for IE (4 out of 10 cases). The variability for IE was high in experiment 2, because the number of repetitions (15) was lower than in experiment 3 (30). Different climatic and physiological sources of variation, reviewed by Pariaud *et al.* (2009a), can modify the expression of disease in greenhouse assessments. All the experiments were performed in the same single greenhouse compartment, where temperature and relative

humidity were homogeneous. Leaf nitrogen content, plant growth stage, age of spores, and presence of other pathogens were controlled and homogenized as much as possible. All these potential sources of variability were included in statistical analyses, and were never found significant.

The level of resistance in cultivars and lines was not evaluated comparatively to a susceptible check. None of the cultivars studied, even Morocco initially included as a susceptible check, happened to be completely susceptible for all components. Since the objective was to measure the variability of resistance in this set of cultivars, the lack of a susceptible check was not a pitfall. There is evidence of some level of resistance in Morocco to leaf rust in field conditions (Denissen, 1993), and it can not be excluded that some level of quantitative resistance against a given isolate exists in this old cultivar.

LP and sporulation components were found dependent on lesion density, decreasing when lesion density increases (data not shown), as reported by Sache (1997) in seedlings and Robert *et al.* (2002, 2004) in adult plants. Accordingly, these components were estimated at a constant lesion density. Comparisons were made between the raw data and components estimated at a constant lesion density: significant differences were found in the ranking of cultivars and in the groups of equal significance level for all the components (data not shown), even for isolates P3 and P4, for which lesion density can be considered homogeneous (Table 4). We concluded, in accordance with Lannou & Soubeyrand (2012) and Pariaud *et al*. (2009a), that whenever lesion density is not taken into account in the analyses, observed differences in the level of these components might be due to a density effect, rather than to genetic differences between cultivars.

Taking into account both the components of resistance involved, and their isolate specificity, conclusions can be drawn about the usefulness of the cultivars and lines tested here as sources of quantitative resistance. Cultivar Balance was highly resistant to isolate P3

in field conditions (Fig. 1), in the greenhouse for LS, and it was moderately resistant to P3 in the greenhouse for IE and LP. Its value as a source of resistance should however be qualified, because of its susceptibility to isolate P5. By contrast, because their high level of resistance for several components was not highly affected by isolate specificity, four of the cultivars and lines tested here appeared interesting for breeding programs. LD7 was one of the most resistant cultivars for all the components. Even if isolate specificity was observed for all the components in this line, it did not significantly decreased the level of resistance with the three isolates. Apache was found highly resistant for LP and all the sporulation components, and it did not show isolate specificity for any of the components. PBI and Trémie were highly resistant for IE, and isolate specificity was detected only in one component, LP and SPS, respectively. Both genotypes showed good level of resistance to isolate P3 in field conditions.

Enhancing durability of quantitative resistance can be achieved by combining, within host genotypes, diversified quantitative resistance loci. Indeed, a lower selection pressure on the pathogen population is expected when the resistance is diversified in the host population (Stuthman *et al*., 2007). To ensure a maximum efficiency, the diversification should be both phenotypic (components of resistance) and genetic. The cultivars investigated in this study displayed diversified phenotypes of quantitative resistance, likely involving different physiogical mechanisms, expected to be governed by different genetic factors. For example, Chung (2010), working with the pathosystem maize-northern leaf blight, detected one QTL that reduced the efficiency of fungal penetration, thus reducing infection efficiency, and another QTL that decrease the speed of hyphal growth in vascular tissues, thus increasing latent period. In order to investigate whether the components of resistance are also genetically diversified, the next step will be to identify the genome regions involved in the expression of resistance on each component by QTL analysis. Most of the numerous quantitative resistance loci identified up to now for different pathosystems result from field assesments of disease

severity scores (St. Clair, 2010), thus based on global phenotypic assessments, without any clue on the diversity of the underlying mechanisms. The assumption of diversification relies only on the diverse genomic location of these resistance loci, which does not implies a diversity of resistance mechanisms. St. Clair (2010) emphasizes the need for approaches and methods to facilitate reliable and precise phenotyping of quantitative traits. As shown in this study, high quality phenotyping can be achieved by measuring components of resistance in controlled conditions. Breeding populations can be characterized both in controlled and field conditions, and identification of QTLs associated to the different components will yield markers useful for the association of diversified quantitative resistance traits in MAS.

Acknowledgments: The present research was financed by the GNIS (Groupement National Interprofessionnel des Semences), project FSOV 2005-2007, and project FSOV 2008-2011. The PhD grant CIFRE of GA was funded by Florimond Desprez, and by the French Ministry of Research and Technology. The invaluable collaboration of breeders from Club5 and CETAC is gratefully acknowledged. We also thank Nicolas Lécutier and Lucette Duveau for the technical help in greenhouse. Seeds of line LD 00170-3 were provided by courtesy of Jean-Michel Delhaye, (Lemaire-Deffontaines) and seeds of linePBI-04-006 were provided by courtesy of Christophe Michelet (RAGT).

Chapitre 2

“Relationship between quantitative resistance components and field resistance”

Relationship between quantitative resistance components and field resistance[1]

INTRODUCTION

Breeding for resistance in cultivated plants has largely relied on the exploitation of the `gene-for-gene' system (Flor, 1971). This `qualitative resistance' confers immunity to the plant, which explains its popularity in breeding programs. It is however easily overcome by the pathogen and, in the recent past, the release of resistant varieties has usually led to rapid adaptation of pathogen populations through accumulation of qualitative pathogenicity factors. Conversely, quantitative resistance is generally controlled by several genes, located in quantitative trait loci (QTL) (Young, 1996) and an increasing number of studies demonstrates that genes implicated in quantitative resistance have diverse structures and functions, and are involved in different resistance mechanisms (St. Clair, 2010). This diversity is believed to be the basis of resistance durability, since it results in both a low selection pressure, and a complex genetic determinism of the resistance that is difficult to overcome by the pathogen (Stuthman *et al*., 2007).

From a phenotypic point of view, quantitative resistance alters the expression of different traits of the host–pathogen interaction, including infection efficiency, latent period, lesion size, and sporulation rate (Parlevliet, 1979; Pariaud *et al*., 2009a). In this paper, the term "resistance component" will refer to the expression of the host resistance with regard to a specific trait of the host–pathogen interaction (e.g. the latent period). A resistance affecting the infection efficiency alters the development of the pathogen before the apparition of a lesion. The latent period measures the rate at which the pathogen develops in plant tissues. Resistance components acting on sporulation alter the lesion size or the amount of spores produced. Evidence from various pathosystems support the idea that the different components of quantitative resistance impact different physiological and molecular mechanisms in the pathogen (Niks & Marcel, 2009; Poland *et al*., 2009).

The resistance components can be accurately determined in controlled conditions, at the scale of a single pathogen life cycle. Both experimental (Shaner *et al*., 1978; Papaïx *et al.*, 2011) and modelling (Papaïx, unpublished Ph.D. thesis) approaches suggest that individual

[1] Projet d'article, co-auteurs: J. Papaix, C. Lannou, H. Goyeau.

components of quantitative resistance result in a decrease of epidemic development in the field conditions. However, it remains unclear how the different resistance components that are usually measured at the scale of a plant relate to the resistance level observed in the field. Correlations between field resistance and resistance components has been found, usually for latent period or lesion size (Broers, 1989a; Denissen, 1993; Herrera-Foessel *et al*., 2007), more sporadically for infection efficiency or spore production (Milus & Line, 1980; Negussie *et al*., 2005). Singh *et al.* (1991), working with 28 wheat cultivars confronted to one leaf rust isolate, found significant linear correlations of AUDPC with latent period, infection frequency and lesion size that explained 97%, 81%, and 81% of the observed variation on AUDPC, respectively. A strong limitation of this study is however that it is based on one isolate only. Since resistance QTLs often have a pleiotropic effect on the pathogen life traits, the correlations observed may actually result from the different pathogen life traits varying in the same direction. The use of a diversified pathogen material would allow a better distinction between the effects of each trait on the global epidemic progression rate. Confronting 15 cultivars with 2 isolates for the same pathosystem, Denissen (1993) found also significant linear correlations between AUDPC or disease severity, on one side, and latent period, infection frequency and lesion size, on the other side, but the correlations explained only 14%, 20%, and 23% of the observed variation, respectively. These examples show that the strength of the correlations can be different among experiments for the same pathosystem, and probably depend on the cultivar x isolate combination used. Moreover, field resistance is usually estimated from a single disease severity scoring, or by the calculation of integrative variable, such as AUDPC. The link between resistance components and the pathogen multiplication rate during the different phases of the epidemic remains to be explored. Moreover, disease severity can be measured as the overall damage, including chlorosis and necrosis, or by considering only the sporulating tissue. Milus and Line (1980) found that resistance reduce the size of the sporulating area without affecting the total size of the lesions in some cultiva-isolate combinations, whereas the opposite happened in others combinations. How the resistance components correlate to both types of disease measures is also to be clarified.

The correlations among resistance components may influence the efficacy of breeding strategies. When components are tightly related, like in the breeding material studied by Singh *et al.* (1991), the selection process can be based on the measurement of only one component, but a low diversity of resistance mechanisms can be expected. On the opposite, components that would be not, or slightly, correlated are more likely to rest on diversified

genetic bases, making pathogen adaptation more difficult, thus enhancing durability of resistance. In that case, the selection process would require the measurement of different components, but the expected durability of the resistance could increase (*e.g.*, Stuthman *et al.*, 2007). Negative correlations among resistance components (trade-offs) would also be valuable because they would impose divergent selective pressure to the pathogen population, rendering pathogen adaptation more difficult (Pariaud *et al.*, 2009a).

The objectives of this paper were 1) to analyse the relationships between the different resistance components measured at the plant scale and the resistance level observed in the field during the course of an epidemic 2) to investigate which of the two field variables, overall diseased tissue or proportion of sporulating tissue, was better correlated to the resistance components and 3) to analyse the correlations among resistance components. A set of cultivars displaying a range of quantitative resistance were confronted to three isolates belonging to different leaf rust pathotypes, both under controlled and field conditions. A statistical model was developed to estimate infection efficiency, latent period, lesion size, sporulation rate per lesion, and sporulation rate per unit of sporulating tissue from measurements on adult plants in the greenhouse. The disease severity (total damage and proportion of sporulating tissue) was measured at three times during the course of field epidemics.

MATERIALS AND METHODS

The analysis presented in this paper is based on a set of three greenhouse and three field experiments. The experimental design was slightly different for each experiment, with the addition of new isolates in greenhouse experiments 2 and 3, and the addition and removal of certain cultivars in the different greenhouse and field experiments (Tables 1 and 2). The reasons for these changes are mainly the improvement in the technical procedures, allowing working with more material, and specific problems encountered with some of the cultivars. Our objective in this study was to perform a global analysis of the whole experimental dataset. For that, we developed specific statistical procedures to account for the structure of the dataset.

Three isolates were chosen as representative of three different leaf rust pathotypes with different virulence combinations. The pathotypes, labelled P3, P4 and P5, were virulent on all the most widely grown cultivars in France and were selected on the basis of the

Table 1. Virulence combinations and frequency over time in France of *Puccinia triticina* isolates used in field and greenhouse experiments.

Isolates			Frequency per year[Z]									
Code (isolate)[W]	Virulent on genes[X]	Gexp[Y]	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
166336 (P3)	*Lr1, Lr3, Lr3bg, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr27+Lr31, Lr37*	G1, G2, G3	0	5.0	7.7	0.9	0	0	0	0	0.4	1.3
106314 (P4)	*Lr1, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr37*	G2, G3	0	0	0	0.9	7.3	12.5	28.7	26.3	44.8	14.5
126-136 (P5)	*Lr1, Lr2c, Lr3, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr23, Lr26, Lr27+Lr31, Lr37*	G3	0	0	0	0.4	2.1	8.2	8.3	13.0	3.4	4.8

[W] : Six-digit code of pathotypes based on an 18-*Lr* gene differential set (Goyeau *et al*., 2006) and name of isolates used.
[X] : Wheat leaf rust resistance genes for which the isolates are virulent at the seedling stage (Goyeau *et al*., 2006).
[Y] : Greenhouse Experiments where isolate was used (experiments conducted in 2007, 2008 and 2009 were named experiment G1, G2 and G3, respectively).
[Z]: Frequency of isolates, as a percentage of the total number of isolates analysed over the period 2000-2008, sampled over 50 locations in France.

Table 2. Leaf Rust resistance genes and registration year of cultivars investigated, and distribution across field and greenhouse experiments.

Cultivar	R-genes [W]	Registration date in France	F experiment [X]	G experiment [Y]
Andalou	*Lr13*	2002	F1, F2, F3	G1, G2, G3
Apache	*Lr13, Lr37*	1998	F1, F2, F3	G1, G2, G3
Balance	*Lr10, Lr13, Lr20, Lr37*	2001	F2, F3	G3
Buster	*Lr13*	Not registered	F3	-
Camp Remy	0 [Z]	1980	F1, F2, F3	G1
Caphorn	*Lr10, Lr13, Lr37*	2001	F1, F2, F3	-
Ciento	*Lr10, Lr13, Lr14a, Lr37*	2007	F1, F2, F3	G1, G2
Ecrin	*Lr13, (Lr14a)*	1985	F1, F2, F3	G1, G2, G3
Frandoc	*Lr13*	1980	F1, F2, F3	-
Instinct	*Lr1, Lr13*	2006	F1, F2, F3	G1
LD 00170-3 (LD7)	*Lr13, Lr37*	Not registered	F1, F2, F3	G2, G3
Morocco	0 [Z]	-	-	G1, G2, G3
PBI-04-006 (PBI)	*Lr13, Lr14a*	Not registered	F1, F2, F3	G2, G3
Sideral	*Lr13, Lr14a*	1991	F2, F3	G1, G2
Soisson	*Lr14a*	1988	F1, F2, F3	G1, G2, G3
Trémie	*Lr10, Lr13*	1992	F1, F2, F3	G1, G2, G3

W : Postulated seedling leaf rust resistance genes (Goyeau *et al*., 2011). Genes indicated in parentheses are likely to be present, but not confirmed. -: No specific resistance gene.

X : Field experiments in which the cultivar was used. Experiments conducted in 2009, 2010, and 2011 were named experiment F1, F2, and F3, respectively).

Y : Greenhouse experiments in which the cultivar was used. Experiments conducted in 2007, 2008, and 2009 were named experiment G1, G2, and G3, respectively).

Z : no *Lr* gene detected

Table 3: Infection types (IT), and associated coefficients used to calculate corrected Disease Severity (cDS).

IT	IT coefficient
S	1.000
S-MS	0.875
MS	0.750
MS-MR	0.500
MR	0.250
MR-R	0.125
R	0.000

contrasted evolution of their frequency in the French populations over the period 2000-2008 (Table 1), respectively low, high and intermediate. The pathotypes were then postulated to represent different aggressiveness levels. They are compared in more details in Appendix 1. For the sake of commodity, the isolates themselves will be referred to as P3, P4 and P5 in the following.

The cultivars were chosen after a preliminary field assessment (described in Azzimonti *et al.*, 2012 / Chapter 1) of the level of quantitative resistance in a set of 86 host lines and cultivars inoculated with isolate P3. Twelve cultivars (Andalou, Apache, Balance, Ciento, Camp Remy, Ecrin, Instinct, Sideral, Soissons and Tremie), and two lines (LD 00170-3, hereafter LD7 and PBI-04-006, hereafter PBI) were selected to represent the range of quantitative resistance observed in the field (Table 2). Highly susceptible cultivars, Buster and Frandoc in the field experiments, and Morocco in the greenhouse experiments, considered as susceptible checks, were also included in the experiments.

Field experiments

Experimental design

Three field experiments were conducted in years 2009, 2010 and 2011, at *Arvalis Institut du végétal* experimental station, located in Boigneville, France. Trials for isolates P3, P4, and P5 were planted separately in a randomized block design with two replications per treatment. Approximately 60 plants were distributed in two consecutive rows of 1.5 m long for each cultivar and line. Rows of spreader cultivar Buster were planted every two entries. Two replicates were planted for each isolate. To initiate the leaf rust epidemics, spreader rows were inoculated, just before heading, using hand-sprayers containing spores of *P. triticina* suspended in Soltrol® oil (Phillips Petroleum). Inoculation was performed on the same day for all replications.

Disease severity scores

Disease severity (DS) was scored according to the modified Cobb Scale where percentage of disease tissue was visually estimated on flag leaves, according to Peterson *et al*. (1948). The qualitative host response to infection, namely infection type, was also evaluated as described in Roelfs *et al*. (1992). Four infection types were used: R (resistant, with no sporulating tissue

in the lesions, or of very small size and surrounded by necrosis and chlorosis), MR (moderately resistant, with a small area of sporulating tissue surrounded by chlorosis or necrosis), MS (moderately susceptible, with a sporulating area of moderate size and no chlorosis or necrosis), and S (susceptible, with large sporulating lesions without chlorosis nor necrosis). Intermediate cases between these four infection types were also identified. A coefficient was attributed to each infection type, from zero for the R infection type to one for the S infection type (Table 3). This coefficient was used to calculate a corrected disease severity cDS, as the product of observed disease severity (DS) by the infection type coefficient. This allowed distinguishing the disease severity accounted for by sporulating tissue only from the overall severity that included all diseased tissues.

Disease severity assessments started when the spreader cultivar reached a severity of 100%, and when the first symptoms began to appear on the flag leaves of the tested cultivars. Three severity assessments were conducted, i.e. every five to seven days until the end of the epidemic. At each time, all replicates were scored.

Greenhouse experiments

Three greenhouse experiments were performed (Table 2). In experiment 1, conducted in 2007, cultivars Andalou, Apache, Ciento, Camp Remy, Ecrin, Instinct, Sideral, Soissons, Tremie, and Morocco were confronted to isolate P3. In experiment 2, conducted in 2008, lines LD7 and PBI, and a second isolate P4, were added to those tested in experiment 1, and cultivars Camp Remy and Instinct were dropped. In experiment 3, conducted in 2009, another cultivar, Balance, and a third isolate, P5, were added to those tested in experiment 2, and cultivars Ciento and Sideral were dropped.

Plant material

All measurements were performed on adult plants with homogeneous growth stages and physiological states. Seeds were sown in Jiffy pots with two seeds per pot. Seedlings at the two-leaf stage were vernalized at 8°C in a growth chamber. To synchronize the development of the different cultivars, sowing times were staggered taking into account earliness in heading time, and the duration of vernalization.

After vernalization, plants were transferred to a greenhouse and left for 7 to 10 days to acclimatize. The most vigorous seedlings were then individually transplanted into pots filled

with 0.7 L of commercial compost (Klasmann® Substrat 4, Klasmann France SARL) to which 6.7 g of slow release fertilizer (Osmocote® 10-11-18 N-P-K, The Scotts company LLC) were added. Moreover, once a week, all plants were watered with nutritive solution (Hydrokani C2®, Hydro Agri Spécialités) at a 1:1.000 dilution rate, starting whenever needed (one, three and four weeks before inoculation for experiments 1, 2 and 3 respectively). During plant growth, natural light was supplemented as needed with 400-W sodium vapor lamps between 6:00 a.m. and 9:00 p.m. The greenhouse temperature was maintained between 15 and 20 °C.

Before inoculation, the plant material was standardized by selecting homogeneous individuals with regards to their growth stage and physiological state. Plants with necrotic symptoms at the stem basis (attributed to *Fusarium* spp.), with apparent physiological disorders, or with extreme heights or growth stages were discarded.

Plant inoculation

The plants were cleared the day before inoculation, to leave only the main stem in experiment 1, three to four stems in experiment 2, and four stems in experiment 3. Growth stages ranged between heading and flowering. At inoculation, plants with different growth stages were evenly distributed among isolates. In experiment 1, all plants were inoculated 124 days after sowing. In experiment 2 and 3, the plants were arranged in three sets and inoculated separately in order to account for the development rates of the different cultivars. The inoculation time was adjusted to the plant stage, between heading and flowering. In experiment 2, Andalou, Ciento, Ecrin, and Sideral were inoculated 121 days after sowing; Apache, Morocco, Soissons and Tremie were inoculated 121 days after sowing; LD7 and PBI were inoculated 119 days after sowing. In experiment 3, Ecrin, LD7, Morocco and Tremie were inoculated 128 days after sowing; Andalou, Apache, PBI, and Soissons were inoculated 127 days after sowing; Balance was inoculated 146 days after sowing. In experiment 1, only the flag leaf of the main stem was inoculated. In experiment 2, two flag leaves per plant were inoculated, on the main stem and the most developed secondary stem. In experiment 3, three flag leaves per plant were inoculated. Inoculation was performed by applying a mixture of freshly produced rust spores and *Lycopodium* spores on the leaf surface with a soft brush. The proportion rust spores : *Lycopodium* spores of the mixture was 1:80 for the flag leaves of the main stems, and 1:160 for the flag leaves of the secondary stems (experiments 2 and 3). The

leaf surface was inoculated along 10 cm, starting at 3 to 5 cm from the stem. The non-inoculated leaf surface was protected with a stencil. Immediately after inoculation, plants were placed in a dew chamber (15°C) for 24 h and then returned to the greenhouse until the end of the experiment, with temperature set between 12°C and 18°C. All plants were placed in the same greenhouse compartment, which was used for the three experiments.

Measurement of component of resistance

In experiments 2 and 3, immediately after inoculation, the half distal portion of the inoculated zone on the leaves of the secondary stems was cut off, saved apart at – 20°C, and used later to count the number (SP) of deposited spores. Spores were counted in a randomly chosen area of 0.7 cm² within the inoculated zone, with a stereo binocular magnifying glass (40X). The number (Les) of sporulating lesions was counted on the part of the inoculated flag leaves that remained attached to the plants. Lesions were counted in a randomly chosen area of 1 cm², at the end of the latent period, *i.e.* when the number of lesions counted each day was stabilized. Infection efficiency (IE) was estimated as Les/Sp (see Section 2.3.1).
The latent period (LP) was measured on the flag leaf of the main stem. The sporulating lesions were counted daily, until their number stabilized, on a randomly chosen area of 1 cm². The latent period was determined as the time when half of the maximum number of sporulating lesions had appeared. This time was estimated by linear interpolation around the 50% count (as in Knott *et al.*, 1991). Since latent period is highly dependent on temperature, LP was expressed in degree-days.

Once the number of lesions had stabilized, each leaf was placed into cellophane bags, and maintained horizontally with a plastic frame. After five days of spore production, the leaf was gently brushed so that the spores fell into the cellophane bags. Spores were transferred into aluminum paper containers, desiccated for 7 to 15 days in a cabinet (9°C, 35% relative humidity), and then weighed. Digital pictures of the leaves were taken with a scanner (400 ppi). The area of the sporulating surface was calculated by image analysis (Optimas 5; Media Cybernetics, Silver Spring, MD, U.S.A.). For each leaf, the area of the inoculated surface was calculated, taking into account the leaf width.

The total number of lesions in the inoculated zone was estimated by multiplying the lesion density, determined in a 2-cm² leaf portion, by the area of the inoculated surface. The lesion size (LS, mm^2) was calculated as the sporulating surface divided by the total number of lesions. The spore production per lesion (SPL, mg) was calculated as the total amount of

spores produced during five days divided by the total number of lesions. The spore production per unit area of sporulating tissue (SPS, mg/mm^2) was calculated as the amount of spores produced within five days divided by the sporulating tissue area.

Statistical analysis

A preliminary analysis using ANOVA models indicated that only the experiment, the cultivar, the isolate, and the cultivar-isolate interaction had significant effects (at a 0.05 threshold) on the measured variables. Other factors assessed during the greenhouse experiment (plant growth stage at inoculation and plant nutritional status) were thus eliminated from the analysis. Since quantitative traits of the host-pathogen interaction can be density-dependant (Lannou & Soubeyrand, 2012), an ANCOVA model, with lesion density as a co-variable, was used to estimate the density-dependent variables (namely LP, SPL, LS, and SPS) at a fixed density of 30 lesions per cm² of leaf (see Lannou & Soubeyrand, 2012).
Then, in a first step, a Generalized Linear Models in a Bayesian framework was developed to provide the estimations of each variable for each cultivar-isolate pair and, in a second step, correlations among resistance components and correlations between resistance components and disease severity in the field were investigated.

Models

The different variables considered were measured for each experiment *e*, cultivar *c*, isolate *i* and replicate *r*. In the following, the suffix "*obs*" indicates observed values. For instance, measured values of latent period are denoted by $LP^{obs}_{e,c,i,r}$.

Model for IE. The infection efficiency was estimated using the counts of deposited spores ($Sp^{obs}_{e,c,i,r}$) and sporulating lesions ($Les^{obs}_{e,c,i,r}$) in experiments 2 and 3 (see Section 2.2.3.). To take into account possible count errors, $Sp^{obs}_{e,c,i,r}$ and $Les^{obs}_{e,c,i,r}$ were first assumed to follow a Poisson distribution with mean $Sp_{e,c,i,r}$ and $Les_{e,c,i,r}$, respectively. Then, $Sp_{e,c,i,r}$ and $Les_{e,c,i,r}$ were assumed to be Binomial distributed, leading to the following model for the deposited spores:

$$\begin{cases} Sp^{obs}_{e,c,i,r} | Sp_{e,c,i,r} \sim \text{Pois}\left(Sp_{e,c,i,r}\right) \\ Sp_{e,c,i,r} | NSp, \pi_{e,c,i} \sim \text{Bin}\left(NSp, \pi_{e,c,i}\right) \end{cases},$$

where NSp and $\pi_{e,c,i}$ are respectively the total number of spores and the probability for a spore to be deposited (see Appendix 1 for detailed information). In the same way, the model for the sporulating lesions was:

$$\begin{cases} Les^{obs}_{e,c,i,r} | Les_{e,c,i,r} \sim \text{Pois}\left(Les_{e,c,i,r}\right) \\ Les_{e,c,i,r} | Sp_{e,c,i,r}, IE^{tot}_{e,c,i} \sim \text{Bin}\left(Sp_{e,c,i,r}, IE^{tot}_{e,c,i}\right) \\ \text{logit}\left(IE^{tot}_{e,c,i}\right) = IE^{expe}_{e} + IE_{c,i} \end{cases},$$

Variable $IE^{tot}_{e,c,i}$, is the infection efficiency of isolate i on cultivar c and for experiment e. Variables IE^{expe}_{e} and $IE_{c,i}$ are the experiment effect and the cultivar-isolate interaction on the logit scale, respectively.

Model for LP. The latent period, $LP_{c,i}$, for each cultivar (c) - isolate (i) pair was estimated by the following normal regression model:

$$LP^{obs}_{e,c,i,r} = LP^{expe}_{e} + LP_{c,i} + \varepsilon^{LP}_{e,c,i,r},$$

where LP^{expe}_{e} is the experiment effect, and $\varepsilon^{LP}_{e,c,i,r}$ is the normally distributed error: $\varepsilon^{LP}_{e,c,i,r} \sim N(0, \sigma^{LP})$.

Model for LS, SPL and SPS. The lesion size, $LS_{c,i}$, the spore production per lesion, $SPL_{c,i}$, and the spore production per sporulating tissue, $SPS_{c,i}$, for each cultivar (c) - isolate (i) pair were estimated by the following normal regression models:

$$LS^{obs}_{e,c,i,r} = LS^{expe}_{e} + LS_{c,i} + \varepsilon^{LS}_{e,c,i,r}, \text{ where } \varepsilon^{LS}_{e,c,i,r} \sim N\left(0, \sigma^{LS}_{c,i}\right),$$

$$SPL^{obs}_{e,c,i,r} = SPL^{expe}_{e} + SPL_{c,i} + \varepsilon^{SPL}_{e,c,i,r}, \text{ where } \varepsilon^{SPL}_{e,c,i,r} \sim N\left(0, \sigma^{SPL}_{c,i}\right),$$

$$SPS^{obs}_{e,c,i,r} = SPS^{expe}_{e} + SPS_{c,i} + \varepsilon^{SPS}_{e,c,i,r}, \text{ where } \varepsilon^{SPS}_{e,c,i,r} \sim N\left(0, \sigma^{SPL}_{c,i}\right).$$

LS_e^{expe}, SPL_e^{expe} and SPS_e^{expe} denote the experiment effects. We used here regression models with standard errors ($\sigma_{c,i}^{LS}$, $\sigma_{c,i}^{SPL}$ and $\sigma_{c,i}^{SPL}$) depending on the cultivar-isolate pair because of the differences in variances observed in previous analyses.

Model for Disease severity. Disease severity scores (DS) were defined as the proportion of the diseased surface in Section 2.1.2.. Consequently, the three scores were assumed to follow a beta distribution with mean value $\overline{DS1_{e,c,i}}$, $\overline{DS2_{e,c,i}}$ and $\overline{DS3_{e,c,i}}$, and scale parameter σ^{DS1}, σ^{DS2} and σ^{DS3}, respectively. The model for the first scoring date is :

$$\begin{cases} DS1_{e,c,i,r}^{obs} | \overline{DS1_{e,c,i}}, \sigma^{DS1} \sim \text{Beta}\left(\overline{DS1_{e,c,i}}, \sigma^{DS1}\right) \\ \quad \text{logit}\left(\overline{DS1_{e,c,i}}\right) = DS1_e^{expe} + DS1_{c,i}. \end{cases}$$

Variables $DS1_e^{expe}$ and $DS1_{c,i}$ are the experiment effect and the cultivar-isolate interaction on the logit scale, respectively. The same model was used for $DS2_{e,c,i,r}^{obs}$ and $DS3_{e,c,i,r}^{obs}$. The models for the corrected disease severity (cDS) were constructed as for DS.

Implementation

Inference on the variables was performed by Bayesian statistical methods, resulting in a joint posterior distribution (Gelman *et al*., 2004). This posterior distribution was computed via a Markov Chain Monte Carlo (MCMC) method using JAGS software (Plummer, 2010). For identification reasons, NSp was fixed to 500 spores but all the other variables received a non-informative prior density. It was systematically verified that this *a priori* had no influence on the posterior densities. Three MCMC-chains of 100,000 iterations were computed. Convergence was assessed using the Gelman and Rubin statistic which compares the within to the between variability of chains started at different and dispersed initial values (Gelman *et al*., 2004). Burn-in was set to 100,000, and thinning every 100 iterations resulted in acceptable mixing and convergence.

Analysis of relationships between the variables

The models described above allowed us quantifying the interactions between isolates and cultivars through any of the variables $\theta_{c,i}$, with θ in $\{IE, LP, LS, SPL, SPS, DS1, DS2, DS3,$ *cDS1, cDS2, cDS3*$\}$. The estimations (medians of the posterior distributions and 95% credibility intervals) of these variables were calculated.

Correlations among the resistance components measured in the greenhouse and between the resistance components and epidemic severity measured in the field were investigated by classical normal regressions among pairs of $\theta_{c,i}$ variables.

RESULTS

The correlations were calculated among the components of resistance, calculated by the models defined above, and between those components and disease severity in the field. Disease severity was either the observed severity (DS) or the corrected severity (cDS), at the different scoring dates (beginning of the epidemic, middle and final stage).

Model validation

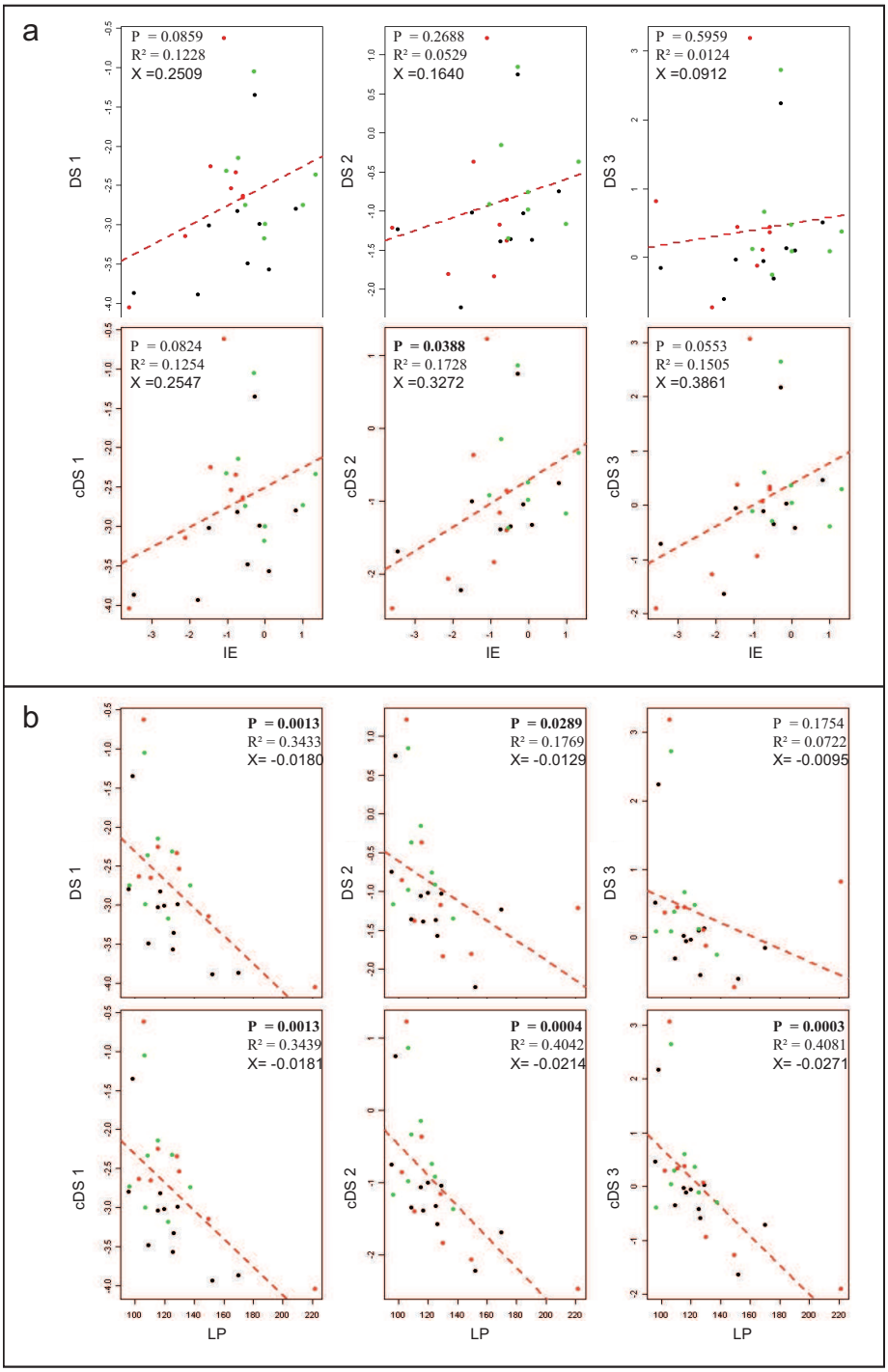
To validate the models, the output data were compared to the measured data (see Appendix 2). Comparisons between the measured components and the estimated components for each cultivar-isolate pair showed good correlations.

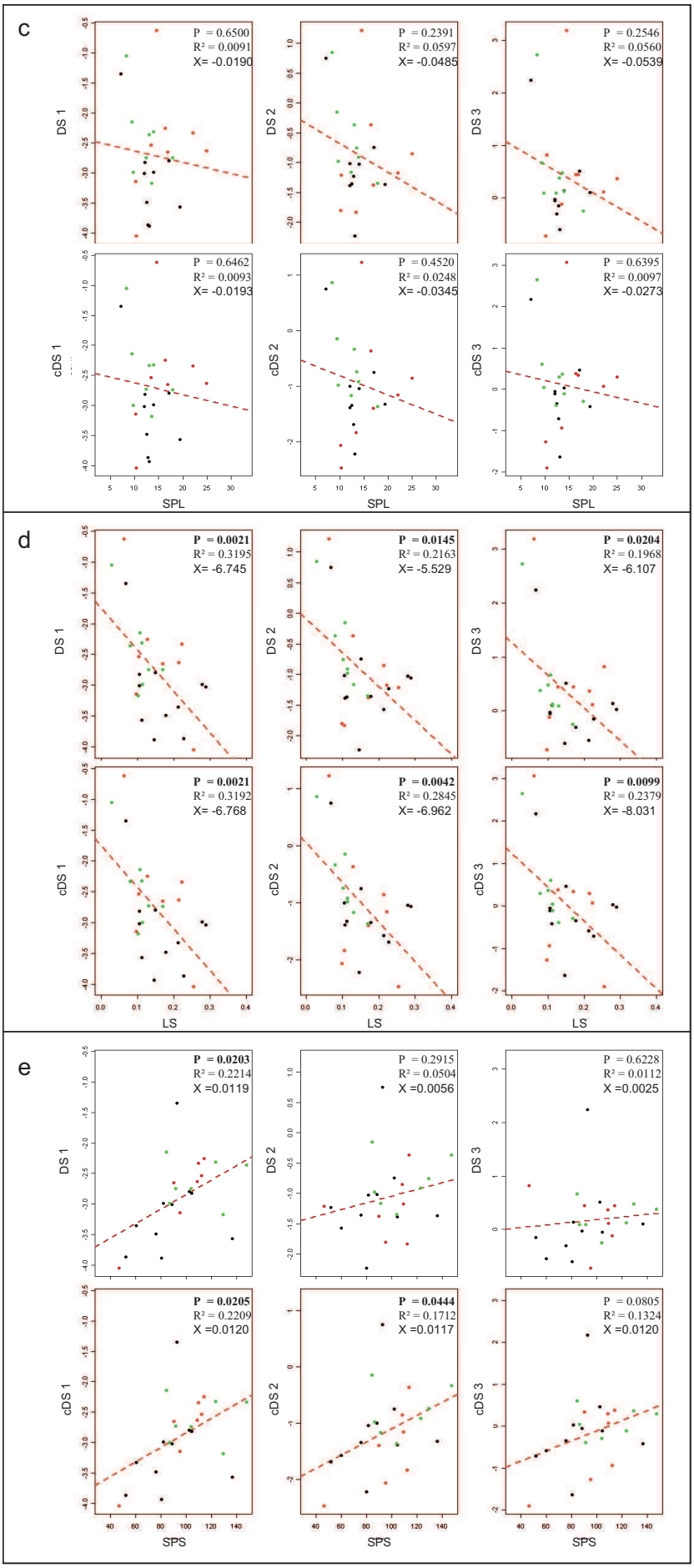
Relationship between resistance components and disease severity

When considering Disease Severity (DS), significant linear correlations between resistance components and disease severity were found for LP, LS and SPS (fig 1b, 1d, and 1e). LP was negatively correlated with DS1 and DS2, but not with DS3. LS was negatively correlated with DS at the three scoring dates. SPS was found positively correlated with DS1 only. No significant correlation was found between disease severity and IE and SPL (fig. 1a and 1c).

When considering corrected Disease Severity (cDS) significant correlations were found between cDS and LP, LS and SPS, as for DS (fig 1b, 1d, and 1e), but the correlation was also found significant between LP and cDS3, between SPS and cDS2, between IE and

Figure 1.. Relationships between field disease severity and resistance components. Disease severity (DSi) and corrected disease severity (cDSi) at each scoring date (i=1, 2, or 3) are plotted against (a) infection efficiency, (b) latent period, (c) spore production per lesion, (d) lesion size and (e) spore production by sporulating surface. The median values of posterior estimation of parameters are indicated for each cultivar-isolate combination. Values for isolates P3, P4, and P5 are figured in black, red, and green, respectively. Red dashed line were drawn from linear regression. P-value, R^2, and slope value (X) are indicated, and figured in bold when the slope was significantly different from 0, with a 0.05 threshold.




c
P = 0.6500
R² = 0.0091
X= -0.0190
P = 0.2391
R² = 0.0597
X= -0.0485
P = 0.2546
R² = 0.0560
X= -0.0539
DS 1
DS 2
DS 3
P = 0.6462
R² = 0.0093
X= -0.0193
P = 0.4520
R² = 0.0248
X= -0.0345
P = 0.6395
R² = 0.0097
X= -0.0273
cDS 1
cDS 2
cDS 3
SPL
SPL
SPL
d
P = 0.0021
R² = 0.3195
X= -6.745
P = 0.0145
R² = 0.2163
X= -5.529
P = 0.0204
R² = 0.1968
X= -6.107
DS 1
DS 2
DS 3
P = 0.0021
R² = 0.3192
X= -6.768
P = 0.0042
R² = 0.2845
X= -6.962
P = 0.0099
R² = 0.2379
X= -8.031
cDS 1
cDS 2
cDS 3
LS
LS
LS
e
P = 0.0203
R² = 0.2214
X =0.0119
P = 0.2915
R² = 0.0504
X =0.0056
P = 0.6228
R² = 0.0112
X =0.0025
DS 1
DS 2
DS 3
P = 0.0205
R² = 0.2209
X =0.0120
P = 0.0444
R² = 0.1712
X =0.0117
P = 0.0805
R² = 0.1324
X =0.0120
cDS 1
cDS 2
cDS 3
SPS
SPS
SPS

cDS2, and was marginally significant between IE and cDS3 (fig. 1a). No significant correlation was found between corrected disease severity and SPL (fig. 1c).

The correlations had similar P and R^2 values when calculated with DS1 or cDS1 but they tended to have smaller P values and higher R^2 values when calculated with cDS2 and cDS3, as compared to DS2 and DS3, whatever the component considered (except for SPL).

When considering the scoring dates, components IE, LP, LS, and SPS were significantly correlated to disease severity at more than one date; except for the DS-SPS correlation, which was significant only at the first date, and for the cDS-IE correlation, which was marginally significant at the last date. In the cases where relationships were significant at more than one date, differences in the strenght of the relationship (measured as changes in the slope value of the linear regression) were detected. For the relationships between cDS and components IE, LP, or LS, the slope value increased during the course of the epidemic. Conversely, for the LP-DS and SPS-cDS relationships, the slope value decreased during the course of the epidemic. Finally, for the LS-DS relationships, the slope value was maximal at the first scoring date, and minimal at the second scoring date.

The strenght of relationships between the field disease severity and the resistance components varied among components. Considering DS and cDS at all scoring dates, LS was the component with the highest slope values, followed by IE, LP, and SPS.

DS and cDS were highly correlated at all scoring dates, and the correlation tended to had higher P values and smaller R^2 values as the epidemic progressed (Fig. 2).

Relationships between components of resistance

Significant linear relationships were found between: IE and LP, IE and SPS, LS and SPS, LP and SPS, LS and SPL, and between SPS and SPL (table 4). The slope between IE and LP was negative (Fig. 3a), i.e. higher infection efficiency was associated to a shorter latent period. The slope between IE and SPS was positive (Fig. 3b), i.e. higher infection efficiency was associated to a higher spore production per unit area of sporulating tissue. A negative slope was detected between LS and SPS (Fig. 3c), with a larger lesion size associated to a lower spore production per unit area of sporulating tissue. The slope between LP and SPS was negative (Fig. 3d), i.e. longer latent period was associated with a lower spore production per unit area of sporulating tissue. Lastly, slopes of SPL with LS or SPS were positive (see appendix 1).

Figure 2. Relationship between disease severity (DSi) and corrected disease severity (cDSi) at each field scoring date (i=1, 2, or 3). The median values of posterior estimation of parameters are indicacated for each cultivar-isolate combination. Values associated to isolates P3, P4, and P5 are figured in black, red, and green, respectively. Red dashed line were drawn from linear regression. P-value, R^2, and slope value (X) are indicated, and figured in bold when the slope was significantly different from 0, with a 0.05 threshold.

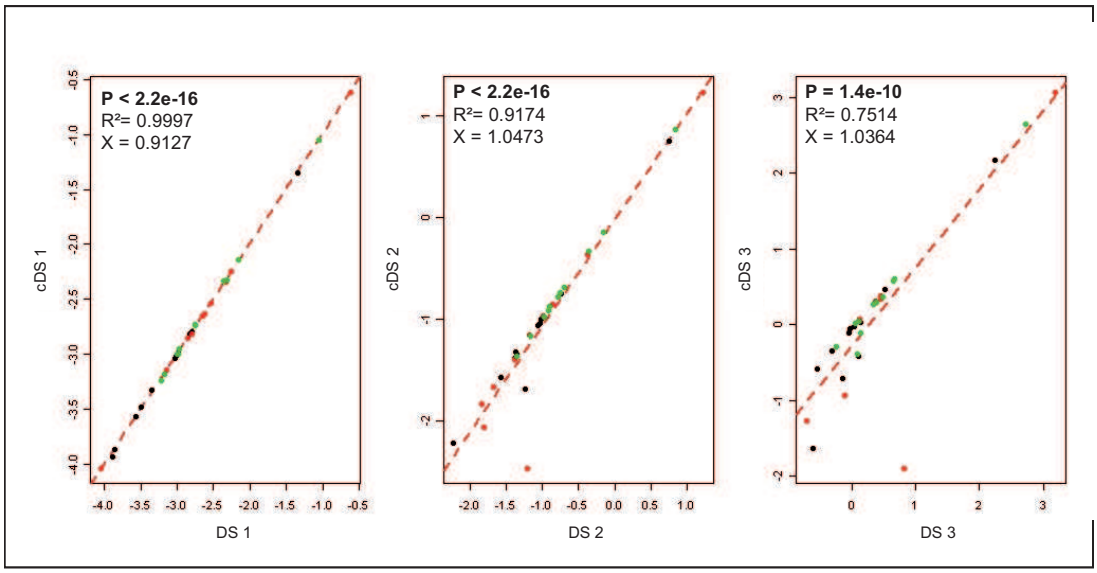
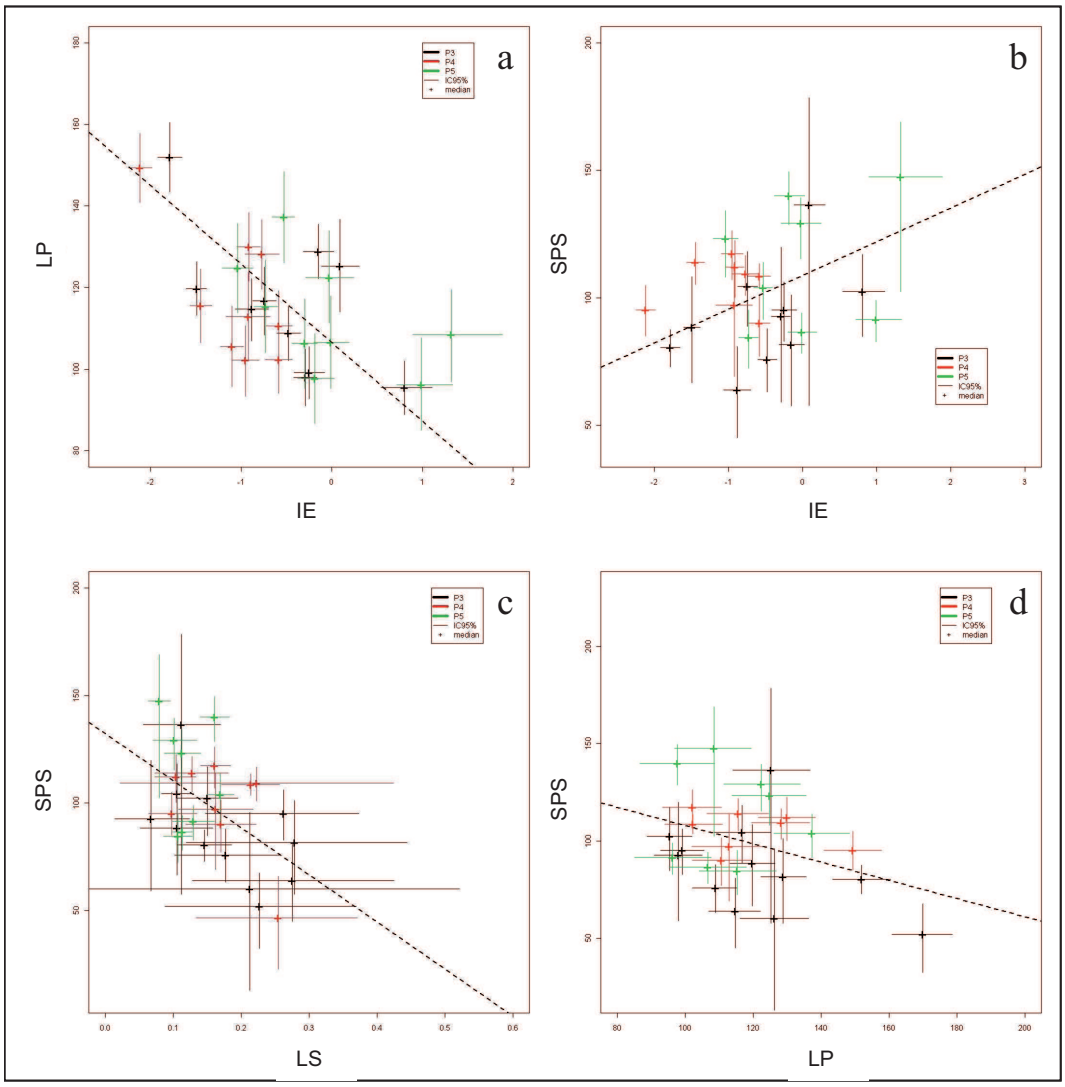


Table 4: Relationship between resistance components (IE = infection efficiency, LP = latent period, SPL = spore production per lesion, LS = lesion size, SPS = spore production per unit of sporulating tissue). Associated probability and R^2 value for the slope are figured in bold when significantly different from 0, with a 0.05 threshold.

variable X	variable Y	P-value	Multiple R^2
IE	**LP**	**0.0000**	**0.6243**
IE	**SPS**	**0.0008**	**0.3559**
LS	**SPS**	**0.0027**	**0.2875**
LP	**SPS**	**0.0068**	**0.2412**
LS	**SPL**	**0.0083**	**0.2237**
SPS	**SPL**	**0.0332**	**0.1629**
LS	LP	0.1144	0.0810
IE	LS	0.1385	0.0767
LP	SPL	0.1633	0.0682
IE	SPL	0.3977	0.0257

Figure 3. Relationship between resistance components (a) IE and LP, (b) IE and SPS, (c) LS and SPS, (d) LP and SPS. The median values and the 95% credibility interval of posterior estimation of parameters are indicacated for each cultivar-isolate combination. Values associated to isolates P3, P4, and P5 are figured in black, red, and green, respectively. Black dashed line was drawn from linear regression. P-values and R^2 value are indicated in Table 4.



DISCUSSION

The present study investigated the links between disease severity measured in the field during the course of an epidemic and quantitative traits of the host-pathogen interaction measured in controlled conditions, at the scale of a single pathogen life cycle. In other words, we characterized the links between the observed plant resistance in the field, and the resistance components covering all stages of pathogen life cycle, as commonly measured in controlled conditions. This relationship has already been investigated on a theoretical basis (Sackett & Mundt, 2005; Lehman & Shaner, 1997), but our study is one of the few to address this question based on a data analysis. We found that most but not all the resistance components were linked to the disease severity, that the strength of the relationship varied among components, and that the strength of the relationship varied between the beginning and the end of the epidemic.

The progression rate of an epidemic is determined by the increase in the proportion of sporulating tissue, which is the only part of the lesions participating to the pathogen multiplication (Van der Plank, 1968). Thus it is logical to use the corrected disease severity as a measure of the plant resistance in the field. This is consistent with the fact that, in our analysis, the corrected disease severity (cDS) had lower P values and higher R^2 values when correlated to the resistance components than the disease severity (DS). Disease severity, which takes into account all visible symptoms produced by the pathogen, is more an indication at a given time of the damage to the plant. When considering DS, LS and LP were the most influent components.

All the individual resistance components were correlated to the corrected disease severity, except SPL. Therefore, all these components significantly contributed to the plant quantitative resistance in the field conditions. A surprising result was that LS was strongly and negatively correlated to cDS (a larger lesion size was associated to a higher level of resistance), whereas this component is usually considered to be positively correlated to host resistance (Ohm & Shaner, 1976; Singh *et al*., 1991; Herrera-Foessel *et al*., 2007). The most likely explanation for this result would be based on the existence of a trade-off between lesion size and spore production (discussed below). The lesion size (LS) was the resistance component the most strongly correlated to cDS, followed by IE, LP, and SPS. LS and LP were commonly found related to the levels of resistance expressed in the field for different pathosystems (Baart *et al*., 1991; Carlisle *et al*., 2002; Herrera-Foessel *et al*., 2007). The results found here for IE and SPS are consistent with other studies (Johnson & Taylor, 1976;

Parlevliet, 1979) but it should be noted that these two components have not been studied as often as LP or LS, and that precise measurements of IE are scarce (Denissen, 1993; Lehman & Shaner, 1997, Chapter 1 of the thesis). The spore production per lesion (SPL) was found related neither to cDS nor to DS. This is surprising, as the number of spores produced per lesion is an indication of the pathogen multiplication rate. However, it has been shown that SPL is a composite trait that can be split into lesion size (LS) and spore production capacity (SPS) (Pariaud *et al*., 2009a). Moreover, we have established above that cDS and DS are negatively linked to LS and positively linked to SPS. This opposing trend is consistent with the absence of relationship between disease severity and SPL. Our results thus confirm that decomposing SPL into LS and SPS is a better approach for detecting differences in resistance to leaf rust.

The different resistance components were not correlated with the same strength to the corrected disease severity at the different stage of the epidemic. The link between cDS and IE, LP, or LS strengthened during the course of the epidemic, suggesting a cumulative effect (Parlevliet, 1979). For example, shorter LP allows indeed the pathogen to complete more multiplication cycles during the epidemic. LP and LS correlate to cDS at all dates, whereas IE correlate from the middle to the end of the epidemic, suggesting that IE is of greater importance to the development of the epidemic after it started. SPS was related to the disease severity from the beginning to the middle of the epidemic, suggesting that spore production is of greater importance to start the epidemic.

In our analysis, six significant linear relationships were found between resistance components. These correlations can be separated according to three different causes. First, SPL was positively correlated to LS and SPS. This was expected, since SPL is a composite trait that aggregates LS and SPS. Second, the correlations between IE, LP, and SPS can be interpreted as a pleiotropic effect of the host resistance since the plants tended to express simultaneously a better level of resistance for these three components. Such pleiotropic expression of the resistance has been found by other authors (Lehman et al., 2005; Cooper *et al*., 2009). However, these correlations had a high P value and a small R^2 value, particularly between SPS and IE or LP, which suggests that pleiotropic effects were not strong. Last, the relationship between LS and SPS can be interpreted as a trade-off, and explained by the effect of a physiological constraint limiting the lesion productivity when its size increases. Since the lesion is fed from the green tissue surrounding the sporulating area, increasing the lesion size might reduce the ratio between the resource and the need for nutriments. A negative link

between lesion size and spore production capacity has sometimes been attributed to a density effect (Parlevliet, 1979). This cannot be the case in our analysis since all resistance components were corrected for density dependence. This trade-off between LS and SPS may explain the negative relationship between the field resistance and LS as well as the absence of relationship between DS and SPL.

Different attempts have been made to relate field resistance with greenhouse measurements of resistance components but with limited success. Composite indices of fitness that aggregate several components have been used (Shaner & Hess, 1978; Day & Shattock, 1997; Flier & Turkensteen, 1999). In this approach the main difficulty is to properly estimate of the weight of each component in the contribution to the overall resistance (Carlisle *et al*., 2002). With our approach, the contribution of each component to the overall resistance is estimated at different times in the epidemic development. Moreover, the use of Generalized Linear Models in a Bayesian framework allowed removing the experiment effect and taking into account the specificity of the quantitative resistance factors by estimating the cultivar-isolate interactions. This information is valuable to predict the expected resistance in the field, based on individually measured components.

The analysis of field resistance at different stages of the epidemic are scarce in the litterature. Most of the time, the epidemic development is expressed by an integrative variable such as AUDPC. Nevertheless, AUDPC underestimates the importance of the first disease severity scores (Simko & Piepho, 2012). Our analysis suggests that the different resistance components may be differently related to the resistance level in the field at the beginning or at the end of the epidemic. Such information might be valuable in integrated strategies associating quantitative resistance with limited chemical protection.

Enhancing the durability of quantitative resistance can be achieved by combining, within host genotypes, diversified quantitative resistance loci. Indeed, a lower selection pressure on the pathogen population is expected when the resistance is diversified in the host population (Stuthman *et al*., 2007). To ensure a maximum efficiency, the diversification should be both phenotypic (resistance components) and genetic. Diversity in resistance components for a subset of the cultivars used here was demonstrated elsewhere (Azzimonti *et al*., 2012). In the present study, the impact of the resistance components during field epidemics and the positive and negative correlations among these components were stablished. In order to promote the use in plant breeding of diversified resistance components with a high impact on field resistance, the next step is to identify the genome regions involved in the expression of each component by QTL analysis.

Chapitre 3

“Diversity and specificity of QTLs involved in five components of quantitative resistance in the wheat leaf rust pathosystem”

Diversity and specificity of QTLs involved in five components of quantitative resistance in the wheat leaf rust pathosystem [2]

INTRODUCTION

Quantitative resistance in plants slows down the rate of epidemic development and, thus, reduces the disease severity in field conditions (Shaner & Hess, 1978; Shaner *et al*., 1978). Quantitative resistance is generally controlled by several genes, located in quantitative trait loci (QTL) (Young, 1996 ; St. Clair, 2010). Each gene contributes at a different degree to the total phenotypic variance (Geiger & Heun, 1989). Those features result in a continuous variation of the plant resistance level. This type of resistance is often described as non-isolate-specific, and thus durable. However, indications of plant genotype x pathogen isolate interactions suggest that this resistance is based on individual interactions between resistance genes and pathogenicity genes in a specific minor gene-for-minor gene fashion (Parlevliet & Zadoks, 1977; Niks & Marcel, 2009). In this context, adaptation of the pathogen, leading to erosion of resistance, is still possible and has been observed in an agricultural context (Mundt *et al*., 2002) or obtained experimentally (Geiger & Heun, 1989; Lehman & Shaner, 1997, 2007). Many studies show that selection for quantitative traits of the host-pathogen interaction influences pathogen evolution in agricultural systems (Pariaud *et al*., 2009a). The likelihood of the erosion of quantitative resistance could be reduced, provided that this resistance rests on diversified mechanisms: indeed diversity leads to a lower selection pressure and a complexity that is difficult to overcome for the pathogen (Stuthman *et al*., 2007). Therefore, with the aim to impede pathogen adaptation, quantitative resistance should involve a combination of physiological mechanisms, for which adaptation of the pathogen is difficult. This requires a characterization of quantitative resistance including both a physical mapping of associated QTLs, and an identification of underlying physiological mechanisms.

The genetic bases of quantitative resistance are usually studied in field experiments, measuring phenotypic traits that assess disease severity as a whole (Gupta *et al*., 2010). These studies allow an assessment of the global action of resistance QTLs on epidemics, but do not indicate anything about the mechanisms or traits involved in resistance. Here, the diversification level of the resistance is inferred from the position of QTLs in the genome and

[2] Projet d'article, co-auteurs : T. Marcel, O. Robert, S. Paillard, H. Goyeau

the interactions between them. Better approaches to assess the diversity of the genetic basis of quantitative resistance are needed (Collard & Mackill, 2008; St. Clair, 2010). The determination of QTL acting on different traits of host-pathogen interaction can be an appropriate way to reveal the genetic diversity underlying quantitative resistance.

Different phenotypic traits describe the outcome of the host-pathogen interaction, each trait being involved in a specific step of the infectious process. Quantitative resistance results from the variations in the values of all these life cycle traits. Infection efficiency, latent period, lesion size, sporulation rate (per lesion or per unit area of sporulating tissue) are the traits usually affected by quantitative resistance (reviewed by Pariaud *et al*., 2009a), and as such they are considered as components of quantitative resistance. Each component can be related to different physiological mechanisms in the plant (Parlevliet, 1979; Bolton *et al*., 2008; Kolmer *et al.*, 2009). Infection efficiency reveals the capacity of the plant in stopping the the pathogen development before the eruption of lesions. Latent period measures the speed of the pathogen development in plant tissues. Components acting on sporulation are linked to the capacity of the plant to reduce lesion size, or the amount of spores produced. Evidences from various pathosystems support the idea that the different components of quantitative resistance involve different physiological and molecular mechanisms, determined by different genetics factors (Niks & Marcel, 2009; Poland *et al*., 2009). Therefore, breeding crops by introgressing QTLs acting on different components of quantitative resistance would diversify the genetic nature of resistance in breeding material in a way that pathogen adaptation would be rendered difficult.

The scarce studies about the action of QTLs on components of quantitative resistance, suggest that the genetic basis of the different components rests on diversified QTLs. Using advanced introgression lines for the maize - *Setosphaeria turcica* pathosystem, Chung *et al*. (2010) found one QTL reducing the efficiency of fungal penetration, therefore acting on infection efficiency; another QTL delayed the invasion and the extension of the pathogen in the vascular tissue of the leaf, therefore acting on latent period. In a population of durum wheat recombinant inbreed lines inoculated with leaf rust, Marone *et al*. (2009) identified QTLs acting specifically on latent period or infection efficiency, but also one QTL affecting both components. Similar results were obtained by Jorge *et al*. (2005) for the poplar – leaf rust pathosystem, where some QTLs affected specifically lesion size or latent period, and other QTLs acted on both components.

Only a few genes contributing to quantitative resistance to plant pathogens have been cloned and functionally validated. Three genes have been cloned up to date: *pi21*, *Yr36* and

Lr34, conferring quantitative resistance to rice blast, yellow rust and leaf rust, respectively (St. Clair, 2010). These genes, as well as other identified candidate genes for resistance QTLs, have diverse gene structures, different than R-genes, and are involved in different molecular and physiological mechanisms (Niks & Marcel, 2009). *pi21* encodes a metal/transport detoxification protein, *Yr36* encodes a kinase and putative START lipid-binding domains and *Lr34* encodes a putative ABC transporter (St Clair., 2010). Poland *et al*. (2009) summarized evidence that support the diversity of biological mechanisms underlying quantitative resistance, including: regulation of morphological and developmental traits, mutation or allelism at genes for basal resistance[3], production of components of chemical warfare, regulation and participation of defence signal transduction, and mutation or allelism of R-genes[4].

Leaf rust in wheat is caused by the biotrophic basidiomycete *Puccinia triticina*. It is considered one of most important crop diseases because of its worldwide distribution (Huerta-Espino *et al*., 2011). Aerial transportation of spores can disperse virulent isolates rapidly and across long distances, increasing the risk of erosion or breakdown of resistance. A large number of QTLs for resistance have been identified in different breeding materials for this pathosystem (Faris *et al*., 1999; Schnurbusch *et al*., 2004; Chu *et al*., 2009; Singh *et al*. 2011). QTLs acting on quantitative resistance were detected on all wheat chromosomes, sometimes in regions where no resistance gene was mapped before (Herrera-Foessel *et al*., 2012), sometimes in clusters of resistance genes against different diseases (Herrera-Foessel *et al*., 2011; Lagudah, 2011). However, almost nothing is known about the effect of these QTLs on the different components of resistance. Only latent period has been studied to some extent, which was determined by one to five genes (Broers & Jacobs, 1989; Das *et al*., 1993; Lee & Shaner, 1985; Lehman *et al*., 2005), or associated to three QTLs (Xu *et al*., 2005).

Recently, we demonstrated that a set of wheat cultivars showing different levels of quantitative resistance in field conditions also had a high variability for five components of quantitative resistance when confronted to three leaf rust isolates under controlled conditions (Azzimonti *et al*., 2012). Cultivars Apache and Balance displayed contrasting profiles of resistance for the components: Apache had a good level of resistance for latent period and sporulation components, with no isolate specificity; Balance was resistant to one isolate for

[3] Basal resistance, after Niks & Marcel (2009), includes two types: i) qualitative, targeting unadapted microbial intruders; ii) quantitative, targeting pathogen spread after successful infection and onset of disease.

[4] R-genes: Major resistance genes, involved in hypersensible response.

latent period and lesion size, but showed high isolate-specificity between two of the isolates, for all the components.

Using these two cultivars with putative diversified quantitative resistance, the objectives of this study were i) to identify QTLs associated to the components of quantitative resistance under controlled conditions, ii) to determine which QTLs have an impact on a field epidemic, and iii) to investigate the specificity of these QTLs against two isolates in field epidemics conditions.

A doubled haploid population derived from the cross between cultivars Apache and Balance was evaluated for reaction to leaf rust at the adult plant stage, in the greenhouse and in the field. In the greenhouse, five components of quantitative resistance were measured in plants confronted to one isolate of leaf rust. In the field, disease severity was monitored during epidemics initiated separately with two different isolates.

MATERIALS AND METHODS

Plant and fungal material

A doubled haploid (DH) population of 91 lines, derived from a cross between the wheat cultivars Apache and Balance, was used in four field trials and two greenhouse experiments. The DH population was provided by Bioplante (O. Robert and V. Laurent).

Two isolates of *P. triticina*, labelled P3 and P5, were chosen because given their frequency in the natural population, respectively low and intermediate, they were postulated to represent different aggressiveness levels (Azzimonti *et al*., 2012). The two isolates were used in field trials, whereas only one isolate, P5, was used in greenhouse experiments, due to technical limitations. Isolates P3 and P5 were derived from the collection of single-lesion spores kept at –80°C. Both isolates were virulent to cultivars Balance and Apache. Isolate P3 was virulent to *Lr1, Lr3, Lr3bg, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr27+Lr31* and *Lr37*. Isolate P5 was virulent to *Lr1, Lr2c, Lr3, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr23, Lr26, Lr27+Lr31 and Lr37.* All inoculations were performed with freshly produced spores, increased on seedlings of the susceptible wheat cultivar Michigan Amber. Maleic hydrazide solution (0.25 g/L) was added into pots to prevent the emergence of secondary leaves and to increase spore production. Seven-day-old seedlings were inoculated by spraying a suspension of spores in Soltrol® oil (Phillips Petroleum). Before the onset of sporulation,

the pots were wrapped into cellophane bags to prevent cross-contamination between different isolates. Spores were collected 11 to 13 days after inoculation, and stored in a cabinet (9°C, 35% relative humidity) until used.

Greenhouse experiments

Greenhouse experiments were conducted in years 2010 and 2011. Seeds were sown in Jiffy pots with two seeds per pot. To synchronize the development of different lines, sowing times were staggered taking into account earliness in heading time, and duration of vernalization. Seedlings at the two-leaf stage were vernalized at 8°C in a growth chamber, and then transferred to the greenhouse for 7 to 10 days to acclimatize. Later, the most vigorous seedlings were individually transplanted into pots filled with 0.7 L of commercial compost (Klasmann® Substrat 4, Klasmann France SARL) to which 6.7 g of slow release fertilizer (Osmocote® 10-11-18 N-P-K, The Scotts company LLC) were added. Moreover, once a week, all plants were watered with nutritive solution (Hydrokani C2®, Hydro Agri Spécialités) at a 1:1,000 dilution rate, starting two weeks before inoculation for both experiments. During plant growth, natural light was supplemented as needed with 400-W sodium vapour lamps between 6:00 a.m. and 9:00 p.m. Greenhouse temperature was maintained between 15 and 20 °C.

The plant material was standardized as much as possible by selecting plants with homogeneous growth stages and physiological states. Plants with necrotic symptoms at the stem basis (caused by *Fusarium* spp.), with apparent physiological disorders, or with extreme heights or growth stages were discarded.

Plants were cleared the day before inoculation, to leave four stems. Growth stages ranged between heading and flowering. Plants were arranged in four sets, inoculated at a 7-day interval. Three flag leaves per plant were inoculated, that of the main stem and those of the two most developed secondary stems. Inoculation was performed by applying a mixture of rust spores and *Lycopodium* spores on the leaf surface with a soft brush. The proportion [rust spores : *Lycopodium* spores] of the mixture was 1:100 for the flag leaves of the main stem, and 1:200 for the flag leaves of secondary stems. These proportions, determined in preliminary experiments, were adjusted to prevent spore crowding and competition effects. The leaf surface was inoculated along 10 cm, starting at 3 to 5 cm from the stem. The non-inoculated leaf surface was protected with a stencil. Immediately after inoculation, plants were placed in a dew chamber (15°C) for 24 h and then returned to the greenhouse until the

end of the experiment, with temperature set between 12°C and 18°C. All plants were placed in the same greenhouse compartment, which was used for the two experiments.

Greenhouse measurement of quantitative resistance components

Infection efficiency (IE) was defined as the ratio of the number of sporulating lesions to the number of deposited spores. IE was measured using the inoculated flag leaves of secondary stems. Immediately after inoculation, the half distal portion of the inoculated zone was cut off, saved apart at – 20°C, and used later to count the number of deposited spores. Spores were counted in a randomly delimitated area of 0.7 cm^2 in the inoculated zone, with a stereo binocular magnifying glass (40X). The sporulating lesions were counted on the proximal half portion of the inoculated flag leaves that stayed attached to the plants. Lesions were counted in a randomly delimited area of 1 cm^2, at the end of the latent period, i.e. when the number of lesions counted each day was stabilized. For each line, the IE was estimated by the mean value of 10 to 12 replicates.

The latent period (LP) was measured on the flag leaf of the main stem. Sporulating lesions were counted daily, until their number stabilized, on a randomly delimited area of 1 cm^2. LP was determined as the time when half of the maximum number of sporulating lesions had appeared. This time was estimated by linear interpolation around the 50% count (Knott *et al*., 1991). Since latent period is highly dependent on temperature, LP was expressed in degree-days. For each line, the LP was estimated by the mean value of 5 or 6 replicates. The last count of sporulating lesions performed for latent period was used to estimate lesion density.

Once the number of lesions had stabilized, leaves were placed into cellophane bags, and maintained horizontally with a plastic frame. After five days, leaves were gently brushed so that the spores fell into the cellophane bags. Spores were transferred into aluminium paper containers, desiccated for 7 to 15 days in a cabinet (9°C, 35% relative humidity), and then weighed. Digital pictures of the leaves were taken with a scanner (400 ppi). The area of the inoculated surface and the sporulating surface were calculated by image analysis (Optimas 5; Media Cybernetics, Silver Spring, MD, U.S.A.).

The total number of lesions in the inoculated zone was estimated by the lesion density, determined in a leaf portion of 2 cm^2, multiplied by the area of the inoculated surface. Lesion size (LS) was calculated as the sporulating surface divided by the total number of lesions. Spore production per lesion (SPL) was calculated as the amount of spores produced in five

days, divided by the total number of lesions. Spore production per unit of sporulating tissue (SPS) was calculated as the amount of spores produced in five days divided by the sporulating surface. LS, SPL and SPS were estimated, for each line, by the mean value of 5 or 6 replicates.

Field experiments

Field experiments were conducted in years 2009 and 2010, in two locations 300 Km apart, Cappelle-en-Pévèle (north of France) and Maisse (Paris Basin). In each location, two trials were planted at least one Km apart, one being inoculated with isolate P3 and the other with isolate P5. Plants were sown in a randomized block design. Approximately 60 plants were distributed on two consecutive rows of 1.5 m long for each DH line. Rows of the spreader cultivar Buster were sown every two or five DH lines in Cappelle and Maisse, respectively. Parental cultivars Apache and Balance, as well as susceptible cultivar Ecrin, were also included. Two replicates were sown at each location, except for Cappelle in 2009 where only one replicate was sown.

To initiate the leaf rust epidemics, spreader rows were inoculated using hand-sprayers containing spores of *P. triticina* suspended in Soltrol® oil (Phillips Petroleum). Inoculation was performed at the heading stage, on the same day for the two trials within each location, and within 2 weeks between locations.

Disease severity was scored according to the modified Cobb Scale (Peterson *et al*., 1948), where percentage of disease tissue was visually estimated on flag leaves. Disease severity assessments started when the susceptible cultivar Ecrin reached almost 100% leaf rust severity. Two to five assessments (table 1) were conducted every five to seven days until the end of the epidemic.

Statistical analyses

The area under the disease progress curve (AUDPC) was calculated with the midpoint rule method (Campbell and Madden, 1990), using the formula: $AUDPC = \Sigma_{(i=1,n-1)} [(t_{i+1} - t_i)(y_i + y_{i+1})/2]$, where t accounts for the time in days of each disease severity assessment date, y accounts for the disease severity score at each date, and n accounts for the number of assessments.

Table 1: Conditions set up for field and greenhouse experiments.

Condition	Location	Year/Replicate	Isolate	Traits [W]	Code for table 2
Field	Cappelle	2009	P3	N1-N4, AUDPC	Cap-09
		2009	P5	N1-N4, AUDPC	Cap-09
	Cappelle	2010 / 1	P3	N1-N2, AUDPC	Cap-10
		2010 / 2	P3	N1-N2, AUDPC	Cap-10
		2010 / 1	P5	N1-N5, AUDPC	Cap-10
		2010 / 2	P5	N1-N5, AUDPC	Cap-10
	Maisse	2009 / 1	P3	N1-N3, AUDPC	Mai-09
		2009 / 2	P3	N1-N3, AUDPC	Mai-09
		2009 / 1	P5	N1-N5, AUDPC	Mai-09
		2009 / 2	P5	N1-N5, AUDPC	Mai-09
	Maisse	2010 / 1	P3	N1-N3, AUDPC	Mai-10
		2010 / 2	P3	N1-N3, AUDPC	Mai-10
		2010 / 1	P5	N1-N3, AUDPC	Mai-10
		2010 / 2	P5	N1-N3, AUDPC	Mai-10
Greenhouse		2010	P5	IE, LP, SPL, LS, SPS	G1
		2011	P5	IE, LP, SPL, LS, SPS	G2

[W] : field traits were Ni (disease severity at scoring date i) and AUDPC (area under the disease progress curve). Greenhouse traits were IE (infection efficiency), LP (latent period), SPL (spore production per lesion), LS (lesion size), and SPS (spore production per unit of sporulating tissue).

Field phenotypic traits (disease severity at time t_i and AUDPC) were analysed as variables function of the main factors: year, location, isolate, DH line. Interaction between these factors was tested whenever possible. Greenhouse phenotypic traits (IE, LP, LS, SPL, SPS) were analysed as a function of factors: year, DH line and experimental factors, with experimental factors being plant growth stage and plant nitrogen content. Plant growth stage at inoculation was recorded according to three categories (« early heading », « late heading/early flowering », and « late flowering »). As an indication of plant nitrogen content, flag leaf colour was visually recorded as « normal », « light green » or « light green striped ». For IE, where two secondary stems were used, a stem factor was included in the analysis. Interaction between these factors was tested whenever possible. The experimental factors (plant growth stage, plant nitrogen content) and the stem factor in the analysis of IE, were never found to be significant ($P>0.01$), and were removed from the models. Factors year, DH line, and their interaction were significant ($P<0.05$), thus the experiments were analysed separately for each year hereafter. Each variable (IE, LP, LS, SPL, and SPS) was analysed as a function of the DH line factor. Lesion density was used as an additional co-variable in the ANOVA models for the analyses of LP, LS, SPL, and SPS, because lesion density can affect these components (Robert *et al*., 2004). Lesion density was found to have a significant effect on all the components ($P<0.01$). To take into account the effect of lesion density in the analyses, each component was thus estimated at a constant lesion density of 20 lesions per cm^2 of leaf, based on the ANOVA model (Lannou & Soubeyrand, 2012).

All statistical analyses were performed with Splus software (Lucent Technologies, Inc.). In all the ANOVAs, all factors were considered as fixed factors and were evaluated with type III sum of square.

Map construction and QTL analysis

Segregation data for 847 molecular markers (678 DArT and 169 SSR markers) in Apache/Balance DH population were provided by O. Robert (Bioplante). Distorted markers (Khi2 5% > 3.84) were eliminated. Also, closely related markers (distance < 0.2cM) were eliminated. A total number of 355 markers were used for map construction with MAPMAKER/EXP 3.0 software (Lander *et al.,* 1987; Lincoln *et al*., 1992). LOD (logarithm of the odds) value of 4.0 and a maximum distance between two consecutive markers of 50 cM were used to determinate linkage groups. For each linkage group, marker loci order was determined using three-point and multi-point analyses. Genetic distances were calculated

using the Kosambi mapping function. Linkage groups were assigned to chromosomes by comparison of the position of the SSR markers with the International Triticeae Mapping Initiative (ITMI) map (Röder *et al*., 1998).

QTL analyses were performed independently for each replicate for field traits, and for each experiment for greenhouse traits. Each phenotypic trait was analysed by composite interval mapping (CIM) (Zeng, 1993, 1994) with the QTL CARTOGRAPHER software ver. 2.5 (Basten *et al*., 1997). LOD significance threshold values were determined by permutation tests, with 1000 permutation rounds. For each QTL, the position of the peak marker was used to position the QTL on the linkage map. The additive effect and the part of the phenotypic variation explained by the QTL were estimated. LOD values of QTLs were classified in three categories: LOD values between 2 and LOD-threshold of 1000 permutations, LOD values between LOD-threshold and 7, and LOD values higher than 7. Each LOD category was named as LOD rank 1, 2 and 3, respectively. QTLs were classified as suggestive when they occurred sporadically and/or when LOD ranks were always, or most of the time, of category 1. Moreover, QTLs were classified as major or minor as the proportion of the phenotypic variation explained by the QTL, estimated by the R^2 mean value, was more or less than 10 %, respectively.

RESULTS

Linkage map characteristics

The assemblage of the linkage map was made with a total of 355 markers, after markers with significant distorded ratio or cosegregation were eliminated from the original set of 847 markers. The markers were assigned to 39 linkage groups, corresponding to the 21 wheat chromosomes (linkage map in suplementary material), with a mean assignation of 1.86 linkage group per chromosome. The only linkage group (5Bb / 7 Bb) that could not be assigned to a single chromosome was assigned to two chromosomes. Chromosomes belonging to the A, B, and D genomes were all represented, but with different levels of coverage. The amount of markers per linkage group varied from 2 to 28, with a mean of 9.1 markers per linkage group. The size of linkage groups varied from 3.7 cM to 207.9 cM, with a mean size of 71 cM per linkage group. The total map length was of 2768 cM, and mean distance between consecutive markers was 8.3 cM. Distribution of markers over the map was

not homogeneous, with a median distance between markers of 21.2 cM, and a minimum and maximum distance between markers of 0.1 cM and 42.3 cM, respectively. The map lenght and marker order were similar to those of the linkage map constructed by Ghaffary *et al*. (2011) for the same DH population.

Frequency distribution of quantitative resistance components, and of field phenotypic traits

All the phenotypic traits measured segregate in the DH population (Figs. 1 and 2). Transgressive segregation was found for all phenotypic traits, in all experiments or replicates.

Frequency distributions of the resistance components were continuous (Fig. 1). However, the range and the shape of the distributions was different between experiments 1 and 2. This is particularly clear for components SPL and SPS, for which the range of mean values distribution was smaller in experiment 2 than in experiment 1 (Fig. 1). Also, factors year of experiment, DH line, and their interaction were significant ($P<0.05$). The mean values of components for parental cultivars changed between experiments, except for values of IE for Apache and LS for Balance. In both greenhouse experiments, cultivar Apache was significantly more resistant than Balance for components IE and LP ($P<0.01$), and Balance was significantly more resistant than Apache for components LS and SPL ($P<0.01$). For component SPS, there were no significant differences between parental cultivars ($P>0.05$).

In field experiments, the frequency distribution of AUDPC was continuous (Fig. 2). The distribution of disease severity scorings was also continuous for all scoring dates, except for the first scoring date with isolate P5 in both replicates at Cappelle location in 2010 experiment, and at Maisse location in 2009 experiment (data not shown). The range and the shape of the distributions of mean AUDPC values changed between locations, year of experiment, isolate, and even between replicates (Fig 2). This was also the case for disease severity at all scoring dates, except at the first dates (data not shown). Also, the factors: year of experiment, site of experiment, isolate, DH line were significant ($P<0.01$), as was the simple interaction between them ($P<0.05$). The mean AUDPC values and disease severity for parental cultivars changed between experiments. The AUDPC values for Apache were always higher than for Balance, whenever measured. It was also the case for disease severity values at all scoring dates, in all experiments (data not shown).

Figure 1: Frequency distribution of the mean values of quantitative resistance components measured in greenhouse experiments 1 and 2 (2010 and 2011 respectively), for the 91 DH lines population derived from the cross Apache x Balance. Quantitative components of resistance were: infection efficiency (IE), latent period (LP), spore production per lesion (SPL), lesion size (LS), and spore production per unit of sporulating tissue (SPS). Values for Apache and Balance are indicated by green and red vertical lines, respectively. Mean values were grouped into 40 categories, and the values indicated on the x-axis are the average trait values for each category.

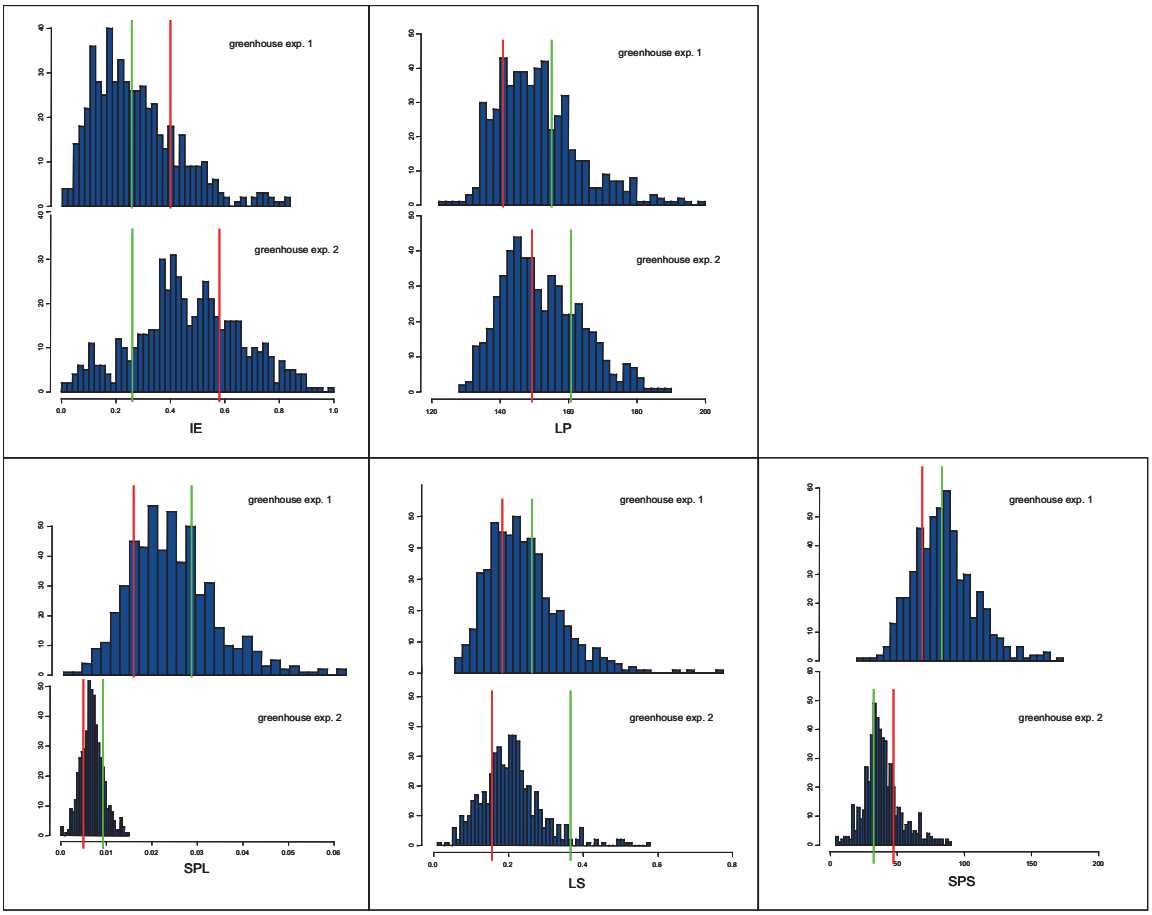
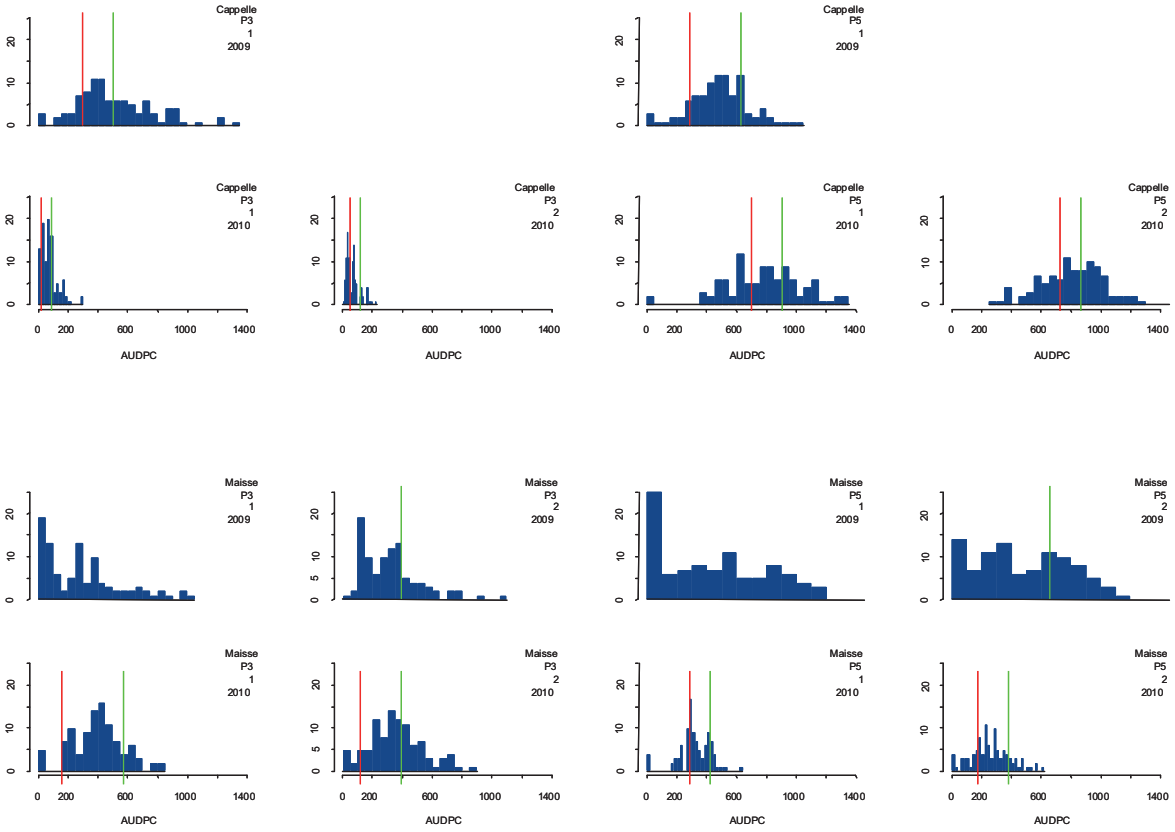


Figure 2: Frequency distribution of AUDPC values for the 91 DH lines population derived from the cross Apache x Balance. Field experiments were conducted at two locations (Cappelle or Maisse), with two isolates (P3 or P5), and two replicates (1 or 2), during two years (2009 and 2010). Values for Apache and Balance are indicated (whenever measured) by green and red vertical lines, respectively. AUDPC values were grouped into 40 categories, and the values indicated on the x-axis are the average trait values for each category.



QTL analysis

QTL analyses were performed independently for each experiment for greenhouse traits and for every replicate for field traits, because significant differences were found between experiments. Distribution of traits was different between field replicates, as well as between greenhouse experiments (Figures 1 and 2). Identification of QTL was based on the evaluation of all QTL analysis (see complementary material for complete description of QTLs found in each QTL analysis). The criterion used to validate QTLs was a LOD value greater than 2.5 in at least two independent experiments, along with a LOD value greater than the LOD-threshold of 1000 permutations in at least one of the experiments.

Based on this criterion, 13 QTLs were identified (Table 2 and Fig. 3). In some cases, the mapping precision was hampered by the fact that the peak marker, for a given QTL could vary between experiments, and that large overlapping confidence intervals did not allow to differentiate between close QTLs. This was the case for *Qlr.inra-2Ab*, *Qlr.inra-2B, Qlr.inra-6Aa,* and *Qlr.inra-7Aa* (Fig. 3). In other cases, the high distance between peak markers did not allow to precisely assign a QTL to a given marker. This was the case for QTLs *Qlr.inra-2D* (Fig. 3). Each QTL was found on a different linkage group, except for QTLs *Qlr.inra-3Bb.1* and *Qlr.inra-3Bb.2*.

Ten QTLs were found for components of resistance in greenhouse experiments (Fig 4). Six of these QTLs were found for only one component of resistance, i.e. *Qlr.inra-2B* and *Qlr.inra-6Aa* for LP, *Qlr.inra-7Aa* for SPL, *Qlr.inra-2D, Qlr.inra-3Bb.1,* and *Qlr.inra-4Bb* for SPS. Three QTLs were found for two components: *Qlr.inra-2Ab* and *Qlr.inra-4Da* for LS and SPL, and *Qlr.inra-3Db* for LS and LP. Only one QTL (*Qlr.inra-3Bb.2*) was found for all components of resistance. All these ten QTLs were also found in field experiments for disease severity. Two of them, *Qlr.inra-2D* and *Qlr.inra-7Aa,* were found for all scoring dates and for AUDPC. The other QTLs were found at different stages of the epidemic: *Qlr.inra-3Bb.2* and *Qlr.inra-4Da* at early dates; *Qlr.inra-3Db* and *Qlr.inra-4Bb* at intermediate dates; and *Qlr.inra-2Ab, Qlr.inra-2B, Qlr.inra-3Bb.1,* and *Qlr.inra-6Aa* at late dates. These QTLs were also found for AUDPC, except for *Qlr.inra-3Bb.2* and *Qlr.inra-3Bb.2*. Lastly, there were only three QTLs found in field experiments and not in greenhouse experiments: *Qlr.inra-1Aa*, which was found for early dates and AUDPC, and *Qlr.inra-5Bb/7Bb* and *Qlr.inra-6B*, which were found for all dates and AUDPC.

QTLs found for both greenhouse components and field disease severity did not contribute to the same extent to the phenotypic variance. The LOD rank of seven QTLs was different between field and greenhouse traits (Fig. 4). For *Qlr.inra-3Bb.1* and *Qlr.inra-3Bb.2,* LOD rank was higher for greenhouse than for field traits. For *Qlr.inra-3Db*, *Qlr.inra-4Bb*, *Qlr.inra-4Da*, *Qlr.inra-6Aa*, and *Qlr.inra-7Aa* LOD rank was higher for field than for greenhouse traits. The mean R^2 of the QTLs as well changed between QTLs found in greenhouse and in the field (see supplementary material). However, noticeable differences in R^2 occured only for *Qlr.inra-3Bb.2*, *Qlr.inra-4Da*, and *Qlr.inra-7Aa*.

Six of the thirteen identified QTLs displayed isolate specificity in field experiments, (Fig 5) four of them being found exclusively with isolate P5 (*Qlr.inra-1Aa*, *Qlr.inra-2B*, *Qlr.inra-3Bb.2*, *Qlr.inra-4Da*), and two of them being found exclusively with isolate P3 (*Qlr.inra-3Bb.1*, *Qlr.inra-4Bb*). However, these last two QTLs were also found for isolate P5 in greenhouse experiments. For the seven QTLs found with both isolates in field experiments, the LOD values and mean R^2 varied between isolates. For *Qlr.inra-2D*, *Qlr.inra-3Db*, and *Qlr.inra-6B*, LOD values and mean R^2 were higher with isolate P5 than with isolate P3. Conversely, *Qlr.inra-2Ab, Qlr.inra-6Aa, and Qlr.inra-7Aa* displayed higher LOD values and mean R^2 with isolate P3 than with isolate P5.

The QTLs were qualified as having major, moderate or minor effect on resistance, when R^2 was greater than 0.2, between 0.2 and 0.1, and lower than 0.1, respectively (Table 2).

Both parental cultivars participated as sources of resistance in the DH population (Table 2), with eight QTLs coming from cultivar Balance, and five QTLs coming from cultivar Apache.

Table 2. Profile of QTLs for quantitative resistance to leaf rust in the Apache - Balance DH population.

A QTL name	B Flanking markers	C QTL Position (cM)	D Max. Conf. Int.	E Experiment	F Epidemic stage	G Component	H Iso-specificity	I LOD rank	J mean R^2	K Effect	L Source	QTLs already identified at the same position (Reference)
Qlr.inra-1Aa	*Pt733361-Pt671596*	0.0 - 9.7	0.0 - 28.0	Cap-09, Cap-10	Early	-	yes	1, 2	0.092	Minor	Apa	
Qlr.inra-2Ab	*Pt0568-un3*	0.0 - 23.5	0.0 - 31.8	Cap-10, Mai-09 / G1, G2	Late	SPL, LS	no	1	0.087	Minor	Apa	QTL for leaf rust, from Apache, near gene cluster *Yr17-Lr37-Sr38* (O. Robert, pers. comm.)
Qlr.inra-2B	*gpw3032-Pt4133*	155.1 - 180.1	147.0 - 186.9	Cap-09, Mai-09 / G1	Late	LP	yes	1, 2, 3	0.143	Moderate	Bal	QTL for yellow rust, from Apache, near *Yr7* (O. Robert, pers. comm.)
Qlr.inra-2D	*Pt8330 - gpw3320*	33.0 - 63.1	27.1 - 104.5	All Field / G1	All	SPS	Quantitative	3	0.226	Major	Bal	QTL for septoria tritici blotch, from Balance, near dwarfing gene *Rht8*, and several QTLs of morphological traits (Tabib Ghaffary et al., 2011)
Qlr.inra-3Bb.1	*Pt741465-Pt1682*	77.1 - 92.2	77.1 - 110.0	Cap-10 / G1	Late	SPS	no	1, 2	0.122	Moderate	Apa	QTL for yellow rust, from Balance (O. Robert, pers. comm.)
Qlr.inra-3Bb.2	*Pt2757-Pt1867*	121.7 - 125.4	105.0 - 136.5	Cap-10 / G2	Early	All	yes	1, 2, 3	0.141	Moderate	Bal	
Qlr.inra-3Db	*Pt664804-gpw4163*	63.2 - 80.5	63.2 - 110.0	Cap-09 / G1, G2	Intermediate	LP, LS	Quantitative	1, 2	0.102	Moderate	Apa	
Qlr.inra-4Bb	*Pt3608-tPt4214*	17.6 - 26.5	0.0 - 44.6	Mai-10 / G2	Intermediate	SPS	no	1, 2	0.1	Minor	Apa	
Qlr.inra-4Da	*cfd54-cfd84*	0.0 - 8.6	0.0 - 8.6	Mai-10 / G1, G2	Early	SPL, LS	yes	1, 2	0.132	Moderate	Bal	
Qlr.inra-5Bb/7Bb	*Pt7720-wmc517*	0.0 - 54.9	0.0 - 69.9	Cap-10, Mai-09, Mai-10	All	-	no	1, 2	0.08	Minor	Bal	
Qlr.inra-6Aa	*Pt671799-Pt1742*	11.3 - 31.9	8.0 - 34.1	Cap-09, Cap-10, Mai-09 / G1	Late	LP	Quantitative	1, 2	0.131	Moderate	Bal	
Qlr.inra-6B	*Pt4716-Pt4388*	0.0 - 16.3	0.0 - 50.0	Mai-09, Mai-10	All	-	Quantitative	1, 2	0.158	Moderate	Bal	
Qlr.inra-7Aa	*Pt0639-Pt7105*	0.0 - 20.6	0.0 - 25.6	Mai-09, Mai-10 / G2	All	SPL	no	2, 3	0.396	Major	Bal	QTL for leaf rust, from Balance, near *Lr20* (O. Robert, pers. comm.)

[A] : QTLs names according to the nomenclature of the Catalogue of gene symbols for wheat (McIntosh et al. 2010).
[B] : Name of the molecular flanking markers. Position of each QTL varied across the different analyses (location, replicate, year) conducted. The markers indicated are the most external ones, combining information from all analyses.
[C]: Position of the markers for QTL with LOD values greater than 2 (LOD rank 1) or superior to LOD threshold value of 1000 permutations (LOD rank 2 or 3). Positions are in centiMorgans, respective to the first marker of each linkage group.
[D] : Maximal confidence interval of QTLs, in centiMorgans. Confidence interval of each QTL varied across the different QTL analyses conducted. The values indicated here correspond to the largest confidence interval, combining information from all analyses.
[E] : Field and greenhouse experiments in which the QTL was detected (see table 1 for the code of each experiment).
[F] : Field epidemic stage at which the QTL was detected, classified as Early, Intermediate, and Late, corresponding to disease severity scoring at first, intermediate or last date. All = QTLs found for all scoring dates.
[G] : Component of quantitative resistance for which QTL was found in greenhouse experiments (IE = infection efficiency, LP = latent period, SPL = spore production per lesion, LS = lesion size, SPS = spore production per unit of sporulating tissue). All = QTLs found for all components.
[H] : Isolate-specificity of QTLs: no = QTL detected for both isolates, yes = QTL detected for isolate P5 only, Quantitative = QTL detected for both isolates, but with differential effect against isolates.
[I] : Minimal and maximal LOD rank across the different analysis. LOD values were classified into categories, 1, 2, and 3 as follows: LOD values comprised between 2 and the LOD threshold of 1000 permutations; LOD values comprised between the LOD threshold of 1000 permutations and 7; and LOD values greater than 7.
[J] : Mean percentage of the total phenotypic variance explained by the postulated QTL.
[K] : Classification of the effect of the QTL, based on mean R^2: Minor ($R^2<0.1$), Moderate ($0.1<R^2<0.2$), Major ($R^2>0.2$).
[L] : Parental source of resistance (Apa=Apache, Bal=Balance).

Figure 3. LOD rank and confidence interval for QTLs of quantitative resistance to wheat leaf rust identified in the Apache x Balance DH population. Location of the QTLs on the linkage groups was determined by composite interval mapping, from independent analyses of different experiments (see supplementary material for detailed information). Each square symbol represents a QTL found for the different experiment x replicate x trait combination. For each QTL, the confidence interval figured result from the superposition of the different confidence intervals obtained from the different analyses. Only the linkage groups carrying resistance loci are represented. Genetic positions (in centimorgans) of the markers are indicated on the right side of the chromosome bars. QTLs are represented with a square symbol positioned at the peak marker. Colours green, yellow and red figure LOD ranks 1, 2, and 3, respectively, associated to non significant, significant, and highly significant QTLs respectively, according to the LOD threshold value of 1000 permutations.

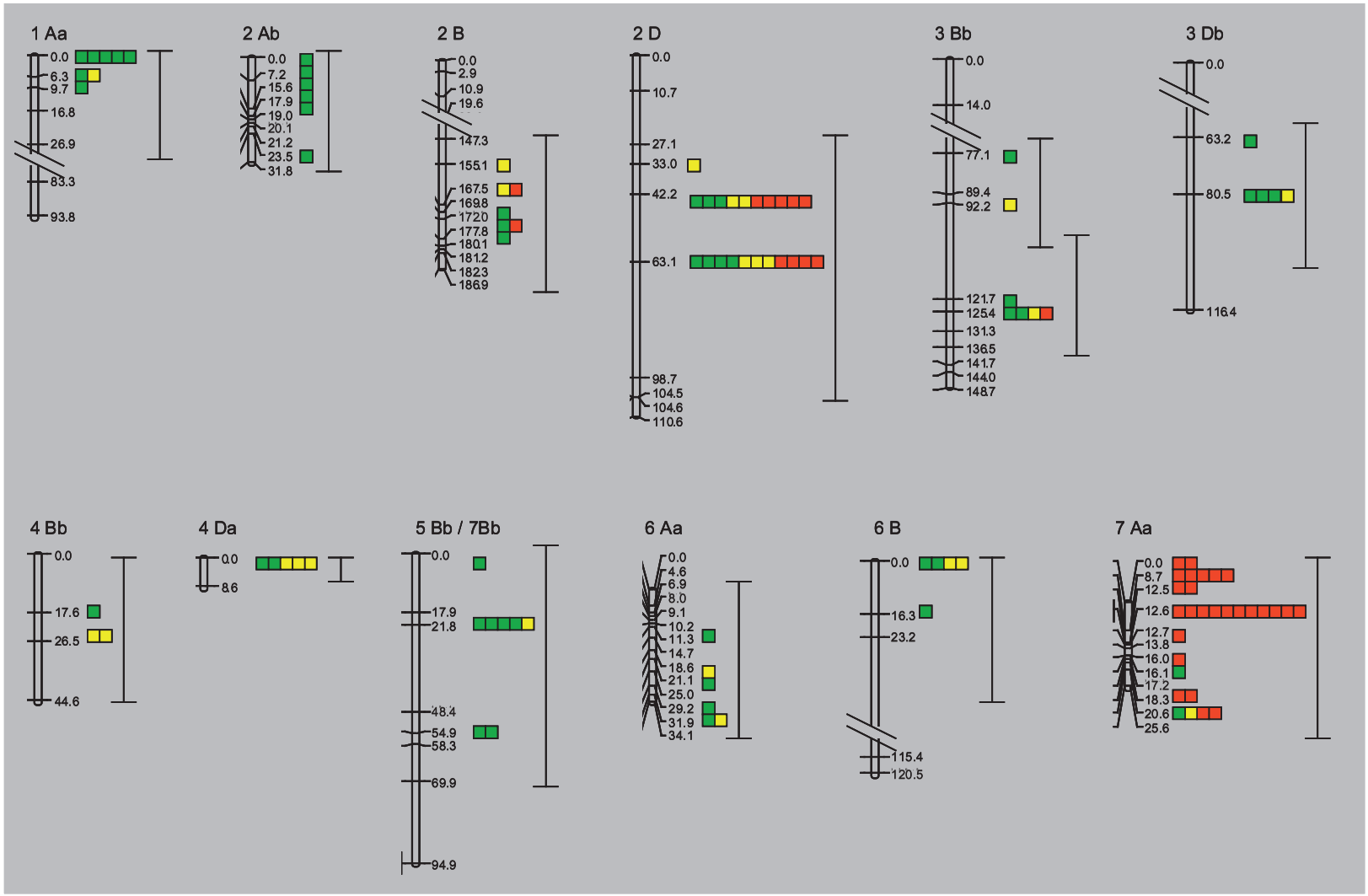


Figure 4. QTLs of quantitative resistance to wheat leaf rust identified in the Apache x Balance DH population, for field traits (black bars) and resistance components measured in greenhouse (dotted bars). The field traits were: Ni(imax), with Ni as the disease severity at scoring date i and imax as the total number of scoring dates; and AUDPC (Area Under the Disease Progress Curve). The resistance components were IE (infection efficiency), LP (latent period), SPL (spore production per lesion), LS (lesion size), SPS (spore production per unit of sporulating tissue). The traits are figured in green, yellow and red, for LOD ranks 1, 2, and 3, respectively, associated to non significant, significant, and highly significant QTLs respectively, according to the LOD threshold value of 1000 permutations. For each QTL, the confidence interval figured result from the superposition of the different confidence intervals obtained from the different analyses. The bars include linkage group regions containing markers, which LOD value exceed the LOD threshold value of 1000 permutations. For QTLs with LOD rank 1, bars represent linkage group regions containing markers with LOD>2.5. Genetic positions (in centimorgans) of the markers are indicated on the right side of the chromosome bars.

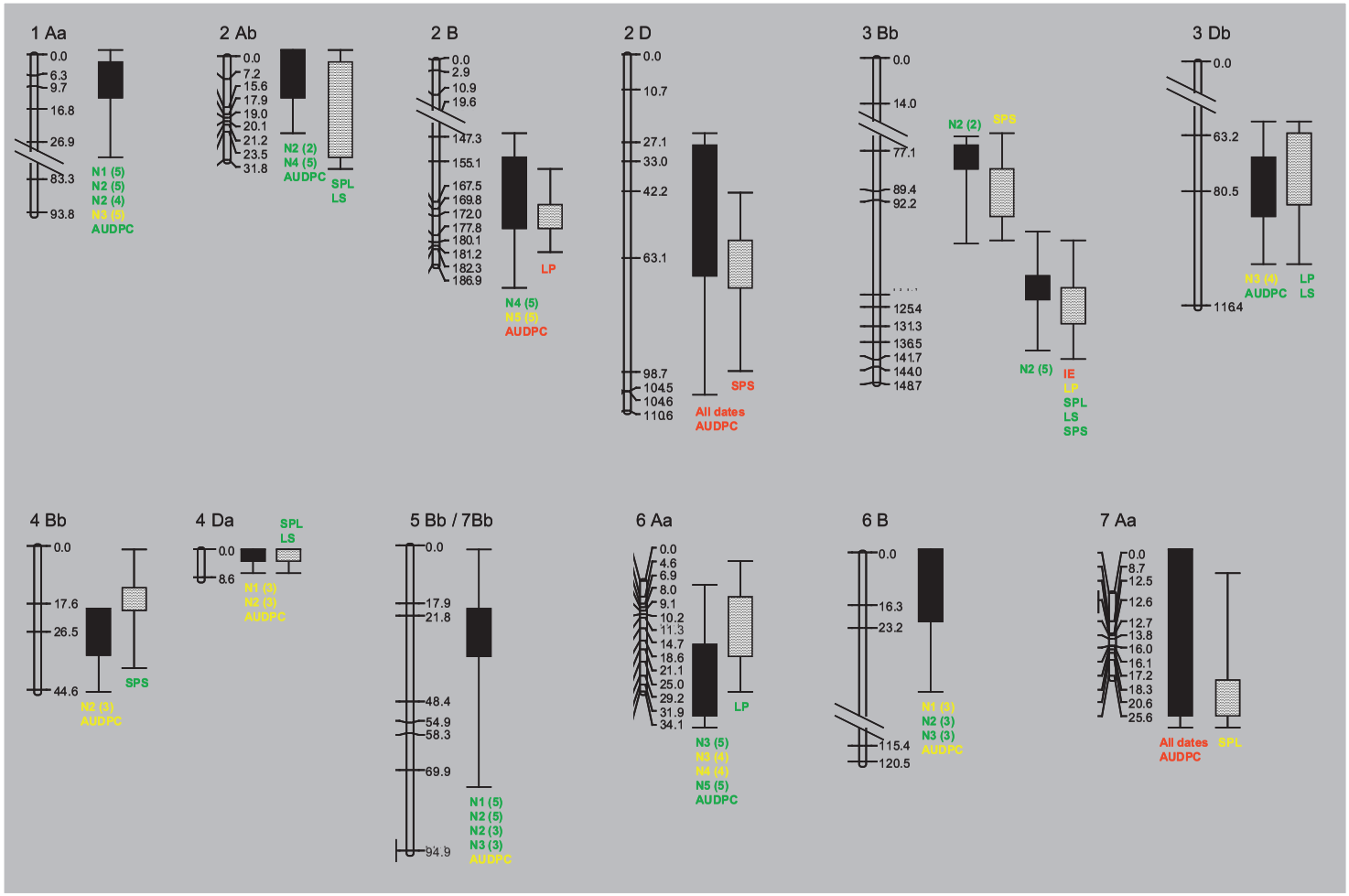
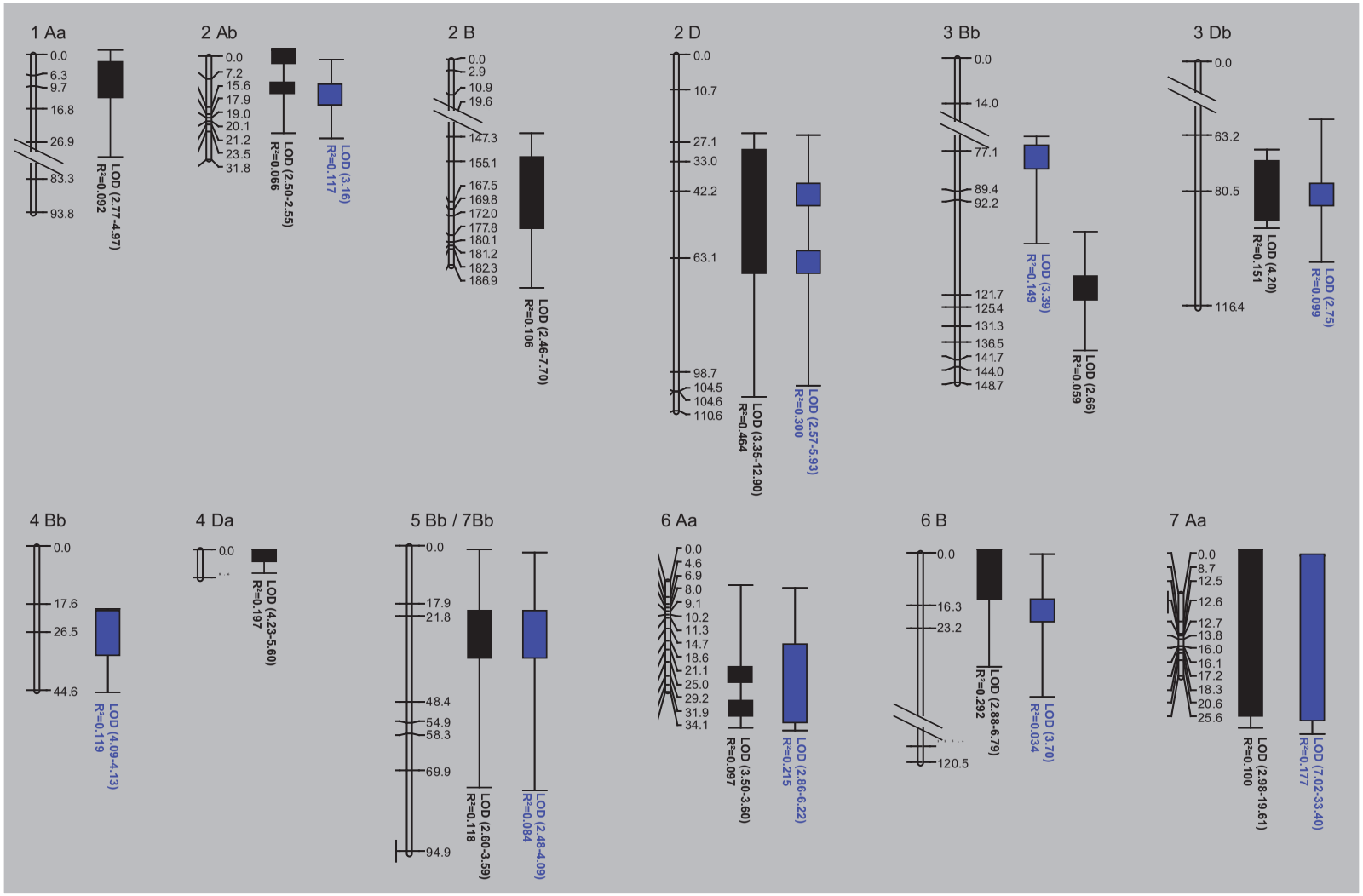


Figure 5. QTLs of quantitative resistance to wheat leaf rust identified in the Apache x Balance DH population, against isolate P5 (black bars) and isolate P3 (blue bars). The minimal and maximal LOD value (when LOD value was higher than 2), and the R^2 value are given. The bars include linkage group regions containing markers, which LOD value exceed the LOD threshold value of 1000 permutations. For QTLs with LOD rank 1, bars represent linkage group regions containing markers with LOD>2.5. For each QTL, the confidence interval figured result from the superposition of the different confidence intervals obtained from the different analyses. Genetic positions (in centimorgans) of the markers are indicated on the right side of the chromosome bars.



DISCUSSION

A high quality phenotyping was achieved through measurement of resistance components in controlled conditions, along with the mapping of the associated QTLs. Assuming that QTLs phenotypically expressed on different components govern different mechanisms of resistance (Chung *et al*., 2010), the present study provided information on the diversification level of the set of QTLs detected here.

Ten of the thirteen QTLs identified were associated to an effect on resistance components measured in the greenhouse. Most of these QTLs were involved in only one or two components, supporting the hypothesis that different genetic factors are involved in the different resistance components (Young, 1996). Six QTLs had an effect on only one component, and three QTLs had an effect on two components (Table 2). The single component QTLs affected LP, SPS, or SPL. The three QTLs impacting simultaneously two components always affected LS, in addition to LP or SPL. *Qlr.inra-3Db* affected both LS and LP, which can correspond to the observation commonly made that a large lesion size tend to be associated with a slow emergence of lesions, i.e. a long latent period. Association of LS with SPL could be expected, as the amount of spores produced by a single lesion (SPL) is determined both by the size of the lesion (LS) and the amount of spores produced by unit of sporulating tissue (SPS). In the case of *Qlr.inra-2Ab* and *Qlr.inra-4Da,* the reduced SPL observed could be attributed to their effect on LS.

There was only one QTL which impacted all components together; however the different components were differentially affected, as the significance level of this QTL, represented by the LOD rank (Fig. 4), and the magnitude of its effect, represented by the mean R^2 (supplementary material), varied across components. *Qlr.inra-3Bd.2* highly impacted IE, and, to a lesser extent, LP; its effect on sporulation components (SPS, LS, and SPL) was low and below the LOD treeshold value of 1000 permutations.

A diversified genetic basis of four different QTLs was found for LP and sporulation components (Table 2), whereas only one QTL was associated to IE . These results were in agreement with the resistance components investigated in the parentals cultivars Apache and Balance , which did not show any level of resistance for IE (Azzimonti *et al*., 2012).

A severe selection of markers was carried out during the map construction, in order to avoid distorted markers. This yielded the required correct assignment of QTLs on regions of linkage groups. However, in three cases (*Qlr.inra-2Ab*, *Qlr.inra-2D and Qlr.inra-7Aa)*, the

assumption of a single QTL could be questioned, because of the large distance between peak markers obtained.

To conclude, in this breeding material we found diversity in QTLs acting on single components, or on several combinations of components, giving a wide array of genes to be used in breeding.

QTL expression is highly dependent on environmental factors (Doerge, 2002; Eeuwijk *et al*., 2010). Components of quantitative resistance can be measured precisely in controlled conditions. However, QTLs identified in greenhouse conditions have to be evaluated in the field to determine their impact on field resistance, therefore their usefulness in breeding. In the present study, all the QTLs found for components in greenhouse conditions were also involved in resistance in field conditions. Most of the QTLs involved on LP or sporulation components had a significant and strong effect on field resistance, according to their high LOD rank (Fig. 4) and their high mean R^2 (supplementary material). The exceptions were *Qlr.inra*-2Ab, Qlr.*inra-3Bd.1* and Qlr.*inra-6Aa*, for which the effect on field resistance was low. Conversely, *Qlr.inra-3Bd.2* had a high effect on IE but its effect on field resistance was low.

According to the criterion chosen in this study, QTLs with LOD values below the significance level, comprised between values of 2 and LOD threshold values of 1000 permutations, were taken into account and classified as LOD rank 1. This criterion was useful, as the accumulation of LOD-rank 1 values allowed to validate the corresponding QTLs expressed in different conditions,. By this means, the effect of *Qlr.inra-3Db*, *Qlr.inra-4Bb*, *Qlr.inra-4Da*, and *Qlr.inra-6Aa* on components could be detected, and compared to their effect on field resistance. *Qlr.inra-2Ab* did not fill our criterion, because it was never found with a significant LOD threshold value of 1000 permutations. However, we decided to keep it, because it was also found previously by O. Robert (personal comunication) for wheat leaf rust, in the same DH population.

Analyzing separately different field scoring dates revealed resistance QTLs involved at different stages of the epidemic. QTLs detected at first, intermediate, and last scoring dates could be qualified as "early", "intermediate", and "late" respectively (Table 2). All these QTLs were also found for AUDPC, except *Qlr.inra-3Bd.1* and *Qlr.inra-3Bd.2*. Therefore, we concluded that these date-specific QTLs also contributed to the reduction of the overall disease. The very few studies aiming at detecting QTLs involved at the different stages of field epidemics, all confirmed that this way of analyzing separetely scoring dates allow to

detect more QTLs involved in field resistance, than a global analysis of AUDPC (Ramburan *et al*., 2004; Mallard *et al.*, 2008; Dedryver *et al*., 2009).

Specific interactions between host and pathogen genotypes are involved in pathogen adaptation for quantitative resistance, leading to an erosion of quantitative resistance (Krenz *et al*., 2008). Therefore, isolate specificity has to be evaluated in breeding material to avoid the use of highly specific breeding sources.

In the present study, information on the specificity of the identified resistance QTLs could be obtained from field data only, greenhouse experiments being conducted with a single pathotype because of space limitations. However, as all the identified QTLs were expressed in field conditions, we were able to evaluate isolate-specificity for the whole set of QTLs detected. Different levels of specificity were found in this DH population on field resistance against isolates P3 or P5. *Qlr.inra-2Ab* and *Qlr.inra-5Bb/7Bb* displayed no isolate specificity, because they were found with both isolates, with similar LOD values and mean R^2 (Fig. 5). *Qlr.inra-2D*, *Qlr.inra-3Db*, *Qlr.inra-6Aa*, *Qlr.inra-6B*, and *Qlr.inra-7Aa* showed a quantitative level of specificity, because even if they were detected with both isolates, their effect was more important against one of the isolates. Finally, *Qlr.inra-1Aa*, *Qlr.inra-2B*, *Qlr.inra-3Bb.2*, and *Qlr.inra-4Da*, were clearly isolate specific against isolate P5. These results were in agreement with the specificity of quantitative resistance to isolates P3 and P5, displayed by the parents of this DH population in the greenhouse in aprevious study (Azzimonti *et al*., 2012): for the parental cultivar Balance, the resistance level was found highly specific, with significant differences between P3 and P5 for all the five resistance components; for the parental cultivar Apache, there was no isolate-specificity for any of the components. Therefore, it could be expected that QTLs provided by Balance displayed isolate specificity, while QTLs provided by Apache did not. From the eight QTLs transmitted by Balance (Table 2), six showed some level of isolate specificity, whereas from the five QTLs transmitted by Apache, three were not isolate specific; the only QTL from Apache that was clearly isolate specific (*Qlr.inra-1Aa*) was expressed in the field epidemic only, and not in the greenhouse.

Based on our results, a profile of each QTL could be drawn (Table 2). These profiles summarize the information about the breeding usefulness of the different QTLs. The usefulness of the QTLs will depend on the objectives of the breeding program. Five of the QTLs found in this study (*Qlr.inra-2Ab*, *Qlr.inra-3Bb.1*, *Qlr.inra-3Db*, *Qlr.inra-4Bb*, and *Qlr.inra-6Aa*) were involved in resistance displayed both i) at a particular stage of the field epidemic, and ii) for a particular component, with no or small difference in the level of

resistance against the two isolates. If the objective is to diversify the genetic basis of resistance, these QTLs can be used. However, if the objective is to enhance the resistance level, QTLs like *Qlr.inra-2D*, *Qlr.inra-6B*, or *Qlr.inra-7Aa* would be preferred, because they had a moderate to major effect on resistance, all along the epidemic development. Finally, the use of QTLs that showed isolate-specificity should be avoided or limited because of the possible rapid pathogen adaptation, especially in the case of *Qlr.inra-3Bb.2*. As this QTL was involved in all resistance components, pathogen adaptation to a resistance based on this single QTL would breakdown resistance for all components at the same time.

The DH population used here was already used to investigate the genetic determinism of quantitative resistance to Septoria tritici bloch (Ghaffary *et al*., 2011), yellow rust and leaf rust (O. Robert, personal communication). Five of the QTLs found here were also found in these studies (Table 2). Four of them were found in the vicinity of known genes (*Qlr.inra-2B*, *Qlr.inra-2D*, and *Qlr.inra-7Aa*), or resistance gene clusters (*Qlr.inra-2Ab*). In the present study, some other significant QTLs were sporadically found, but they were dropped because they did not fullfill our validation criterion (see suplementary material). It would be interesting to investigate the presence of these environemental fluctuant QTLs in other breeding populations.

Proposal of the QTLs found here for practical breeding will require to improve the linkage map with the addition of more markers, to get a better precision for QTLs positions. Moreover, the introgression of interesting QTLs into Near Isogenic Lines would allow to study their effect and characteristics with much better precision, and to compare directly the effect of the QTLs in components and in the field resistance, as successfully performed by Marcel *et al*. (2008) for barley leaf rust. Lastly, fine mapping of the most interesting QTLs would allow to find tightly linked markers to use in marker assisted selection (Collard *et al*., 2005).

Durability of resistance will be enhanced if adaptation of the pathogen can be delayed. In the present agronomical context, diversification in the mechanisms of genetic resistance seems to be a reliable alternative to avoid pathogen adaptation. The analysis of QTLs involved in components of resistance developped here, allowed us to found the genetic bases of diversified mechanisms, that can be further used in breeding.

Discussion générale

In common with other workers, the author accepts the convenience of cataloguing resistance in two types. Nature, I am sure, never intended this division.

Clifford, B. C. 1975. Stable resistance to cereal diseases : Problems and progress.
Rep. Welsh Plant Breed. Stn. 1974, pp. 107-13

Discussion générale

Cette thèse a comme enjeu le développement de sources durables de résistance à la rouille brune du blé. La diversification de sources de résistance non ou peu spécifiques permettrait d'augmenter la durabilité de la résistance quantitative. Nous avons proposé de baser la diversification des sources de résistance sur la caractérisation des composantes. En conséquence, l'objectif global de la thèse était de déterminer, pour un ensemble de sources potentielles de résistance :

1) la diversité, la variabilité, et la spécificité des composantes de la résistance, à l'échelle de la plante ;

2) l'impact du niveau de résistance des composantes, à l'échelle de l'épidémie au champ ;

3) le déterminisme génétique des composantes de la résistance.

Pour atteindre ces objectifs, nous avons confronté un ensemble de variétés et de lignées hôtes, à trois isolats pathogènes. Dans le premier chapitre, nous avons mis en évidence une grande diversité des composantes affectées, une variabilité importante pour toutes les composantes, ainsi que différents niveaux de spécificité dans les sources de résistance étudiées. Nous avons aussi démontré qu'une haute spécificité au niveau des composantes peut entraîner une spécificité de la résistance à l'échelle épidémique (Annexe chapitre 1, Appendix 1).

Dans le deuxième chapitre, la modélisation des composantes a permis d'établir que l'ensemble des composantes intervient dans la détermination du niveau de résistance à l'échelle épidémique, mais avec une magnitude différentiée selon les composantes, et que l'importance d'une composante pour la détermination du niveau global de résistance change selon les étapes de l'épidémie. Nous avons aussi démontré que les trois isolats utilisés ont un profil d'agressivité contrasté vis-à-vis des différentes composantes, et que ces différences peuvent entraîner des différences d'agressivité lors du développement épidémique (Annexe chapitre 2, Appendix 1).

Dans le troisième chapitre, nous avons identifié des déterminants génétiques pour les différentes composantes, ce qui nous a permis de démontrer que la diversité au niveau phénotypique en composantes est liée à une diversité génotypique. Nous avons aussi déterminé que la majorité des QTLs étaient impliqués dans la résistance à des étapes précises de l'épidémie.

L'intégration de l'analyse de la spécificité de la résistance quantitative à chaque étape de notre étude (composantes, champ, QTLs), nous a permis d'apprécier son influence dans le matériel étudié. Les cultivars utilisés représentent toute une gamme de spécificité, de l'absence d'interaction spécifique (Apache) à des interactions spécifiques pour presque toutes les composantes (Balance, LD7 et Morocco). Cette spécificité s'est répercutée sur l'épidémie au champ seulement dans les cas où la spécificité a affecté d'une façon importante la plupart des composantes (LD7). La population HD étudiée était parfaite pour mesurer le niveau de spécificité de la résistance dans son support génétique, car elle était issue des parents présentant une résistance très spécifique (Balance) et la moins spécifique (Apache) par rapport à aux isolats retenus. L'analyse de la spécificité dans le déterminisme génétique à deux échelles (qualitative : présence ou absence du QTL avec les deux isolats; quantitative : LOD value et R^2 du QTL majeur avec un des isolats) nous a permis d'estimer précisément le niveau de spécificité pour chaque QTL. Nous avons trouvé, ici aussi, une gradation dans le niveau de spécificité, en allant des QTLs qui agissent avec un seul isolat, en passant par des QTLs avec des effets plus ou moins grands selon l'isolat, jusqu'à des QTLs impliqués dans la résistance envers les deux isolats.

Nos résultats démontrent, pour un même ensemble de matériel, l'importance et la variation de la spécificité i) au niveau des composantes mesurées sur un cycle infectieux ii) au niveau de la sévérité de maladie mesurée lors de l'épidémie au champ et iii) au niveau du déterminisme génétique. L'intégration de ces trois niveaux d'étude nous a permis d'établir que l'estimation de l'impact de la spécificité sur la durabilité d'une source de résistance, doit être nuancée en fonction du niveau de cette spécificité. Ces résultats corroborent ceux obtenus pour d'autres pathosystèmes, chez lesquels le niveau de spécificité de la résistance quantitative peut être très variable. Nous concluons que la caractérisation de sources de résistance quantitative doit inclure une estimation de leur niveau de spécificité selon les trois niveaux explicités ci-dessus, car ainsi que l'a suggéré Johnson (1976) "it is not simply the presence or absence of differential interactions that matters but their magnitude relative to the differences in resistance between cultivars".

La mesure des composantes agissant dans toutes les étapes du cycle infectieux, avec un bon niveau de précision, a été déterminante pour détecter des variations parfois subtiles. Ceci nous a permis de mettre en évidence une gradation très fine du niveau de résistance pour chaque composante dans l'ensemble du matériel étudié (chapitre 1), et de relier cette gradation avec le niveau de résistance au champ (chapitre 2). En particulier, la méthode développée ici

pour la mesure de l'efficacité de l'infection nous a permis d'apprécier correctement son importance dans la mise en place de la résistance lors du cycle infectieux, ainsi que son incidence sur le niveau de résistance à différentes étapes de l'épidémie. Mesurer précisément l'efficacité de l'infection nous a également permis de:

i) choisir deux cultivars (PBI et Trémie) comme de bons candidats pour la sélection, grâce à leur haute résistance pour cette composante ;

ii) déterminer que l'efficacité de l'infection participe au maintien du niveau de résistance lors des étapes tardives de l'épidémie au champ, au moment où l'effet d'autres composantes commence à s'estomper. En revanche, nous n'avons pas pu avoir un aperçu du déterminisme génétique de l'efficacité de l'infection, car les cultivars choisis pour cartographier les QTLs agissant sur les composantes n'avaient pas un bon niveau de résistance pour l'efficacité d'infection.

De même, la décomposition de la sporulation par lésion en taille de lésion et sporulation par unité de surface sporulant, nous a permis de :

i) déterminer que l'impact de ces trois composantes sur le niveau de résistance au champ est différent.

ii) trouver une relation négative entre la taille de lésion et la sporulation par surface sporulante, ce qui reflète une contrainte exercée par la plante qui pourrait être exploitée en sélection. Cette relation négative nous a permis d'expliquer la relation entre la taille de lésion et le niveau de résistance au champ, ainsi que l'absence de relation entre la sporulation par lésion et la résistance au champ.

Prendre en compte la dépendance des composantes à la densité des lésions a aussi été indispensable pour une détermination correcte du niveau de résistance pour les composantes. En effet, ignorer l'effet de la densité de lésions aurait entraîné :

- plus de chevauchement dans la classification des cultivars en termes du niveau de résistance pour chaque composante (tableau 3, chapitre 1) ;

- l'absence d'interactions spécifiques avec les isolats pour certaines variétés (figure 2, chapitre 1) ;

- l'absence de certaines relations significatives et un R^2 faible pour les corrélations composantes - résistance au champ (figure 1, chapitre 2);

- l'absence de certaines relations significatives et un R^2 faible pour les corrélations entre composantes (figure 3, chapitre 2) ;

- moins de QTLs détectés pour les composantes de sporulation (figure 4, chapitre 3).

Nous concluons que l'analyse des composantes doit prendre en compte l'effet de densité-dependance, y compris lors d'expériences réalisées à densité d'inoculation supposée, ou vérifiée, constante.

Dans cette thèse, nous avons caractérisé pour la première fois la résistance quantitative à la rouille brune du blé d'un ensemble de variétés et lignées issues du matériel français utilisé en sélection. Si cela avait déjà été fait pour le matériel de sélection d'autres régions du monde (par exemple, au CIMMYT), notre travail vient combler un vide important, visant à élargir l'utilisation de la résistance quantitative dans la sélection variétale française. La principale conclusion de notre étude dans ce domaine est que le matériel proposé par les sélectionneurs français contient une grande diversité phénotypique pour la résistance quantitative à la rouille brune, ce qui donne de bonnes bases pour sélectionner des résistances plus durables.

Les résultats obtenus pendant cette thèse, ainsi que leur confrontation et leur discussion avec les sélectionneurs du CIMMYT pendant mon séjour au Mexique, permettent de proposer le schéma de sélection suivant, qui intègre la caractérisation des composantes de la résistance.

1. Caractérisation en serre des composantes, sur des plantes adultes, pour les génotypes hôtes porteurs de résistance quantitative, par confrontation à des isolats représentatifs de la population pathogène. Cette étape requiert un investissement important (travail en serre avec des plantes adultes, mesure précise des composantes), mais il se fait une seule fois. Le choix d'isolats représentatifs de la population pathogène permet de réduire le nombre d'isolats à tester.

2. Caractérisation au champ des génotypes hôtes pour déterminer l'impact des composantes en conditions épidémiques.

3. Choix des parents pour les populations de cartographie.
C'est à cette étape que la caractérisation du matériel pour les composantes va se révéler décisive. La gamme des composantes affectées, le degré de variation dans le niveau de résistance pour chaque composante, le degré de spécificité (dans la quantité des composantes affectées spécifiquement, et dans l'ampleur de la différence de résistance envers différents

isolats), et, finalement, l'impact de ces composantes sur le niveau de résistance au champ, vont permettre de choisir les parents à utiliser selon les objectifs du programme de sélection.

4. Détermination, dans les populations de cartographie, des QTLs agissant sur les composantes de la résistance, en serre, et sur le niveau de résistance au champ, par confrontation à des isolats représentatifs de la population pathogène.
Les deux contraintes importantes de cette étape sont la quantité de plantes à suivre dans les expériences en serre (donc le nombre de familles de la population), et le nombre de marqueurs moléculaires disponibles pour cartographier les QTLs. Suivant l'exemple des analyses QTL faites à partir des expériences en serre réalisées pendant cette thèse, nous sommes en mesure d'indiquer qu'un bon niveau de détection des QTLs agissant sur les différentes composantes peut être atteint avec une population d'environ 100 lignées, et une carte avec un nombre modeste de marqueurs (moins de 500). Ici aussi, une seule expérimentation en serre, avec un minimum de 10 répétitions par lignée, pourrait donner des résultats corrects. Au champ, il serait préférable de faire deux expérimentations dans des sites différents, avec deux répétitions.

5. Choix des QTLs les plus intéressants, selon les objectifs du programme de sélection et leur cartographie fine, afin d'identifier des marqueurs à utiliser en sélection assistée par marqueur.

Dans cette thèse, nous avons achevé les étapes 1 à 4, sur un intervalle de trois ans, avec trois personnes travaillant à plein temps. Cette évaluation ne tient pas compte du travail effectué en amont pour mettre au point la méthodologie de mesure des composantes, ni du suivi annuel des populations pathogènes. Le schéma de sélection proposé n'est fondamentalement pas très différent des schémas de sélection déjà implémentés par les sélectionneurs. Des schémas similaires ont déjà été implémentés avec succès au CIMMYT.

Cependant, l'éclaircissement de certains points est nécessaire pour améliorer l'efficacité du schéma décrit ci-dessus.

Dans le premier chapitre, nous avons identifié quatre génotypes utilisables en sélection, avec l'objectif de diversifier la résistance quantitative, tout en augmentant son niveau. La résistance d'Apache est non spécifique pour la latence et la sporulation. Nous avons trouvé cinq QTLs intéressants pour cette variété (chapitre 3). LD7 a un très bon niveau de résistance pour toutes les composantes. La spécificité de la résistance de cette lignée est

forte, mais le niveau de résistance reste très élevé, même envers l'isolat auquel la lignée est la plus sensible. L'analyse du déterminisme génétique de la résistance de cette lignée pourrait donner les QTLs les moins spécifiques. PBI et Trémie présentent une résistance élevée et non spécifique pour l'efficacité d'infection, et une résistance moyenne pour les autres composantes. Le niveau de résistance au champ de ces quatre génotypes est élevé (Apache, LD7) ou moyen (PBI, Trémie).

En collaboration avec l'ensemble des établissements de sélection du blé en France, et avec le soutien financier du Fonds de Soutien à l'Obtention Végétale en blé tendre (FSOV), nous avons produit des populations HD ou SSD issues de ces quatre génotypes, croisés avec la variété sensible Écrin. Le niveau de résistance de ces populations a été mesuré dans des essais au champ sur plusieurs sites en 2011 et 2012. Nous poursuivrons la caractérisation de QTLs sur ces populations au cours d'un projet de post-doc qui vient d'être accepté. Ce projet a comme objectifs la cartographie des QTLs de ces quatre populations, et la cartographie fine de certains QTLs trouvés dans la population HD issue du croisement des variétés Apache et Balance. En outre il permettra de développer l'approche de modélisation initiée dans cette thèse, afin de mieux comprendre le lien entre le niveau de résistance pour les composantes et le niveau de résistance observé en conditions épidémiques.

Annexes

Annexe Chapitre 1

Appendix 1: Determination of isolate specificity in field levels of quantitative resistance at different dates of the epidemic.

Appendix 1: Determination of isolate specificity in field levels of quantitative resistance at different dates of the epidemic.

In the first chapter of the thesis, isolate-specific interactions were found for all the cultivars tested, except for Apache. The objective of this appendix was to search for isolate-specific interactions for these cultivars in the level of resistance during the course of epidemic in field conditions.

All these cultivars, plus other cultivars not used in greenhouse experiments, were confronted to the same three isolates in field conditions.

MATERIALS AND METHODS

The design of the field experiments is fully described in Chapter 2 of this Thesis.

Disease severity (DS) was scored according to the modified Cobb Scale where percentage of disease tissue was visually estimated on flag leaves, according to Peterson *et al*. (1948). The qualitative host response to infection, namely infection type, was also evaluated as described in Roelfs *et al*. (1992). Four infection types were used: R (resistant, with no sporulating tissue in the lesions, or of very small size and surrounded by necrosis and chlorosis), MR (moderately resistant, with a small area of sporulating tissue surrounded by chlorosis or necrosis), MS (moderately susceptible, with a sporulating area of moderate size and no chlorosis or necrosis), and S (susceptible, with large sporulating lesions without chlorosis nor necrosis). Intermediate cases between these four infection types were also identified. A coefficient was attributed to each infection type, from 0 for the R infection type to 1 for the S infection type (Table 3). This coefficient was used to calculate a corrected disease severity, cDS, as the product of observed disease severity (DS) by the infection type coefficient. This allowed distinguishing the disease severity accounted for by sporulating tissue only from the overall severity that included all diseased tissu.

Disease severity assessments started when disease severity on the spreader cultivar reached 100%, and when the first symptoms began to appear on the flag leaves of the tested cultivars. Three severity assessments were conducted, *i.e*., every five to seven days until the end of the epidemic. At each assessment time, all replicates were scored.

All statistical analyses, performed with Splus software (Lucent Technologies, Inc.), were based on linear models. Each variable (DS_1, DS_2, DS_3, cDS_1, cDS_2, cDS_3) was analysed as a function of the cultivar, the isolate and their interaction. In all ANOVAs, cultivar, isolate and their interaction were considered as fixed factors. Effects were evaluated with type III sum of squares and a significance level set at P=0.05. Multiple comparisons of means were performed with Tukey-Kramer range test with significance level set at P=0.05.

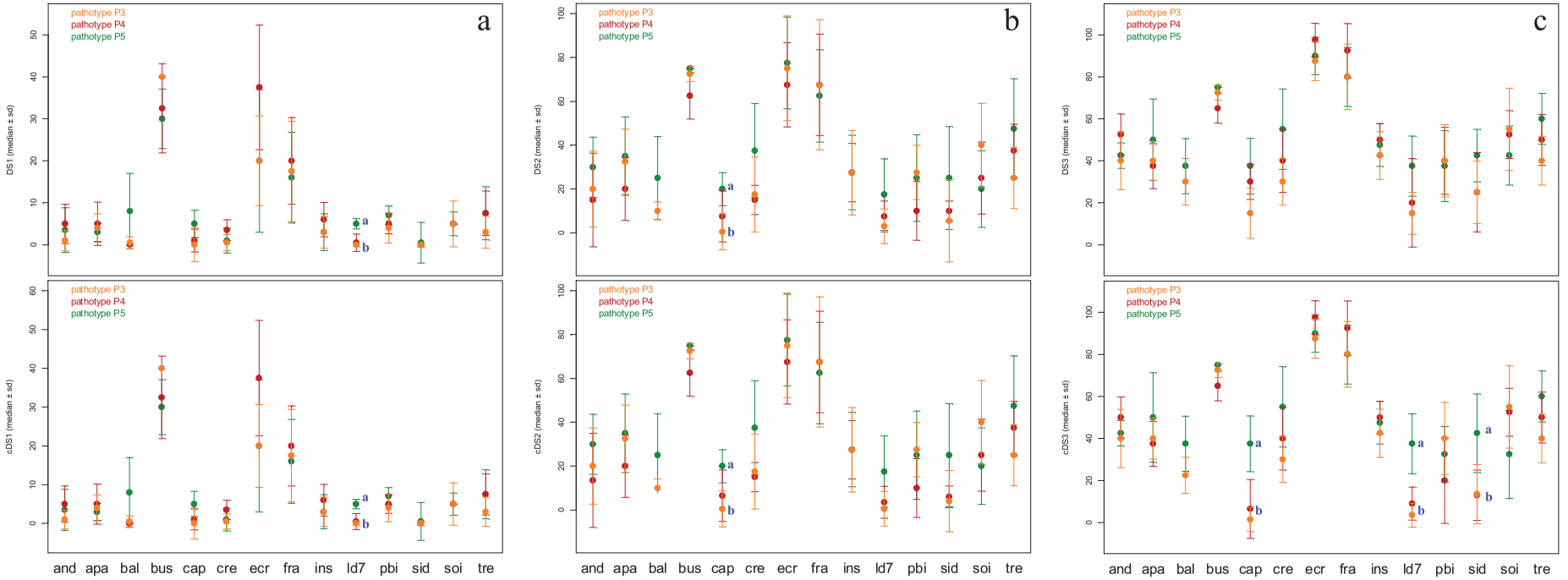
RESULTS

The cultivar, isolate and their interaction factors were all significant ($P<0.05$). Significant differences between isolates were found for the level of resistance in cultivars LD7, Caphorn, and Sideral (Figure 1). All these cultivars were more susceptible against isolate P5 than against the other two isolates. LD7 was more susceptible against isolate P5 at the dates 1 and 3. Caphorn was more susceptible against isolate P5 at the dates 2 and 3. Sideral was more susceptible against isolate P5 only at the date 3. Significant differences between isolates for the cultivars LD7 and Caphorn at dates 1 and 2 were expressed in both disease severity and corrected disease severity. At date 3, significant differences between isolates were only found for corrected disease severity.

DISCUSSION

Isolate specifics interactions were not common in field epidemic conditions for most of the cultivars tested, whereas all the cultivars tested in the greenhouse had isolate interactions for resistance components. LD7 was the only cultivar, from those tested for components, that showed isolates interactions in field conditions.

Figure 1. Cultivar-by-isolate differential interactions for field disease severity traits at three different times of epidemic developement. Disease severity (DS_i) and corrected disease severity (cDS*i*) at (a) date 1, (b) date 2, and (c) date 3. For each cultivar, the median ± standard deviation is represented in yellow, red and green for isolates P3, P4 and P5, respectively; the letters indicate significant differences between isolates ($P < 0.05$, Tukey-Kramer test).The cultivar names are abbreviated as: and = Andalou, apa = Apache, bal = Balance, bus = Buster, cap = Caphorn, cie = Ciento, cre = Camp Remy, ecr = Ecrin, fra = Frandoc, ins = Instinct, ld7 = LD 00170-3, mor = Morocco, pbi = PBI-04-006, sid = Sideral, soi = Soissons, and tre = Tremie.



Annexes Chapitre 2

Appendix 1. Characterisation of aggressiveness profiles of isolates

Appendix 2. Validation of the models and supplementary materials

Appendix 1. Characterisation of aggressiveness profiles of isolates.

Introduction

Three isolates were chosen as representative of three different leaf rust pathotypes (labelled P3, P4 and P5) with different virulence combinations. The three pathotypes, virulent on all the most widely grown cultivars in France ,were selected on the basis of their contrasted frequency in the French populations over the period 2000-2008 (Table 1), respectively low, high and intermediate. The pathotypes were then postulated to represent different aggressiveness levels. For the sake of commodity, the isolates themselves will be referred to as P3, P4 and P5 in the following.

Using a subgroup of the cultivars used here and the same three isolates, we were unable to find different aggressiveness profiles, based on both the components affected and the specificity of the interaction, even if one of the isolates seemed more aggressive than the others (Azzimonti et al., 2012)a. The objective of this Annex was to explain the contrasted evolution of the frequency of the three isloates in natural populations; we analysed the aggressiveness profiles of the isolates, taking advantage of the extended set of cultivars and of the modeling approach (see Chapter 2).

Materials and methods

Analysis of variable relationships

The models described in Chapter 2 allowed us quantifying the interactions between isolates and cultivars through any of the variables $\theta_{c,i}$, with θ in

$\{IE, LP, LS, SPL, SPS, DS1, DS2, DS3$, *cDS1,c DS2,c DS3* $\}$.

Comparison of isolates

Hereafter we present the construction of the criterion that compares a resistance component among isolates. We first computed $\mathrm{prob}(\theta_{c,i} > \theta_{c,i'})$, i≠i', the posterior probability that

component θ was greater for isolate i on cultivar c than for isolate i' on cultivar c. Then, we only retained the cultivars where this probability was greater than 0.5 (i.e. on which isolate i had a greater infection efficiency than isolate i'). Finally, the criterion was divided by the number of cultivar – isolate pairs for which the infection efficiency was estimable, so that its value was kept <1. This led to a criterion $C(\theta,i)$ defined as the proportion of cultivars for which isolate i had the greatest estimated value of θ. $C(\theta,i)$ varied in (0,1) and is equal to 0 when θ is always smaller for isolate i than for the other isolates, across all cultivars, and to 1 when θ is always greater:

$$C(\theta, i) = \frac{\sum_{i' \neq i} \sum_c \delta\left(\text{prob}\left(\theta_{c,i} > \theta_{c,i'}\right) > 0.5\right)}{\text{number of estimable } \theta_{c,i}},$$

where $\delta\left(\text{prob}\left(\theta_{c,i} > \theta_{c,i'}\right) > 0.5\right)$ is equal to 1 when $\text{prob}\left(\theta_{c,i} > \theta_{c,i'}\right)$ is greater than 0.5 and to 0 otherwise.

Results

Aggressiveness profile of isolates .

The graphic representation of correlation between traits (Figs. 1 and 3, Chapter 2) shows that the three isolates were not always equally distributed. For example, in correlation graphics of field epidemics traits with IE (Fig. 1a, Chapter 2), isolate P4 tended to had small IE, isolate P5 tended to had high IE values, and isolate P3 was distributed along the whole range of IE values. Also, in the correlation between LS and SPS (Fig. 3c), isolate P5 tended to had low LS with high SPS, isolate P3 tended to had high LS with small SPS, and isolate P4 tended to had medium LS and SPS.

The aggressiveness profiles of the three isolates were derived from the analysis of $C(\theta,i)$, (Fig. 1). The $C(\theta,i)$ value of 0.5 was used as a threshold value. When $C(\theta,i) > 0.5$, the isolate had a higher trait value than the other isolates in more than half of the cultivars used; the isolate was considered more aggressive than the other isolates. This criterion is the same for all traits, except for LP and LS, where smaller values indicate higher aggressiveness.

Aggressiveness profiles for components of resistance.

Isolate P3 was more aggressive than the other isolates for IE and less aggressive for SPL, LS and SPS (Fig. 1a). The components that changed the most for isolate P3 in relation to

the other isolates were LS and SPS. Isolate P4 was more aggressive than the other isolates for SPL and SPS, but less aggressive for IE and LP. The components that changed the most for isolate P4 in relation to the other isolates were IE and LP. Isolate P5 was more aggressive than the other isolates for IE, LP, LS and SPS, but less aggressive for SPL. The components that changed the most for isolate P4 in relation to the other isolates were IE and LP.

Aggressiveness profiles for disease severity and corrected disease severity.

Aggressiveness profiles for field epidemic traits were constructed using the same threshold value of 0.5 for criterion $C(\theta,i)$,. For disease severity (Fig. 1b), isolate P3 was usually less aggressive than the other isolates; isolate P4 was usually less aggressive than the other isolates, except for DS1; and isolate P5 was usually more aggressive than the other isolates. The same general pattern of aggressiveness was found for corrected disease severity (Fig 1c). However, small differences were found. For isolate P3, when considering Disease Severity, aggressiveness increased along with the epidemic progress, but when considering corrected Disease Severity, aggressiveness was highest at cDS2 and lowest at cDS3. For isolate P4, when considering Disease Severity, aggressiveness decreased along with the epidemic progress, but when considering corrected Disease Severity, aggressiveness was lowest at DS2-IT. For isolate P5, aggressiveness increased between DS1 and DS2, and stayed at the same level for DS3, but when considering corrected Disease Severity, aggressiveness increased along with the epidemic progress.

Discussion

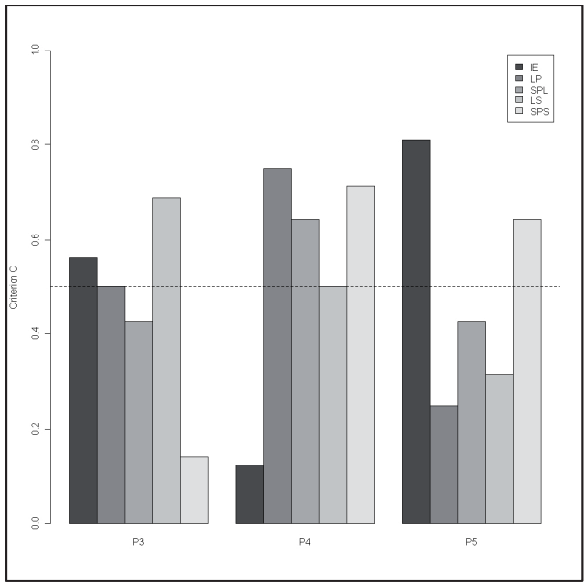
Aggressiveness profiles found here for isolates do not explain entirely the field frequency distribution of their cooresponding pathotypes. The disappearance of pathotype P3 from field populations could be explained by its low aggressiveness. Conversely, the high and medium-to-low frequency of pathotypes P4 and P5, respectively, in field populations, do not match its medium-to low and high aggressiveness levels, respectively. Two causes can be attributed to the differences found between aggressiveness levels and field frequency. First, only one isolate belonging to each pathotype was tested, and it could be possible that the aggressiveness level of the isolates used was not representative of the aggressiveness level of their corresponding pathotypes. It has been demonstrated that different aggressiveness levels can be found among isolates of the same pathotype (Lionel 1999, Flier 1999). Second, environmental factors, besides aggressiveness, can influence field distribution of the pathogen

(Johnson 1976), and could contribute to the discrepancy found between aggressiveness level and field distribution of pathotypes P4 and P5.

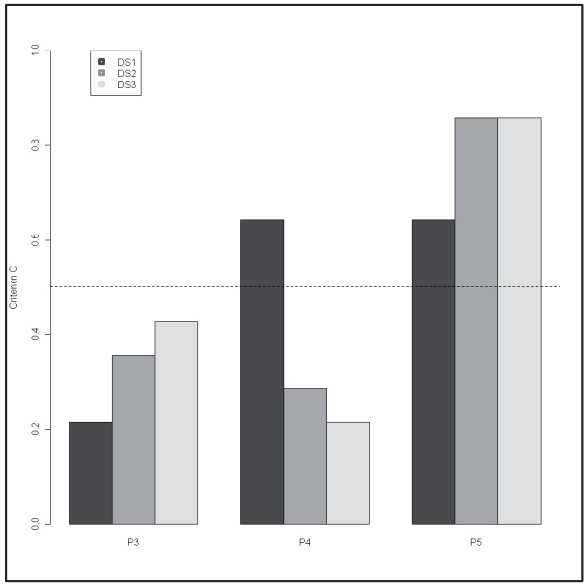
Our results demonstrated that field aggressiveness levels can be explained by differences in aggressiveness levels on components of the host-pathogen interaction. The low field aggressiveness level of isolate P3 could be due to its low aggressiveness for all sporulation components (SPL, LS, and SPS). The low field aggressiveness level of isolate P4 could be due to its low aggressiveness for IE and LP. The high field aggressiveness level of isolate P5 could be due to its high aggressiveness for IE, LP, LS and SPS.

Figure 1: Profiles of aggressiveness of isolates P3, P4, and P5 for (a) components of resistance, (b) disease severity and (c) corrected disease severity. Criterion $C(\Theta,i)$ is defined as the proportion of cultivars for which the isolate i had the greatest estimated value of Θ, with Θ been each component of resistance [infection efficiency (IE), latent period (LP), spore production per lesion (SPL), lesion size (LS) and spore production by sporulating surface (SPS)]; disease severity (DSi) and corrected disease severity (cDSi) at different times of epidemic developement (i=1, 2, or 3). Horizontal dashed line indicate $C(\Theta,i)$=0.5, used as a treeshold value to consider an isolate less or more aggressive than the others.

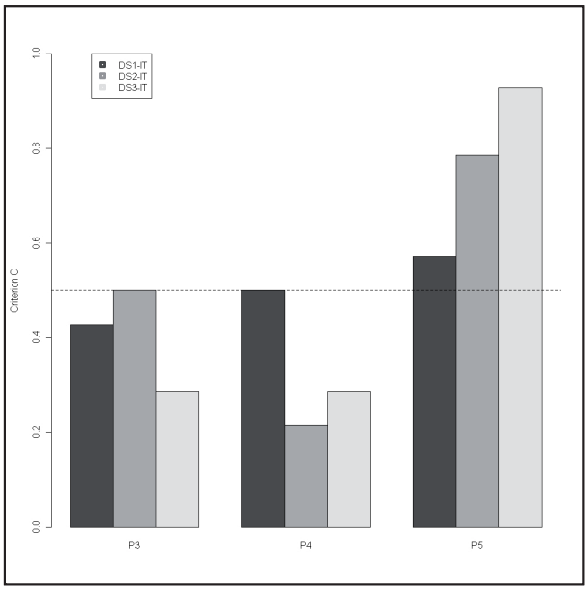
a



b



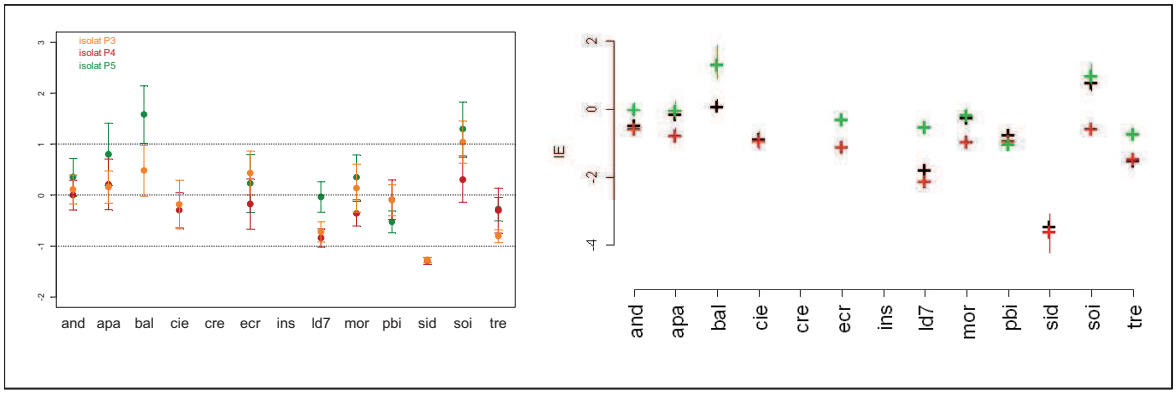
c



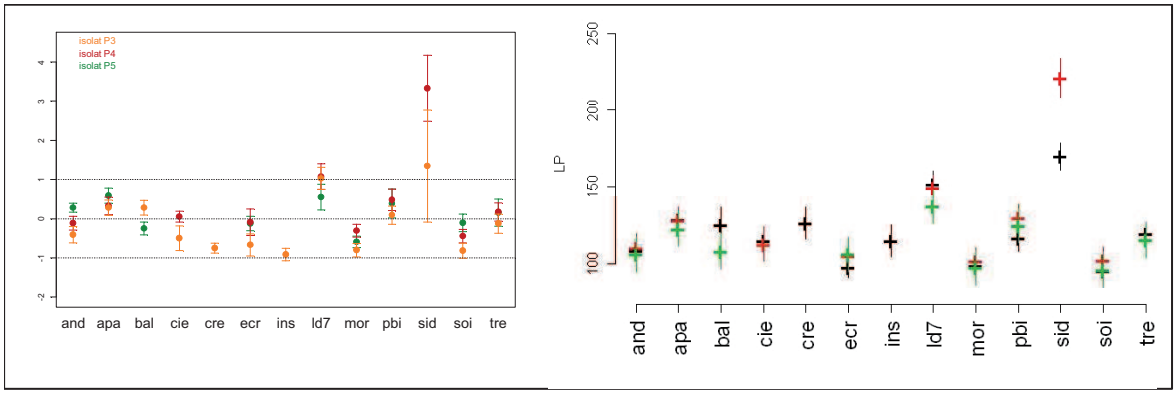
Appendix 2. Validation of the models and supplementary materials.

Figure 1. Comparison between observed (mean +/- confidence interval at 95%) and estimated (median +/- confidence interval at 95%) aggressivenenss components for each cultivar-isolate combination. (a) infection efficiency, (b) latent period, (c) lesion size, (d) spore production per unit of sporulating tissue, and (e) spore production per lesion. Left, measured values; right, estimated values. Black, red, and green symbols stand for isolates P3, P4, and P5, respectively.

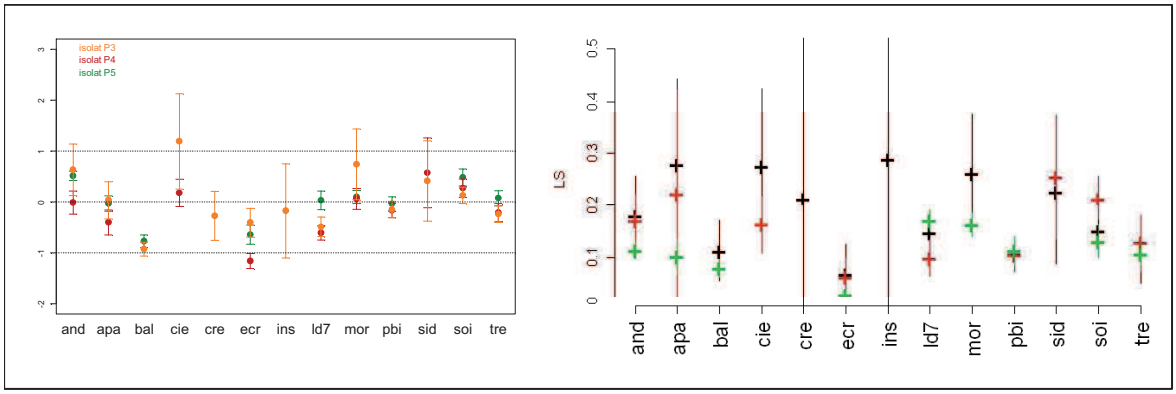
a



b



c



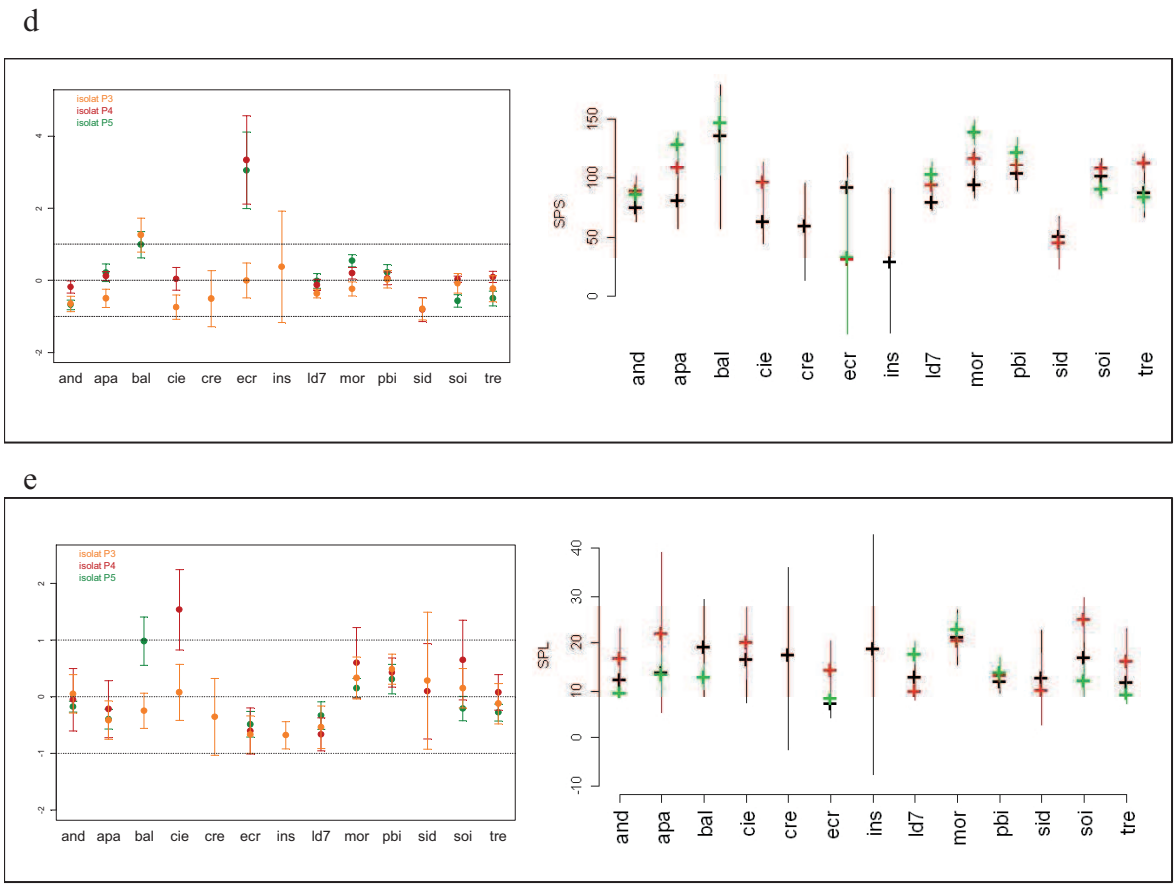
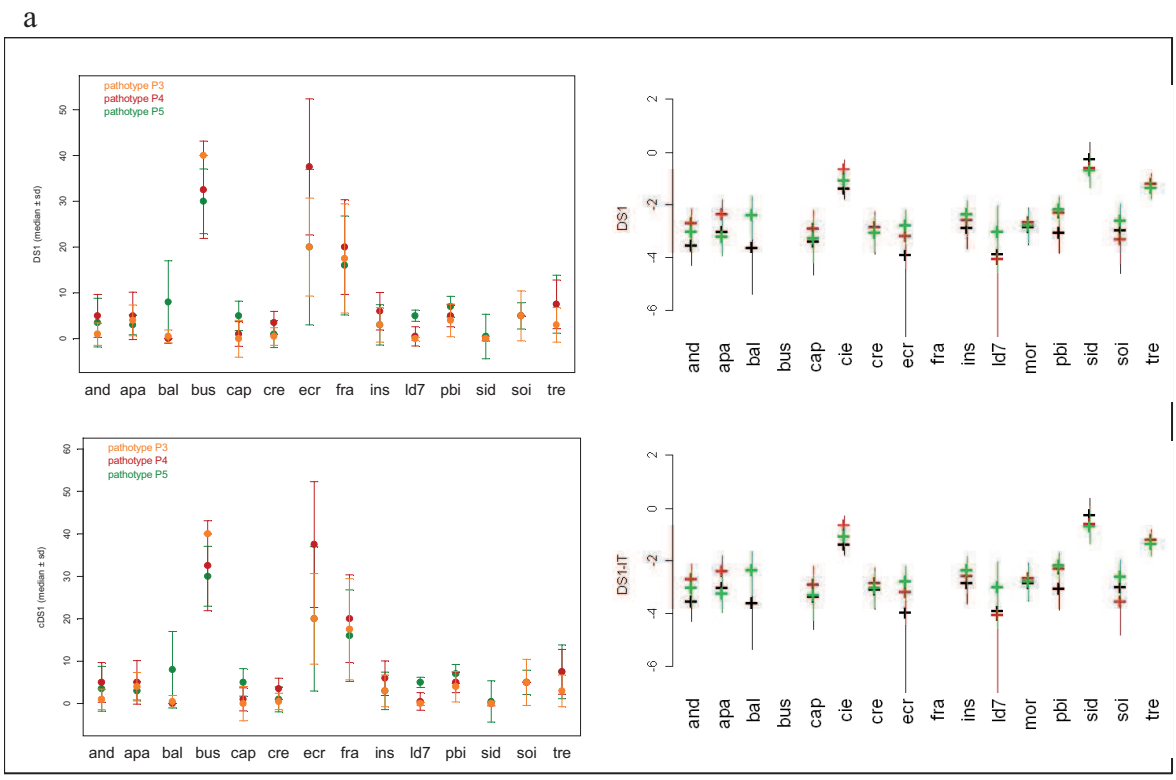
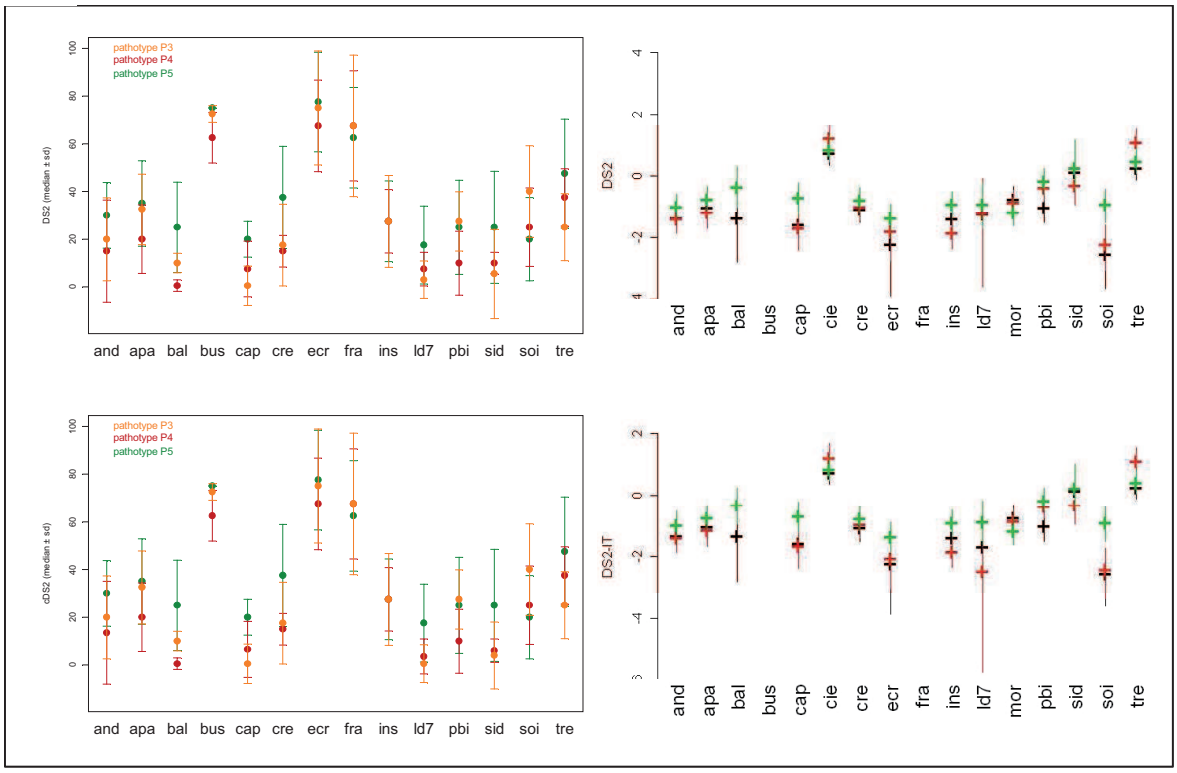


Figure 2. Comparison between observed (mean +/- confidence interval at 95%) and estimated (median +/- confidence interval at 95%) field traits for each cultivar-isolate combination. (a) assessment date 1, (b) assessment date 2 , (c) assessment date 3. Left, measured values; right, estimated values. Black, red, and green symbols stand for isolates P3, P4, and P5, respectively.



b


pathotype P3
pathotype P4
pathotype P5
DS2 (median ± sd)
cDS2 (median ± sd)
DS2
DS2-IT
and apa bal bus cap cie cre ecr fra ins ld7 mor pbi sid soi tre

c

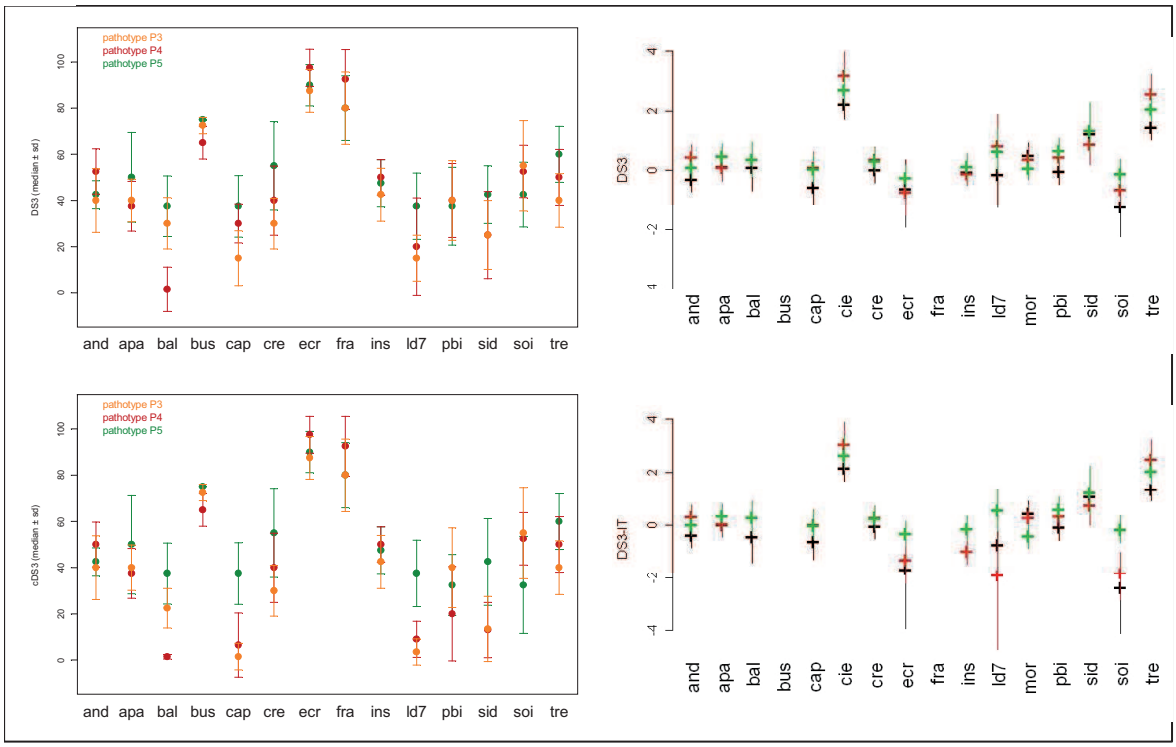
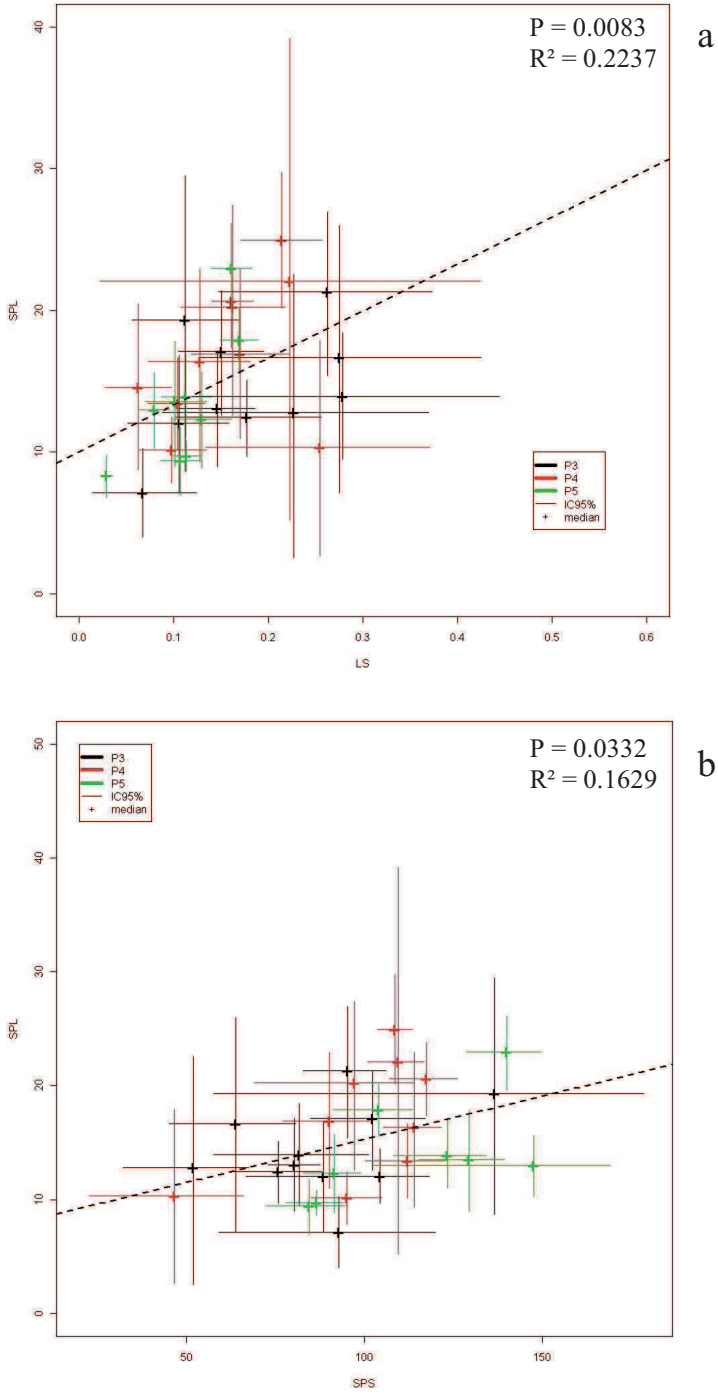

pathotype P3
pathotype P4
pathotype P5
DS3 (median ± sd)
cDS3 (median ± sd)
DS3
DS3-IT
and apa bal bus cap cie cre ecr fra ins ld7 mor pbi sid soi tre

Figure 3. Correlation between variables related to spore production. (a) spore production per lesion (SPL) vs. lesion size (LS), (b) SPL vs SPS (spore production per unit of sporulating tissue). The median values and the 95% confidence interval of posterior estimation of parameters are indicated for each cultivar-isolate combination. Black, red, and green symbols stand for isolates P3, P4, and P5, respectively. Black dashed line was drawn from linear regression (associated probability and R^2 value for the slope are indicated).



Annexe Chapitre 3

Supplementary materials

Supplementary materials

Table 1: Description of all QTLs founded by composite interval marker analysis. For each QTL, the experiment-replicate-trait conditions where it was found with a LOD value >2 are given.

QTL name	Linkage group	Pick Marker	Marker position (cM)	Trait[a]	Isolate	Experiment	Additivity	LOD value	LOD rank	R^2
Qlr.inra-1Aa	1Aa	*Pt733361*	0.0	N2(4)	P5	Cappelle 2009	-5.25	2.95	1	0.104
Qlr.inra-1Aa	1Aa	*Pt733361*	0.0	N1(5)	P5	Cappelle 2010 Rep. 1	-1.63	3.09	1	0.090
Qlr.inra-1Aa	1Aa	*Pt733361*	0.0	AUDPC	P5	Cappelle 2010 Rep. 1	-138.83	3.43	1	0.083
Qlr.inra-1Aa	1Aa	*Pt733361*	0.0	N2(5)	P5	Cappelle 2010 Rep. 2	-4.63	3.04	1	0.086
Qlr.inra-1Aa	1Aa	*Pt733361*	0.0	N2(5)	P5	Cappelle 2010 Rep. 1	-5.51	2.77	1	0.064
Qlr.inra-1Aa	1Aa	*Pt3870*	6.3	N3(5)	P5	Cappelle 2010 Rep. 2	-9.93	4.97	2	0.136
Qlr.inra-1Aa	1Aa	*Pt3870*	6.3	AUDPC	P5	Cappelle 2010 Rep. 2	-128.95	3.09	1	0.086
Qlr.inra-1Aa	1Aa	*Pt671596*	9.7	N3(5)	P5	Cappelle 2010 Rep. 1	-10.67	3.62	1	0.088
Qlr.inra-2Ab	2Ab	*Pt0568*	0.0	AUDPC	P5	Maisse 2009 Rep. 1	-158.93	2.55	1	0.056
Qlr.inra-2Ab	2Ab	*Pt740658*	7.2	SPL	P5	G2	0.00	2.60	1	0.082
Qlr.inra-2Ab	2Ab	*Pt6431*	15.6	N4(5)	P5	Cappelle 2010 Rep. 1	-8.45	2.50	1	0.075
Qlr.inra-2Ab	2Ab	*Pt5027*	17.9	N2(2)	P3	Cappelle 2010 Rep. 2	-7.75	3.16	1	0.117
Qlr.inra-2Ab	2Ab	*Pt8464*	19.0	SPL	P5	G1	0.00	2.07	1	0.067
Qlr.inra-2Ab	2Ab	*un3*	23.5	LS	P5	G1	-0.05	3.46	1	0.122
Qlr.inra-2B	2B	*gpw3032*	155.1	N5(5)	P5	Maisse 2009 Rep. 1	14.22	4.26	2	0.079
Qlr.inra-2B	2B	*gwm120*	167.5	AUDPC	P5	Maisse 2009 Rep. 2	262.21	7.70	3	0.168
Qlr.inra-2B	2B	*gwm120*	167.5	AUDPC	P5	Maisse 2009 Rep. 1	265.76	6.47	2	0.150
Qlr.inra-2B	2B	*gpw4043*	172.0	N5(5)	P5	Maisse 2009 Rep. 2	12.61	3.67	1	0.082
Qlr.inra-2B	2B	*Pt9350*	177.8	N4(5)	P5	Maisse 2009 Rep. 2	10.60	3.01	1	0.064
Qlr.inra-2B	2B	*Pt9350*	177.8	LP	P5	G1	13.31	10.77	3	0.369
Qlr.inra-2B	2B	*Pt4133*	180.1	AUDPC	P5	Cappelle 2009	113.32	2.46	1	0.091
Qlr.inra-2D	2D	*Pt8330*	33.0	N1(3)	P5	Maisse 2010 Rep. 2	6.54	4.30	2	0.155
Qlr.inra-2D	2D	*Pt6419*	42.2	N3(3)	P5	Maisse 2010 Rep. 2	13.55	3.76	1	0.153
Qlr.inra-2D	2D	*Pt6419*	42.2	N1(4)	P3	Cappelle 2009	6.86	4.97	2	0.300
Qlr.inra-2D	2D	*Pt6419*	42.2	N2(2)	P3	Cappelle 2010 Rep. 1	11.13	3.30	1	0.133
Qlr.inra-2D	2D	*Pt6419*	42.2	N4(5)	P5	Cappelle 2010 Rep. 2	18.00	7.09	3	0.284
Qlr.inra-2D	2D	*Pt6419*	42.2	AUDPC	P3	Cappelle 2010 Rep. 1	42.40	3.23	1	0.124
Qlr.inra-2D	2D	*Pt6419*	42.2	N3(5)	P5	Cappelle 2010 Rep. 2	18.45	12.84	3	0.464
Qlr.inra-2D	2D	*Pt6419*	42.2	N2(5)	P5	Cappelle 2010 Rep. 2	8.45	8.18	3	0.279
Qlr.inra-2D	2D	*Pt6419*	42.2	N2(5)	P5	Maisse 2009 Rep. 2	3.55	5.17	2	0.124
Qlr.inra-2D	2D	*Pt6419*	42.2	AUDPC	P5	Cappelle 2010 Rep. 2	279.43	10.83	3	0.395
Qlr.inra-2D	2D	*Pt6419*	42.2	N2(5)	P5	Cappelle 2010 Rep. 1	13.54	12.90	3	0.395
Qlr.inra-2D	2D	*gpw3320*	63.1	N2(4)	P3	Cappelle 2009	9.08	2.67	1	0.096
Qlr.inra-2D	2D	*gpw3320*	63.1	N1(5)	P5	Cappelle 2010 Rep. 1	2.69	7.55	3	0.247
Qlr.inra-2D	2D	*gpw3320*	63.1	N3(5)	P5	Cappelle 2010 Rep. 1	20.04	9.14	3	0.309
Qlr.inra-2D	2D	*gpw3320*	63.1	AUDPC	P5	Cappelle 2010 Rep. 1	272.81	11.02	3	0.322
Qlr.inra-2D	2D	*gpw3320*	63.1	N1(5)	P5	Cappelle 2010 Rep. 2	2.49	6.66	2	0.242
Qlr.inra-2D	2D	*gpw3320*	63.1	N3(3)	P3	Maisse 2010 Rep. 2	17.90	5.93	2	0.133
Qlr.inra-2D	2D	*gpw3320*	63.1	AUDPC	P3	Maisse 2010 Rep. 2	83.26	3.18	1	0.063
Qlr.inra-2D	2D	*gpw3320*	63.1	N5(5)	P5	Maisse 2009 Rep. 2	12.08	3.35	1	0.075
Qlr.inra-2D	2D	*gpw3320*	63.1	SPS	P5	G1	21.38	11.33	3	0.359
Qlr.inra-2D	2D	*gpw3320*	63.1	N4(5)	P5	Cappelle 2010 Rep. 1	16.26	6.60	2	0.282
Qlr.inra-2D	2D	*gpw3320*	63.1	AUDPC	P3	Maisse 2010 Rep. 1	78.53	2.57	1	0.044
Qlr.inra-3Bb.1	3Bb	*Pt741465*	77.1	N2(2)	P3	Cappelle 2010 Rep. 2	-11.33	3.39	1	0.149
Qlr.inra-3Bb.1	3Bb	*Pt1682*	92.2	SPS	P5	G1	-11.72	3.84	2	0.095
Qlr.inra-3Bb.2	3Bb	*Pt2757*	121.7	N2(5)	P5	Cappelle 2010 Rep. 1	5.25	2.66	1	0.059
Qlr.inra-3Bb.2	3Bb	*Pt1867*	125.4	LP	P5	G2	-8.68	4.82	2	0.177
Qlr.inra-3Bb.2	3Bb	*Pt1867*	125.4	SPL	P5	G2	0.00	2.82	1	0.089
Qlr.inra-3Bb.2	3Bb	*Pt1867*	125.4	IE	P5	G2	0.17	7.24	3	0.236
Qlr.inra-3Bb.2	3Bb	*Pt1867*	125.4	LS	P5	G2	0.05	3.56	1	0.146
Qlr.inra-3Db	3Db	*Pt664804*	63.2	LP	P5	G1	5.45	2.68	1	0.093
Qlr.inra-3Db	3Db	*gpw4163*	80.5	AUDPC	P3	Cappelle 2009	-193.08	2.75	1	0.099
Qlr.inra-3Db	3Db	*gpw4163*	80.5	N3(4)	P5	Cappelle 2009	-42.74	4.20	2	0.151
Qlr.inra-3Db	3Db	*gpw4163*	80.5	LS	P5	G2	-0.04	2.58	1	0.091
Qlr.inra-3Db	3Db	*gpw4163*	80.5	LP	P5	G2	5.46	2.26	1	0.074
Qlr.inra-4Bb	4Bb	*Pt3608*	17.6	SPS	P5	G2	-6.91	2.28	1	0.078
Qlr.inra-4Bb	4Bb	*tPt4214*	26.5	N2(3)	P3	Maisse 2010 Rep. 2	-12.54	4.13	2	0.119
Qlr.inra-4Bb	4Bb	*tPt4214*	26.5	AUDPC	P3	Maisse 2010 Rep. 2	-106.00	4.09	2	0.103
Qlr.inra-4Da	4Da	*cfd54*	0.0	N1(3)	P5	Maisse 2010 Rep. 1	6.62	5.60	2	0.197
Qlr.inra-4Da	4Da	*cfd54*	0.0	N2(3)	P5	Maisse 2010 Rep. 1	7.37	4.23	2	0.137
Qlr.inra-4Da	4Da	*cfd54*	0.0	AUDPC	P5	Maisse 2010 Rep. 1	60.05	4.32	2	0.138
Qlr.inra-4Da	4Da	*cfd54*	0.0	LS	P5	G1	0.05	2.71	1	0.099
Qlr.inra-4Da	4Da	*cfd54*	0.0	SPL	P5	G2	0.00	2.86	1	0.092
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*Pt7720*	0.0	AUDPC	P5	Cappelle 2010 Rep. 1	136.28	3.01	1	0.082
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*Pt8040*	21.8	AUDPC	P3	Maisse 2009 Rep. 1	154.83	4.09	2	0.084
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*Pt8040*	21.8	N2(3)	P3	Maisse 2009 Rep. 1	10.66	2.81	1	0.056
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*Pt8040*	21.8	N3(3)	P5	Maisse 2010 Rep. 1	9.47	3.11	1	0.118
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*Pt8040*	21.8	N2(5)	P5	Maisse 2009 Rep. 2	2.98	3.30	1	0.087
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*Pt8040*	21.8	N2(5)	P5	Cappelle 2010 Rep. 1	6.58	3.59	1	0.092

Qlr.inra-5Bb/7Bb	5Bb/7Bb	*wmc517*	54.9	N1(5)	P5	Cappelle 2010 Rep. 1	1.50	2.60	1	0.075
Qlr.inra-5Bb/7Bb	5Bb/7Bb	*wmc517*	54.9	AUDPC	P3	Maisse 2010 Rep. 2	73.78	2.48	1	0.048
Qlr.inra-6Aa	6Aa	*Pt671799*	11.3	LP	P5	G1	-5.38	3.29	1	0.093
Qlr.inra-6Aa	6Aa	*Pt667780*	18.6	N3(4)	P3	Cappelle 2009	15.85	5.49	2	0.187
Qlr.inra-6Aa	6Aa	*Pt731250*	21.1	N3(5)	P5	Cappelle 2010 Rep. 2	18.32	3.50	1	0.097
Qlr.inra-6Aa	6Aa	*Pt731861*	29.2	AUDPC	P3	Cappelle 2009	177.15	2.86	1	0.113
Qlr.inra-6Aa	6Aa	*Pt1742*	31.9	N4(4)	P3	Cappelle 2009	17.69	6.22	2	0.215
Qlr.inra-6Aa	6Aa	*Pt1742*	31.9	N5(5)	P5	Maisse 2009 Rep. 2	11.91	3.60	1	0.080
Qlr.inra-6B	6B	*Pt4716*	0.0	N2(3)	P5	Maisse 2010 Rep. 2	10.91	2.88	1	0.122
Qlr.inra-6B	6B	*Pt4716*	0.0	AUDPC	P5	Maisse 2010 Rep. 2	100.34	4.68	2	0.190
Qlr.inra-6B	6B	*Pt4716*	0.0	N3(3)	P5	Maisse 2010 Rep. 2	13.51	3.83	1	0.154
Qlr.inra-6B	6B	*Pt4716*	0.0	N1(3)	P5	Maisse 2010 Rep. 2	9.15	6.79	2	0.292
Qlr.inra-6B	6B	*Pt4388*	16.3	N3(3)	P3	Maisse 2009 Rep. 1	11.78	3.70	1	0.034
Qlr.inra-7Aa	7Aa	*Pt0639*	0.0	N3(5)	P5	Maisse 2009 Rep. 1	18.78	15.33	3	0.540
Qlr.inra-7Aa	7Aa	*Pt0639*	0.0	N4(5)	P5	Maisse 2009 Rep. 1	40.71	17.60	3	0.518
Qlr.inra-7Aa	7Aa	*Pt1023*	8.7	N1(3)	P3	Maisse 2010 Rep. 2	8.27	8.73	3	0.306
Qlr.inra-7Aa	7Aa	*Pt1023*	8.7	N3(3)	P3	Maisse 2010 Rep. 2	23.98	14.33	3	0.411
Qlr.inra-7Aa	7Aa	*Pt1023*	8.7	N3(5)	P5	Maisse 2009 Rep. 2	16.04	15.55	3	0.420
Qlr.inra-7Aa	7Aa	*Pt1023*	8.7	N2(3)	P3	Maisse 2009 Rep. 2	18.23	17.53	3	0.501
Qlr.inra-7Aa	7Aa	*Pt1023*	8.7	N3(3)	P3	Maisse 2009 Rep. 2	20.02	11.42	3	0.372
Qlr.inra-7Aa	7Aa	*Pt6495*	12.5	AUDPC	P3	Maisse 2009 Rep. 2	220.61	10.61	3	0.362
Qlr.inra-7Aa	7Aa	*Pt6495*	12.5	N5(5)	P5	Maisse 2009 Rep. 2	29.69	14.39	3	0.468
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N3(3)	P3	Maisse 2009 Rep. 1	51.29	33.40	3	0.694
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	AUDPC	P3	Maisse 2009 Rep. 1	298.17	12.20	3	0.313
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N1(3)	P3	Maisse 2010 Rep. 1	11.25	11.43	3	0.416
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	AUDPC	P3	Maisse 2010 Rep. 1	169.82	11.08	3	0.290
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N1(3)	P3	Maisse 2009 Rep. 2	10.86	25.55	3	0.631
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N2(3)	P3	Maisse 2010 Rep. 2	19.97	10.28	3	0.284
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	AUDPC	P3	Maisse 2010 Rep. 2	207.07	14.00	3	0.372
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N2(5)	P5	Maisse 2009 Rep. 1	3.91	8.15	3	0.292
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N2(5)	P5	Maisse 2009 Rep. 2	6.64	14.03	3	0.404
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	N4(5)	P5	Maisse 2009 Rep. 2	31.64	16.72	3	0.547
Qlr.inra-7Aa	7Aa	*gpw4050*	12.6	AUDPC	P5	Maisse 2009 Rep. 2	464.16	19.61	3	0.595
Qlr.inra-7Aa	7Aa	*Pt0790*	12.6	N3(3)	P3	Maisse 2010 Rep. 1	17.62	7.02	3	0.177
Qlr.inra-7Aa	7Aa	*Pt5533*	13.8	N1(3)	P3	Maisse 2009 Rep. 1	9.75	11.67	3	0.337
Qlr.inra-7Aa	7Aa	*Pt3403*	16.0	N1(5)	P5	Maisse 2009 Rep. 1	0.63	3.00	1	0.125
Qlr.inra-7Aa	7Aa	*Pt1557*	17.2	N2(3)	P3	Maisse 2009 Rep. 1	29.87	17.18	3	0.475
Qlr.inra-7Aa	7Aa	*Pt1557*	17.2	AUDPC	P5	Maisse 2009 Rep. 1	468.75	15.45	3	0.444
Qlr.inra-7Aa	7Aa	*Pt7105*	20.6	N2(3)	P3	Maisse 2010 Rep. 1	14.08	9.67	3	0.263
Qlr.inra-7Aa	7Aa	*Pt7105*	20.6	N5(5)	P5	Maisse 2009 Rep. 1	39.15	19.00	3	0.690
Qlr.inra-7Aa	7Aa	*Pt7105*	20.6	SPL	P5	G2	0.00	3.86	2	0.133
Qlr.inra-7Aa	7Aa	*Pt7105*	20.6	N3(3)	P5	Maisse 2010 Rep. 1	9.04	2.98	1	0.100

[a]Field phenotypic traits for disease severity
(N*i*(*j*): disease severity at assessment date *i*, *j* being the number of assessment dates in the whole experiment)
AUDPC: area under the disease progress curve.
Components of quantitative resistance assessed in greenhouse. IE: infection efficiency, LP: latent period, SPL: spore production per lesion, LS: lesion size, SPS: spore production per unit of sporulating tissue.

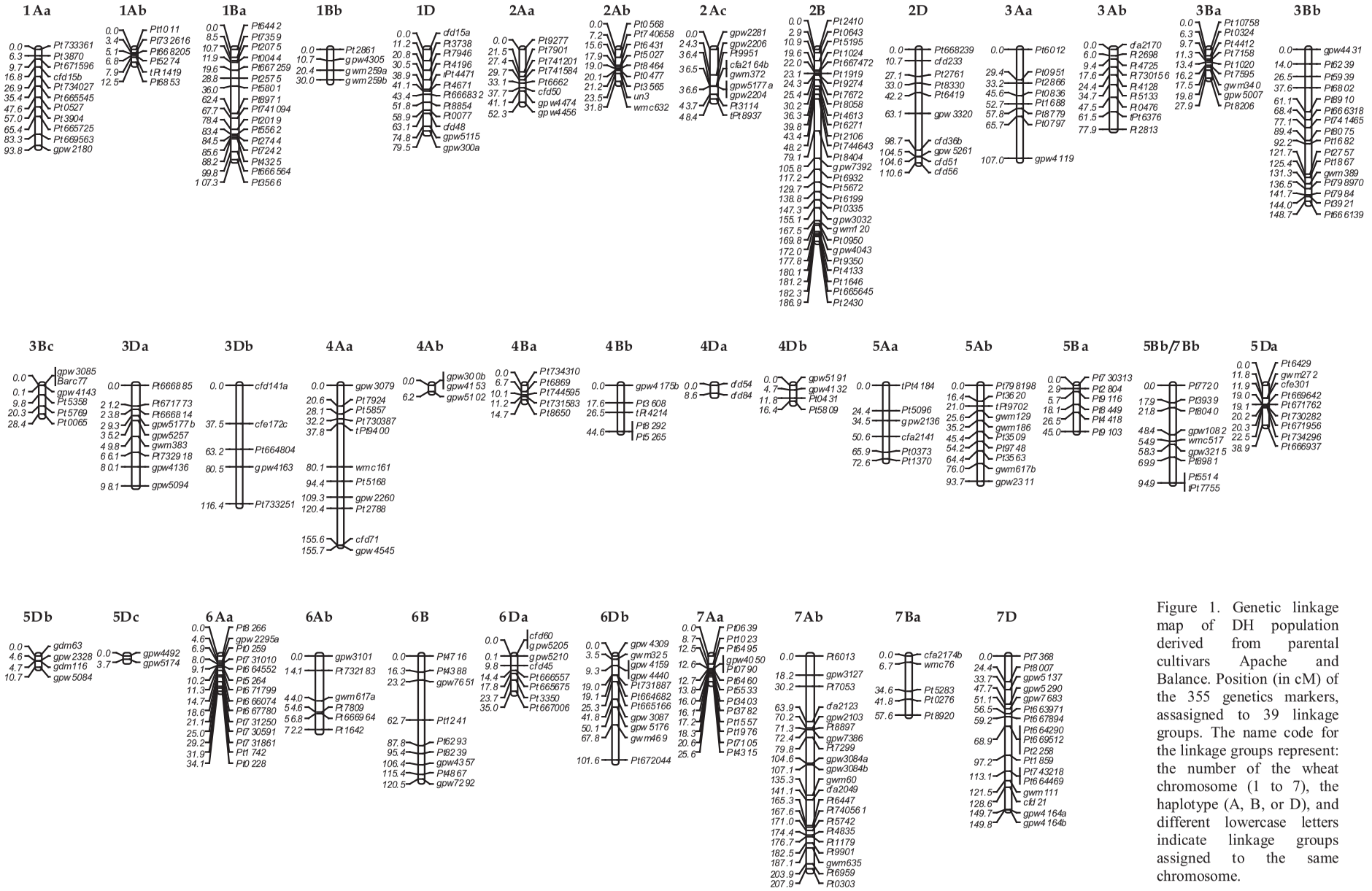


Figure 1. Genetic linkage map of DH population derived from parental cultivars Apache and Balance. Position (in cM) of the 355 genetics markers, assasigned to 39 linkage groups. The name code for the linkage groups represent: the number of the wheat chromosome (1 to 7), the haplotype (A, B, or D), and different lowercase letters indicate linkage groups assigned to the same chromosome.

References bibliographiques

References bibliographiques

Ahmed HU, Mundt CC, Hoffer ME, Coakley SM. 1996. Selective influence of wheat cultivars on pathogenicity of *Mycosphaerella graminicola* (anamorph *Septoria tritici*). *Phytopathology* 86, 454-458.

Azzimonti G, Lannou C, Sache I, Goyeau H. 2012. Components of quantitative resistance to leaf rust in wheat cultivars: diversity, variability and specificity. *Plant Pathology* - accepted

Baart PGJ, Parlevliet JE, Limburg H. 1991. Effects of infection density on the size of barley and wheat leaf rust colonies before and on the size of uredia after the start of sporulation. *Journal of Phytopathology* 131, 59-64.

Ballini E, Morel JB, Droc G, Price A, Courtois B, Notteghem JL, Tharreau D. 2008. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Molecular Plant-Microbe Interactions* 21, 859-868.

Basten CJ, Weir BS, Zheng ZB. 1997. QTL cartographer. A reference manual and tutorial for QTL mapping. Department of Statitics, North Carolina State University: Raleigh.

Bolton MD, Kolmer JA, Garvin DF. 2008. Wheat leaf rust caused by *Puccinia triticina*. *Molecular Plant Pathology* 9, 563-575.

Broers LHM. 1989a. Influence of development stage and host genotype on three components of partial resistance to leaf rust in spring wheat. *Euphytica* 44, 187-195.

Broers LHM. 1989b. Race-specific aspects of partial resistance in wheat to wheat leaf rust, *Puccinia recondita f.sp. tritici*. *Euphytica* 44, 273-282.

Broers LHM, Jacobs T. 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. 2. Number of segregating factors and evidence for transgressive segregation in F3 and F5 generations. *Euphytica* 44, 207-214.

Brown JKM, Tellier A. 2011. Plant-parasite coevolution: Bridging the gap between genetics and ecology. *Annual Review of Phytopathology* 49, 345-367.

Calenge F, Faure A, Goerre M, Gebhardt C, Van de Weg WE, Parisi L, Durel CE. 2004. Quantitative trait toci (QTL) analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. *Phytopathology* 94, 370-379.

Campbell, CL, Madden LV. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York City.

Carlisle DJ, Cooke LR, Watson S, Brown AE. 2002. Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. *Plant Pathology* 51, 424-434.

Caten CE. 1974. Intra-racial variation in *Phytophthora infestans* and adaptation to field resistance for potato blight . *Annals of Applied Biology* 77, 259-270.

Cheatham MR, Rouse MN, Esker PD, Ignacio S, Pradel W, Raymundo R, Sparks AH, Forbes GA, Gordon TR, Garrett KA. 2009. Beyond yield: Plant disease in the context of ecosystem services. *Phytopathology* 99, 1228-1236.

Chin KM, Wolfe MS. 1984. Selection on *Erysiphe graminis* in pure and mixed stands of barley. *Plant Pathology* 33, 535-546.

Chu CG, Friesen TL, Xu SS, Faris JD, Kolmer JA. 2009. Identification of novel QTLs for seedling and adult plant leaf rust resistance in a wheat doubled haploid population. *Theoretical and Applied Genetics* 119, 263-269.

Chung CL, Longfellow JM, Walsh EK, Kerdieh Z, Van Esbroeck G, Balint-Kurti P, Nelson RJ. 2010. Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize - *Setosphaeria turcica* pathosystem. *BMC Plant Biology* 10, 103.

Collard BCY, Jahufer MZZ, Brouwer JL, Pang ECK. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142, 169-196.

Collard BCY, Mackill DJ. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B-Biological Sciences* 363, 557-572.

Cooper M, Van Eeuwijk FA, Hammer GL, Podlich DW, Messina C. 2009. Modeling QTL for complex traits: detection and context for plant breeding. *Current Opinion in Plant Biology* 12, 231-240.

Das MK, Rajaram S, Kronstad WE, Mundt CC, Singh RP. 1993. Associations and genetics of three components of slow rusting in leaf rust of wheat. *Euphytica* 68, 99-109.

Day JP, Shattock RC. 1997. Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. *European Journal of Plant Pathology* 103, 379-391.

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann M, Ellis J, Foster GD. 2012 The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* 13, 414-430.

Dedryver F, Paillard S, Mallard S, Robert O, Trottet M, Nègre S, Verplancke G, Jahier J. 2009. Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. *Phytopathology* 99, 968-973.

Denissen CJM. 1991. Influence of race and post infection temperature on two components of partial resistance to wheat leaf rust in seedlings of wheat. *Euphytica* 58, 13-20.

Denissen CJM, 1993. Components of adult plant resistance to leaf rust in wheat. *Euphytica* 70, 131-140.

Doerge RW. 2002. Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews Genetics* 3, 43-52.

Dowkiw A, Bastien C. 2007. Presence of defeated qualitative resistance genes frequently has major impact on quantitative resistance to *Melampsora larici-populina* leaf rust in *P. xinteramericana* hybrid poplars. *Tree Genetics & Genomes* 3, 261-274.

Drijepondt CS, Pretorius ZA,.1989. Greenhouse evaluation of adult-plant resistance conferred by the gene *Lr34* to leaf rust of wheat. *Plant Disease* 73, 669-671.

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS, 1999. Candidate gene analysis of quantitative disease resistance in wheat. *Theoretical and Applied Genetics* 98, 219-225.

Flier WG, Turkensteen LJ. 1999. Foliar aggressiveness of *Phytophthora infestans* in three potato growing regions in the Netherlands. *European Journal of Plant Pathology* 105, 381-388.

Flor HH. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology* 9, 275-296.

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH, Holloway T, Howard EA, Kucharik CJ, Monfreda C, Patz JA, Prentice IC, Ramankutty N, Snyder PK. 2005. Global consequences of land use. *Science* 309, 570-574.

Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmersond M, Morales MB, Ceryngier P, Liira J, Tscharntke T, Winqvist C, Eggers S, Bommarco R, Pärt T, Bretagnolle V, Plantegenest M, Clement LW, Dennis C, Palmer C, Oñate JJ, Guerrero I, Hawro V, Aavik T, Thies C, Flohre A, Hänke S, Fischer C, Goedhart PW, Inchausti P. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology* 11, 97-105.

Geiger HH, Heun M. 1989. Genetics of quantitative resistance to fungal diseases. Annual Review of Phytopathology 27, 317-341.

Gelman A, Carlin JB, Stern HS, Rubin DB. 2004. *Bayesian Data Analysis*, 2nd edition. Chapman and Hall/CRC.

Ghaffary SMT, Robert O, Laurent V, Lonnet P, Margalé E, van der Lee TAJ, Visser RGF, Kema GHJ. 2011. Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache. *Theoretical and Applied Genetics* 123, 741-754.

Goyeau H, Berder J, Czerepak C, Gautier A, Lanen C, Lannou C. 2012. Low diversity and fast evolution in the population of *Puccinia triticina* causing durum wheat leaf rust in France from 1999 to 2009, as revealed by an adapted differential set. *Plant Pathology* 61, 761-772.

Goyeau H, Halkett F, Zapater MF, Carlier J, Lannou C. 2007. Clonality and host selection in the wheat pathogenic fungus *Puccinia triticina*. *Fungal Genetics and Biology* 44, 474-483.

Goyeau H, Lannou C. 2011. Specific resistance to leaf rust expressed at the seedling stage in cultivars grown in France from 1983 to 2007. *Euphytica* 178, 45-62.

Goyeau H, Park R, Schaeffer B, Lannou C. 2006. Distribution of pathotypes with regard to host cultivars in French wheat leaf rust populations. *Phytopathology* 96, 264-273.

Gupta PK, Langridge P, Mir RR. 2010. Marker-assisted wheat breeding: present status and future possibilities. *Molecular Breeding* 26, 145-161.

Herrera-Foessel SA, Lagudah ES, Huerta-Espino J, Hayden MJ, Bariana HS, Singh D, Singh RP. 2011. New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. *Theoretical and Applied Genetics* 122, 239-249.

Herrera-Foessel SA, Singh RP, Huerta-Espino J, Crossa J, Djurle A, Yuen J, 2007. Evaluation of slow rusting resistance components to leaf rust in CIMMYT durum wheats. *Euphytica* 155, 361-369.

Herrera-Foessel SA, Singh RP, Huerta-Espino J, Rosewarne GR, Periyannan SK, Viccars L, Calvo-Salazar V, Lanet C, Lagudah ES. *Lr68*: a new gene conferring slow rusting resistance to leaf rust in wheat. *Theoretical and Applied Genetics* 124, 1475-1486.

Huerta-Espino J, Singh R, Germán S, McCallum BD, Park RF, Chen WQ, Bhardwaj SC, Goyeau H. 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. *Euphytica* 179, 143-160.

Imhoff MW, Leonard KJ, Main CE. 1982. Patterns of bean rust lesion size increase and spore production. *Phytopathology* 72, 441-446.

Isenring R. 2010. *Pesticide and the loss of biodiversity. How intensive pesticide use affects wildlife populations and species diversity*. PAN Europe, London.

Jagger LJ, Newell C, Berry ST, Maccormack R, Boyd LA. 2011. Histopathology provides a phenotype by which to characterize stripe rust resistance genes in wheat. *Plant Pathology* 60, 640-648.

Johnson R, Taylor AJ. 1976. Spore yield of pathogens in investigations of race-specificity of host resistance. *Annual Review of Phytopathology* 14, 97-119.

Jorge V, Dowkiw A, Faivre-Rampant P, Bastien C, 2005. Genetic architecture of qualitative and quantitative *Melampsora larici-populina* leaf rust resistance in hybrid poplar: genetic mapping and QTL detection. *New Phytologist* 167, 113-127.

Kelm C, Ghaffary MST, Bruelheide H, Röder MS, Miersch S, Weber WE, Kema GHJ, Saal B. 2011. The genetic architecture of seedling resistance to *Septoria tritici* blotch in the winter wheat doubled-haploid population Solitär × Mazurka. *Molecular Breeding* 29, 813-830.

Knott EA, Mundt CC. 1991. Latent period and infection efficiency of *Puccinia recondita* f.sp. *tritici* populations isolated from different wheat cultivars. *Phytopathology* 81, 435-439.

Kolmer JA, Ordonez ME, Groth JV. 2009. The Rust Fungi. In: *Encyclopedia of Life Sciences (ELS).* John Wiley & Sons, Ltd: Chichester.

Krenz JE, Sackett KE, Mundt CC. 2008. Specificity of incomplete resistance to *Mycosphaerella graminicola* in wheat. *Phytopathology* 98, 555-561.

Lagudah ES. 2011. Molecular genetics of race non-specific rust resistance in wheat. *Euphytica* 179, 81-91.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L. 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181

Lannou C, Soubeyrand S. 2012. Measure of life-cycle traits of a biotrophic pathogen. In: Stevenson KL, Jeger MJ, eds. *Exercises in plant disease epidemiology*, second edition, St. Paul, USA: APS.

Lee TS, Shaner G. 1985. Oligogenic inheritance of length of latent peiod in 6 slow leaf-rusting wheat cultivars. *Phytopathology* 75, 636-643.

Lehman JS, Hanson KA, Shaner G. 2005. Relationship among genes conferring partial resistance to leaf rust (*Puccinia triticina*) in wheat lines CI 13227 and L-574-1. *Phytopathology* 95, 198-205.

Lehman JS, Shaner G. 1996. Genetic variation in latent period among isolates of *Puccinia recondita* f. sp. *tritici* on partially resistant wheat cultivars. *Phytopathology* 86, 633-641.

Lehman JS, Shaner G. 1997. Selection of populations of *Puccinia recondita* f. sp. tritici for shortened latent period on a partially resistant wheat cultivar. *Phytopathology* 87, 170-176.

Lehman JS, Shaner G, 2007. Heritability of latent period estimated from wild-type and selected populations of *Puccinia triticina*. *Phytopathology* 97, 1022-1029.

Leonard KJ. 1969. Factors affecting rates of stem rust increase in mixed plantings of susceptible and resistant oat varieties. *Phytopathology* 59, 1845-1850.

Lincoln SE, Daly MJ, Lander ES. 1992. Constructing genetic maps with MAPMAKER/EXP ver. 3.0, 3rd edn. Whitehead Institute Technical Report, Whitehead Institute: Cambridge, Mass.

Mallard S, Nègre S, Pouya S, Gaudet D, Lu ZX, Dedryver F. 2008. Adult plant resistance-related gene expression in 'Camp Remy' wheat inoculated with *Puccinia striiformis*. *Molecular Plant Pathology* 9, 213-225.

Marcel TC, Gorguet B, Ta MT, Kohutova Z, Vels A, Niks RE. 2008. Isolate specificity of quantitative trait loci for partial resistance of barley to *Puccinia hordei* confirmed in mapping populations and near-isogenic lines. *New Phytologist* 177, 743-355.

Marone D, Del Olmo AI, Laidò G, Sillero JC, Emeran AA, Russo MA, Ferragonio P, Giovanniello V, Mazzucotelli E, De Leonardis AM, De Vita P, Blanco A, Cattivelli L, Rubiales D, Mastrangelo AM. 2009. Genetic analysis of durable resistance against leaf rust in durum wheat. *Molecular Breeding* 24, 25-39.

Milus EA, Line RF. 1980. Characterization of resistance to leaf rust in Pacific Northwest wheats. *Phytopathology* 70, 167-172.

Mundt C, Cowger C, Garrett K. 2002. Relevance of integrated disease management to resistance durability. *Euphytica* 124, 245-252.

Negussie T, Pretorius ZA, Bender CM. 2005. Components of rust resistance in lentil. *Euphytica* 142, 55-64.

Niks RE, Marcel TC, 2009. Nonhost and basal resistance: how to explain specificity? *New Phytologist* 182, 817-828.

Ohm HW, Shaner GE. 1976. Three components of slow leaf-rusting at different growth stages in wheat. *Phytopathology* 66, 1356-1360.

Palloix A, Ayme V, Moury B. 2009. Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytologist* 183, 190-199.

Papaïx J, Goyeau H, Du Cheyron P, Monod H, Lannou C. 2011. Influence of cultivated landscape composition on variety resistance: an assessment based on wheat leaf rust epidemics. New Phytologist 191, 1095-1107.

Pariaud B, Ravigné V, Halkett F, Goyeau H, Carlier J, Lannou C, 2009a. Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology* 58, 409-424.

Pariaud B, Robert C, Goyeau H, Lannou C. 2009b. Aggressiveness components and adaptation to a host cultivar in wheat leaf rust. *Phytopathology* 99, 869-878.

Parlevliet JE. 1979. Components of resistance that reduce the rate of epidemic development. *Annual Review of Phytopathology* 17, 203-222.

Parlevliet JE. 1986. Pleiotropic association of infection frequency and latent period of two barley cultivars partially resistant to barley leaf rust. *Euphytica* 35, 267-272.

Parlevliet JE. 2002. Durability of resistance against fungal, bacterial and viral pathogens; present situation. *Euphytica* 124, 147-156.

Parlevliet JE, Zadoks JC. 1977. Inegrated concept of disease resistance – New view including horizontal and vertical resistance in plants. *Euphytica* 26, 5-21.

Peterson RF, Campbell AB, Hannah AE. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research, Section C* 26: 496–500

Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ. 2009. Shades of gray: the world of quantitative disease resistance. *Trends in Plant Science* 14, 21-29.

Plummer M. 2010. *JAGS Version 2.2.0 manual* (November 7, 2010). URL http: //mcmc-jags.sourceforge.net.

Qi X, Niks RE, Stam P, Lindhout P. 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. *Theoretical and Applied Genetics* 96, 1205-1215.

Ramburan VP, Pretorius ZA, Louw JH, Boyd LA, Smith PH, Boshoff WHP, Prins R. 2004. A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar Kariega. *Theoretical and Applied Genetics* 108, 1426-1433.

Richardson K, Vales M, Kling J, Mundt C, Hayes P. 2006. Pyramiding and dissecting disease resistance QTL to barley stripe rust. *Theoretical and Applied Genetics* 113, 485-495.

Robert C, Bancal MO, Lannou C. 2002. Wheat leaf rust uredospore production and carbon and nitrogen export in relation to lesion size and density. *Phytopathology* 92, 762-768.

Robert C, Bancal MO, Lannou C. 2004. Wheat leaf rust uredospore production on adult plants: Influence of leaf nitrogen content and *Septoria tritici* blotch. *Phytopathology* 94, 712-721.

Robert C, Bancal MO, Ney B, Lannou C. 2005. Wheat leaf photosynthesis loss due to leaf rust, with respect to lesion development and leaf nitrogen status. *New Phytologist* 165, 227-241.

Robinson RA, Sutherland WJ. 2002. Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology* 39, 157-716.

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MT, Leroy P, and Ganala MW. 1998. A microsatellite map of wheat. *Genetics* 149, 2007-2023.

Roelfs AP, Singh RP, Saari EE. 1992. *Rust diseases of wheat: concepts and methods of disease management*. CIMMYT: Mexico DF.

Sache I. 1997. Effect of density and age of lesions on sporulation capacity and infection efficiency in wheat leaf rust (*Puccinia recondita* f.sp. *tritici*). *Plant Pathology* 46, 581-589.

Sache I., de Vallavieille-Pope C. 1993. Comparison of the wheat brown and yellow rusts for monocyclic sporulation and infection processes, and their polycyclic consequences. *Journal of Phytopathology* 138, 55-65.

Sackett KE, Mundt CC. 2005. The effects of dispersal gradient and pathogen life cycle components on epidemic velocity in computer simulations. *Phytopathology* 95, 992-1000.

Samborski DJ. 1985. Occurrence and virulence of *Puccinia recondita* in Canada in 1984. *Canadian Journal of Plant Pathology* 7, 428-430.

Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, Keller B. 2004. Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. *Theoretical and Applied Genetics* 108, 477-484.

Shaner G. 1980. Probits for analyzing latent period data in studies of slow rusting resistance. *Phytopathology* 70, 1179-1182.

Shaner G, Hess FD. 1978. Equations for integrating scusceptible components of slow leaf-rusting resistance in wheat. *Phytopathology* 68, 1464-1469.

Shaner G, Ohm HW, Finney RE. 1978. Response of susceptible and slow leaf-rusting wheats to infection by Puccinia recondita. *Phytopathology* 68, 471-475.

Simko I, Piepho HP. 2012. The Area Under the Disease Progress Stairs: Calculation, advantage, and application. *Phytopathology* 102, 381-389.

Singh RP, Huerta-Espino J. 2003. Effect of leaf rust resistance gene *Lr34* on components of slow rusting at seven growth stages in wheat. *Euphytica* 129, 371-376.

Singh RP, Huerta-Espino J, Bhavani S, Herrera-Foessel SA, Singh D, Singh PK, Velu G, Mason RE, Jin Y, Njau P, Crossa J. 2011. Race non-specific resistance to rust diseases in CIMMYT spring wheats. *Euphytica* 179, 175-186.

Singh RP, Payne TS, Rajaram S.1991. Characterization of variability and relationships among components of partial resistance to leaf rust in CIMMYT bread wheats. Theoretical and Applied Genetics 82, 674-860.

St. Clair, DA. 2010. Quantitative disease resistance and quantitative resistance loci in breeding. *Annual Review of Phytopathology* 48, 247-268.

Stukenbrock EH, Mcdonald BA. 2008. The origins of plant pathogens in agro-ecosystems. *Annual Review of Phytopathology* 46, 75-100.

Stuthman DD, Leonard KJ, Miller-Garvin J. 2007. Breeding crops for durable resistance to disease. *Advances in Agronomy* 95, 319-367.

Talukder ZI, Tharreau D, Price AH. 2004. Quantitative trait loci analysis suggests that partial resistance to rice blast is mostly determined by race–specific interactions. *New Phytologist* 162, 197-209.

Thabuis A, Lefebvre V, Bernard G, Daubèze AM, Phaly T, Pochard E, Palloix A. 2004. Phenotypic and molecular evaluation of a recurrent selection program for a polygenic resistance to *Phytophthora capsici* in pepper. *Theoretical and Applied Genetics* 109, 342-351.

Van der Plank JE, 1968. *Disease Resistance in Plants*. New York, USA: Academic Press.

Van Eujwijk FA, Bink MCAM, Chenu K, Chapman SC. 2010. Detection and use of QTL for complex traits in multiple environments. *Current Opinion in Plant Biology* 13, 193-205.

Villaréall LMMA, Lannou C. 2000. Selection for increased spore efficacy by host genetic background in a wheat powdery mildew population. *Phytopathology* 90, 1300-1306.

Wang GL, Mackill DJ, Bonman JM, Mccouch SR, Champoux MC, Nelson RJ. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a surably resistant rice cultivar. *Genetics* 136, 1421-1434.

Wang LJ, Wang YJ, Wang Z, Marcel TC, Niks RE, Qi XQ. 2010. The phenotypic expression of QTLs for partial resistance to barley leaf rust during plant development. *Theoretical and Applied Genetics* 121, 857-864.

Wolfe MS. 1973. Changes and diversity in populations of fungal pathogens. *Annals of Applied Biology* 75, 132-136.

Xu XY, Bai GH, Carver BF, Shaner GE, Hunger RM. 2005. Mapping of QTLs prolonging the latent period of *Puccinia triticina* infection in wheat. *Theoretical and Applied Genetics* 110, 244-251.

Young, ND. 1996. QTL mapping and quantitative disease resistance in plants. *Annual Review of Phytopathology* 34, 479-501.

Zadoks JC, Bouwman JJ. 1985. Epidemiology in Europe. In *The cereal rusts*, vol. 2. Roelfs AP & Bushnell WR (eds.), Orlando: Academic Press.

Zeng ZB. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Sciences of the United States of America* 90, 10972-1096.

Zeng ZB. 1994. Precision mapping of quantitative trait loci. *Genetics* 136, 1457-1468.